Handbook T-XIII
CIERMMI Women in Science
Medicine and Health Sciences

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ECORFAN CIERMMI Women in Science

Volume XIII

The Handbook will offer volumes of selected contributions from researchers who contribute to the scientific dissemination activity of the Colegio de Ingenieros en Energías Renovables de Querétaro A.C. in their areas of research in Medicine and Health Sciences. In addition to having a total evaluation, in the hands of the directors of the Colegio de Ingenieros en Energías Renovables de Querétaro A.C., the quality and timeliness of its chapters, each individual contribution was refereed to international standards (RESEARCH GATE, MENDELEY, GOOGLE SCHOLAR and REDIB), the Handbook thus proposes to the academic community, recent reports on new developments in the most interesting and promising areas of research in the Medicine and Health Sciences.

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CIERMMI Women in Science T-XIII
Medicine and Health Sciences
Handbooks

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Prologue

Women in health and medical science. It is important to recognize and respect the impact that women have had on scientific evolution. This space provides generous information on recent scientific findings of women in medical and health sciences. It is gratifying to be a participant in the interdisciplinary goal of CIERMMI, which surely intends to consolidate in the future as a transdisciplinary space, the main objective of science. As researchers, our hobby, not our job, is to solve doubts, the "heart of the matter", to give the core answer to a problem by checking and re-checking and reaffirming and demonstrating to the scientific community again and again, if necessary, the veracity of our results. This manual presents a space for discussion and reflection on topics of interest developed by women in science. Owners of a prodigious and exhaustive pen, they take us by the hand explaining molecular and behavioral topics. And so, they take us into the importance of screening tests for human papillomavirus, a common sexually transmitted infection. Three research groups offer us data derived from studies on young university students. In two of them the population is of medical students and they analyze health risk behaviors, emotional state and in another one specifically anxiety during the covid 19 pandemic. Let us remember that doctors look after the health of their patients, but who looks after them? they themselves, hence the importance of emotional well-being for their health and that of others, even in the face of a pandemic. The third study in young university students provides us with data on proinflammatory cytokines: leptin and visfatin associated with obesity. It is worth mentioning that sedentary lifestyles and lifestyle changes lead us to chronic inflammatory states such as obesity, a pathological process that in Mexico presents higher figures than the world average. Another group of innovators discusses how early trauma is a conditioning factor of psychopathologies in adult women; a current argument in vogue that affects the individual and harms not only the family, but also society.

Continuing with the wealth of findings present in the manual, we find the evaluation of physical activity and fitness and sedentary behaviors in perimenopausal women; analysis that addresses different points of the present day, for example, in Mexico there is a greater proportion of women than men and menopausal problems are increasing because the population is aging in a technological era linked to sedentary lifestyles. So much so that aging is also accompanied by an increase in the incidence of neurodegenerative processes such as Parkinson's disease. According to the analysis of our research group, neuroglobin is a promising molecule that protects dopaminergic neurons of the substantia nigra, the main brain area damaged in this pathology and the one in charge of controlling body movement, from death. Thanks to the above, we were able to contribute a grain of sand for the development of research on new adjuvant treatments for this disease. And speaking of processes that occur in the skull, experts explain to us how the growth and development of the craniofacial region and the stomatognathic apparatus (mouth and jaws), which in short helps us to speak, eat, socialize and breathe and therefore a deficit in its development can be catastrophic. As well as ectopic eruption and intercanine distance in children, which is the subject of study by another research team. Finally, and to strengthen this manual, a group of scientists examined the uses and adverse effects of general plant toxicology, transdisciplinary information of great utility, for example, to help justify the use of homeopathy in animals, including the self-styled human hominid primate. After appreciating the wealth of results exposed thanks to the sustained effort of the knowledge makers present, I am certain that it will be of your interest to read the cautious information in this manual. Let's get to work.

Vieyra-Reyes, Patricia. PhD

Introduction

The Colegio de Ingenieros en Energías Renovables de Querétaro A.C., A.C. (CIER-QUERÉTARO), and its chapters of Renewable Energy, Industrial Maintenance, Mechatronics and Computer Science, technical sponsors of the International Interdisciplinary Congress on Renewable Energy, Maintenance, Mechatronics and Computer Science, CIERMMI 2021 has as general objective to establish a space for discussion and reflection on issues related to the areas of: renewable energy, industrial maintenance, mechatronics and computer science with the participation of students, professors, researchers and national and international speakers, promoting the formation and consolidation of research networks. Contributing to provide a space for dissemination and discussion of the presentations of students, graduates, academics and researchers, representatives of various higher education institutions, research centers in our country, as well as educational institutions beyond our borders. Promoting the formation of research networks between different institutions. Offering a space for undergraduate, master's, doctoral and postdoctoral students, in which they can present the progress of the research they carry out in their different educational centers. Providing a space in which study groups and members of academic bodies, linked to the curricular program of renewable energy, industrial maintenance, mechatronics and computer science careers, can present the research work developed within their institution and in collaboration with other national or international educational institutions. Establishing a training space for the attendees, through the development of specific lectures and conferences.

This volume, Women in Science T-XIII-2021 contains 10 refereed chapters dealing with these issues, chosen from among the contributions, we gathered some researchers and graduate students from the 32 states of our country. We thank the reviewers for their feedback that contributed greatly in improving the book chapters for publication in these proceedings by reviewing the manuscripts that were submitted.

As the first chapter, *Terán, Cisneros and Gutiérrez* present Knowledge of health personnel about HPV screening tests: a systematic review, as second chapter, *González, Meraz, Chávez and González* will discuss Health Risk Behaviors and Emotional State of Medical Students as third chapter, *Aguilar, Morado, Villada and Tovar* present Early trauma as conditioning of psychopathology in adult women, as fourth chapter, *Torre, Therio, Carrillo and Mendoza* propose Growth and development of the craniofacial region and the stomatognathic apparatus, as the fifth chapter, *Villarreal, Enriquez, Hernández and Medina*, perform Assessment of physical activity, sedentary behaviors and physical fitness in perimenopausal women, as the sixth chapter, *Díaz, González, Uvalle and Mederos* develop Pro-Inflammatory cytokines: Leptin and visfatin associated to obesity in young university students, as seventh chapter, *Enríquez, Vieyra, Ramos and Trujillo*, will discuss Presence of neuroglobin in the substantia nigra in a murine model of parkinson's disease: an immunohistochemical study, in eighth chapter, *Martínez, Tavizon, Carlos and Mauricio* present Prevalence of ectopic eruption and intercanine distance in children aged 6 to 12 years. Cycle 2019-2020, as the ninth chapter, *Caceres, Zárate, Flores and Bustillos*, performed Anxiety in medical students, during a COVID-19 pandemic and as the last chapter, *González, Hernández, Martínez and González*, focus on Overwiev of general plant toxicology uses and adverse effects.

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Capítulo 7 Presencia de neuroglobina en la *sustancia negra* en un modelo murino de enfermedad de Parkinson: un estudio inmunohistoquímico

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Abstract

Neuroglobin (NGB) is a protein with antioxidant and antiapoptotic activity against conditions such as oxidative stress, oxygen / glucose deprivation and neuronal apoptosis. Its presence has been documented in different brain areas including the midbrain, a site of key importance for global motor control by the presence of dopaminergic neurons in the substantia nigra located inside and whose progressive loss culminates in the most common neurodegenerative movement disorder, Parkinson's disease (PD). PD is a condition characterized by motor disturbances such as resting tremor, muscle rigidity, bradykinesia and deterioration gait and balance. There are few studies that inquire about the role of this protein in this disease, including its expression in the substantia nigra. The present study evaluated the presence of NGB in a murine model of PD induced by 6-OHDA injury using immunohistochemistry. The results show a significant difference of NGB aggregates in the substantia nigra in compared to controls (p=0.003) These findings provide the first *in vivo* experimental evidence of an adaptive NGB response in a model of PD, supporting its probable neuroprotective action in the main area involved in the pathophysiology of this disease.

Neuroglobin, Substantia nigra, Neurodegeneration, Immunohistochemistry

Resumen

La neuroglobina (NGB) es una proteína con actividad antioxidante y antiapoptótica frente a condiciones como el estrés oxidativo, la privación de oxígeno / glucosa y la apoptosis neuronal. Se ha documentado su presencia en diferentes áreas cerebrales, incluyendo el mesencéfalo, un sitio de importancia clave para el control motor global por la presencia de neuronas dopaminérgicas en la sustancia negra ubicada en su interior y cuya pérdida progresiva culmina en el trastorno neurodegenerativo del movimiento más común, la enfermedad de Parkinson (EP). La EP es una enfermedad caracterizada por alteraciones motoras como temblor en reposo, rigidez muscular, bradicinesia y deterioro de la marcha y el equilibrio. Hay pocos estudios que indaguen sobre el papel de esta proteína en esta enfermedad, incluyendo su expresión en la sustancia negra. El presente estudio evaluó la presencia de NGB en un modelo murino de EP inducido por lesión de 6-OHDA utilizando inmunohistoquímica. Los resultados muestran una diferencia significativa de agregados de NGB en la sustancia negra en comparación con los controles (p=0.003). Estos hallazgos proporcionan la primera evidencia experimental in vivo de una respuesta adaptativa de NGB en un modelo de EP, apoyando su probable acción neuroprotectora en la principal área involucrada en la fisiopatología de esta enfermedad.

Neuroglobina, Substancia nigra, Neurodegeneración, Inmunohistoquímica

7.1 Introduction

Neuroglobin (NGB) is a member of the globin superfamily and its presence in neurons of the central (CNS) and peripheral (SNP) nervous system was demonstrated in 2000 (Burmester, Weich, Reinhardt, & Hankeln, 2000), becoming a paradigm for current molecular biology, since its discovery has been shown to have an essential function in vertebrates as a neuroprotector.

NGB is described as a particularly conserved protein; in mice and humans it differs only by 6% in amino acid positions and, substitution rates are three to four times lower than those of vertebrate hemoglobin and myoglobin (Pesce et al., 2003), disclosing that its function does not allow great changes in its sequence.

NGB is a 150 - 160 amino acid long hemoprotein with a molecular mass of 17 kDa (Qiu & Chen, 2014) placed in a monomeric structure similar to myoglobin and α and β chains of hemoglobin, however, its iron atom is hexacoordinated, so the binding of the ligand to the center of the metal requires the dissociation of the distal His (E7) 64-Fe bond (Ascenzi et al., 2016); for this reason, its affinity for oxygen measured by the p50 value, that is, the oxygen pressure required to saturate 50% of the protein binding sites, is similar to that of myoglobin (Pesce et al., 2002). In addition, NGB has high stability, and its melting temperature is 100° C (Hamdane et al., 2005).

The intrinsic affinity of this globin for low molecular weight diatomic gases is like other globins, but the relatively low level of its expression in brain neurons limits its potential to function as an oxygen reservoir, especially during periods of acute ischemia. *In vitro* studies suggest that the neuroprotective role of NGB is due to its ability to uptake reactive oxygen species (ROS) and nitrogen (RNS), however, other studies have proposed that NGB is part of a signaling chain that transmits the redox state of the cell to protect it against oxidative stress and inhibit its apoptosis (Hua, Antao, Corbett, & Witting, 2010).

The antioxidant properties of NGB are related to its affinity for nitric oxide (NO) (Brunori et al., 2005; Lee, McClintock, Santore, Budinger, & Chandel, 2002) and it has been shown to act as a scavenger of ROS and RNS in different animal models under hypoxic conditions (Liu et al., 2015; Qiu & Chen, 2014), in which exist a low oxygen level and an excess of reactive species. NGB also has interactions with proteins related to antioxidants such as Cyt c (Mitochondrial electronic transporter) (Fago, Hundahl, Malte, & Weber, 2004).

Human studies have correlated NGB genetic polymorphisms with susceptibility to neurodegeneration. One of these studies showed that decreased NGB expression in the elderly is associated with an increased risk of Alzheimer's disease (AD) (Baez et al., 2016). In another preclinical study using transgenic mice, it was found that the intracerebral ventricular injection of NGB decreased the formation of Aβ peptides, the mitochondrial dysfunction, apoptosis, and neuronal death (Chen et al., 2012).

Other studies suggested that the neuroprotective effects of NGB involve the inhibition of caspase-3 and 9, activation of the PI3K / Akt pathway (Baez et al., 2016), and removal of protein aggregates (Lechauve et al., 2009); Another actions of NGB involved mitochondrial mechanisms related to apoptosome assembly through a redox reaction with Cyt c. Therefore, NGB can be considered as a potential target to decrease neuronal damage, and its upward expression after brain injury probably reflects endogenous neuroprotective mechanisms (Baez et al., 2016).

In the CNS of mice and humans, NGB is predominantly expressed in neurons. Although the evidence suggests mRNA and protein expression in neurons from different brain regions, NGB expression is different at regional and cellular level (Fago et al., 2004). For example, NGB is highly expressed in the hypothalamus, particularly in the anterior and lateral hypothalamic area (mammillary region), paraventricular nucleus, and arcuate nucleus; in dorsomedial hypothalamic nucleus and preoptic area; laterodorsal and pontine tegmental nucleus and anterior medial basomedial and posterodorsal tonsil nucleus, as well as in midbrain (Schneuer et al., 2012).

NGB has been also found in different regions including cortex, thalamus, cerebellum, hippocampus, and hypothalamus (Van Leuven, Van Dam, Moens, De Deyn, & Dewilde, 2013). These areas are important in sensation processing, memory and learning, and are often affected in hypoxic and ischemic shock, and traumatic injuries (Burmester & Hankeln, 2004).

On particular interest is that the presence of NGB in the midbrain. The midbrain is a key development component of appropriate goal-directed behaviors, which is due to calculations based on the integration of different aspects of motivation and cognition to develop and execute appropriate action plans. Midbrain dopaminergic neurons play a central role in these behaviors, including reward, cognition, and motor control; the latter being fundamental the role of neurons belonging to the *substantia nigra* (SN) (Haber, 2014). Although the *substantia nigra* was first recognized in 1786 with the description of the distribution of brain neuromelanin, it was linked to the motor system much later because of its association with Parkinson's disease (PD). Collectively, the work of several researchers demonstrated that cells of the *substantia nigra* contained dopamine (a catecholaminergic neurotransmitter that participates in the regulation of various functions such as motor behavior, emotion and affectivity) and that these cells were dopamine depleted in Parkinson's disease (Haber, 2014).

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by motor disturbances that include slow voluntary movements, resting tremor, muscle stiffness, impaired gait and balance (Stacy, 2009). This disorder affects up to 3% of the population over 60 years old (Tysnes & Storstein, 2017); Furthermore, in Mexico a prevalence of 40-50 cases per 100,000 inhabitants / year has been estimated.

It has been calculated that Parkinson's disease currently affects 4.1-4.6 million people over 50 years of age around the globe, calculating that by the year 2030 this number will be doubled, which entails a public health problem(Tysnes & Storstein, 2017). This progressive neurodegenerative disorder is mainly caused by the loss of dopaminergic cells in the substantia nigra (SN) (Hornykiewicz, 2006). However, it has been widely accepted that the early stages of this pathology are related to brain stem problems, followed later by α-synuclein deposition in the cerebral cortex (Braak et al., 2003; McCann, Cartwright, & Halliday, 2016). α-synuclein is a presynaptic protein that can be soluble in the cytosol or bound to cell membranes. It has been linked to synaptic plasticity and intraneuronal vesicular transport, as well as to the release and reuptake of dopamine (Apostolova et al., 2010). Likewise, it has been hypothesized that it could fulfill the function of a molecular chaperone collaborating in the folding and unfolding of synaptic proteins called SNARE (receptors for soluble binding proteins of NSF (sensitive factor to N-ethylmaleimide), which would be fundamental for neurotransmitter release, vesicle recycling, and synapse integrity. Mutations in α-synuclein caused by oxidative stress, nitrite aggregates, the presence of heavy metals and toxins increase its intracellular concentration and aggregates in a fibrillar way in the soma of vulnerable neurons, forming inclusions called Lewy bodies. Lewy bodies lead to neuronal dysfunction occurring in PD, favoring increased vulnerability to oxidative stress and ultimately to the appearance of apoptosis (Demey I & Allegri R, 2008). In advanced stages of PD, a selective loss of dopaminergic neurons from the *substantia nigra pars compacta* of the ventral midbrain (German, Manaye, Smith, Woodward, & Saper, 1989; German, Manaye, Sonsalla, & Brooks, 1992). Neurodegeneration is accompanied by the loss of neuromelanin neurons leading to depigmentation of the area (Fedorow et al., 2005; Gibb & Lees, 1991). The loss of nigral dopamine neurons leads dopamine depletion in the striatum and generates a wide range of motor dysfunctions (Bellucci et al., 2016). PD is also associated with non-motor and non-dopaminergic symptoms extended beyond the nigrostriatal dopaminergic pathway and often occur years or even decades before clinical diagnosis (Bellucci et al., 2016).

According to Kleinknecht *et al.* (Kleinknecht et al., 2016), human NGB showed a protective effect against α-synuclein aggregates in yeast and mammalian cells. NGB expression reduced the number of cells with α-synuclein aggregates almost twice compared to the controls, as well as the number of aggregates per cell. When performing lactate dehydrogenase (LDH) measurements to determine whether there was an effect of NGB on cell toxicity (the release of LDH in the cell culture medium is an indicator of damage to the plasma membrane and is used as a marker of cytotoxicity), Kleinknecht *et al*., found LDH levels were similar for all cells in the test; indicating that NGB acts as a suppressor of α-synuclein aggregation without causing significant cytotoxicity (Kleinknecht et al., 2016).

Until now, although the neuroprotective properties of NGB have been proven under different pathological conditions, only *in vitro* investigations have been carried out in PD. Our study represents the first *in vivo* report of the presence of NGB in the main damaged area in this disease. For this purpose, a murine model of lesion with 6-OHDA in the substantia nigra was used using stereotaxic surgery and the presence of NGB in brain sections was subsequently investigated by immunohistochemistry. We hypothesized that an increase of NGB in the substantia nigra indicates a response to cell damage.

The following sections detail the methodology for the induction of the experimental model of Parkinson's disease through the lesion of the dopaminergic neurons of the substantia nigra with 6-OHDA using stereotaxic surgery and the subsequent processing of brain tissue as well as detection of the presence of NGB by immunohistochemistry, the results are detailed exposing the representative images and the accumulated count to finally expose our conclusions.

7.2 Methodology

7.2.1 Ethical implications

Experiments followed the principles and procedures outlined by the National Institutes of Health; the guide for the care and use of laboratory animals and the local IRB of the Universidad Autónoma del Estado de México. The study observed Mexican standard NOM-062-ZOO-1999, regarding technical specifications for the production, care and use of laboratory animals.

7.2.2 Murine model of Parkinson's disease

Eight male Wistar rats, weighing 200-300 g, were used for the study. The animals were kept under standardized conditions in 12:12 light / dark cycles, controlled room temperature (22 ± 2 ° C), and food and water *ad libitum*.

Surgery was performed under anesthesia using a cocktail of 90 mg / kg of ketamine (Pisa, Mexico) and 10 mg / kg of xylazine (Pisa, Mexico) administered by intraperitoneal injection (27-gauge needle and 1 cc syringe). Additional anesthesia was supplemented as necessary during the surgical precedures.

The lesion with 6-OHDA was performed according to Jáidar et al. (Dunnett & Iversen, 1980). Briefly, 20 µl of 6-hydroxydopamine (6-OHDA) (Sigma, 4 µg / µl in 0.9% NaCl, 0.5% C6H8O6) were injected into the right *substantia nigra* (2 µl / min) according to the following stereotaxic coordinates: anteroposterior, 3.9; lateral, 1.8; ventral, 6.7 mm (Figure 7.1). One week after injection, rats were treated with amphetamine (4 mg / kg i.p.) and ipsilateral turns were counted for 90 minutes with an automatic apparatus (device). Animals that showed > 500 ipsilateral turns were used for the experiments that were performed 15 days after injury (Figure 7.2). This rotational score corresponds to> 97% of SN dopaminergic cell injury (Dunnett & Iversen, 1980; Grant & Clarke, 2002). Controls were injected with vehicle only (sham op).

Figure 7.1 Stereotaxic surgery for induction of murine model of Parkinson's disease

Figure 7.2 Automatic lap counter for turning test

7.2.3 Obtaining samples

Euthanasia

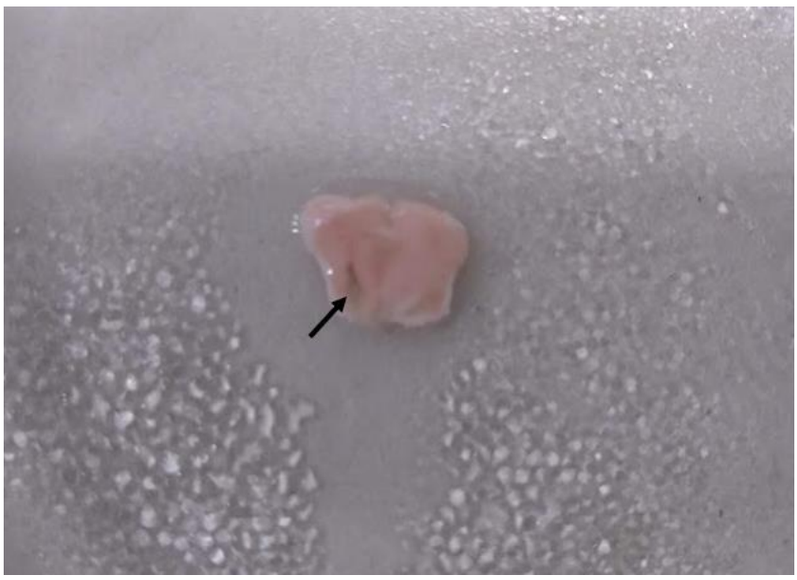
2 mL of sodium pentobarbital (Pfizer anesthetic) was administered intramuscularly and after the absence of vital signs, perfusion was carried out.

Perfusion

After euthanasia, the abdominal cavity was opened, followed by clamping of the vena cava and abdominal aorta. A cannula was introduced intracardially directed to the ascending aorta, fixed with a pressure clamp, and the cardiac atria were cut. 200 mL of phosphate buffer were perfused, followed by 250 mL of 4% paraformaldehyde. Once the perfusion was completed, the head was removed using a guillotine and the brain extruded.

After removing the brain from the bone cavity, it was placed in a vial containing 20 mL of 10% sucrose solution for 24 hours, and then replaced by 20% sucrose for a further period of 24 hours. The next 24 hours, the brain was maintained with sucrose at 30%. Subsequently, a coronal cut was made at the level of the midbrain (Figure 7.3) and coronal slices of 40 µm were made using cryostat (820 jung histocut microtome, Leica, USA).

Figure 7.3 Brain section at ventral midbrain level, showing the lesion in substantia nigra (black arrow)



7.2.4. Immunohistochemistry

Midbrain slices (anteroposterior, 3.9; lateral, 1.8; ventral, 6.7 mm) were washed three times in PBS pH 7.0, and then were immersed in 0.28% of periodic acid solution for 1 minute at room temperature Next, they were immediately washed again and then incubated in antigen retriever for 30 minutes at 60º C and maintained at room temperature. The slices were washed again and placed in a blocking solution (PBS - Triton 0.01% + Bovine Serum Albumin 2.5%). At the end of the previous step, the sections were incubated in a 1: 500 solution of primary antineuroglobin antibody (Sigma) in blocking solution for 24 hours at 4 ° C, then they were washed 3 times with PBS pH 7.0 and were placed in a solution 1,500 biotinylated secondary antibody 2 hours at room temperature. The washes were repeated, and the slices were immersed in Complex AB (Streptovidin-Peroxidase) for 45 min at room temperature, the washes were repeated once more and finally submerge the sections in developing solution for 5 minutes. For microscopic observation, the sections were mounted on slides, allowed to dry at room temperature, and synthetic resin was placed on them to seal the coverslip.

7.2.5 Neuroglobin quantification

Counts of NGB aggregates were identified with a brown color precipitate using a 100X objective in a brightfield microscope in a standardized area of 0.031 mm^2. The counts were made for triplicate in each of the areas to be evaluated in the sections for each animal. 18 fields were analyzed overall. The data were then analyzed in (SPSS®) Statistics. An exploratory / descriptive study of the counts was carried out, which included measures of central tendency, dispersion, and a normality test. The Independent Samples t test was used to compare means. The measured variable was the density of brown NGB aggregates per unit area (0.031 mm^2). Statistical differences were accepted when $p < 0.05$.

7.3 Results

A reconstruction of the sections of the area was performed with Bregma coordinates -6.00 mm and interaural 2.96 mm, according to Paxinos (Figure 7.4). Photographs were taken with 4X objective for reconstruction and 40X for cell/aggregates counting using a bright field microscope, later the reconstruction was performed using the Image Composite Editor program. In figure 7.5 the specific marking can be seen as small brown marks corresponding to NGB, evaluated in the *substantia nigra* on the left side, in the control and the injured, respectively.

Figure 7.4 Reconstruction of substantia nigra sections (blue box)

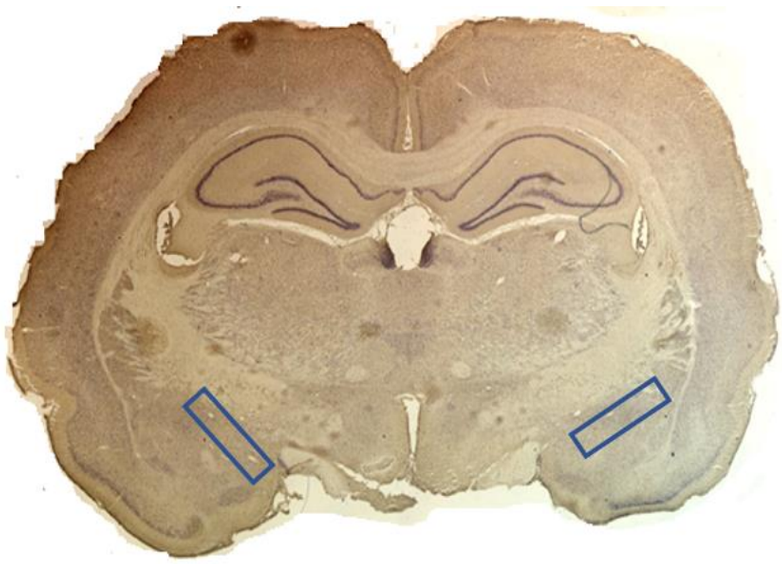
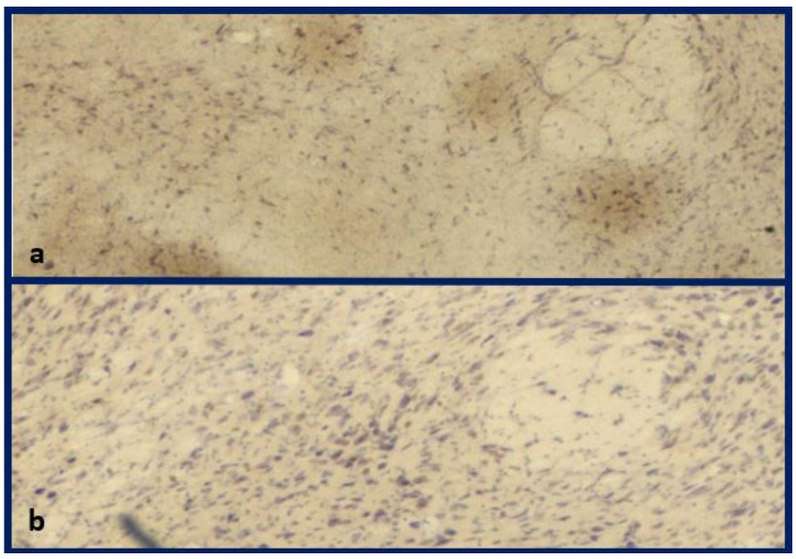
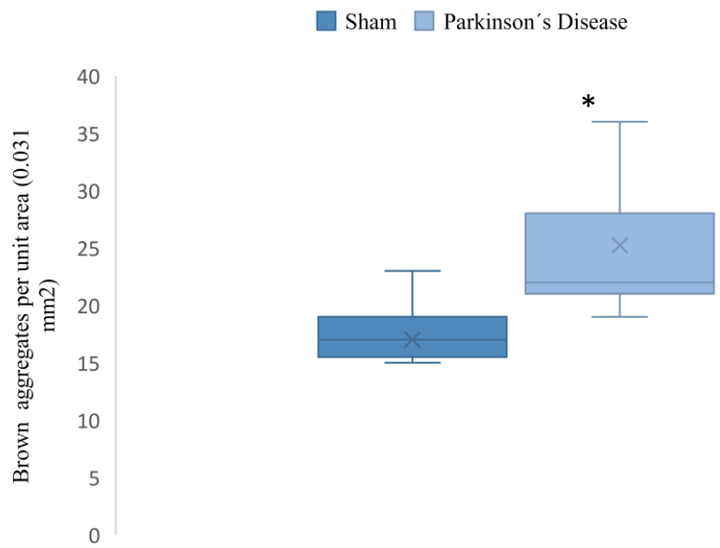


Figure 7.5 Immunohistochemistry of neuroglobin in substantia nigra of murine model of Parkinson's disease. Points correspond to NGB aggregates. a. Sham. b. Parkinson Disease



According to statistical study, we found a greater presence of NGB when the 6-OHDA lesion is present ($\overline{X}$ = 25.22 ± 2.005) *vs*. control ($\overline{X}$ = 17 ± 1.179), according to the density of NGB aggregates found in the target area (0.031 mm^2) in the triplicate counts that were carried out (t (16) = -3.535 p < 0.003) (Graphic 7.1).

Graphic 7.1 Comparison of means of NGB aggregates density. The average number of aggregates in a standardized area of 0.031 mm^2 are shown. Parkinson's disease ($\overline{X}$ = 25.22 ± 2.005) vs sham ($\overline{X}$ = 17 ± 1.179) (t (16) = -3.535 p < 0.003)



7.4 Discussion

NGB has a wide presence in the central nervous system, which has been demonstrated in previous studies. According to Wistub (Wystub et al., 2003), this protein is present in the cortex, thalamus, hippocampus, and hypothalamus, among other areas. However, there are few studies that showed NGB presence in neurodegenerative diseases. In a report where its presence was evaluated in affected areas in Huntington's disease, (striatum, thalamus and cortex) it was found a greater presence of the protein in striatum where the disease is evident, however, NGB had a lesser expression in thalamus and cortex, despite the striatum being the main area affected in the disease (Cardinale et al., 2018). In our study, we found the presence of NGB in the *substantia nigra* in control subjects (sham op), in an area where it had not been previously described (Fig. 5) however, its presence was much lower than in the experimental subject ($\overline{X}$ = 25.22 ± 2.005 vs $\overline{X}$ = 17 ± 1.179, t (16) = -3.535 p < 0.003). Due to the great epidemiological importance of Parkinson's disease, analyzing the presence of a protein with proven neuroprotective functions is of relevance and interest. Overall, our study shows the first report of NGB presence in the *substantia nigra* and a statistically significant increase of the protein when the lesion was present. corresponding to the main area affected in PD due to the loss of dopaminergic neurons. We suggest that an increase in NGB in this midbrain area could indicate a upregulation dependent on damage and neurodegeneration.

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7.6 Conclusions

The increase in life expectancy at a global level brings with it a progressive increase in the incidence of degenerative diseases such as Parkinson's disease, whose pathophysiology is far from being clarified. The study of proteins with neuroprotective functions, like NGB, can play a relevant role. The present study shows upregulation of the protein when progressive and irreversible death of dopaminergic neurons in substantia nigra occurs.

More studies are needed on NGB in Parkinson's disease, not only in the substantia nigra but in other affected areas such as the motor cortex and the striatum, it is also important to determine in future studies the cell type that expresses the protein, as well as its subcellular location in order to clarify its role in this condition.

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