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Impact of 6-OHDA injection and microtrauma in the rat *substantia nigra* on local brain amyloid beta protein concentrations in the affected area

Joshua A. Roldán-Kalil[1], Sara E. Vendrell-Gonzalez[2], Natalia Espinosa-Ponce[2], Jadier Colón-Vasques[1], Jescelica Ortiz-Rivera[1], Vassiliy Tsytsarev[3], Janaina M. Alves[2] and Mikhail Inyushin[1]

[1]Department of Physiology, [2]Department of Microbiology and Immunology, Universidad Central del Caribe School of Medicine, Bayamón, Puerto Rico and [3]Department of Neurobiology, University of Maryland School of Medicine, Baltimore, MD, USA

Summary. Amyloid beta peptides (Aβ) are key indicators of Alzheimer's disease and are also linked to cognitive decline in Parkinson's disease (PD) and other neurodegenerative disorders. This study explored the accumulation of Aβ in a standard 6-Hydroxydopamine (6-OHDA) model of PD. We unilaterally injected 6-OHDA into the *substantia nigra* of Wistar rats to induce dopaminergic cell degeneration and death, a characteristic of PD. The goal was to detect Aβ protein in tissues and blood vessels showing inflammation or degeneration from the 6-OHDA injection. Our results showed that 6-OHDA injection produced a statistically significant rise in Aβ concentration at the injection site 60 minutes after injection, which was slightly reduced 24 hours post-injection but still significantly higher than in controls. We also tried Gp120 injection in the same zone but it only produced effects comparable to control needle trauma. The presence of Aβ in tissues and blood vessel walls after injection was confirmed through ELISA tests and was supported by immunohistochemical staining of injection areas. We found that the increased Aβ concentration was visible in and around blood vessels and inside blood vessel walls, and also, to a lesser extent in some cells, most probably neurons, in the area. This research highlights the connection between dopaminergic cell poisoning and the accumulation of Aβ, offering insights into the progression of PD to cognitive disorders and dementia.

Key words: Amyloid beta, Gp120, Parkinson's disease model, Substantia nigra, 6-hydroxydopamine

Corresponding Author: Mikhail Inyushin, Department of Physiology, Universidad Central del Caribe School of Medicine, Bayamón 00960, Puerto Rico. e-amil: mikhail.inyushin@uccaribe.edu
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Introduction

Amyloid beta peptides (Aβ) are recognized as a hallmark of Alzheimer's disease (AD), however, Aβ accumulation is associated also with the neurocognitive decline of patients with Parkinson's disease (PD) and some other neurodegenerative complications (Akhtar et al., 2017; Fan et al., 2021; Mihaescu et al., 2022). Research indicates that Aβ accumulation becomes more prominent in the later stages of PD (Compta et al., 2011; Irwin et al., 2013; Wales et al., 2013; Lim et al., 2018; Isaev et al., 2019; Inyushin et al., 2020). Yet, even in the early stages, following the progression of α - synucleinopathy, Aβ can be detected in the brain. Interestingly, α-synuclein fragments were first described two decades ago as being a component of beta-amyloid plaques from AD patients, affecting plaque stability (Iwai et al., 1995; Kallhoff et al., 2007). Approximately 20 years into PD, about half of the patients with Parkinson's disease dementia (PDD) exhibit extensive neuropathologies akin to those seen in AD, including misfolded Aβ plaques and tau neurofibrillary tangles, primarily in the frontal cortex and striatum (Kalaitzakis et al., 2008). The extent of damage caused by Aβ and its impact on PDD progression, however, remains a topic of discussion among researchers (Jendroska et al., 1996; Compta et al., 2011; Irwin et al., 2013; Fiorenzato et al., 2018; Melzer et al., 2019). Additionally, 53% of PD patients show signs of insoluble Aβ accumulation around the blood vessels, known as cerebral amyloid angiopathy (CAA) (Bertrand et al., 2008). Despite the significant degeneration and inflammation in the PD striatum and frontal cortex, distinguishing the role of Aβ in AD from that in PD remains challenging (Villemagne et al., 2009; Tufekci et al., 2012; Walker et al., 2024). Platelets have been suggested as key contributors to these conditions, releasing Aβ in/near blood vessels and adjacent brain cells. This may be the basis for amyloid beta accumulation in PD, as we suggested previously

(Inyushin et al., 2020). Thus, we hypothesized that PD pathologies, which have been shown to elicit proinflammatory immunological responses and neuronal damage in the brain, could explain the local accumulation of Aβ in the affected zone.

The objective of the present study was to further establish the presence of Aβ in tissues and blood vessels undergoing degeneration and/or inflammation. Neurotoxin 6-hydroxydopamine 6-OHDA (6-OHDA) was stereotaxically injected into rat brains to promote dopaminergic cell damage in the *substantia nigra* (SN) (Markina et al., 2023). The ELISA procedure detected increased levels of Aβ in tissues shortly after being injected with 6-OHDA. Immunohistochemical staining confirmed the presence of Aβ in and around blood vessels adjacent to tissues undergoing cell degeneration, similar to previous findings (Kucheryavykh et al., 2017; Martins et al., 2019). Also, we used injections of Gp120 protein to the same stereotaxic coordinates at low concentrations, as an active control, and needle trauma to the same coordinates as an additional control. It is known that Gp120 is the main external protein of the HIV capsid and the main determinant for cytokine burst in HIV patients, producing active inflammation in this cohort (Levast et al., 2017). However, in rats, it is just a protein that may produce some non-specific response. This allowed us to use it as an additional positive control. We also used needle trauma as a positive control, and the contralateral SN as a negative control.

There is no doubt that the accumulation and aggregation of Aβ is associated with PD. The precise mechanisms remain unclear; however, our data suggest that the accumulation of Aβ, including within brain vessels, may be associated with previous damage to SN neurons. If neurodegenerative changes and Aβ accumulation in PD are indeed more closely related than previously thought, this certainly deserves more attention. Understanding the details of this mechanism may reveal new diagnostic and therapeutic approaches to PD.

Materials and methods

Ethics Statement

All procedures involving rodents were conducted in accordance with the National Institutes of Health (NIH) regulations concerning the use and care of experimental animals and approved by the Universidad Central del Caribe Institutional Animal Care and Use Committee (IACUC, #035-2021-09-00-PHA). Surgical procedures were performed using sterile/aseptic techniques following institutional and NIH guidelines. To minimize discomfort, animals were anesthetized for all procedures involving surgery.

Animal Handling

Male and female Sprague Dawley rats weighing 280-400 g (3 to 4 months old) from the Universidad Central del Caribe Animal Facility were used in our experiments. In total, 26 animals were used to study beta-amyloid accumulation with ELISA, and three were used for the immunocytochemistry study. All animals were subjected to directed stereotaxic brain injections. The experimental groups received 2 μL of 6-OHDA or Gp120 injection. Contralateral hemispheres not injected with 6-OHDA or Gp120 during the stereotaxic procedure, as well as rat brains injected with 2 μL of 0.9% sterile saline (needle trauma), served as the negative and "sham" controls for this study, respectively. After the desired time intervals, rats were euthanized by decapitation. The brains were removed from the cranium for analysis.

Stereotaxic Surgery

Sprague Dawley adult rats were anesthetized with isoflurane and positioned in a Kopf stereotaxic apparatus. Betadine solution was added to the area of the incision. Medial scalp and periosteum incisions were subsequently performed to reach the skull. Bregma and Lambda cranial sutures were identified and their coordinates were established. Using a Dremel tool (Dremel Manufacturing Co. Racine, Wisconsin, US), Burr holes were executed above the cranial area corresponding to the SN portion of the brain on the hemisphere of choice. The stereotaxic coordinates for rat SN were AP: -5.8 mm, ML: -2.0 mm relative to bregma (DV: -8.0 mm) (Paxinos and Watson, 2006). Insertion of the Hamilton syringe, as well as syringe content release and retraction, was performed in a 10-to-20-minute window to ensure minimal damage to the brain regions through which the needle passed. Administration of 6-OHDA into the SN was carried out at a rate of 0.1 μL every 30 seconds to prevent tissue displacement and damage while releasing the syringe contents. A total volume of 2 μL of (2 mg/ml) 6-OHDA hydrobromide was injected, as recommended (Alvarez-Fischer et al., 2008). In additional experiments, injections of 2 μL of Gp120 (2 mg/ml) were used, in the same region and within the same timescale. Both substances were dissolved in 0.9% sterile saline containing ascorbic acid (0.2%).

Tissue preparation and cryosection

Upon dissection, rat brains injected with 6-OHDA and HIV protein Gp120 were fixed in 4% paraformaldehyde solution. After 24 hours, the brain tissues were removed from the paraformaldehyde solution and rinsed with phosphate-buffered saline solution (PBS: NaCl, 137 nM; KCl, 2.70 nM; $NaHPO_4$, 1.77 nM; pH 7.4). Then it was washed five times with PBS solution to ensure the complete elimination of formaldehyde solution from the rat brain. The part containing the midbrain was cut from the brain for further processing. The brain section was then cryoprotected by submerging it in solutions with

increasing concentrations of glucose dissolved in PBS. Solutions were obtained by mixing 5% and 20% sucrose solutions at ratios 2:1, 1:1, and 2:1. Brain tissue was left in each solution until it sank to the bottom of the test tube. The rat brain section was again washed with PBS five times for 30 seconds after each increasing solution of sucrose. After the 2:1 (25%: 5%) sucrose solution, the rat brain section was subjected to a 30% glucose concentration solution and kept submerged in the solution for 24 hours. Next, 30 μm slides were prepared from rat brain sections, using a cryo-microtome (CM 1850; Leica Microsystems GmbH, Wetzlar, Germany) at -18°C.

Immunofluorescence staining and confocal microscopy

The acquired slides were subsequently air-dried for 30 min and subjected to immunohistochemistry staining using a protocol previously established in our laboratory (Inyushin et al., 2020). In short, wells were drawn on the slides and around the sectioned tissues using a "Liquid Blocker Pen" (ab2601, Abcam, Cambridge, UK). Sections were then treated with a permeabilization solution consisting of 0.03% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) and 1% dimethyl sulfoxide (DMSO; MP biomedicals, Santa Ana, CA, USA) in PBS for 10 minutes. Afterward, sections were treated with a blocking solution containing 5% normal goat serum, 5% normal horse serum (Vector Laboratories, Burlingame, CA, USA), and 2% w/v bovine serum albumin (BSA; Sigma-Aldrich, St Louis, MO, USA) in permeabilization solution for 1h.

Following the blocking step, the sections were processed with antibodies to identify astrocytes. A mouse monoclonal antibody against Aβ (MOAB-2, 1: 200; Abcam, Cambridge, MA, USA, cat. #ab126649), and a rabbit monoclonal antibody against glial fibrillary acidic protein- Alexa Fluor® 594 (GFAP- Alexa Fluor® 594) (1: 200; Abcam, Cambridge, MA, USA, cat. no. ab201732) were used, all incubated overnight at 4°C without light. After three washes with permeabilization solution for 10 min, the secondary antibodies (fluorescein-labeled horse anti-mouse IgG, Vector lab, FI-2000-1.5) were added at a dilution of 1: 200 for 2h and covered with aluminum foil, to prevent light. After this period, the sections were once again washed three times for 10 minutes, this time with PBS solution.

Fluoroshield mounting medium (Sigma-Aldrich, cat. No. F6057) with 4′,6-diamidino-2-phenylindole (DAPI) was added to the tissue section and a cover slip was placed on top. Images were acquired using an Olympus Fluoview FV1000 scanning inverted confocal microscope system equipped with a 4×, 10×, 20× or 40×/1·43 oil objective (Olympus, Melville, NY, USA). The images were analyzed using ImageJ software (version 1.8.0_112, http://imagej.nih.gov/ij) with the Open Microscopy Environment Bio-Formats library and plugin (http://www.openmicroscopy.org/site/support/bio-formats5.4/), allowing for the opening of Olympus files. The images were evaluated using custom colorization.

Amyloid beta ELISA Assay

From the brains used for this assay, with tissues exposed to either 6-OHDA or HIV-Protein Gp120, the region of interest (about 1x1x1 mm) was extracted manually with a blade, placed in round bottom microfuge tubes, and stored at -80°C for later use. Protein extraction from the collected tissues was then performed as follows: RIPA (sigma) and Protease Inhibitor solution (Sigma) (1:100) were added to the microfuge tubes containing the tissues, which were placed on ice for 20 min, and then macerated. These were then centrifuged for 30 min at 4°C. The protein content was extracted with a pipette and placed into a different microfuge tube for later use. More than one microfuge tube was obtained from each sample. Before using for Aβ quantification assay, the total protein concentration was determined using the Bradford assay. During the assay, the extracted tissue was thawed out and used in a rat-specific sandwich ELISA kit (cat. #LS-F24023-1; LifeSpan Biosciences Inc., Seattle, WA, USA) to measure the concentration (pg/mL) of Aβ1-40 peptide present in the prepared sample solutions. Samples were diluted 1:100 using the "Sample Diluent" solution provided in the kit. The rest of the assay was performed following the instruction manual. Briefly, samples with known Aβ1-40 peptide concentrations were used for calibration purposes, as well as samples containing supernatant solutions obtained from negative control and treated tissues, were pipetted onto the microtiter plate. Next, a solution containing 1X Biotinylated detection antibody was added and incubated for 1h. After completing the instructed number of washes, horseradish peroxidase-labeled anti-rabbit antibody and its corresponding 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate was added to detect protein in the wells. The optic density at 450 nm of samples and standards in the plate was read using Spectra Max ID3 equipment (Molecular Devices, San Jose, CA, USA).

Statistical Analysis

Data was statistically analyzed using GraphPad Prism software (version 10.2.0). Pairwise comparisons between groups were calculated using unpaired T-tests (and nonparametric tests to compare variances). Data was considered significantly different if $p<0.01$. Significance levels were marked by asterisks (Fig. 2).

Results

After injection of 6-OHDA to the substantia nigra, Aβ is visible mainly in and around blood vessel walls

Monoclonal antibodies against Aβ in our experiments have shown that, one hour after the injection of 6-OHDA into the SN, in this injection zone, Aβ is present mainly in and around blood vessels (Fig. 1,

green and yellow). The control side shows no significant accumulation of Aβ (Fig. 3C,F).

We used antibodies against the glial fibrillary acid protein (GFAP); these antibodies demonstrated red fluorescence in astrocytes (Fig. 1). Because astrocytes provide the morphological support for vascular function and their processes surround vessels, they make the position of vessel walls visible. In Fig. 1A, the green color associated with Aβ is mainly visible inside the vessel and in the vessel wall due to astrocyte processes (endfeet), which make all the vessel visible. The mixture of red (astrocyte GFAP) and green (Aβ) produces a yellow coloring of the vessel wall. Fig. 1B-E are at higher amplification and present different blood vessels in the zone that was sectioned, showing the distribution of Aβ (green) in and around the vessel. In these figures, Aβ is present inside the vessel, around it, and also inside the wall, where it coincides with astrocyte endfits. The blue color represents DAPI staining of the cell nucleus. Besides blood vessels, in some SN slices, beta-amyloid can be seen coinciding with some cells (Fig. 1F). These cells are not astrocytes because they have no GFAP staining inside and are relatively large (20-40 microns), which is larger than any glial cells, suggesting that these cells are most probably neurons.

Injection of 6-OHDA into the substantia nigra produces elevation of Aβ1-40 in the zone

Injection of 2 or 4 μg of 6-OHDA into the SN is known to produce chemical trauma in dopaminergic neurons, first leading to transient augmentation of dopamine release by the damaged neurons during the first hours of injection and then to steady depletion of dopamine levels and degeneration of neurons, leading to the constant reduction of dopamine production (Alvarez-Fischer et al., 2008). In our ELISA experiments, we used five groups of four animals each, each tissue was assayed twice and then averaged, giving n=4 for each group of measurements. One hour after 6-OHDA injection, the Aβ1-40 concentration region reached 2440±131 pg/ml, while in the contralateral zone without injection, the concentration was 612±102 pg/ml. Thus, 6-OHDA injection produces Aβ1-40 accumulation in the SN with high statistical significance (N=4, t=21.99, df=6; $p<0.0001$, see Fig. 2). On the other hand, in animals with 6-OHDA injection to the SN, 24 hours after injection, the amount of Aβ1-40 was 1862±171 pg/ml,

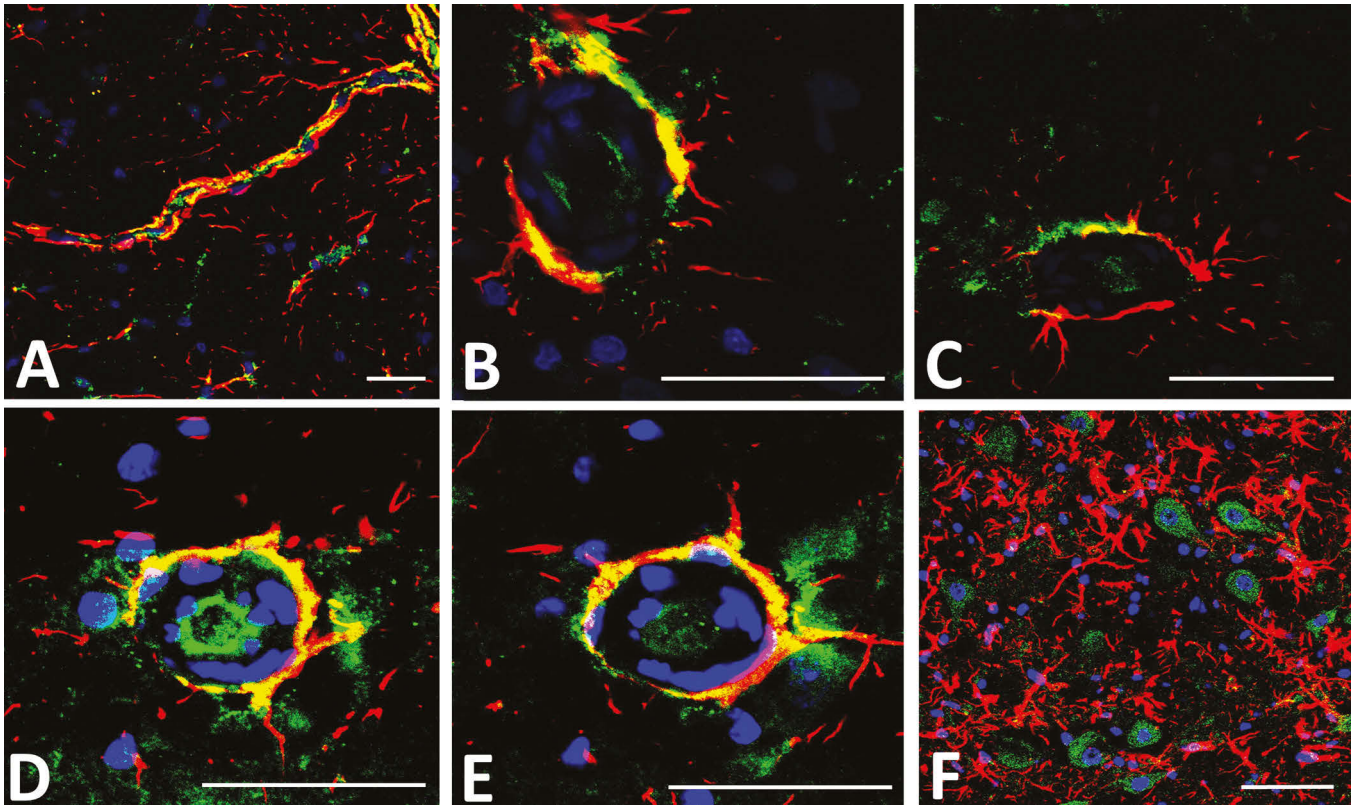

Fig. 1. Beta-amyloid (Aβ) in the *substantia nigra* after injection of 6-hydroxydopamine (6-OHDA). **A-D.** Accumulation of Aβ in blood vessel walls at different amplifications. F-accumulation of Aβ (green) in neurons. **B-E** are at higher amplification and present different blood vessels in the zone that was cut across, showing the distribution of Aβ (green) in and around the vessel marked by astrocyte enfeet. The blue represents DAPI staining of the cell nucleus; **B, D, E** mark the nucleus of endothelial cells and nearby astrocytes. Red-GFAP (astrocyte marker), green–Aβ, blue–DAPI nuclear staining. The yellow mark is a coincidence of red and green. F. Neurons with Aβ (green) surrounded by astrocytes (red) in the injection zone. Scale bar: 100 μm.

which was still significantly higher than the "negative" control (which was 532.6 ± 35 pg/ml, N=4, t=15.20, df=6; $p<0.0001$); however, the amount of Aβ1-40 was statistically lower than 1 hour after injection (N=4, t=5.355, df=6; $p=0.0017$, Fig. 2).

We also suggested that the injection of Gp120 protein (external protein of the HIV capsid) into the brain, e.g., the SN, may give rise to Aβ1-40. After injection, the amount of Aβ1-40, determined by ELISA was effectively elevated (1764±78) and significantly higher than the negative control (552±100) (N=4, t=19.11, df=6, $p<0.0001$). However, in animals with Gp120 injection into the SN, 24 hours after injection, the amount of Aβ1-40 was statistically similar to the negative control. Also, needle trauma with saline injection statistically gave the same Aβ1-40 increase as the injection of Gp120 (Fig. 2); after 24 hours, it also returned to the baseline level. Thus, we found that trauma itself transiently affects the amount of Aβ1-40 in the zone but not the Gp120 protein. Thus, the injection of Gp120 into the same coordinates as 6-OHDA only produces a transient rise in Aβ levels, similar to needle trauma with saline injection, while the distribution of Aβ was practically the same (Fig. 3A,B,D), emphasizing the role of blood vessels in the Aβ level response to any trauma (Martins et al., 2019). No visible accumulation of Aβ could be seen on the contralateral side (Fig. 3 E).

The effect of 6-OHDA (1h) was, nevertheless, statistically different than the 1-hour sham control (needle trauma) (N=4,t=10.16, df=6; $p<0.0001$). This allows us to say, that injection of 6-OHDA into the SN produces a statistically significant rise of Aβ1-40 in the zone, significantly bigger than simple needle trauma. While it became slightly reduced after 24 hours, it still was at least as big as immediately after the needle trauma, showing that the trauma to neurons persists.

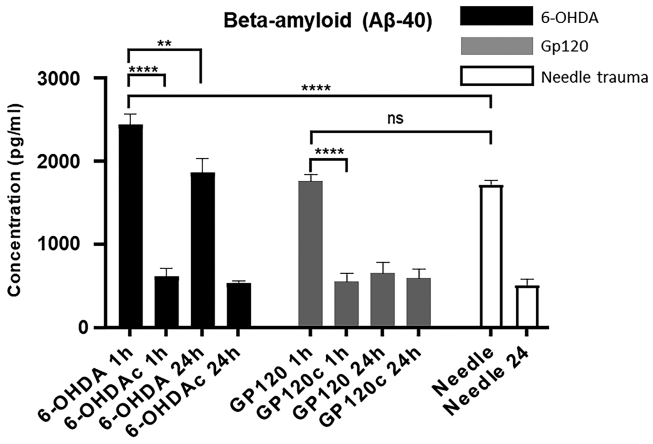

Fig. 2. ELISA assay measurements of Aβ1-40 in tissue samples from the midbrain area containing the substantia nigra (SN), 1 hour and 24 hours after 6-OHDA or Gp120 injection, and 1 hour after needle trauma (saline injection). Measurements of samples from the contralateral zone with no injection are marked by the letter "c". Asterisks show the level of statistical significance: $^{**}p<0.01$, $^{****}p<0.0001$, ns if no significance. Each bar represents Mean ± SD.

Discussion

PD is a progressive neurological disorder that affects both movement and non-movement functions. Symptoms gradually worsen, with non-motor issues like cognitive, behavioral, sleep, and sensory problems becoming more prominent as the disease advances. The motor symptoms result from the loss of dopaminergic neurons in the SN, a midbrain region that supplies dopamine to the basal ganglia. The exact reasons behind this cell loss are not well understood but are associated with the buildup of alpha-synuclein protein fibrils, which create spherical eosinophilic Lewy bodies within the cells and are suggested to be immunity-related and induced at sites of inflammation (Spillantini et al., 1997; Alam et al., 2022). However, synuclein fibrils are present in many disorders, including AD (Kilarski et al., 2009) and, actually, synuclein was described first as a component of Alzheimer's brain plaques second only to Aβ itself (Uéda et al., 1993; Wales et al., 2013). PDD is a common occurrence in its late stages.

While Aβ peptides are significant markers of AD, they also have been associated with cognitive impairments in PD and other neurodegenerative conditions (Jendroska et al., 1996; Compta et al., 2011; Irwin et al., 2013; Fiorenzato et al., 2018 ; Melzer et al., 2019). Up to 50% of patients with PDD also develop many Aβ-amyloid plaques and tau-containing neurofibrillary tangles, often leading to a secondary diagnosis of AD. Moreover, diagnosing neuro-degenerative disease in the early stages is itself a rather complex task, and is often based on physiological biomarkers (Plachez et al., 2023). Not much is known about why dopaminergic neurons are specifically destroyed in PD. The question arises as to whether Aβ accumulation is also associated with dopamine neuron degeneration in the SN?

This research examined Aβ accumulation using the conventional 6-OHDA PD model that reproduces several motor and non-motor symptoms of PD (Masini et al, 2021). 6-OHDA is an analog of dopamine and norepinephrine. This substance does not cross the blood-brain barrier and must be injected directly into the brain, where it acts selectively on dopaminergic neurons to induce oxidative stress through synthesizing reactive oxygen species (ROS). This in turn causes cell injury and death by inhibiting mitochondrial complex I. It is known that the model induced by 6-OHDA does not include all PD symptoms, although it does reproduce the main cellular processes involved in PD, such as oxidative stress, neurodegeneration, neuroinflammation, and neuronal death by apoptosis (Hernandez-Baltazar et al., 2017); over time it produces significant morphological changes in the striatum (Sun et al., 2024). We administered a unilateral injection of 6-OHDA into the SN of Wistar rats, aiming to replicate the dopaminergic neuronal injury typical of PD, and the response to this

damage. The study's objective was to identify the appearance of Aβ in tissues and blood vessels experiencing inflammation or degeneration due to the 6-OHDA injection. Results indicated a notable, although temporary, increase in Aβ levels in the affected region, with a slight decrease observed 24 hours post-injection, yet significantly higher than that in controls (Fig. 2).

It is known that Aβ levels in rodents transiently elevate and aggregate after trauma, however, if the animals are alive, they reduce rapidly (Washington et al., 2014; Martins et al., 2019). This is because in rodents, extracellular beta-amyloids only exist in soluble form, unlike in humans, and the flow of liquids rapidly reduces beta-amyloid depositions (Jankowsky et al, 2007). To form insoluble plaques (like in Alzheimer's) after trauma, animals must be genetically modified with human genes to produce human beta-amyloid and, in these genetic models, angiopathy is similar to that seen in humans. Still, it is chimeric, containing a human amyloid core and rodent amyloid periphery (van Groen et al., 2006). Thus, because time is important, instead of a simple saline injection with a needle, we used another control, with Gp120, to see how long the non-toxic injection effect lasted.

As we already mentioned, inflammation is a trigger of Aβ production (Inyushin et al., 2020). Also, it is known that HIV infection is associated with increased production of Aβ in humans and may be linked to HIV-associated neurocognitive disorder (HAND) (Fulop et al., 2019). We tried Gp120 because, in humans, it produced marked inflammation and is an important component of Aβ pathogenesis in HAND (Levast et al., 2017; Fulop et al., 2019). However, Gp120 is a protein that may cause only a non-specific response in rats. Indeed, the effect of the Gp120 injection lasted less than 24 hours, replicating the effect of needle trauma, the Aβ level was reduced to the basal level the day after the control protein injection (Fig. 2).

We also used a sham control (needle trauma to the zone) and a simple positive control with saline injection. We found that both positive controls (saline and Gp120) show the same effect on Aβ induction as needle trauma (Fig. 2). Only 6-OHDA produced a more pronounced elevation of Aβ than the controls, which lasted at least 24 hours (Fig. 2). This may be interpreted as Aβ production in response to chemical injury of neurons.

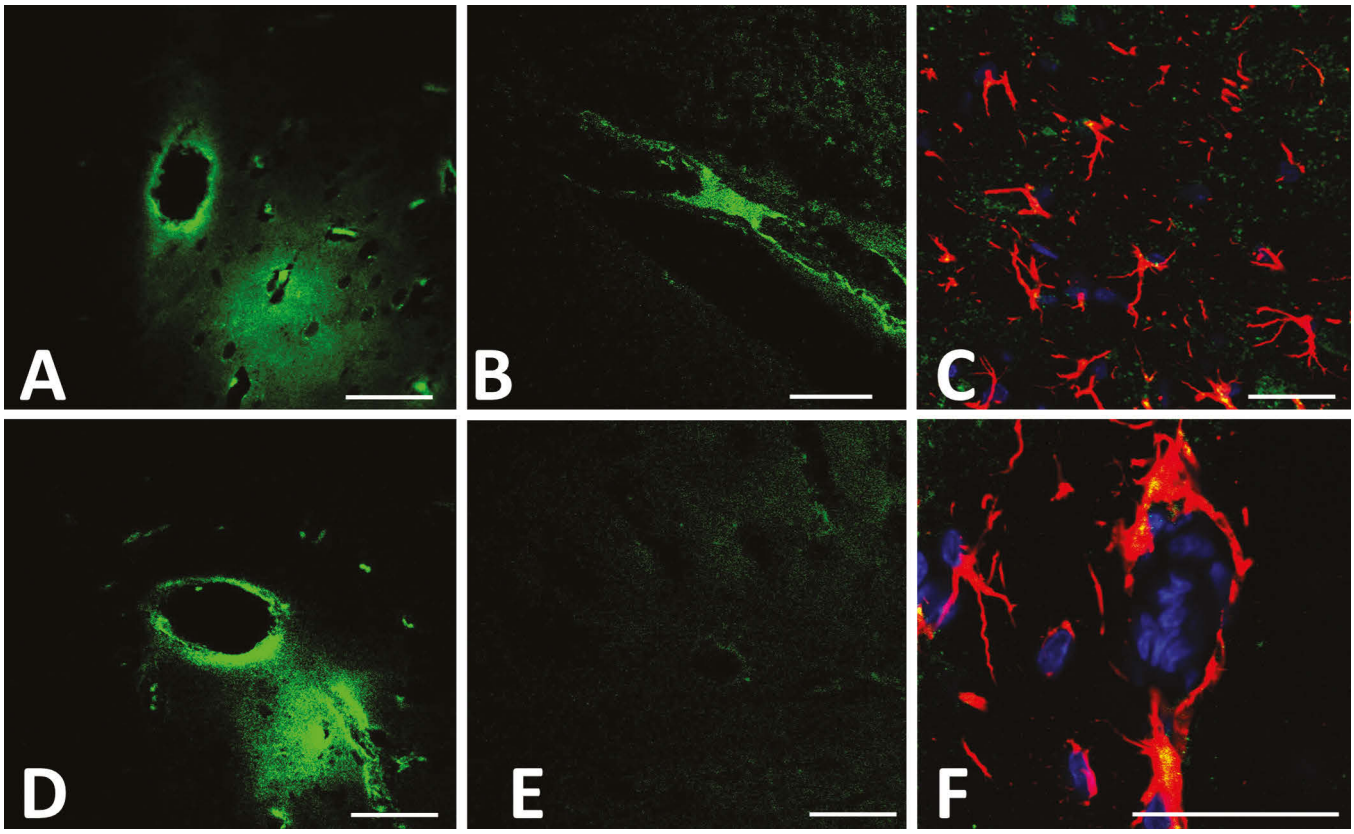

Fig. 3. A, B, D show the Aβ distribution in *substantia nigra* (SN) tissue after Gp120 injection (2 μL of Gp120 (2 mg/ml), dissolved in 0.9% sterile saline containing 0.2% ascorbic acid. Aβ is marked by a mouse monoclonal antibody (green). In **A, B, D,** the main Aβ concentration can be attributed to blood vessel walls and nearby space. **E.** The image from the contralateral SN side after Gp120 injection shows no apparent staining. **C, F,** images of the contralateral SN side after 6-OHDA injection, also show no apparent staining for Aβ. A blood vessel can be seen in **F**, surrounded by astrocytes but no beta-amyloid can be seen. Scale bar: 100 μm.

Immunohistochemical staining confirmed Aβ presence in tissue and blood vessel walls 60 min after injection. Notably, the surge in Aβ was detected in and around blood vessels and in their walls, and to a lesser degree within certain cells, likely neurons (see results), within the impacted area (Fig. 1). Interestingly, one hour after the injection of Gp120, the distribution of Aβ was similar (Fig. 3), while less intensive, emphasizing the role of blood vessels in Aβ supply (Martins et al., 2019; Inyushin et al., 2020).

Conclusions

We found that poisoning dopaminergic neurons with 6-OHDA produces Aβ elevation in the SN, mainly in and around blood vessels and their walls, as well as in some nearby tissue. These elevated levels remain, slightly reduced, after 24 hours. Injection of other proteins or saline to the same coordinates produces only a transient rise in Aβ, representing the response to needle trauma in both cases. While the data on the appearance of Aβ is robust, and beta-amyloid distribution corresponds with previous data, additional confirmation of Aβ distribution may be needed because of the limited sample set. This study underscores the possible link between dopaminergic neuron damage by toxic substances and Aβ accumulation, shedding light on the potential progression from PD to cognitive dysfunctions and dementia because of long-lasting damage to neurons.

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Author Contributions. Conceptualization, M.I., V.T., and J.A..; methodology, J.A.; formal analysis, X.X.; investigation, J.R-K., S.V-G., N.E-P, and J.K-V.; data curation, J.O-R.; writing—original draft preparation, M.I, S.V-G., and J.R-K; writing—review and editing, V.T., J.A., M.I., J.O-R.; visualization, M.I and J.K-V.; supervision, M.I.; project administration, M.I.;. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board. The animal study protocol was approved by the Institutional Review Board of the Universidad Central Del Caribe (UCC), (IACUC, #035-2021-09-00-PHA, 2021).

Data Availability Statement. All data presented in this study are included in this published article. Additional data can be received upon request from the main author.

Conflicts of Interest. The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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