Loss of Panx1 Function in Zebrafish Alters Motor Behavior in a Lab-on-Chip Model of Parkinson's Disease

Arezoo Khalili[1], Nickie Safarian[1], Ellen van Wijngaarden[1], Georg Zoidl[1], and Pouya Rezai[1]

[1]York University

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Abstract

Pannexin 1 (Panx1) forms ATP-permeable membrane channels that play essential roles in purinergic signaling in the nervous system. Several studies suggest a link between Panx1-based channels activity and neurodegenerative disorders including Parkinson's disease (PD), but experimental evidence is limited. Here, we applied behavioral and molecular screening of zebrafish larvae to examine the role of Panx1 in both pathological and normal conditions, using electrical stimulation in a microfluidic chip and RT-qPCR. A zebrafish model of PD was produced by exposing wildtype (panx1a+/+) and Panx1a knock-out (panx1a-/-) zebrafish larvae to 250µM 6-hydroxydopamine (6-OHDA). After 72hrs treatment with 6-OHDA a reduced electric-induced locomotor activity was observed in 5 days post fertilization (dpf) panx1a+/+ larvae. The 5dpf panx1a-/- larvae were not different from affected. The RT-qPCR data showed a reduction in tyrosine hydroxylase (TH) expression level in both panx1a+/+ and panx1a-/- groups. However, TH expression of 6-OHDA exposed panx1a-/- larvae was not decreased when compared to untreated mutants. Extending 6-OHDA treatment duration to 120hrs caused a significant reduction in the locomotor response of 7dpf panx1a-/- larvae when compared to the untreated panx1a-/- group. The RT-qPCR data also confirmed a significant decrease in TH expression levels after 120hrs treatments with 6-OHDA for both genotypes. Our results suggest that the absence of Panx1a channels compromised dopaminergic signaling in 6-OHDA-treated zebrafish larvae. We here propose that zebrafish Panx1a models offer great opportunities to shed light on the physiological and molecular basis of PD. Panx1a might play a preventive role on PD progression, and therefore deserves further investigation

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Arezoo Khalili[1], Nickie Safarian[2], Ellen van Wijngaarden[1], Georg Zoidl[2], Pouya Rezai[1,*]

[1] Department of Mechanical Engineering, York University, Toronto, ON, CANADA

[2] Department of Biology, York University, Toronto, ON, CANADA

* Corresponding Author: BRG 433B, 4700 Keele St, Toronto, ON, M3J 1P3, Canada; Tel: 416-736-2100 ext. 44703; Email: prezai@yorku.ca

Abstract

Pannexin 1 (Panx1) forms ATP-permeable membrane channels that play essential roles in purinergic signaling in the nervous system. Several studies suggest a link between Panx1-based channels activity and neurodegenerative disorders including Parkinson's disease (PD), but experimental evidence is limited. Here, we applied behavioral and molecular screening of zebrafish larvae to examine the role of Panx1 in both pathological and normal conditions, using electrical stimulation in a microfluidic chip and RT-qPCR. A zebrafish model of PD was produced by exposing wildtype (*panx1a*$^{+/+}$) and Panx1a knock out (*panx1a*$^{-/-}$) zebrafish larvae to 250µM 6-hydroxydopamine (6-OHDA). After 72hrs treatment with 6-OHDA a reduced electric-induced locomotor activity was observed in 5 days post fertilization (dpf) *panx1a*$^{+/+}$ larvae. The 5dpf*panx1a*$^{-/-}$

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Keywords: Microfluidics; Zebrafish; Panx1a knockout; Parkinson's disease model; Electric-induced response; RT-qPCR

Abbreviations

PD: Parkinson's disease

AD: Alzheimer's disease

Panx: Pannexin

6-OHDA: 6-hydroxydopamine

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

KO: Knockout

WT: Wild type

RD: Response duration

TBF: Tail beat frequency

dpf: days post-fertilization

RT-qPCR: Quantitative Real Time-PCR

TL: Tupfel long fin

TH: Tyrosine hydroxylase

PDMS: Polydimethylsiloxane

fps: Frames per second

SEM: Standard error of mean

L-DOPA: L-3,4-dihydroxyphenylalanine

1. Introduction

Parkinson's disease (PD) is one of the most prevalent neurological disorders which affects about 1% of the world population over 60 years old[1]. The etiology of PD is complex. Although the causes and risk factors of PD are still unknown, several factors including specific genes and environmental cues seem to play a role in inducing PD[2], [3]. Recent studies demonstrated the involvement of gap junctions and connexin hemichannels in a variety of neurological diseases, including Alzheimer's disease (AD) and PD[4], [5]. Pannexin (Panx) channels have also displayed aberrant functioning in neurodegenerative disease and may be etiologic in PD[6]. Panx1 is one of the known members of the Panx family which are ubiquitously expressed in many organs[7]. They show a widespread distribution in neurons and astrocytes of all major subdivisions of the brain including those affected by PD. However, their roles in the activity of astrocytes and neurons remains to be fully characterized[8]–[10]. There is evidence supporting a role of Panx1 channels in oxidative stress,

which is considered as one of the main contributors to the development of a variety of diseases such as AD and PD[11]–[13]. Yet, the involvement of Panx1 in the etiology of PD remains to be fully characterized.

In addition to humans and mice, the expression of Panx1 channels has been identified in other species including zebrafish[14]–[16]. Zebrafish larvae are widely used for studying genetic[17], behavioral activities[18]–[20] and neurodegenerative disorders[17], [21], [22]. They offer many advantages including small size[18], rapid development[23], genetic homology to humans[24] and optical transparency[25] that facilitate their use for fundamental and large-scale research. The optical transparency and rapid neurodevelopment throughout embryogenesis in zebrafish facilitates study of dopaminergic-related diseases such as PD[26]–[35]. Zebrafish PD models have been produced relying on either genetic manipulations[29], [34], [35] or exposure to different neurotoxins such as 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)[26], [30], [33].

Here, an association of Panx1 and PD was investigated by comparing molecular and behavioral properties of Panx1a knockout (KO) (*panx1a*$^{-/-}$) and wild type (WT) (*panx1a*$^{+/+}$) zebrafish using the 6-OHDA model. We have previously reported different microfluidic techniques to study the electric-induced behavioral responses of zebrafish larvae, quantitatively[20], [36]–[39]. The lab-on-chip approach allows to address challenges of behavioral studies such as providing a controllable stimulus to evoke the behavioral responses of larvae and quantifying their quick movements. Here, the electrical stimulus offers several advantages for behavioral studies as its duration, magnitude and direction can be accurately modulated to evoke locomotor responses in zebrafish larvae on demand. Using the electric-induced response duration (RD) and tail beat frequency (TBF) as quantifying parameters, we previously discovered significant difference between behavioral responses of 5-7 days post-fertilization (dpf)*panx1a*$^{+/+}$ and*panx1a*$^{-/-}$ larvae, suggesting the potential involvement of Panx1a in electric-induced locomotor response of zebrafish larvae[20]. This result was exploited to study the role of Panx1a channels for early stages of the development of Parkinson related disorders. Here, the electric-induced RD and TBF of*panx1a*$^{+/+}$ and*panx1a*$^{-/-}$ zebrafish larvae in response to 6-OHDA provided insight into Panx1a channels' involvement in the etiology of PD. In support of the behavioral analysis quantitative Real Time-PCR (RT-qPCR) tested the differential expression of tyrosine hydroxylase expression was also employed to study the molecular events underlying the behavioral response of zebrafish larvae. This study opens broad areas of application including on-demand behavioral investigations of gene functions and chemical toxicity, as proposed in this application for studying the roles of Panx1a in the etiology of PD.

2. Materials and Methods

2.1. Zebrafish Husbandry and Chemical Exposure

This study used zebrafish larvae of the Tupfel long fin (TL) strain and the *panx1a*$^{-/-}$ line described previously[40]. Larvae were raised in egg water (prepared from Instant Ocean, Blacksburg, USA) supplemented with 0.1% methylene blue (M291-110 Fisher Scientific, USA). Larvae were maintained in a controlled environment at a temperature of 28°C with a 14:10 hour light to dark cycle. Adult TL and *Panx1a*$^{-/-}$ zebrafish were bred, housed, and raised based on our recent protocol[41].

6-OHDA (Sigma-Aldrich, Oakville, ON, Canada) was used at 250µM based on previous studies[20], [33], [42]. A total of 15 embryos per well were placed in a 12-well plate and 6-OHDA was administered every day starting at 2dpf for either 72hrs (testing at 5dpf) or 120hrs (testing at 7dpf). Considering the light sensitivity of 6-OHDA, the multi-well plates were covered with aluminum foil to avoid light exposure. To keep conditions consistent the untreated control groups were shielded from light in parallel with the test group.

The instructions and specifications outlined in the Canadian Council for Animal Care (CCAC), ACC protocol *GZ 2020-7 R3* and York University biosafety permit *PR 02-19* were diligently followed.

2.2. RNA Isolation and Quantitative Real Time-PCR

Total RNA was extracted from whole larvae using RNeasy Plus Mini Kit (Qiagen, Toronto, ON, Canada).

One microgram of total RNA input was reverse transcribed to cDNA using the iScript Reverse Transcription Supermix (Bio-Rad Laboratories, Mississauga, ON, Canada). The cDNA equivalent of 15ng total RNA was analyzed in triplicate by RT-qPCR using the SsoAdvanced SybrGreen PCR mix (Bio-Rad) and the MACHINE Biorad. The raw data were exported from the CFX Manager Software (Bio-Rad, Canada), and the relative gene expression was calculated using the Relative Expression Software Tool (REST-2009)[43]. The 18srRNA gene served as the reference gene to verify changes in the expression of the target gene tyrosine hydroxylase (TH; which is the marker enzyme for dopaminergic neurons). The qPCR primers for TH were: forward, 5'- TTGTGTCCGAGAGCTTTGAG-3‘ and reverse, 5‘-AAGCATTCTGGATCTTGGAGG-3‘; for 18srRNA were: forward, 5'- TCGCTAGTTGGCATCGTTTATG -3‘ and reverse, 5‘-CGGAGGTTCGAAGACGATCA -3‘.

2.3. Behavioral Screening with Microfluidics

The behavioral experiments were performed with a three-layer polydimethylsiloxane (PDMS) microfluidics device demonstrated previously[20], complemented with key auxiliary components such as syringe pumps (LEGATO 111, KD Scientific Inc., USA), a sourcemeter (Model 2410, Keithley, USA) and an upright Leica stereomicroscope (Stereomicroscope Leica MDG41, Singapore) with a camera (GS3-U3-23S6M-C, Point Grey Research Inc., Canada) to enable manipulation, stimulation, and imaging of zebrafish larvae (Fig. 1A).

The microfluidic device consisted of a series of microchannels (Fig. 1B). 3D master molds for the top and bottom layers were first designed using SolidWorks (SolidWorks Corp., USA) and printed (Objet260 Connex3 printer (Stratasys Ltd., USA)) to allow for PDMS casting. The top mold contained the inlet, outlet, main channel, electrodes, larva head trap, and larva tail screening pool. The bottom mold contained a L-shaped valving channel. The PDMS base and curing agent were mixed at a ratio of 10:1, degassed for half an hour and poured into the molds and with the necessary tubes added. After curing for approximately 6 hours on a hotplate at a temperature of 60°C, the molds were removed, and a 0.2mm thick PDMS middle layer membrane was bonded between the top and bottom PDMS layers.

During an experiment, a larva was transferred into the device via the inlet in the top layer and moved along the main channel using the syringe pump with a controlled flow rate of 2 mL/min until reaching the trapping region (TR) (Fig. 1C). The narrowed section of the TR adjoined to the open screening pool acted to immobilize the upper body and head of the larvae while allowing the tail to move freely in the screening pool. The valve, situated in the bottom layer, was then pressurized, causing the middle membrane to deflect and create a physical barrier that prevented the larva from swimming out of the TR. Following a one-minute recovery period, shown to be sufficient to return cardiac activity to baseline, the electric stimulus of 3µA was applied for 20 s using the sourcemeter[39]. The larva's locomotor response could be recorded with a camera at 2x magnification on the Leica stereomicroscope (Fig. 1B). The tested larva was removed from the device via the outlet before repeating the experiment to reach the designated sample sizes for each condition.

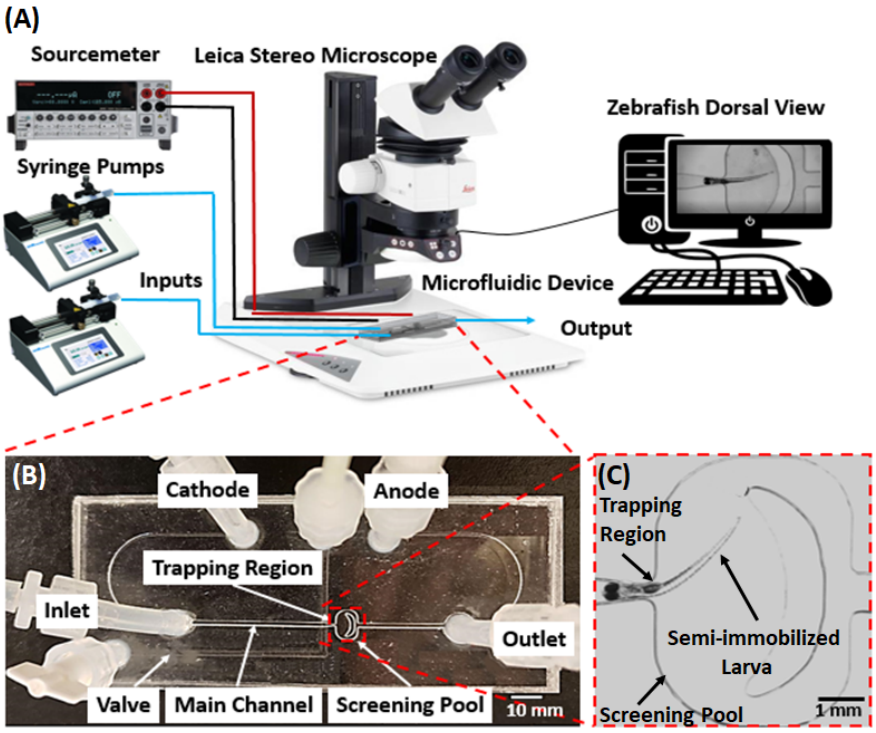


Fig. 1. Microfluidic device and experimental setup for screening the electric-induced response of semi-immobilized 5-7dpf zebrafish larva. (A) The experimental set up made up of a microscope, two syringe pumps, sourcemeter, computer, camera and microfluidic device connected via tubing and wires (B) The device consisting of a sloped inlet, rounded side channels, main channel, trapping region (TR), screening pool, outlet, two electrodes and valve channel. (C) Close up view of the TR and screening pool showing a semi-immobilized 5dpf larva.

2.4. Video and Image Analysis

The tail movement was analyzed using an open-source software, Kinovea (www.kinovea.org, France). The software facilitates dynamic analysis for a variety of applications. After importing a tail movement video recorded at a speed of 160 frames per second (fps) using the camera mounted on the microscope, the tail tip position could be efficiently tracked over the duration of the video. The software output a data file that can be used to calculate the RD and TBF of the movement tracked. Tracking was monitored to ensure that the software was correctly identifying the tail position to ensure accuracy. If required, the tracking position could be manually adjusted using the convenient point tracking tool. The RD value was taken to be the time between the beginning and end of tail movement. The start of movement coincided with the start of electric stimulation with the end of movement varying between fish. The TBF was calculated as the ratio of the number of complete cycles to the RD. Any small flick movement patterns were excluded to keep tail analysis methods consistent with previous methods of behavioral screening in the field[20], [28]. To be counted as a part of a cycle, a tail movement had to pass the designated threshold of $\pm 0.25mm$ relative to the axial centerline of the TR. A full cycle is composed of four threshold passes.

2.5. Data Analysis

Common statistical processing was applied to better understand the distribution of the data and discern the presence of significant differences. All errors are reported in terms of the Standard Error of the Mean (SEM). Shapiro-Wilk and Mann-Whitney U tests were used to check the normal distribution and presence of significant differences between behavioral data of various groups, respectively. For RT-qPCR, the statistical

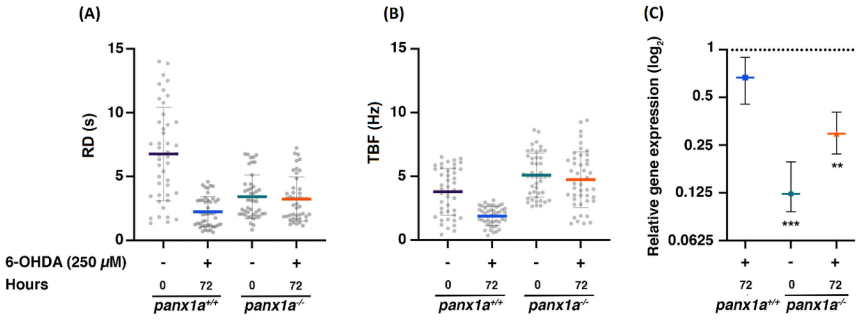
significance was tested by a Pair Wise Fixed Reallocation Randomisation Test © and plotted using SEM estimation[43]. Sample sizes were determined by power analysis. An upper limit of 0.05 and a significance level of 80% was applied.

3. Results and Discussion

A lab-on-chip approach was used to test the hypothesis that loss of Panx1 function alters an experimental PD phenotype. To investigate this hypothesis, 2dpf *panx1a*$^{+/+}$ and*panx1a*$^{-/-}$ larvae were exposed to 250µM 6-OHDA for either 72 or 120hrs. The research compared behavioral responses and changes of TH expression of *panx1a*$^{+/+}$ and*panx1a*$^{-/-}$ larvae exposed to 6-OHDA with the non-treated control groups.

3.1. Panx1a Function Impacts Molecular and Behavioural Changes Induced by 6-OHDA Treatment in 5dpf Zebrafish Larvae

The initial evidence for a potential involvement of Panx1a in zebrafish larvae electric-induced response was detected when*Panx1a*$^{-/-}$ larvae responded with shorter RD and higher TBF compared to the *panx1a*$^{+/+}$ group. The effect of 6-OHDA on locomotor activity was evaluated by examining the behavioral phenotypes of RD and TBF of larvae exposed to electrical stimulus (Fig. 2A and 2B, with all p-values presented in Table 1). Exposure to 6-OHDA for 72hrs caused a 68% and 50% decrease in the RD and TBF of *panx1a*$^{+/+}$ control larvae. The results were consistent with previously published data, where 6-OHDA treatment was associated with mobility deficits[44], [45]. However, these changes in the locomotor activity were absent in*panx1a*$^{-/-}$ larvae after 6-OHDA treatment, suggesting that 6-OHDA treatment does not induce locomotor deficits in 5dpf larvae in the absence of Panx1a functions. We concluded that Panx1 channels and 6-OHDA might function through similar signaling pathways, so that in the absence of Panx1 the 6-OHDA target is disrupted as well[6], [46].



Φιγ. 2. Ελεςτριc-ινδυcεδ (A) PΔ ανδ (B) TBΦ οφ 5δπφ πανξ1α$^{+/+}$ *ανδ πανξ1α*$^{-/-}$ *ζεβραφιcη λαραε εξποcεδ το 250µM 6-OHΔA φορ 0 ανδ 72ηρc. (15 λαραε περ εξπεριμενταλ cονδιτιον ιν τηρεε ινδεπενδεντ τριαλc, N = 45). (˚) Τηε PT-χΠ˜P δατα cηοωινγ 6-OHΔA-ινδυcεδ cηανγε ιν τψροcινε ηψδροξψλαcε (TH) εξπρεccιον οφ λαραε τρεατεδ ωιτη 250µM 6-OHΔA φορ 0 ανδ 72ηρc. Δοττεδ λινε ατ Ψ=1 ρεπρεcεντc τηε TH εξπρεccιον λεελ ιν πανξ1α*$^{+/+}$ *cοντρολ γρουπ. Εαcη αλυε ρεπρεcεντc Μεαν ± SEM of three independent experiments (N=150/group). All data were compared with the panx1a*$^{+/+}$ *control group. **: p<0.01, ***: p<0.001.*

Ταβλε 1. δμπαριcον οφ ελεςτριc-ινδυcεδ PΔ ανδ TBΦ οφ 5δπφ πανξ1α$^{+/+}$ *ανδ πανξ1α*$^{-/-}$ *λαραε τρεατεδ ωιτη 250µM 6-OHΔA φορ 0 ανδ 72ηρc· π-αλυεc ωερε cαλcυλατεδ υcινγ τηε Μαννν-Ωηιτνεψ ϒ τεcτc.*

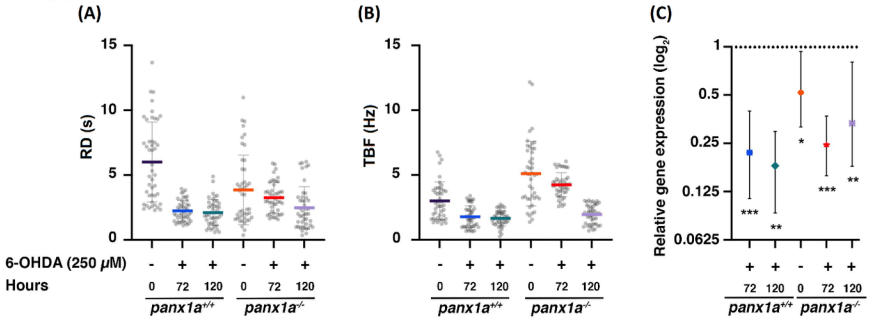
p-value	p-value	Genotype	-/-	+/+
Parameter	**Genotype**	**6-OHDA exposure duration**	**0**	**72hrs**
RD	+/+	**0**	<0.0001	<0.0001
TBF			0.003	<0.0001
RD	-/-	**72hrs**	0.395	0.005
TBF			0.375	<0.0001

The expression level of tyrosine hydroxylase (TH) was quantified to examine if changes in the dopaminergic pathway were affecting the behavioural responses in both two genotypes. TH is the enzyme responsible for catalyzing the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) (Molinoff PB, Axelrod J. Biochemistry of catecholamines. Annual Review of Biochemistry. 1971;40:465–500). In neurons TH is localized in the pre-synaptical compartment and acts as the rate-limiting enzyme of catecholamine biosynthesis. Evidence from the RT-qPCR data presented in Fig. 2C demonstrated that the TH expression level at 2dpf in untreated*panx1a*$^{-/-}$ larvae was significantly lower compared to *panx1a*$^{+/+}$ controls (*p-value* <0.0001). 6-OHDA treatment for 72hrs decreased the expression of TH in both *panx1a*$^{+/+}$(*p-value*= 0.049) and *panx1a*$^{-/-}$(*p-value*= 0.002) groups. No attenuation of TH expression after 6-OHDA treatment was found in *panx1a*$^{-/-}$ larvae when compared to untreated age-matched mutants (*p-value*= 0.064). This result suggested that a significant reduction in TH expression occurs when the Panx1a function is lost and that 6-OHDA treatment could not further aggravate the situation.

3.2. Dopaminergic Degeneration Minds the Duration of Exposure to 6-OHDA

The first week of zebrafish development is rapid and vital that every day counts for critical steps in forming a functional organism [46]. To assess the impact of age and treatment duration on the extent of developmental changes caused by 6-OHDA treatment, we repeated the experiments using 7dpf larvae. We exposed 2dpf*panx1a*$^{+/+}$ and*panx1a*$^{-/-}$ larvae to 250µM 6-OHDA for either 72 or 120hrs and tested their performance at 7dpf (Fig. 3A and 3B). All p-values were presented in Table 2. A 72hrs treatment significantly reduced the RD and TBF of 7dpf *panx1a*$^{+/+}$ larvae by 63% and 40%, respectively. The results were consistent with the data obtained for 5dpf larvae in section 3.1. The electric-induced RD and TBF of 7dpf *panx1a*$^{-/-}$ larvae were unchanged upon 72hrs treatment with 6-OHDA, like the behavioral response of 5dpf mutants.

Extending the treatment from 72 to 120hrs had no effect on 7dpf*panx1a*$^{+/+}$ larvae, suggesting that both exposure times led to the same and significant levels of motor deficiency and cell death of dopaminergic cells. The results suggested that *panx1a*$^{+/+}$ larvae showed the greatest sensitivity to the neurotoxin within the first 5 days of development. In contrast to *panx1a*$^{+/+}$ larvae, 7dpf*panx1a*$^{-/-}$ larvae RD (24%) and TBF (52%) declined when the 6-OHDA exposure time was extended from 72 to 120 hour. This result showed that the duration of exposure of mutants to the neurotoxin dictated the behavioral outcomes. We concluded that the deregulation of the dopaminergic pathway in*panx1a*$^{-/-}$ larvae shown previously[40] is the probable cause of the 6-OHDA susceptibility at 7dpf. This idea was tested using the rate-limiting enzyme TH as a surrogate for changes to dopaminergic signaling. A significant reduction in TH expression levels was observed after toxin treatments for both genotypes which was aligned with the behavioral outcomes (Fig. 3C). We concluded that the absence of functional Panx1a channels compromised dopaminergic signaling in 6-OHDA treated zebrafish larvae following a reduction in the expression of the critical rate-limiting enzyme TH.



Φγ. 3. Ελεστρις-ινδυσεδ (A) PΔ ανδ (B) TBΦ οφ 7δπφ πανξ1a$^{+/+}$ *ανδ πανξ1a*$^{-/-}$ *ζεβραφισηλαρα ε εξποσεδ το 250µM 6-OHΔA φορ 0, 72 ανδ 120ηρς. (15 λαρα ε περ εξπεριμενταλ γονδιτιον ιν τηρεε ινδεπενδεντ τριαλς, N = 45). (˚) Τηε PT-χΙΤΡ δατα σηοωινγ 6-OHΔA-ινδυσεδ γηανγε ιν τψροσινε ηψδροξψλασε (TH) εξπρεσσιον οφ λαρα ε τρεατεδ ωιτη 250µM 6-OHΔA φορ 0, 72 ανδ 120ηρς. Δοττεδ λινε ατ Ψ=1 ρεπρεσεντς τηε TH*

*εξπρεσσιον λεελ ιν πανξ1α$^{+/+}$ ςοντρολ γρουπ. Εαςη αλυε ρεπρεσεντς Μεαν ± SEM of three independent experiments (N=150/group). All data were compared with the panx1a$^{+/+}$ control group. *: p<0.05, **: p<0.01, ***: p<0.001.*

Ταβλε 2. δμπαρισον οφ ελεςτρις-ινδυςεδ ΡΔ ανδ ΤΒΦ οφ 7δπφ πανξ1α$^{+/+}$ ανδ πανξ1α$^{-/-}$ λαραε τρεατεδ ωιτη 250μΜ 6-ΟΗΔΑ φορ 0, 72 ανδ 120ηρς· π-αλυες ωερε ςαλςυλατεδ υσινγ τηε Μαννν-Ωηιτνεψ Υ τεστς.

p-value	p-value	Genotype	+/+	+/+	-/-	-/-
Parameter	**Genotype**	**6-OHDA exposure duration**	**0**	**72hrs**	**0**	**120hrs**
RD	+/+	**72hrs**	**<0.0001**	—	—	—
TBF			**<0.0001**	—	—	—
RD		**120hrs**	**<0.0001**	**0.375**	—	**0.583**
TBF			**<0.0001**	**0.775**	—	**0.055**
RD	**-/-**	**0**	**0.003**	—	—	**0.011**
TBF			**<0.0001**	—	—	**<0.0001**
RD		**72hrs**	—	**0.011**	**0.939**	**0.003**
TBF			—	**<0.0001**	**0.173**	**<0.0001**

4. Conclusion

Currently, there is no standard treatment for PD; Therefore, investigation of relevant factors underlying the pathophysiological progression of this disease is required for translational research.

Here, we aimed to investigate the molecular, and behavioral responses of *panx1a* $^{-/-}$ zebrafish larvae to shed some light on the association of Panx1a in the etiology of PD. Although a significant decrease was observed in the behavioral response and TH expression of 5 pdf *panx1a*$^{+/+}$ larvae, our results demonstrated a resistance against 6-OHDA-induced locomotor deficits in 5 pdf *panx1a*$^{-/-}$ larvae. However, treatment of both genotypes with 6-OHDA for 120hrs was accompanied by motor decline and TH expression reduction in 7dpf larvae that might be attributed to the deregulations of the dopaminergic pathway in*panx1a*$^{-/-}$ larvae at this age. The key findings of this study have the potential to foster new lines of research that will resolve changes of molecular and cellular mechanisms caused by 6-OHDA which are likely to represent the earliest insults driving a vertebrate towards PD. These studies will shed light on the roles of Panx1 channels in PD. Finally, the versatility of the lab-on-chip architecture used in this study will allow to test a wide variety of environmental toxins for their ability to cause PD-like phenotypes.

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Data Availability

Data is available on request from the authors.

Ethics Approval Statement

All experiments carefully followed the required guidelines set out by the Canadian Council for Animal Care (CCAC), based on Animal Care Committee (ACC) protocol GZ 2020-7 R3 and York University Biosafety Permit PR 02-19.

Conflict Of Interest Statement

The authors do not claim any conflict of interest.

Author's Contributions

Arezoo Khalili: Methodology, Investigation, Formal analysis, Validation, Data curation, Visualization, Writing - original draft.

Nickie Safarian: Methodology, Investigation, Visualization, Writing - original draft.

Ellen van Wijngaarden: Investigation, Writing - original draft.

Georg R. Zoidl: Conceptualization, Validation, Resources, Writing - review and editing.

Pouya Rezai: Conceptualization, Methodology, Validation, Resources, Writing - review and editing, Supervision, Funding acquisition.

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