Rotenone as a nerutoxin model for Parkinson's disease

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Introduction

Parkinson's disease(PD) is one of the most common neurodegenerative disorders, the hallmark being dopamine (DA) neuron loss resulting in locomotory deficits. Scientists identified that a major factor contributing to the development of the disease is damage from the environment such as long term exposure of neurotoxins or heavy metals.

To provide insight on the disease, multiple models have been developed to test multiple aspects of the disease. The majority of works are to test PD-linked drugs for DA reduction and movement deficits.

Rotenone is a commercially available pesticide that is associated with PD. Rotenone has succesfully been used as an environmental toxin model for larval zebrafish [1]. The objective of this research is to assess the viablity of using adult zebrafish as a model for PD since the disease is more relevant in older patients.

Materials and methods

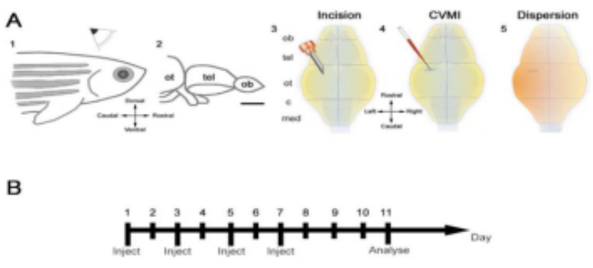
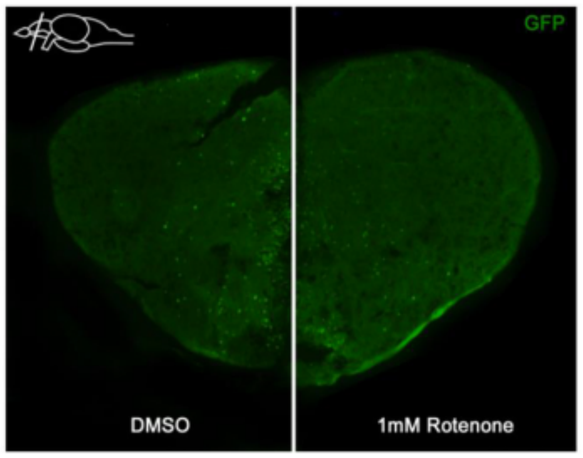


Figure 1: Procedure for cerebroventricular microinjection of Rotenone into adult zebrafish. CVMI technique consists of making a small incision in the skull making way for a microinjection needle to penetrate, dispersing the drug into the ventricular fluid (A).

The injections were performed every two days, a total of 4 times. 4 days after the last injection, the fish were recorded using a Nikon camera and Tracker© software to asses the distance travelled for behavioural assays; and then sacrificed and brain-dissected. The brains were sectioned and cells were visualized after staining with an anti-GFP antibody using the ZEISS LSM880 confocal microscope.(B).

A trangenic line *dat:eGFP* was used which GFP was driven by the gene promoter of Dopamine Transporter. This allowed dopamine neurons to be labelled with GFP.

Results



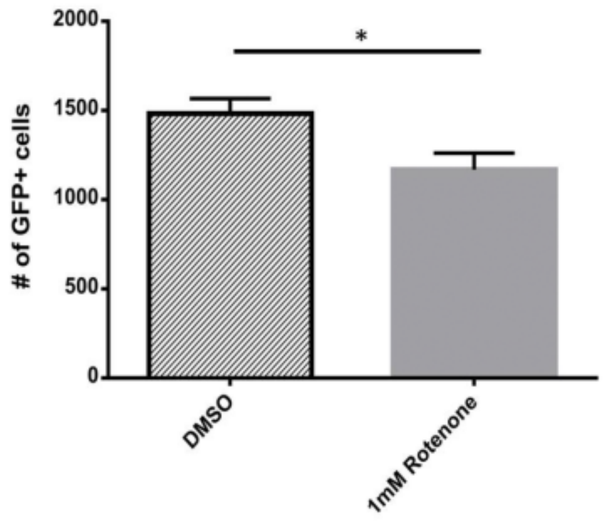
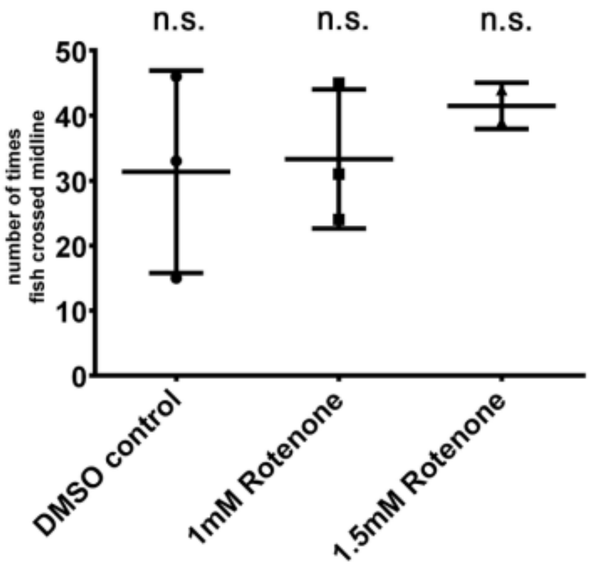


Figure 2: Immunostains showing a 20% decrease in telencephalic DA level of adult zebrafish injected with Rotenone. Frozen sections (20µm thickness) of 10 month old dat:eGFP zebrafish injected with 1mM, Rotenone or 0.1% DMSO and immunostained with anti-GFP (green) (n=3).



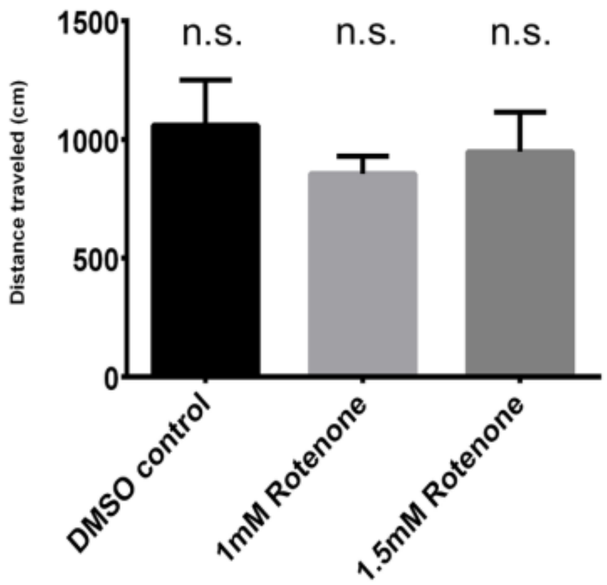


Figure 3: Midline crossings and distance show that adult zebrafish injected with Rotenone have no distinct behavioral phenotype nor locomotory deficits. Adult zebrafish injected with either 1mM Rotenone, 1.5mM Rotenone or 0.1% DMSO were placed in a standalone tank and were tracked for position every 10 milliseconds in a total time of 3 minutes to calculate the distance traveled (right), or monitored at 4 minute intervals for the number of times they crossed the midline of the tank (left) (n=3).

Discussion

In the literature, conventional methods where fish were treated with drugs dissolved in water had little effects on DA loss. CVMI was used as a novel approach to increase delivery efficiency.

The telencephalic neuron population is of interest because it is believed the DA clusters here are responsible for locomotion and this region is close to where the drug dispersed, making it a vulnerable cluster.

A significant reduction of DA neurons (~20%) was observed but to see if this translated to locomotry deficits 2 behavioural assays were conducted. Zebrafish being a very hyperactive species should exhibit differences in locomotion with significant reduction of DA neurons, therefore a decrease in distance travelled by the fish can be a symptom of bradykensia which is a hallmark of PD. However, the results of the distance travelled and midline crossing assays depict no distinct behavioural phenotype. This suggests a 20% decrease was not enough to induce locomotory deficits.

This may be a limitation of the drug's kinetics, as rotenone is only retained in the skull for a few hours. It may also be due to the fact that a large number of neurons are still present to attenuate the loss. Another possibilty is the telencephalic DA clusters are not involved in locomotion but instead, the clusters projecting from the diencephalon to the subpallium are [2].

Future Works

To induce more loss of DA neurons, injection with increased dosing would be done to increase exposure time of rotenone to the brain

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CVMI of other neurotoxins with faster kinetics, which are more robust, may counteract Rotenone's limitations, and be more effecient.

Furthermore, other DA populations may also be implicated in the locomotory pathway so future analysis may shed further insight on aspects of this disease.

References:

[1] Li N, Ragheb K, Lawler G, Sturgis J, Rajwa B, Melendez JA, Robinson JP. 2003. Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *J Biol Chem.* 278(10):8516-25.

[2] Martel, Simon, et al. "Rotenone Neurotoxicity Causes Dopamine Neuron Loss in Zebrafish." University of Ottawa Journal of Medicine, vol. 5, no. 2, Nov. 2015, p. 16, doi:10.18192/uojm.v5i2.1413.