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Nicotine-Induced Neuroprotection in Drosophila Models of Parkinson's Disease

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NICOTINE-INDUCED NEUROPROTECTION IN *DROSOPHILA* MODELS OF PARKINSON'S DISEASE

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Abstract

Parkinson's disease (PD) causes rigidity, tremors, and posture impairments. There is no cure for this disease and its symptoms intensify with age. In this study, fruit flies were induced with PD using rotenone and the flies in treatment group were provided with food that contained nicotine to determine if nicotine causes neuroprotection and alleviates symptoms of PD. A climbing assay was used to assess the severity of symptoms of PD in the control and experimental groups. The PD induced flies that received nicotine did not show a significant difference in motor ability and therefore did not experience neuroprotection.

1. Introduction

Parkinson's disease is a neurodegenerative disease that destroys the dopaminergic neurons in the substantia nigra of the brain. It also causes a decrease in the level of dopamine in the striatum (Lei et al., 2009). Physical symptoms include rigidity, tremors, and posture impairments (Quick et al., 2007). The symptoms of PD intensify with age and the disease has no known cure. There are medications that help alleviate the symptoms of PD, but they do not prevent or treat the condition and they have adverse side effects. This

is why it is critical to try to find a drug that can cause neuroprotection for PD patients (Lei et al., 2009).

It is important to study neuroprotection and neurodegeneration on living organisms because their complex processes can affect outcomes of treatments. *Drosophila,* known more commonly as the fruit fly, is a useful model organism because it has, a very short generation time and it is easy to manipulate its environment to test different variables. Fruit flies are similar to humans in that they can carry out complex behaviors (Botella et al., 2009).

In a study by Trinh et al. (2010), the climbing index of PD induced fruit flies was calculated after the flies consumed tobacco-free food, cornmeal molasses or food containing tobacco. The flies in the experimental group experienced neuroprotection and an increased climbing index. In addition, flies in the experimental group experienced an increased lifespan from 45 days to 65 days. Flies in the control group did not experience an increased climbing index or neuroprotection and had a lifespan of 40 days (Trinh et al., 2010).

Other studies have sought to determine if nicotine is the ingredient in the tobacco that causes the protective effect. In a study by Lei et al. (2009), mice were induced with PD using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Mice in the experimental group were injected with nicotine and subsequently scored higher on behavioral tests, and experienced restoration of their degenerated neurons (Lei et al., 2009).

A study by Morens et al. (1995) found that the risk of PD decreases about 50% in smokers. Nicotine was suspected to be the compound causing this result because it

increases striatal dopamine release (Dani et al., 2006). It also protects the brain from degeneration due to toxic insults in different experimental designs (O'neill et al., 2002). Nicotine was used to test against nigrostriatal damage in nonhuman primates (Quik et al., 2007). The experiments were designed to mimic long-term smoking in humans. This was achieved through nicotine exposure in drinking water for a year on primates that were induced with slow nigrostriatal damage over the previous six months using MPTP. It was shown that striatal markers linked to dopamine were more abundant in nicotine-treated monkeys compared to monkeys not receiving nicotine treatments. Not only did the nicotine treatment improve molecular measures of dopamine levels it also normalized the abnormal activity of the dopamine pathways. These finding support the idea that nicotine is one of the key ingredients in tobacco that lowers the risk of PD in smokers (Quik et al., 2007).

Nicotine has been shown to help improve motor activity in people with PD. When 15 patients with early to moderate PD were administered nicotine intravenously and through a patch, their motor activity improved by several measures (Kelton et al., 2000). This result corroborates findings that PD-related tremors and rigidity decrease due to smoking (Quik and Kulak, 2002). It has been reported multiple times that tobacco users have a 20-80% decreased risk of developing PD when compared to people who do not use tobacco (Balfour et al., 1996; Baron, 1996; Haack et al., 1981;Morens et al., 1995). The responsible compound in tobacco has yet to be discovered. One hypothesis for the mechanism of action is that tobacco products reduce enzymatic activity. Additionally, nicotine receptor activation stimulates dopamine release. This accelerates neural firing and helps the brain function more efficiently (Quik and Kulak, 2002).

Some studies have suggested that there is no correlation between using tobacco products and a decreased risk of developing PD. A study by Kandinov et al. (2007) observed patients diagnosed with PD from Tel Aviv Sourasky Medical Center. It was found that there was no correlation between patient's use of cigarettes and the rate of progression of PD. The authors' findings were limited in that tobacco use was specific to smoking cigarettes (Kandinov et al., 2007).

The central focus of my thesis is to determine whether or not nicotine causes a neuroprotective effect in *Drosophila* models of PD. I hypothesize that nicotine is the main ingredient in tobacco that causes a neuroprotective effect, which may result in a slower progression of PD.

2. Materials and methods

2.1 Preparation of Flies

To conduct this experiment, 100 *Drosophila* were used. The flies were sexed and only the males were used for this study to reduce variability in behaviors and prevent new offspring from being produced. Twenty flies were placed in each of two vials and were provided with 8.9 μL (.5 nM) of nicotine, 10 mL of 5% sucrose, and 1% agar food. The remaining sixty flies were provided with food that lacked nicotine. They were housed in three vials and provided with 10 mL of 5% sucrose and 1% agar food. Both the control and experimental flies were given time to consume their food for a period of seven days.

After seven days had passed, the sixty flies in the control group were transported into three different vials of twenty flies. Flies in two of the three vials were induced with

PD using different concentrations of rotenone. One group of twenty flies was provided with food containing 10 mL of 5% sucrose and 1% agar. The other two groups of 20 flies were provided with either 30 mM or 40 mM of rotenone (one concentration per group) in their food containing 10mL of 5% sucrose and 1% agar.

The 40 flies that had been exposed to nicotine were divided into two different groups after the seven days had past. All of the flies in this group were induced with PD using rotenone. Each of the flies was provided with either 30 mM or 40 mM of rotenone (one concentration per group) and provided with 10 mL of 5% sucrose and 1% agar food.

2.2 Climbing Assay

After seven days, the climbing index of all flies was measured. The climbing chambers were made by taping two clear plastic vials together vertically. A height of 8 cm was measured and marked around the circumference of the tube. Five climbing chambers were made for each treatment group. The flies were transported into their climbing chambers and were given a minute to acclimate prior to the assay.

To perform the assay, the bottom of the tube was tapped to stimulate climbing and a timer was started. After 10 seconds the flies that successfully completed the 8cm climb were counted. All of the flies were tested simultaneously starting with the control group. Control and experimental groups were tested sequentially to allow time for rest and recovery of flies between the ten trials that were conducted per group. The number of flies that were successful per trial was recorded as a percentage of total flies per treatment group.

3. Results

The flies treated with 30 mM of rotenone exhibited a lower climbing ability in the assay compared to the control flies. However the difference was not statistically significant using an alpha of 0.05 (p= 0.054). The flies treated with 40 mM of rotenone were more adept at climbing than the flies treated with 30mM of rotenone, but again the difference was not significant (p=0.708).

The flies treated with 0.5 nM of nicotine plus 30 mM of rotenone had a greater climbing ability compared to the flies treated with 30 mM of rotenone alone, but the difference was not significant (p=0.708). The flies treated with 0.5 nM of nicotine plus 40 mM rotenone had the lowest climbing ability of all of the treatment conditions.

Trial Number	Control	30 mM Rotenone	40 mM Rotenone	30 mM Rotenone plus 0.5nM Nicotine	40 mM Rotenone plus 0.5 nM Nicotine
1	0.6	0.67	0.58	0.2	0.21
2	0.4	0.33	0.66	0.6	0.28
3	0.5	0.22	0.33	0.4	0.42
4	0.6	0.22	0.33	0.4	0.21
5	0.3	0.33	0.58	0.5	0.29
6	0.2	0.22	0.33	0.3	0.21
7	0.5	0.33	0.41	0.4	0.14
8	0.5	0.44	0.25	0.3	0.21
9	0.4	0.22	0.25	0.4	0.14
10	0.4	0	0.42	0.4	0.07
Mean	0.44	0.3	0.42	0.39	0.22

Fig. 1. The proportion of flies that successfully climbed 8cm in 10 seconds and the mean values for each treatment group

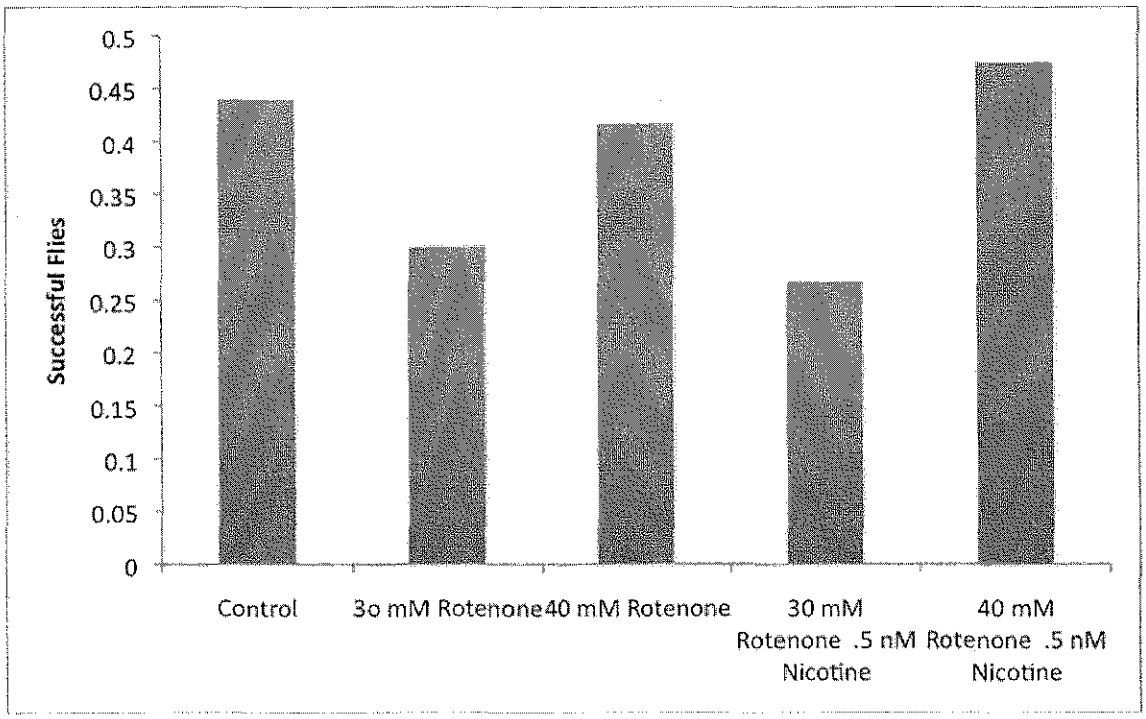

Fig. 2. The effects of rotenone and nicotine on the climbing success of flies. The treatment conditions are shown on the x-axis, and the proportion of successful flies able to climb 8 cm in 10 seconds is shown on the y-axis.

Control	3o mM Rotenone	40 mM Rotenone	30 mM Rotenone .5 nM Nicotine	40 mM Rotenone .5 nM Nicotine
0.44	0.5	0.43	0.44	0.36

Fig. 3. The proportion of flies that died prior to the climbing assay.

4. Discussion

There was evidence of a lower climbing ability of flies treated with rotenone compared to the control flies, although the difference between the groups was not significant. Adding a 35 mM concentration of rotenone to help the PD model. Using a larger

number of flies in each treatment group should also be attempted. Using young adult flies may decrease the number of deaths during the experiment.

There are many other methods that could be used to induce PD in flies besides rotenone. To study the function and contribution of genes involved in PD, mutational analysis, genetic screens and RNAi technology could be used (Botella et al., 2009). Many genes that are involved in PD have counterparts in flies, but others may be absent. The gene *α-synuclein* has been linked to PD and has a mechanism of toxicity that needs investigation, but this gene is not present in flies. To incorporate this gene into flies, the human-related transgene is cloned into a plasmid. This plasmid contains the 'upstream activating sequence' that serves as a target for GAL4, a transcription activator. If the fly had GAL4 present then it could express this gene and thus been used as a model of PD (Botella et al., 2009).

The flies treated with 30 mM of rotenone and 0.5 nM of nicotine showed evidence of higher climbing abilities when compared to the flies treated with 30 mM of rotenone alone, although there was no significant difference between the groups. If the dosage of nicotine was increased, this may produce a significant difference.

Direct observation of neuroprotection, rather than testing motor ability, would be useful for future studies. The brain of each fly could be dissected in 1x PBS and fixed for 30 minutes in paraformaldahyde. After treating the brains with 10% serum and staining them against tyrosine hydroxylase, observations could be made under a confocal microscope to determine the integrity of dopamine secreting neurons (Trinh et al., 2010).

The fast generation time of flies helped to allow for greater numbers of individuals to test on, but it also allowed for a limited amount of time to test the flies. Some of the treatment groups experienced high mortality and this reduced the sample sizes. If a different model organism was used with a longer life expectancy such as rats or monkeys, this problem may have been avoided. However different animal models may show discrepancies in the neuroprotective effect of nicotine. Mice, for example are not effective models for testing neuroprotection due to nicotine exposure. In contrast, rats, flies and nonhuman primates are effective at modeling human diseases and have reproducible neuroprotection (Quick et al., 2007).

Our results did not show that nicotine causes neuroprotection in *Drosophilla* models of PD. We need to improve the induction of PD in the flies and look for evidence of neuroprotection in ways other than testing the climbing ability. More testing should be conducted in the future to see if nicotine or other compounds can cause neuroprotection and help reduce the progression of PD in humans.

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