

Manganese Toxicity in the Dopamine Synthesis Pathway in *Drosophila melanogaster*

Brandon Adair and Tamra Jones.
Department of Biology, Carroll College

Introduction

- The *ple* gene encodes the enzyme Tyrosine Hydroxylase (TH) which catalyzes the first, rate limiting, step of dopamine (DA) biosynthesis.
- Low and declining levels of DA is a characteristic of Parkinson's Disease.
- Manganese Toxicity (Manganism) has been reported to have symptoms resembling Parkinson's Disease.
- In this study, a possible link between Manganese toxicity and expression of *ple*, was explored in the model organism *Drosophila melanogaster*.
- *Drosophila melanogaster* are easy to culture and observe through development making them a popular model organism in Developmental Biology.
- **Hypothesis:** Exposure of *Drosophila* larvae to environmental Manganese will result in decreased *ple* expression in the larvae and motor deficits in adults.

Methods

- **Primer synthesis:** Primers for *ple* were designed using IDT software.
- **Culturing *Drosophila melanogaster*:** Cultures were maintained in glass vials with a potato-based media hydrated with water (control) or 0.1mM Manganese Chloride (treatment).
- **RNA extraction:** Qiagen's RNeasy Mini Kit was used to extract RNA. The RNA was then treated with DNase.
- **Reverse transcription:** cDNA was synthesized from messenger RNA using the RevertAid RT kit.
- **Polymerase chain reaction (PCR):** PCR was performed using GoTaq.
- **Gel electrophoresis** was performed on RT-PCR reactions and relative band intensities were analyzed using ImageJ.

Results

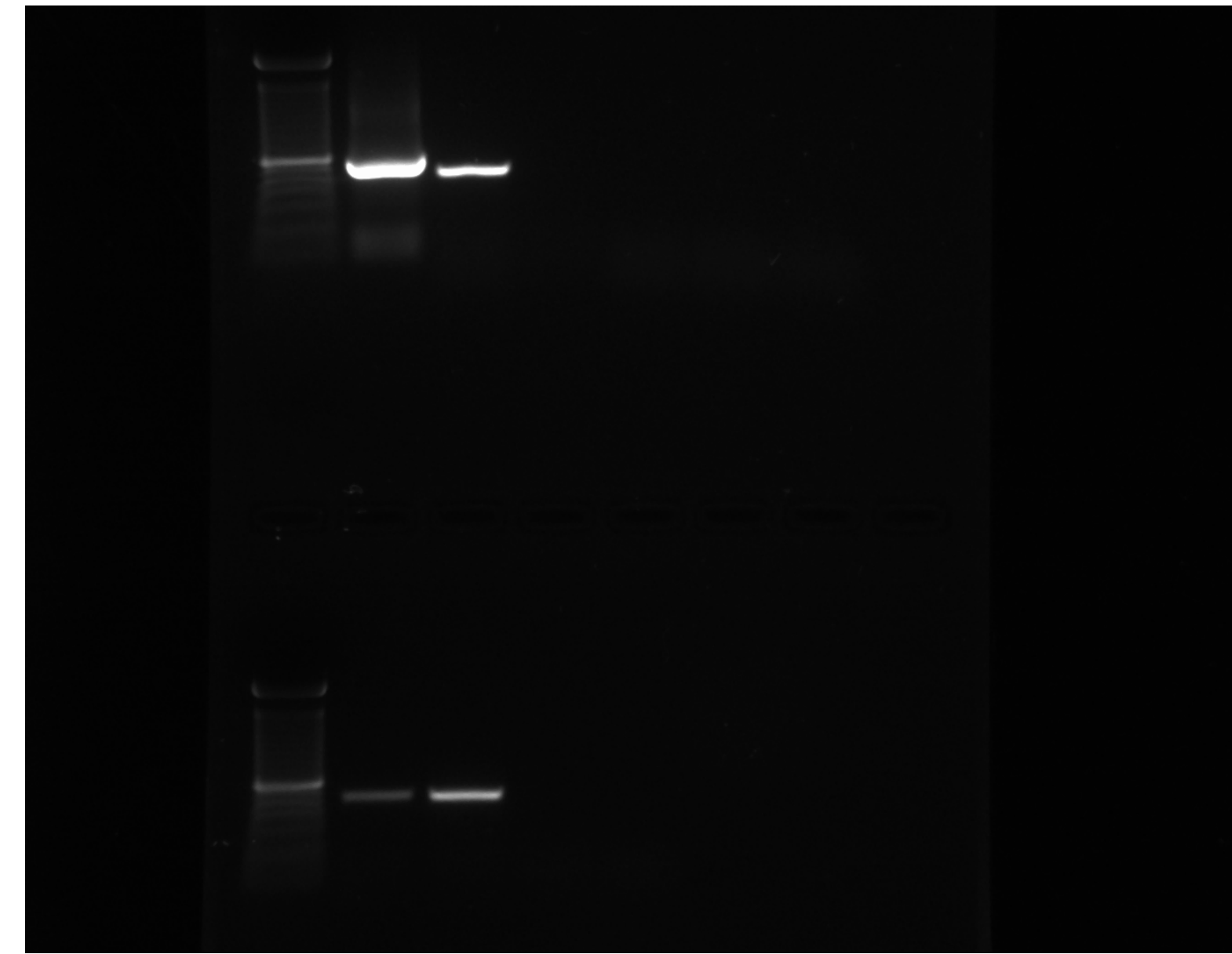


Figure 1: Round 1 Gel 1 PCRs. *GAPDH* followed by control (top) and both experimental (bottom) groups showing *ple* expression.

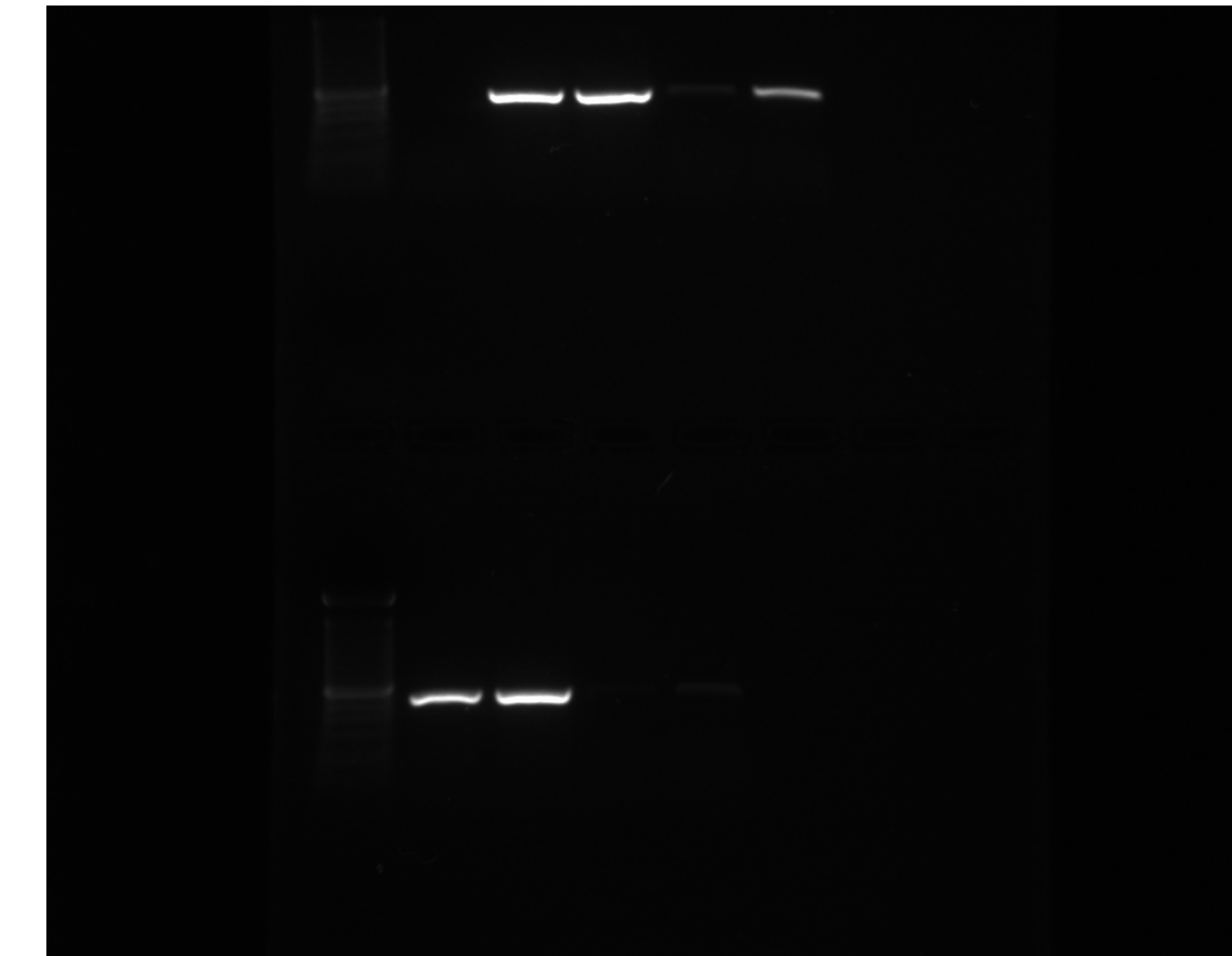


Figure 2: Round 1 Gel 2 PCRs. Both controls (top) and experimental (bottom) groups show *DM GAPDH* expression.

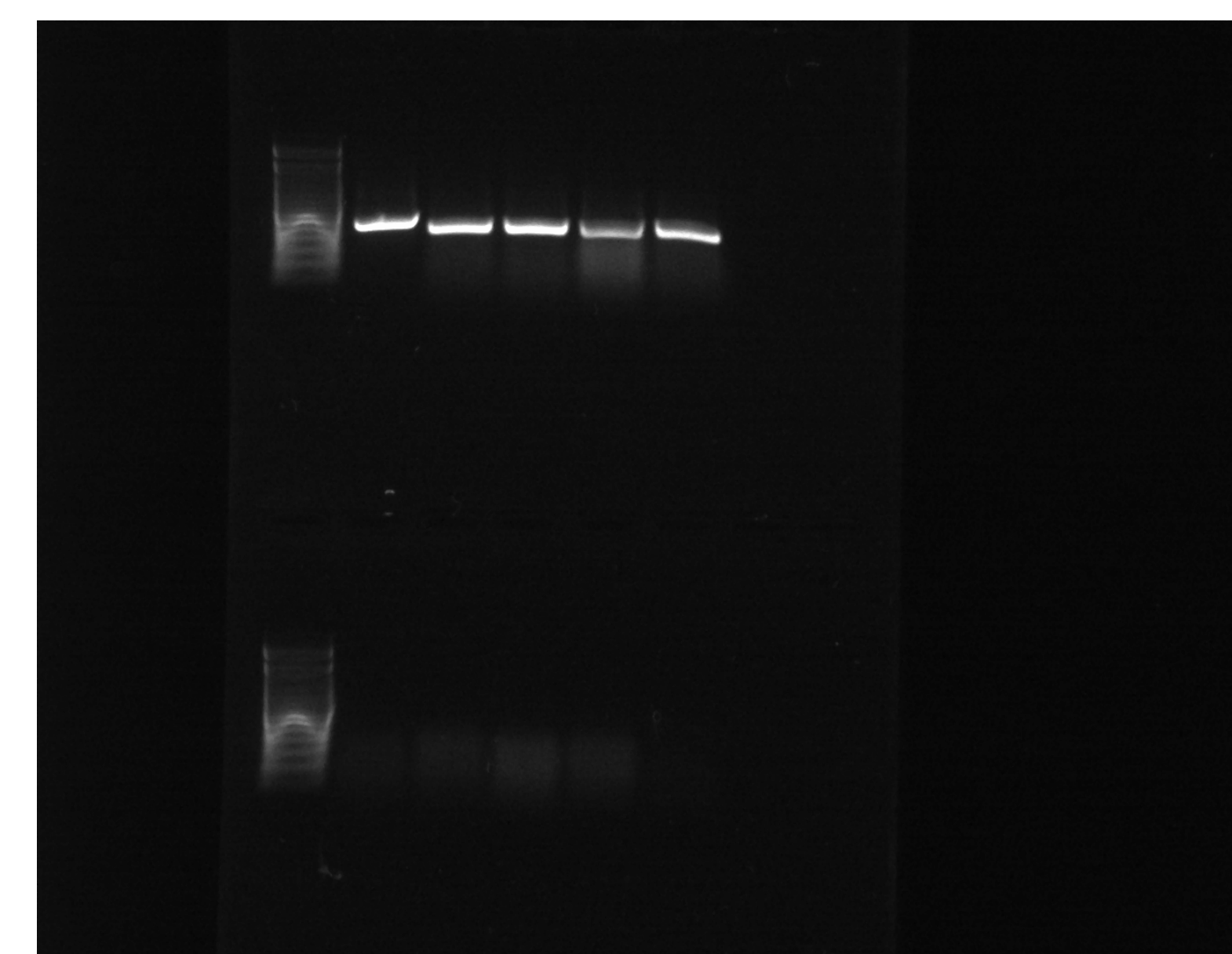


Figure 3: Round 2 Gel 1 PCRs. *Gapdh* followed by both control and experimental groups showing *ple* expression.

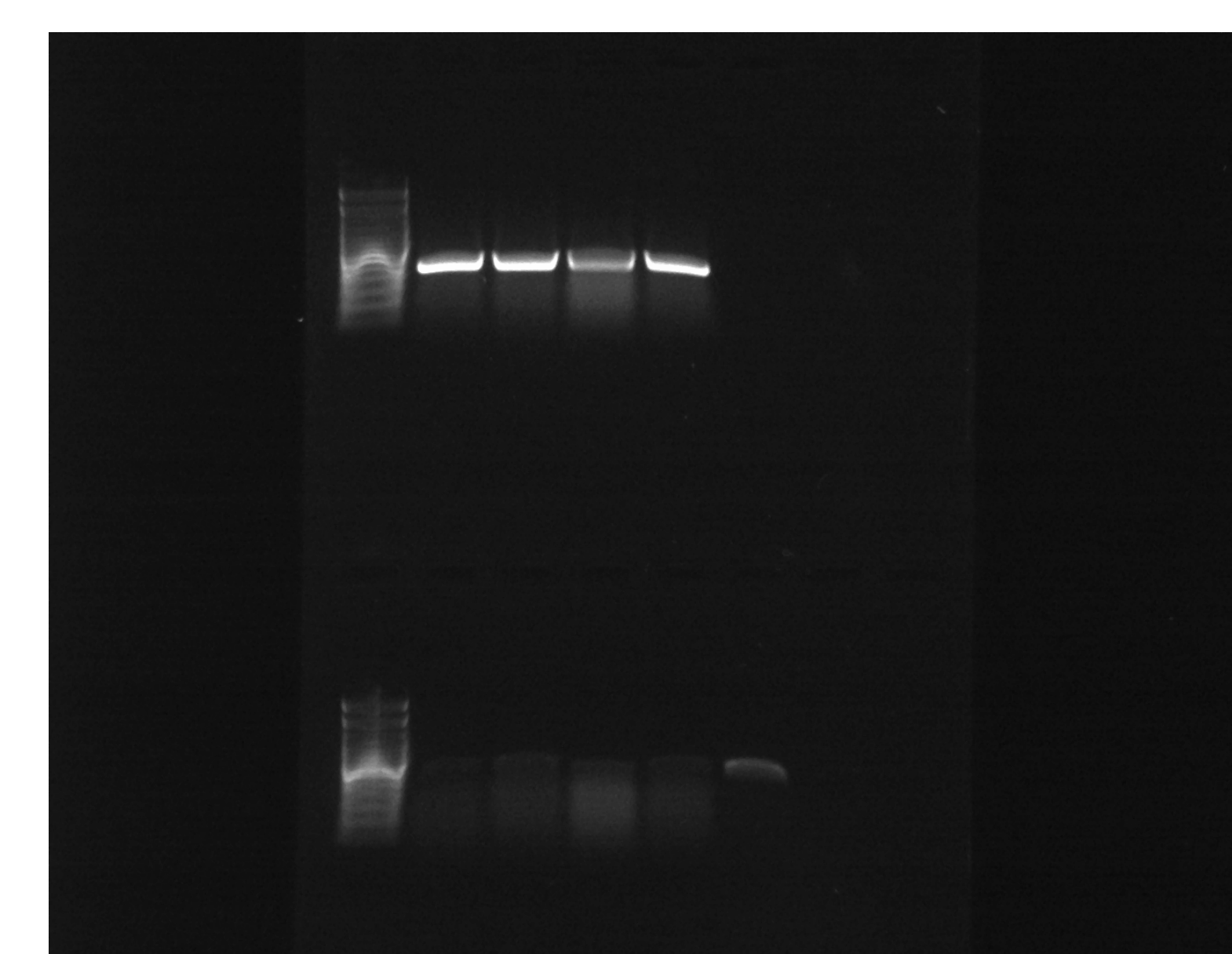


Figure 4: Round 2 Gel 2 PCRs. Both control and experimental groups (top) showing *ple* expression.

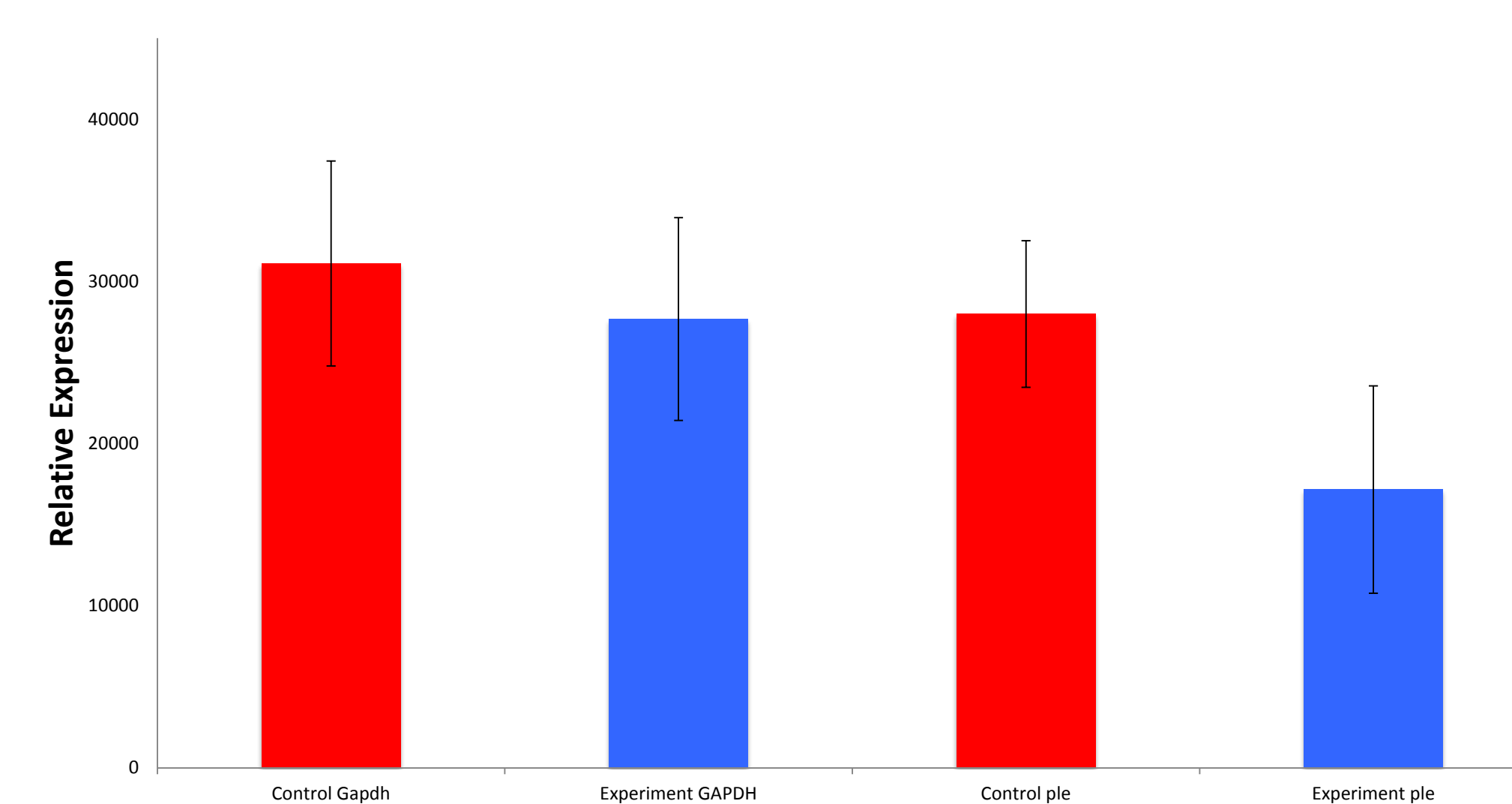


Figure 5: Bar graph showing average *Gapdh* and *ple* expression. There was no significant difference in expression between the control and treatment groups (p-values: 0.7133 (*Gapdh*), 0.7147 (*ple*))

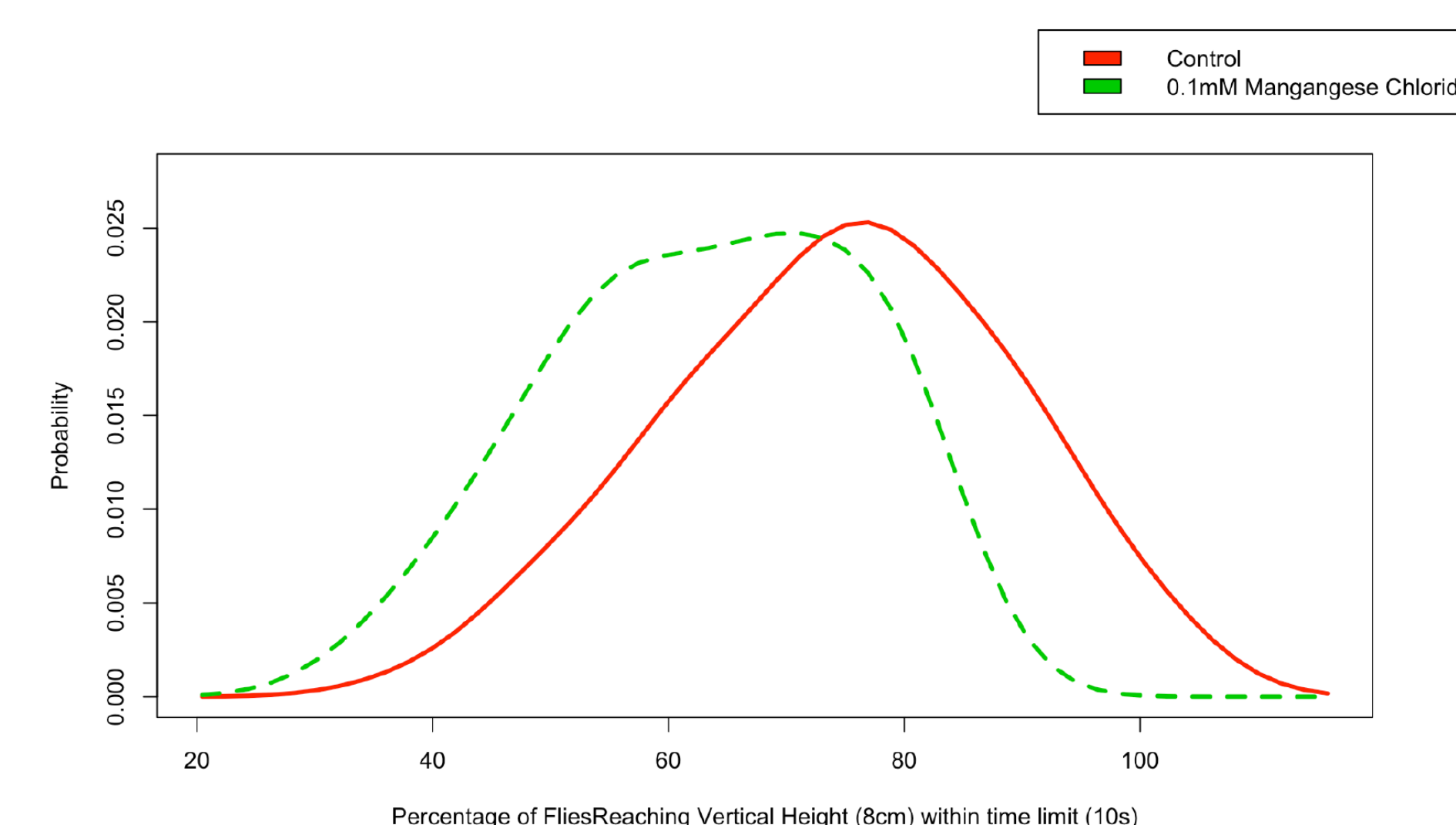


Figure 6: Distribution of performance index percentages for both control and Manganese Chloride treatment groups. A significant difference was observed (p-value = 9.77×10^{-6})

Methods (Cont.)

- **Negative Geotaxis Assay:** After the control and treated larvae developed into adults, 30 flies were selected at random from each group with 10 randomly assigned to one of three vials. Flies were tapped to the bottom of the vial and then allowed to climb. A performance index was calculated by measuring the percentage of flies in each vial that could climb above 8-cm by 10 seconds after the tap. This was reproduced ten times for each vial, giving n=60 for control and treatment groups.

Conclusions

- Image J analysis of gels for *ple* expression between controls and Manganese treated groups was found not to be statistically significant (Figure 5, $p = 0.71$).
- The negative geotaxis assay showed a statistically significant decrease in motor function ($p = 9.77 \times 10^{-6}$) in 0.1 mM Manganese Chloride treated *Drosophila melanogaster* when compared to controls (Figure 6).
- These combined results suggest that reported Parkinson's-like symptoms in Manganism patients are not linked to Tyrosine Hydroxylase in the biosynthesis pathway of Dopamine.
- **Future Directions:** The second step in the Dopamine biosynthesis pathway is the conversion of L-DOPA into Dopamine via the enzyme Aromatic L-amino acid Decarboxylase, which is encoded by the *Dmel/Ddc* gene. Analysis of *Dmel/Ddc* gene expression could strengthen our current finding that deficits in the Dopamine biosynthesis pathway are not the cause of similar symptoms between Manganism and Parkinson's patients.

Acknowledgements

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