Effects of varying CO_2 exposure time on the recovery of wild-type Oregon-R and mutant *ort[1]* *Drosophila melanogaster*

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ABSTRACT

The purpose of this study was to determine how time of exposure to CO_2 affected the recovery time of wild-type and *ort[1]* *Drosophila melanogaster.* Previous studies have suggested that mutant *D. melanogaster* take a longer time to recover from CO_2 exposure compared to the wild-type. In order to test the effects of CO_2 exposure on recovery time in both wild-type and *ort[1]* *D. melanogaster,* we had exposure times of 80 seconds, 200 seconds and 320 seconds. Each treatment consisted of 10 replicates of wild-type or *ort[1]* *D. melanogaster* (the wild type was the control). After *D. melanogaster* were exposed to CO_2 for the designated treatment time, individual flies were transferred to separate vials where they were monitored for movement and once movement occurred, that recovery time was recorded. In all three treatments, mutant *ort[1]* had a statistically significant longer mean recovery time than the wild type (p value = 1.5×10^{-5}). As the treatment time increased, the observed recovery time for both mutant *ort[1]* and wild-type *D. melanogaster* also increased.

INTRODUCTION

Drosophila melanogaster, more commonly known as the "fruit fly", has been used as a universal model organism in many fields of research. Scientists who study living organisms such as *D. melanogaster* most often require that the organism be immobilized either through chilling, etherisation, or exposure to CO_2 (Colinet and Renault 2012). Although CO_2 is the most frequently used anaesthesia for studying *D. melanogaster*, it has been found to have several negative side effects on the reproductive cycle (Colinet and Renault 2012). Yet, there has been little research done on the consequences of CO_2 anaesthesia on specific physiological/behavioural traits.

Suh *et al.* (2004) state that CO_2 is known as a "*Drosophila* stress odorant". *Drosophila* were seen exhibiting avoidance behaviour at CO_2 concentrations as low as 0.1% above the original surrounding CO_2 levels (Suh *et al.* 2004*).* Colinet and Renault (2012) also found that

CO_2 induced a stress response in *Drosophila melanogaster* due to the increased haemolymph acidity and the lack of a heartbeat. CO_2 exposure impairs oxygen delivery to the tissues of the body, therefore weakening oxidative phosphorylation and ATP production within the mitochondria (Colinet and Renault 2012). Furthermore, Badre *et al*. (2005) found that rapid paralysis occurs as well because the CO_2 molecules block the olfactory receptors, more specifically known as the gustatory receptor *Gr21a* in the synapse of the skeletal neuromuscular junction (Figure 1). Therefore, immobilization is due to CO_2 compromising the ability of these postsynaptic receptors to interact with the motor nerve terminal neurotransmitter, glutamate (Badre *et al.* 2005). However, as CO_2 exposure is eliminated, the recovery process begins. The signal transduction pathway at the neuromuscular junction is no longer impaired, allowing movement to occur (Nilson *et. al* 2006).

The objective of our study was to examine the effects of varying length of time of CO_2 exposure on the recovery times of both Oregon-R wild-type and ort^1 mutant *D. melanogaster*. Iovchev *et al*. (2002) conducted a similar experiment, which involved anaesthetizing mutant ort^1 *D. melanogaster* with diethyl ether and found that after exposure, mutants had a prolonged recovery time compared to the wild type. Thus, we predict that the mutant ort^1 *D. melanogaster* will have an increased recovery time compared to the wild type. We also predict that the recovery time for both mutant and wild-type *D. melanogaster* will increase as the length of CO_2 exposure increases. The reason is that when the length of CO_2 exposure increases, the delay in synaptic transmission is prolonged as CO_2 interferes with the olfactory receptors for a longer period of time (Badre *et al.* 2005). To explore the difference between mutant and wild-type *D. melanogaster*, we proposed three hypotheses:

H_{o1}: The length of time of CO_2 exposure has no effect on the recovery time of *D. melanogaster.*

H_{a1}: The length of time of CO_2 exposure has an effect on the recovery time of *D. melanogaster*.

H_{o2}: The presence of the *ort*[1] mutation has no effect on the recovery time of *D. melanogaster* after varying length of time of CO_2 exposure.

H_{a2}: The presence of the *ort*[1] mutation has an effect on the recovery time of *D. melanogaster* after varying length of time of CO_2 exposure.

Ho_3: The effect of varying length of time of CO_2 exposure on the recovery time of *D. melanogaster* is the same in the wild-type and mutant strain.

H_{a3}: The effect of varying length of time of CO_2 exposure on the recovery time of *D. melanogaster* is not the same in the wild-type and mutant strain.

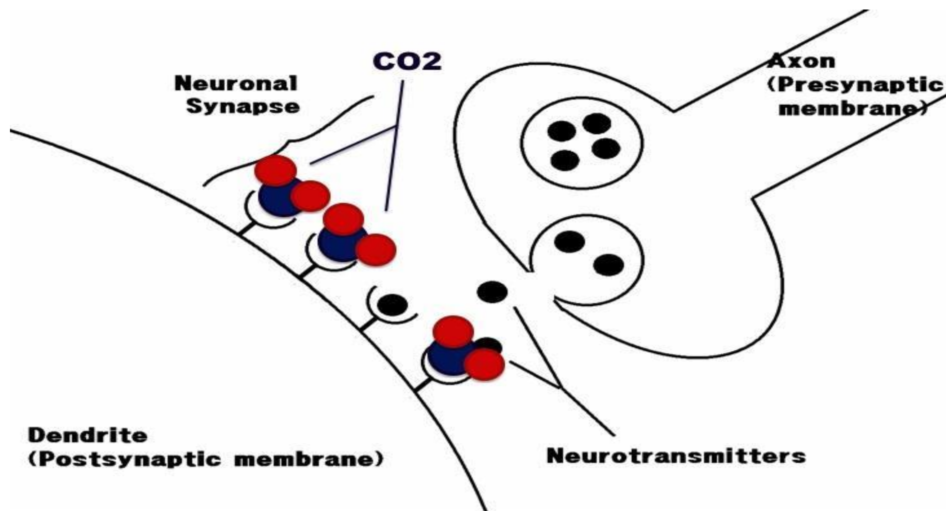

Figure 1. A simplified overview of a synapse where CO_2 blocks the postsynaptic receptors and prevents the binding of neurotransmitters (Wiki Commons Neuronal Synapse (2011).

Overall, studying the effects of CO_2 exposure on *D. melanogaster* will provide a better understanding of their short-term behavioural response that may potentially be similar in other insects (Faucher *et al.* 2013). By studying chemical stimuli that are present in the natural environment, one can gain insight on how they may cause an organism to move towards food and mates or away from toxins and predators (Faucher *et al.* 2013).

METHODS

We studied the recovery time of *D. melanogaster* after various times of CO_2 exposure. We used CO_2 as the source of anaesthesia. We were also supplied with six vials of *D. melanogaster*, which all contained roughly 15 flies along with food stock (Figure 2). We administered CO_2 to each vial for either 80, 200, or 320 seconds. Lheritier (1948) suggests that the minimum duration of gas exposure needed to get the full effect of anaesthesia should be at least 15 seconds. Therefore, we used three treatments of CO_2 that were considerably above the minimal exposure requirement. In addition to the minimum required exposure time, Brooks (1957) found that exposure to CO_2 above three minutes had detrimental effects on the growth and reproduction of insects. These findings led us to select our three CO_2 exposure times of 80, 200 and 320 seconds.

Each treatment consisted of 10 replicates for both wild-type and *ort*^{1} mutant *D. melanogaster.* Each treatment was exposed to constant environmental factors, including room temperature and light intensity. We initially exposed the first *ort*^{1} group to CO_2 (5 pounds per square inch) for 80 seconds (.Figure 3). Immediately after exposure, we started the timer and transferred 10 anaesthetized flies into 10 empty vials (Figure 4). We observed each fly and watched for recovery, which was defined as any movement from a single flinch of the leg, to standing or walking in the vial. Once recovery occurred, the time was recorded.

We repeated this procedure at 80 seconds CO_2 exposure for 10 wild-type *D. melanogaster.* We then repeated the procedure for 200 seconds and 320 seconds for both wild-type and *ort*^{1} mutant *D. melanogaster.* We calculated the means and 95% confidence intervals for mean CO_2 recovery time for each of the six treatments and used a two-way ANOVA test to analyze our data.

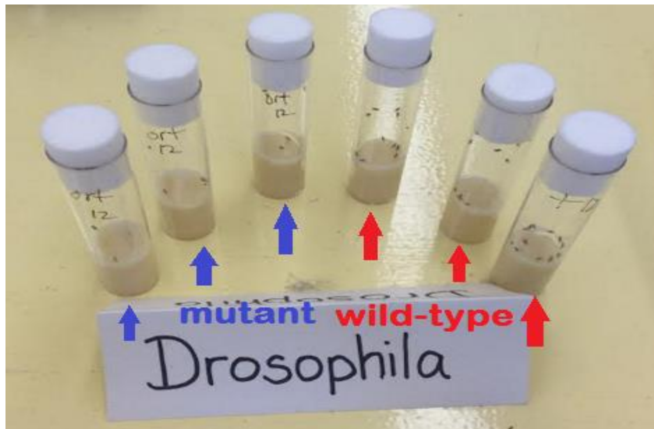

Figure 2. The six vials that were provided by the University of British Columbia Biology department. The blue arrows indicate the *ort*[1] mutant *D. melanogaster* and the red arrows indicate wild-type *D. melanogaster.*

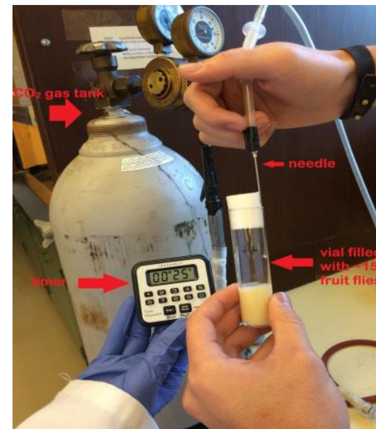

Figure 3. The experimental setup for CO_2 exposure to a vial with about 15 *D. melanogaster,* along with a timer to keep track of the exposure time. This setup was used for all three treatments (80 seconds, 200 seconds and 320 seconds) for both wild type and mutant.

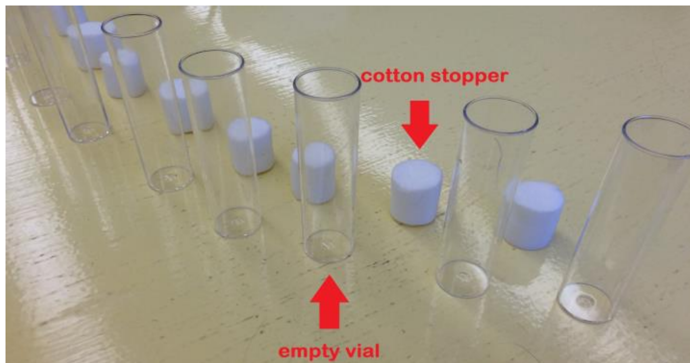

Figure 4. The empty vials where individual *D. melanogaster* were transferred after the allotted CO_2 exposure time limit, including stoppers for each vial.

RESULTS

In our experiment, we calculated the mean recovery times for wild-type and mutant *ort*[1] *D. melanogaster* after exposure to CO_2 for 80 seconds, 200 seconds and 320 seconds. The mean recovery times for wild-type *D. melanogaster* for the three treatments were 74 seconds, 170 seconds, and 216 seconds respectively (Figure 5). Moreover, the 95% confidence intervals for each of the three treatments were +/- 9 seconds, +/- 46 seconds and +/-14 seconds respectively. In addition, the mean recovery time for mutant *ort*[1] *D. melanogaster* for the three different treatments was calculated to be 135 seconds, 199 seconds and 310 seconds respectively (Figure

5). The 95% confidence intervals for the *ort*[1] mutant *D. melanogaster* were +/- 26 seconds, +/- 22 seconds and +/- 48 seconds respectively. Furthermore, using a two- way ANOVA test, the *p*-values for H_1, H_2 and H_3 were calculated to be 4.70×10^{-13}, 1.4710×10^{-5} and 0.134 respectively.

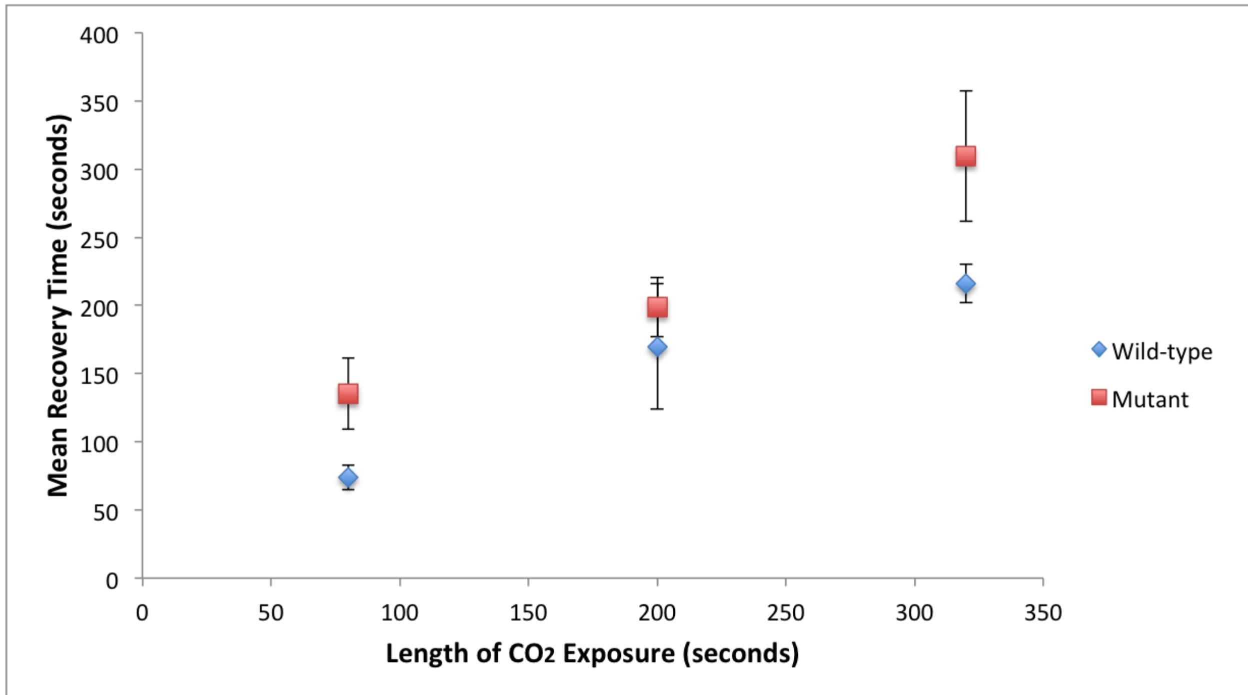

Figure 5. The effect of CO_2 exposure on the mean recovery time recorded in seconds for wild-type and mutant *D. melanogaster* are displayed for each CO_2 exposure treatment (80 seconds, 200 seconds, and 320 seconds). The sample size (n) for each treatment included 10 replicates. The error bars represent 95% Confidence Intervals. The *p* values calculated using a two-way ANOVA for H_1, H_2 and H_3 are: $p = 4.70 \times 10^{-13}$, $p = 1.4710 \times 10^{-5}$ and $p = 0.134$ respectively.

As illustrated in Figure 5, the mean recovery time for mutant *ort*[1] *D. melanogaster* was greater than that of the wild type at each of the three different treatment times; the greatest difference between the recovery times occurred at 320 seconds of CO_2 exposure. Figure 5 also shows that as the exposure time increased, the mean recovery time for both mutant *ort*[1] and wild-type *D. melanogaster* also gradually increased. The variance observed in our data was greater for the mutant strain at 80 seconds and 320 seconds compared to the corresponding wild-type strain. However, the variance seen at 200 seconds of CO_2 exposure for the wild-type was larger than the

variance observed for the mutant strain in the corresponding treatment. Moreover, the 95% confidence intervals of the mean recovery time of both the wild-type and mutant *ort*[1] strain overlap at 200 seconds. On the other hand, the 95% confidence intervals in at 80 seconds and 320 seconds of CO_2 exposure do not overlap.

DISCUSSION

We reject H_{o1} and H_{o2} because the obtained *p* values were less than 0.05, suggesting that our results are statistically significant. We reject H_{o1} and provide support for H_{a1}: CO_2 exposure has an effect on the recovery time of *D. melanogaster.* Similarly, we reject H_{o2} and provide support for H_{a2}: the presence of the *ort*[1] mutation has an effect on the recovery time of *D. melanogaster* after exposure to CO_2. However, we fail to reject H_{o3} and fail to provide support for H_{a3}, the effect of CO_2 exposure on the recovery time of *D. melanogaster* is not the same in the wild type and mutant, because the calculated *p* value was greater than 0.05. Consistent with our findings for CO_2, Iovchev *et al.* (2002) also found that an increase in chemical stimuli, in their case diethyl ether, caused a prolonged recovery time in both the wild-type and *ort*[1] mutant strain. Similarly, we also found that the *ort*[1] mutant took longer to recover than the corresponding wild type within each treatment.

Other researchers also found that an increased length of CO_2 exposure on *D. melanogaster* would result in a longer recovery time (Nilson *et al*. 2006). Similarly, these findings were consistent with what we found as our H_{a1} suggests that different length of time of exposure to CO_2 has an effect on the recovery time of *D. melanogaster.* Moreover, we failed to reject H_{o3} as the same effects were observed in both the mutant and wild-type. The reason that both the wild type and mutant had the same effect with varying length of time of CO_2 is because when *D. melanogaster* are exposed to CO_2, the neuromuscular junction has a reduced

responsiveness to glutamate, resulting in paralysis for both strains (Badre *et al.* 2005). They also found that CO_2 exposure affects immobilization and causes a decline in cardiac contraction as well; in essence the central nervous system is affected. More specifically, the exposure to CO_2 affects the central nervous system as it inhibits the release of glutamate and prevents it from interacting with the postsynaptic receptors (Figure 1). Upon exposure to CO_2, we observed this paralysis in both mutant and wild-type *D. melanogaster.*

Our findings for H_{a2} correspond to the results obtained by researchers in the past that the *ort¹* mutation does indeed affect the recovery time of *D. melanogaster*. The mutation that occurs in *ort¹ D. melanogaster* is said to arise from a deletion within the *hclA* gene sequence located on the third chromosome (O'Tousa *et al.* 1989). It was believed that this mutation solely affected the interaction of histamine and the ligand-gated chloride channels within the visual system (Nassel 1999). However, histamine has been found to be a major neurotransmitter in synaptic transmission within the insect central nervous system (Nassel 1999). Further, Iovchev *et al.* (2002) also found that histamine and glutamate are important neurotransmitters for *D. melanogaster*. The *hclA* gene encodes for histamine-gated chloride channel subunits, and the impaired function of this gene corresponds to a lack of histamine receptors, which in turn causes behavioural changes such as a prolonged recovery time when exposed to certain toxins (Iovchev *et al.* 2002). Therefore, the observed prolonged recovery in the *ort¹* mutant strain could potentially be due to the deletion in the *hclA* gene.

Although we tried to minimize sources of error and uncertainty, there were factors that may have provided variation in our results. For example, some *D. melanogaster* may have received more exposure to air than others while they were being transferred to the empty vials. Due to the direct diffusion of oxygen to all cells via their tracheal tubes, *D. melanogaster*

respond quickly to even minimal exposure of oxygen in the air (Semenza 1999). However slight, it is a possibility that the oxygen in the air will cause the organisms to recover from the anaesthesia. To minimize differences in CO_2 pressure, we tried to turn the knob by the same amount during each treatment and had one person monitoring the gauge. However, since the tank had an analogue gauge, we could not be certain that all vials of *D. melanogaster* were exposed to exactly the same CO_2 concentration. We also tried to minimize uncertainty by using each timer for a specific task. This way, there would be less variation in the recovery times obtained. Since our timers were running continuously, we may have made an error in recording the recovery time, which may have caused us to overestimate the recovery time. To minimize another human error, we had a general consensus of what the first movement looked like.

The greatest source of biological variation arose from gender. Although our qualitative observations suggested that the majority of flies were male, there were still some flies that were female. Not taking gender into account could have skewed our results because researchers in the past found that both mutant and wild-type females took a longer time to recover after exposure to CO_2 (Iovchev *et al.* 2002). For this reason, we suggest that if further research is to be done on recovery time after exposure to anaesthetics, all organisms involved should be of the same sex. It may also be beneficial to compare wild type and *ort*^{1} mutant recovery times of males and females after exposure to anaesthetics to see if males and females significantly differ in their response to CO_2.

CONCLUSION

This study tested the recovery time of both mutant *ort*^{1} and wild-type *D. melanogaster* after exposure to CO_2 for 80 seconds, 200 seconds or 320 seconds. Increased time of CO_2 exposure increases the recovery time of *D. melanogaster* wild type and the *ort*^{1} mutant. Our

results also suggest that the presence of the *ort*[1] mutation increases the recovery time of *D. melanogaster* after varying time of CO_2 exposure.

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