

Altered Gravity Induces Oxidative Stress in *Drosophila melanogaster*. Sharmila Bhattacharya¹, and Ravikumar Hosamani^{1,2}. ¹Space Biosciences Division, NASA Ames Research Center; ²NASA Postdoctoral Program (administered by Oak Ridge Associated Universities).

Altered gravity environments can induce increased oxidative stress in biological systems. Microarray data from our previous spaceflight experiment (FIT experiment on STS-121) indicated significant changes in the expression of oxidative stress genes in adult fruit flies after spaceflight. Currently, our lab is focused on elucidating the role of hypergravity-induced oxidative stress and its impact on the nervous system in *Drosophila melanogaster*. Biochemical, molecular, and genetic approaches were combined to study this effect on the ground.

Adult flies (2–3 days old) exposed to acute hypergravity (3g, for 1hr and 2hrs) showed significantly elevated levels of Reactive Oxygen Species (ROS) in fly brains compared to control samples. This data was supported by significant changes in mRNA expression of specific oxidative stress and antioxidant defense related genes. As anticipated, a stress-resistant mutant line, *Indy*³⁰², was less vulnerable to hypergravity-induced oxidative stress compared to wild-type flies. Survival curves were generated to study the combined effect of hypergravity and pro-oxidant treatment. Interestingly, many of the oxidative stress changes that were measured in flies showed sex specific differences. Collectively, our data demonstrate that altered gravity significantly induces oxidative stress in *Drosophila*, and that one of the organs where this effect is evident is the brain.

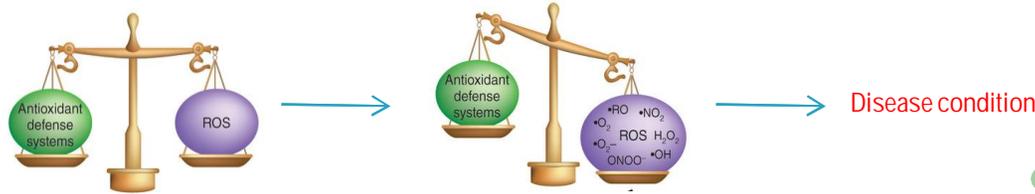
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INTRODUCTION



❖ OXIDATIVE STRESS

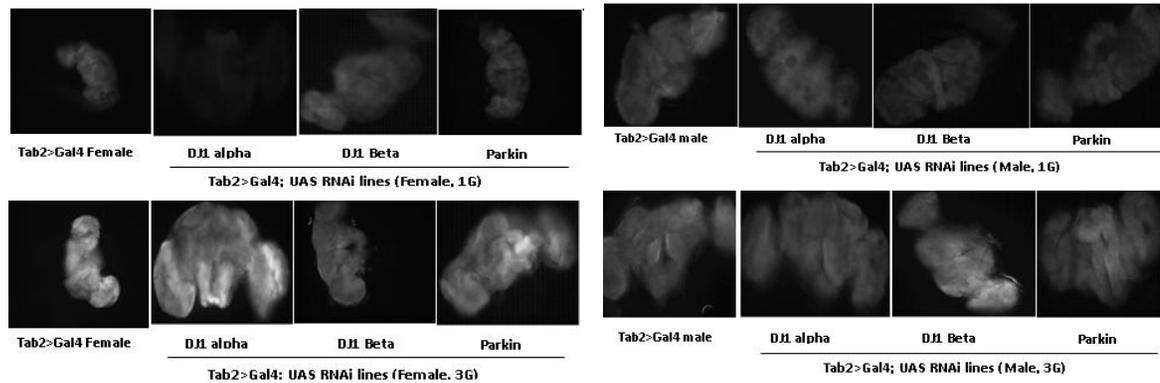


Diabetes, Cancer, Ageing, cardiovascular disease and neurological disease etc.

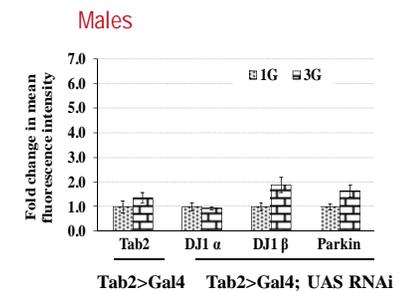
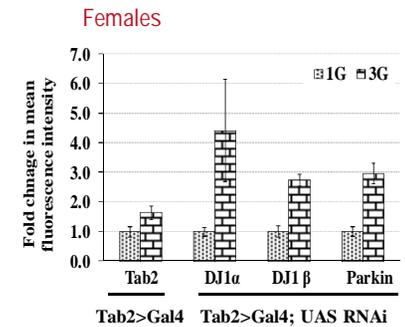
Normal physiological homeostasis Imbalanced physiological homeostasis

- ❖ Evolutionarily basic physiological oxidative stress pathway conserved across species
- ❖ Main difference in antioxidant defense in *Drosophila* and mammalian models: Glutathione Reductase is replaced by the Thioredoxin system in *Drosophila*
- ❖ Hypergravity can induce significant oxidative stress

❖ PREVIOUS DATA FROM OUR LAB



- ❖ 3G exposure caused significant ROS level in fly brain
- ❖ Females are more vulnerable to 3G-induced ROS





HYPOTHESIS AND EXPERIMENTAL PLAN

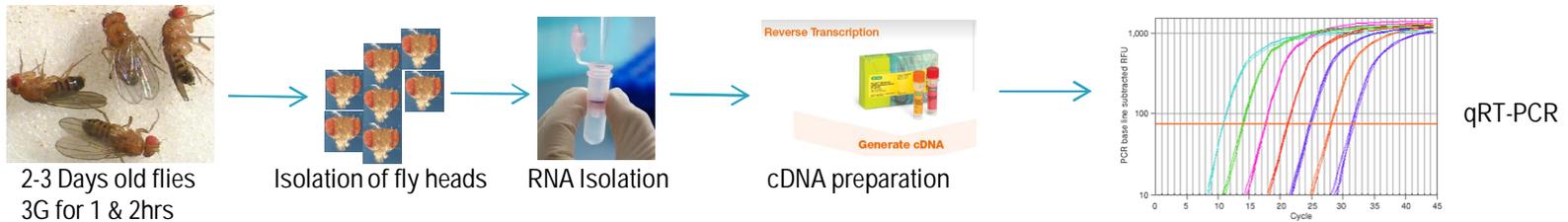


❖ HYPOTHESIS

“Acute hypergravity can induce significant changes in the expression of oxidative stress genes in the head region of fly”

❖ EXPERIMENTAL DESIGN

- W^{1118} – white eye, control genetic background for $Indy^{302}$
- $Indy^{302}$ - I' m not dead yet, encodes the fly homolog of a mammalian di and tricarboxylate transporter, the mutant is stress- and aging-resistant
 - Fly Indy is highly expressed in the gut, fat bodies, and oenocytes
 - The activity of ETC complexes I and III , H_2O_2 production ↓, Unchanged ATP production, Mitochondrial density per cell (PCG-1-alpha) ↑
Combination of decreased electron flux + ↑mitochondrial density leads to a net decrease in ROS with balanced ATP levels.



❖ SPECIFIC OXIDATIVE STRESS GENES

Catalase
SOD1
SOD2
GSTD8
GSTE1

✓ First line enzymatic antioxidant defense

Prx 2540-1
Prx 2540-2
Prx 6005
Pxn
Pxt
Pxd
Jafrac1
Jafrac2

✓ First line of defense
✓ Peroxireodoxins
✓ Peroxidase activity

TrxR1
TrxR2
TrxT

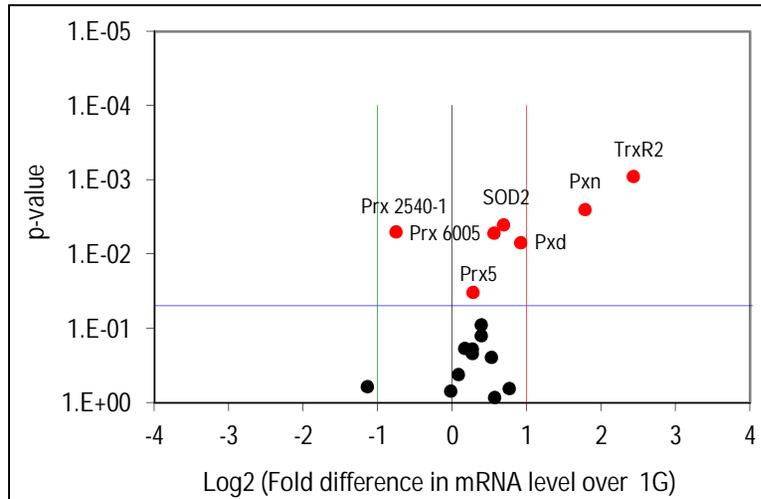
✓ Second line of defense
✓ Thioredoxin system
✓ Glutathione homeostasis
✓ Substitute for GR in mammals



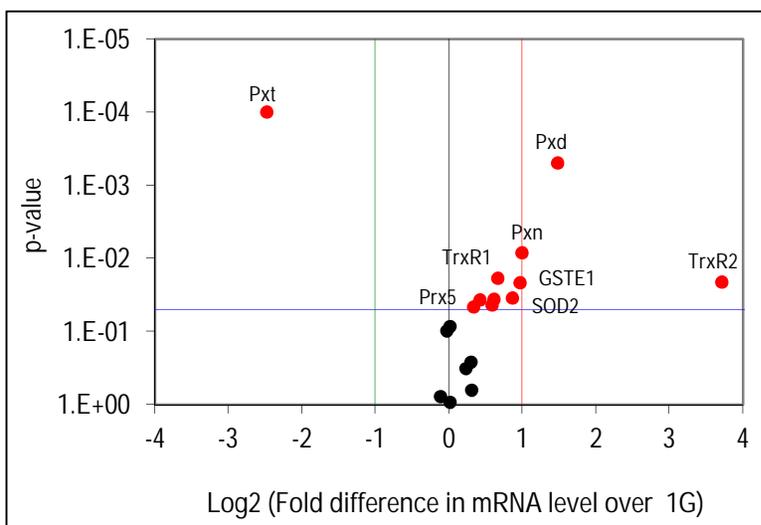
W^{+1118} females exposed to 3G for 1hr and 2hr caused significant changes in oxidative stress related gene expression



W^{+1118} , 3G for 1hr



W^{+1118} , 3G for 2hr



W^{+1118} , 3G for 1hr
FC mRNA level over 1G

Genes	Log ₂ (FC)	p-value
Catalase	0.26	2.1E-01
SOD1	0.38	8.9E-02
SOD2	0.69	4.0E-03
GSTD8	0.38	1.2E-01
GSTE1	0.76	6.3E-01
TrxR1	0.52	2.4E-01
TrxR2	2.43	9.0E-04
TrxT	0.07	4.1E-01
Prx 2540-1	-0.76	5.0E-03
Prx 2540-2	0.16	1.8E-01
Prx 6005	0.56	5.2E-03
Prx 5	0.28	3.3E-02
Pxn	1.78	2.5E-03
Pxt	-1.15	6.0E-01
Pxd	0.92	7.0E-03
Jafrac1	-0.03	6.9E-01
Jafrac2	0.26	1.9E-01
Foxo	0.57	8.4E-01

W^{+1118} , 3G for 2hr
FC mRNA level over 1G

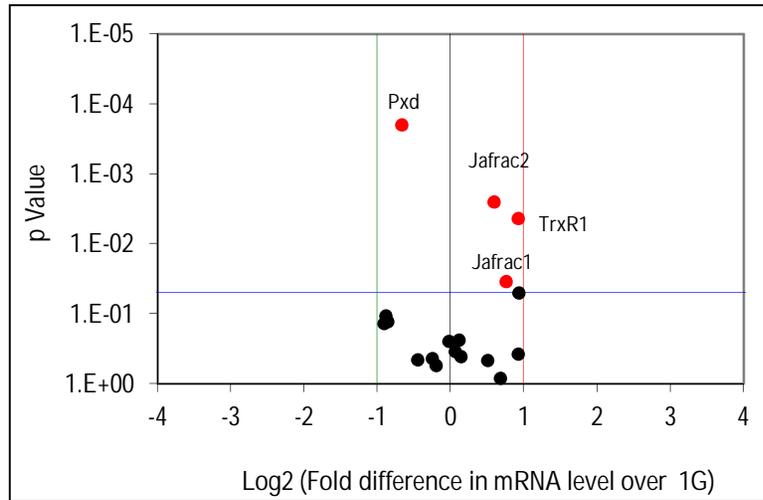
Genes	Log ₂ (FC)	p-value
Catalase	0.23	3.1E-01
SOD1	-0.03	9.5E-02
SOD2	0.86	3.5E-02
GSTD8	0.97	2.1E-02
GSTE1	0.42	3.7E-02
TrxR1	0.67	1.9E-02
TrxR2	3.72	2.1E-02
TrxT	0.59	4.4E-02
Prx 2540-1	0.31	6.2E-01
Prx 2540-2	0.30	2.6E-01
Prx 6005	0.01	8.3E-02
Prx 5	0.33	4.6E-02
Pxn	0.99	8.3E-03
Pxt	-2.47	1.0E-04
Pxd	1.48	5.0E-04
Jafrac1	-0.12	7.6E-01
Jafrac2	0.01	9.0E-01
Foxo	0.61	3.6E-02



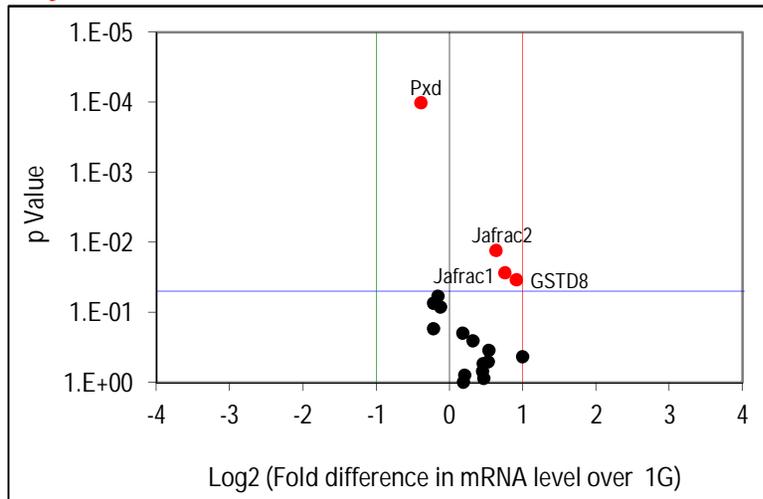
Indy³⁰² females exposed to 3G for 1hr and 2hr showed fewer changes in oxidative stress gene expression compared to its control line



Indy³⁰², 3G for 1hr



Indy³⁰², 3G for 2hr



Indy³⁰², 3G for 1hr
FC mRNA level over 1G

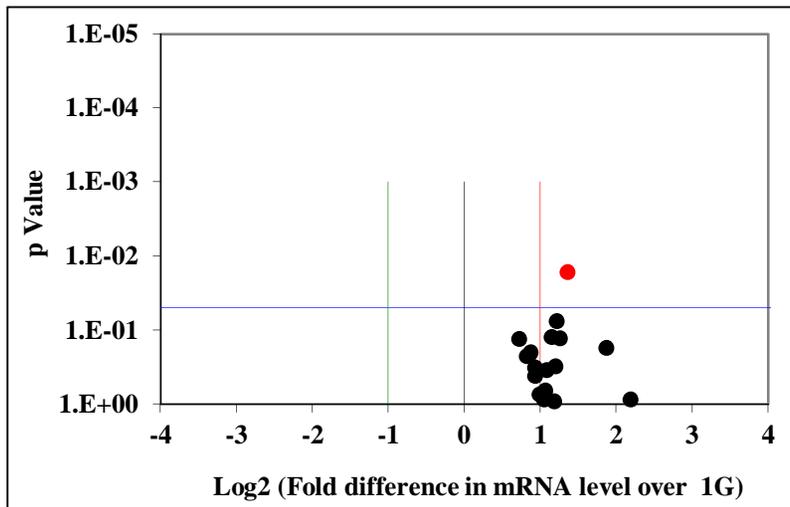
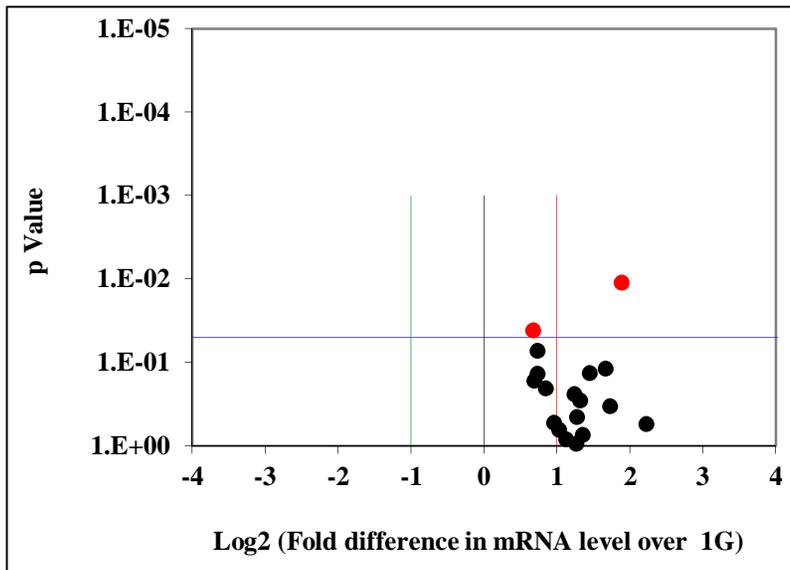
Genes	Log ₂ (FC)	p-value
Catalase	-0.92	1.4E-01
SOD1	0.11	2.3E-01
SOD2	-0.25	4.3E-01
GSTD8	-0.45	4.5E-01
GSTE1	-0.86	1.3E-01
TrxR1	0.93	4.3E-03
TrxR2	-0.20	5.5E-01
TrxT	0.92	3.8E-01
Prx 2540-1	0.68	8.2E-01
Prx 2540-2	0.51	4.6E-01
Prx 6005	0.14	4.1E-01
Prx 5	0.06	3.4E-01
Pxn	-0.89	1.1E-01
Pxt	-0.03	2.5E-01
Pxd	-0.67	2.0E-04
Jafrac1	0.76	3.4E-02
Jafrac2	0.59	2.5E-03
Foxo	0.93	5.0E-02

Indy³⁰², 3G for 2hr
FC mRNA level over 1G

Genes	Log ₂ (FC)	p-value
Catalase	0.52	5.0E-01
SOD1	0.18	9.7E-01
SOD2	0.31	2.5E-01
GSTD8	0.91	3.4E-02
GSTE1	-0.14	8.2E-02
TrxR1	0.44	5.3E-01
TrxR2	0.16	1.9E-01
TrxT	0.19	7.8E-01
Prx 2540-1	-0.23	7.2E-02
Prx 2540-2	-0.23	1.7E-01
Prx 6005	0.53	3.4E-01
Prx 5	-0.17	5.8E-02
Pxn	0.44	6.9E-01
Pxt	0.45	8.6E-01
Pxd	-0.40	1.0E-04
Jafrac1	0.76	2.7E-02
Jafrac2	0.63	1.3E-02
Foxo	0.99	4.2E-01



W⁺¹¹¹⁸ and Indy 302 males exposed to 3G for 1hr caused less significant changes in oxidative stress related gene expression



W⁺¹¹¹⁸, 3G for 1hr
FC mRNA level over 1G

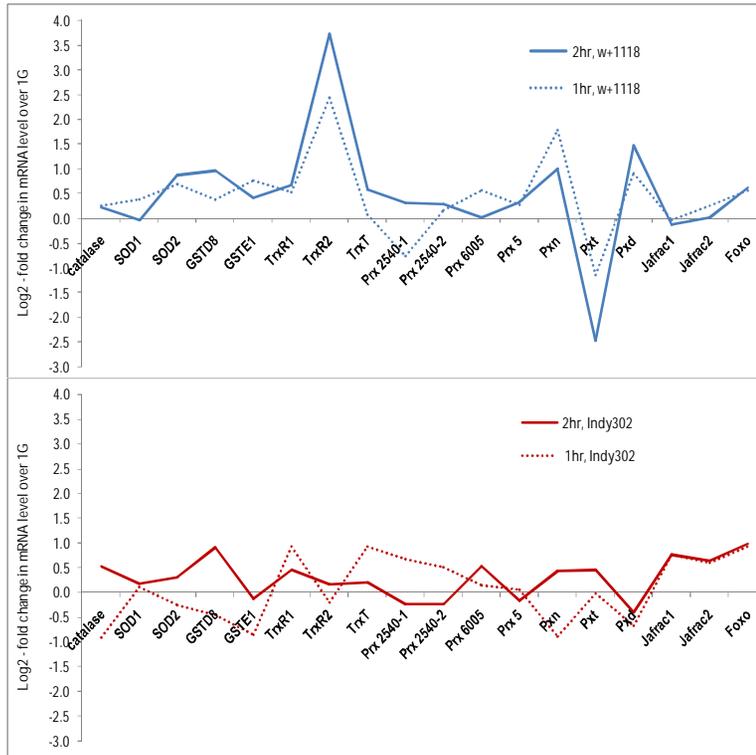
Genes	Log ₂ (FC)	p-value
Foxo	0.73	1.4E-01
Cat	1.27	4.5E-01
SOD1	0.68	4.2E-02
SOD2	0.95	5.3E-01
GSTD8	0.68	1.7E-01
GSTE1	1.23	2.4E-01
Jafrac1	0.73	7.2E-02
Jafrac2	1.35	7.4E-01
Pxd	2.22	5.4E-01
Pxt	1.72	3.4E-01
Pxn	1.66	1.2E-01
Prx5	1.44	1.3E-01
Prx 25401	1.02	6.5E-01
Prx 25402	1.89	1.1E-02
Prx 6005	0.84	2.0E-01
TrxR1	1.12	8.3E-01
TrxR2	1.31	2.9E-01
TrxT	1.26	9.4E-01

Indy³⁰², 3G for 1hr
FC mRNA level over 1G

Genes	Log ₂ (FC)	p-value
catalase	0.82	2.3E-01
SOD1	1.20	3.1E-01
SOD2	0.87	2.0E-01
GSTD8	0.99	7.4E-01
GSTE1	1.18	9.2E-01
TrxR1	1.36	1.7E-02
TrxR2	0.85	2.2E-01
TrxT	1.26	1.3E-01
Prx 2540-1	2.19	8.7E-01
Prx 2540-2	1.87	1.7E-01
Prx 6005	0.93	3.2E-01
Prx 5	1.08	3.5E-01
Pxn	0.93	4.1E-01
Pxt	1.05	8.6E-01
Pxd	0.72	1.3E-01
Jafrac1	1.07	6.6E-01
Jafrac2	1.15	1.3E-01
Foxo	1.22	7.5E-02



SUMMARY



w⁺¹¹¹⁸ Females (Control)

- At 2hr – Expression of more genes altered compared to 1hr with 3G exposure.
- At 2hr – Consistent upregulation of SOD2, GSTD8, Prx5 and TrxR2 suggests mitochondrial oxidative stress induced by hypergravity.
- At 2hr- Upregulation of GSTs suggests formation of lipid hydroperoxides.
- At 2hr- Upregulation of TrxR1 and TrxR2 implicates generation of GSH and Thioredoxins.
- At 2hr- Upregulation of Prx5, Pxd and Pxn suggests hydroperoxides.

Indy³⁰² Females

- At both-time points – Expression of fewer oxidative stress related genes compared to *w⁺¹¹¹⁸*
- In keeping with Indy flies being more resistant to stressors, and as shown here including under hypergravity conditions.
- At both time points – Upregulation of Jafrac1 and Jafrac2 suggests *Indy³⁰²* flies may recruit a different set of enzymes to decompose peroxides.

Overall summary

- Acute hypergravity can significantly alter oxidative stress genes in females.
- Peroxides may be a significant category of free radicals produced by hypergravity.
- Acute hypergravity induced oxidative stress is better handled by the stress resistant, aging resistant *Indy³⁰²* mutant flies compared to wild type controls.

Future studies

- Will conduct biochemical assays to correlate generation of peroxides and glutathione with gene function and with behavioral assays in wild type and mutant flies.
- Will use transgenic and gene mutants to characterize the role of the thioredoxin system under altered gravity conditions in female flies.

Groups	Number of genes significantly altered over 1G (p<0.05)
<i>w⁺¹¹¹⁸</i> 1hr,	7
<i>w⁺¹¹¹⁸</i> 2hr,	11
<i>Indy³⁰²</i> 1hr,	4
<i>Indy³⁰²</i> 2hr,	4

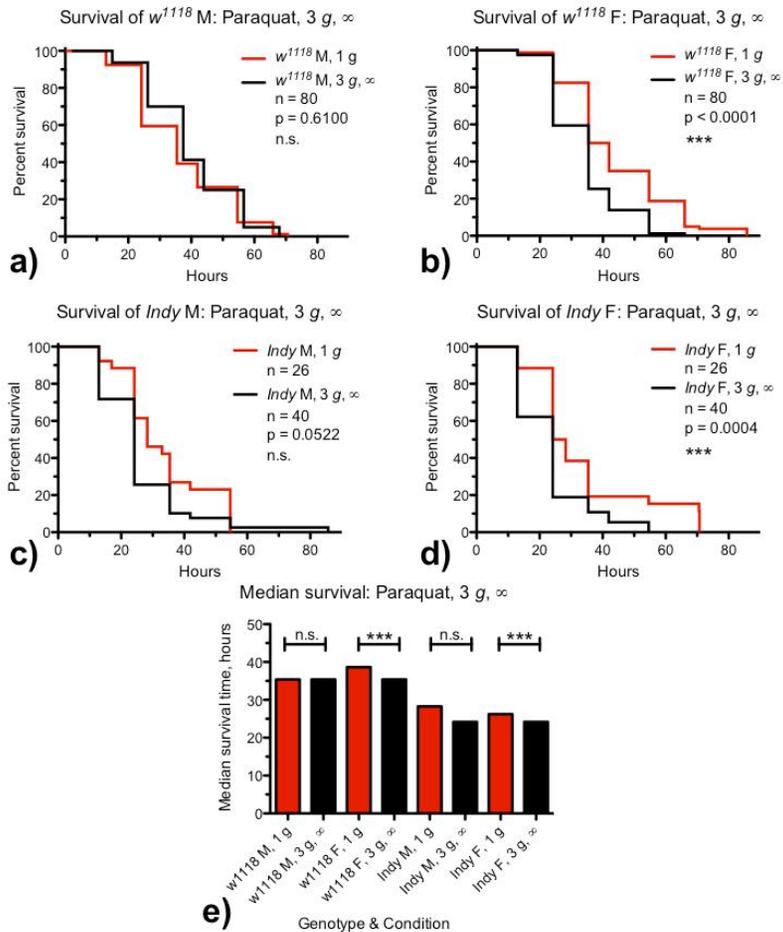


Figure 6. Chronic hypergravity reduces female w^{1118} and *Indy*³⁰² *Drosophila*'s survival to paraquat.

Age-isolated and sex-segregated *Drosophila* were kept on food containing paraquat at 3 g for the duration of the assay. Control *Drosophila* were kept on the same food but at 1 g for the entire assay. Chronic hypergravity only significantly reduced survival time for female *Drosophila*, though both w^{1118} and *Indy*³⁰² genotypes were affected. e) Statistical significances are reported as determined by log-rank tests of survival curves. See Table 2 for a numerical enumeration of results. ***p < 0.001

-Just keep w1118 data
 -Just double check log-ranked test for survival curves

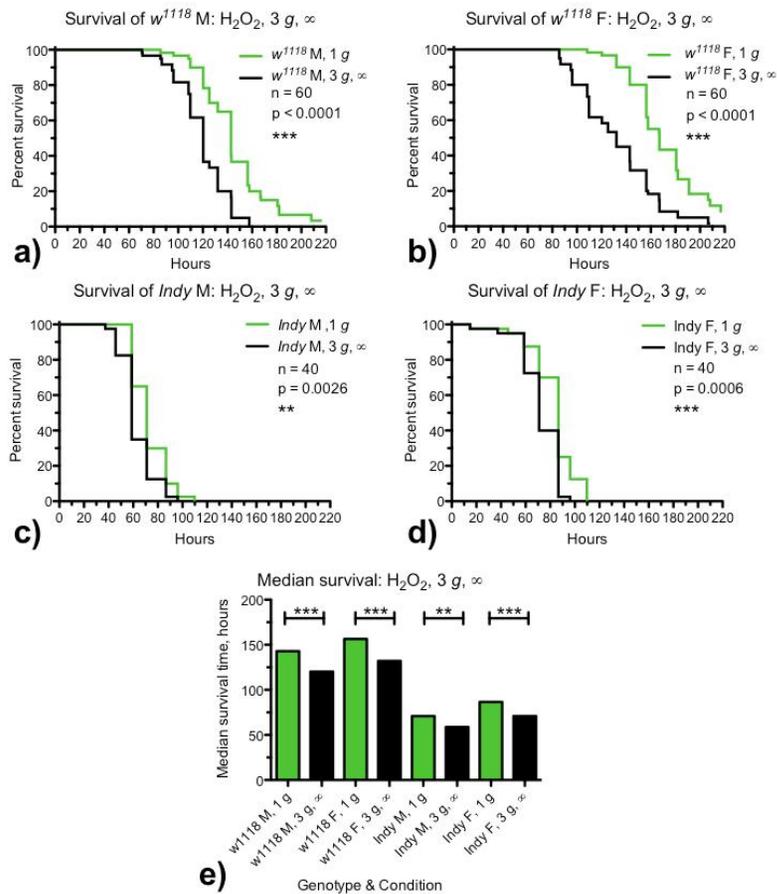


Figure 5. Chronic hypergravity reduces w^{1118} and *Indy*³⁰² *Drosophila*'s survival to hydrogen peroxide.

Age-isolated and sex-segregated *Drosophila* were kept on food containing hydrogen peroxide 3 g for the duration of the assay. Control *Drosophila* were kept on the same food but at 1 g for the entire assay. Chronic hypergravity significantly reduced survival time for both w^{1118} and *Indy*³⁰² genotypes. e) Statistical significances are reported as determined by log-rank tests of survival curves. See Table 2 for a numerical enumeration of results. ***p < 0.001, **p < 0.01.

-Just keep w1118 data
 -Just double check log-ranked test for survival curves

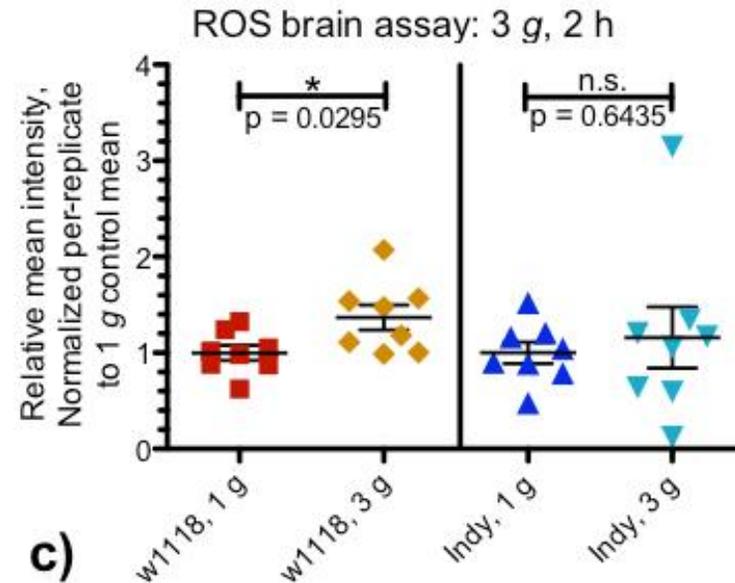
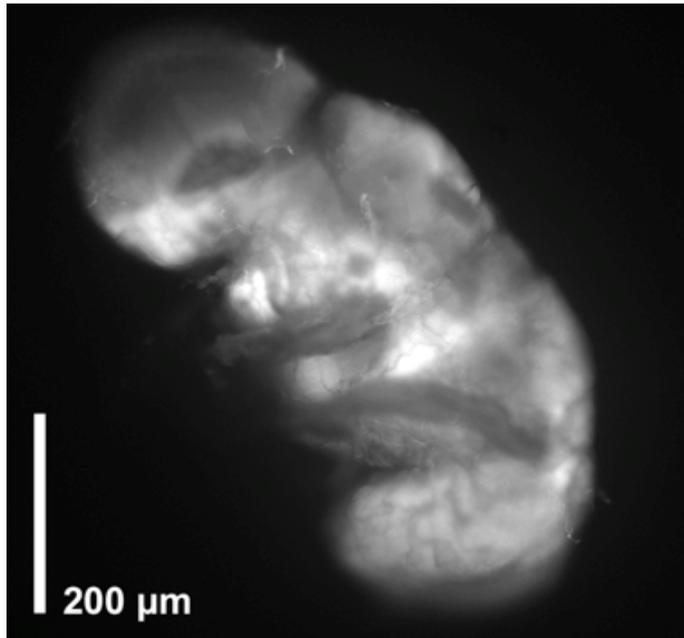


Figure 1. Acute hypergravity increased DCFH-DA-labeled ROS fluorescence in w^{1118} .

The brains of *Drosophila* exposed to acute hypergravity (3 g for 2 hours) were dissected and incubated in DCFH-DA which labels ROS via fluorescence. a) Representative images of brain dissections under DIC white light (left) and UV light (right). Image exposure, contrast, and levels are enhanced for clarity. Scale bar is approximate. b) UV light auto-oxidizes DCFH on a short timescale, rapidly saturating the image, as seen in this *Indy*³⁰² brain kept at 1 g. c) We found a 36.8% increase in average fluorescence intensity in w^{1118} *Drosophila* exposed to 3 g (relative fluorescence intensity = 136.8%, SEM = 13.04%, $p = 0.0295$, $n = 8$) compared to those kept at 1 g (fluorescence intensity normalized to 100%, SEM = 7.774%, $n = 8$). *Indy*³⁰² *Drosophila* exposed to 3 g showed no significant increase in fluorescence intensity (relative fluorescence intensity = 115.9%, SEM = 31.80%, $p = 0.6435$, $n = 8$) compared to those kept at 1 g (fluorescence intensity normalized to 100%, SEM = 11.07%, $n = 8$). Statistical significance was calculated using Student's t-test. * $p < 0.05$