



Using Conditional Autophagy Inhibition In Mice To Study Neurological Disorders

Triebold M.N.¹; Anderson C.E.¹; Einat H.^{2,3}; Anderson G.W.¹

¹ College of Pharmacy, University of Minnesota, USA.

² School of Behavioral Sciences, Tel Aviv-Yaffo Academic College, Israel. ³ Dept. of Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Israel



Introduction

- Autophagy, a cell survival promoting process and cellular mechanism involved in the clearance of aggregated cytosolic proteins, has been studied in relation to neurodegenerative disorders such as Parkinson's disease, depression and affective disorders.
- Recently autophagy was suggested to be involved in the therapeutic action of antidepressant and mood stabilizing drugs.
- Previous data from our collaborator's lab demonstrated that repeated administration of compounds that enhance autophagy via different pathways, including rapamycin, trehalose and nicardipine result in mood stabilizing-like effects and in changes in autophagy-related protein levels indicative of enhanced autophagy in the frontal cortex of mice (1,2).
- Further understanding of relationship between autophagy and affective disorders can be achieved using molecular approaches.
- Specifically, the present study was designed to explore the behavioral consequences of a conditional deletion of *Atg5*, a gene required for autophagy.
- Western blot analysis confirmed conditional knockout of *Atg5*
- A battery of behavioral tests after induction suggested that mania-like symptoms resulted from knockout of *Atg5*
- Based on our previous pharmacological data, we hypothesize that conditional deletion of *Atg5* will result in behavioral changes related to affective-like pathology as well as Parkinsonian symptoms.

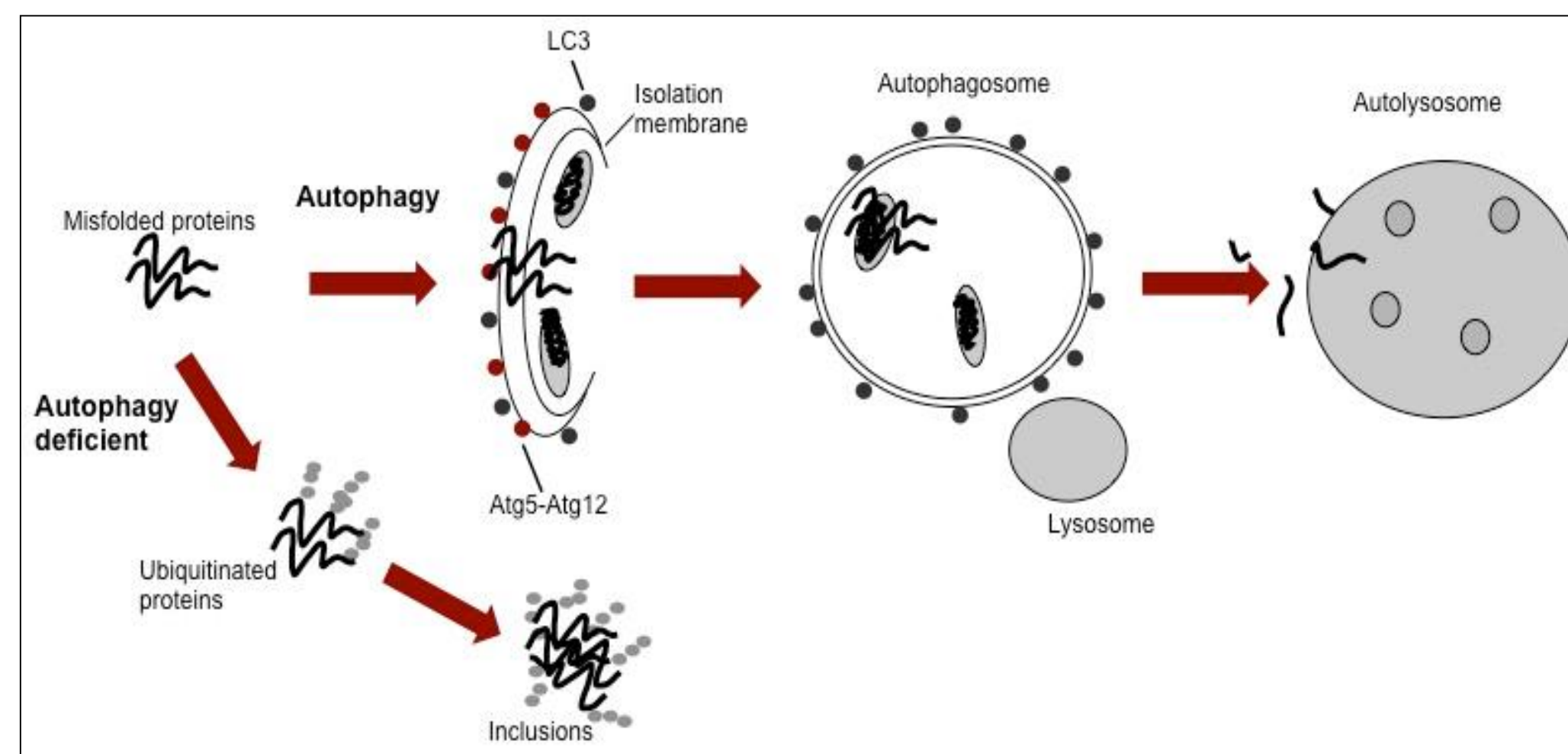


Figure 1. Effects of *Atg5* on formation of autophagosome.

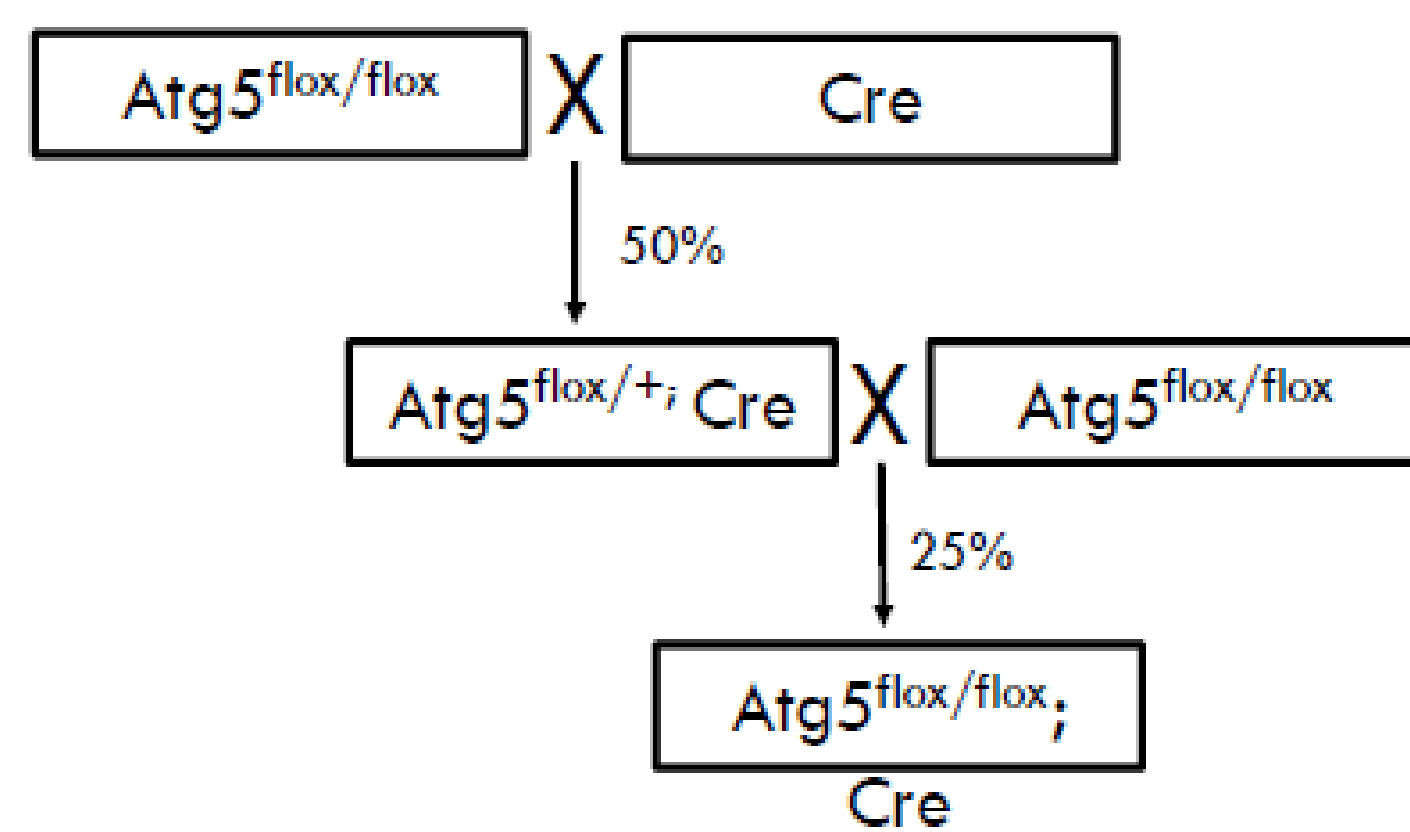


Figure 2. Breeding Scheme Summary. Creating an inducible knockout *Atg5* gene in a mouse model.

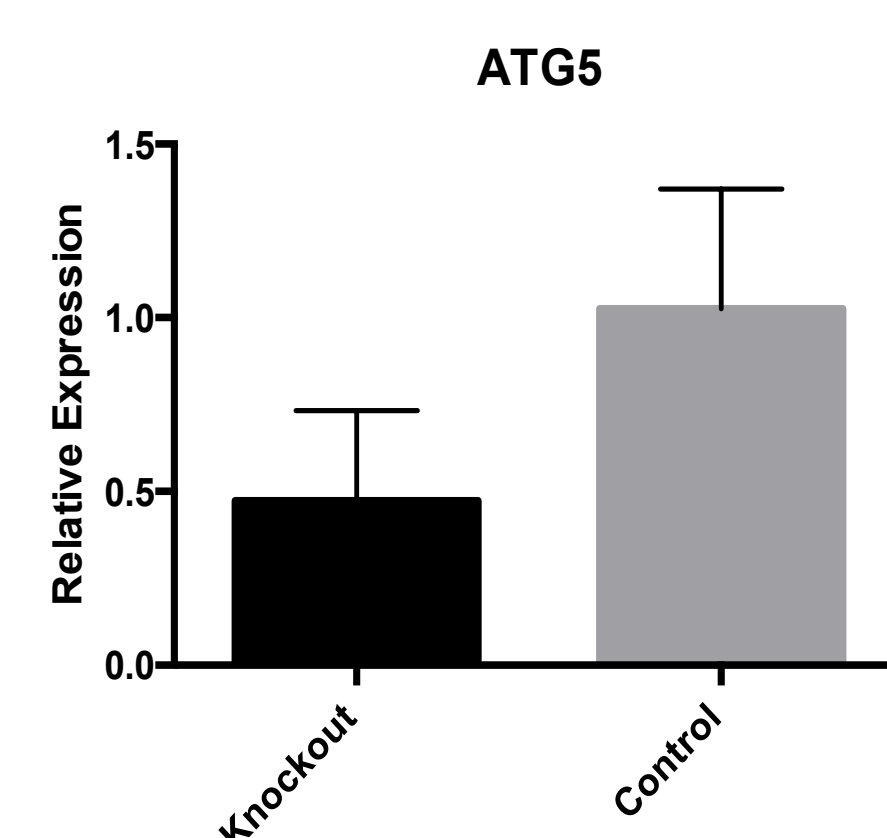


Figure 3. Relative *Atg5* protein concentration in the cortex. *Atg5* protein is 2.2 fold higher in control mice cortex. [p=0.046]

Experimental design and methods

- Mice carrying a floxed *Atg5* allele were cross-bred with transgenic mice carrying a Cre-recombinase enzyme driven off an inducible, neuronally expressed promoter.
- Resultant progeny were genetically selected to carry both the floxed *Atg5* allele and the Cre recombinase, allowing induction of gene deletion to occur at a defined age using tamoxifen.
- The neurodegenerative behaviors of the mice at approximately 9 months post-induction were assessed using the following tests:
 - Open field – mice placed in new environment and activity time in ten minutes analyzed
 - Grip strength – mice placed on metal grid, which was flipped upside down for 60 seconds. Time to fall recorded.
 - Rotarod performance – Rotamex machine speeds up by 1 RPM/3 seconds; time to fall recorded
 - Clasping analysis – mice were held by tails and assessed for clasping reflex
 - Gait analysis – hind and front feet painted different colors; footprints on white paper analyzed
- Results were analyzed using one-tailed student t-tests.
 - p<0.05 considered significant.



Figure 4. Rotamex Machine Used for Rotarod Performance Test.

Qualitative Results

Clasping Analysis:

Table 1. Clasping Analysis Results

	WT Males	WT Females	KO Males	KO Females
Normal Phenotype	3	8	1	4
Mild Abnormal Phenotype	0	0	2	4
Strong Abnormal Phenotype	0	0	3	1
Total Number	3	8	6	9



WT KO

Figure 14. Abnormal Clasp Reflex In Male *Atg5* Knockout. Knockout mice displayed mild to severe clasping abnormalities when held by their tails, as compared to wild type mice.

Gait Analysis:



WT

KO

Figure 15. Gait Analysis of a Representative Wild Type and Severely Affected Knockout Mouse. Knockout displayed splayed hind legs and an abnormal walking pattern. This knockout was from an earlier cohort, and was female.

Quantitative Results

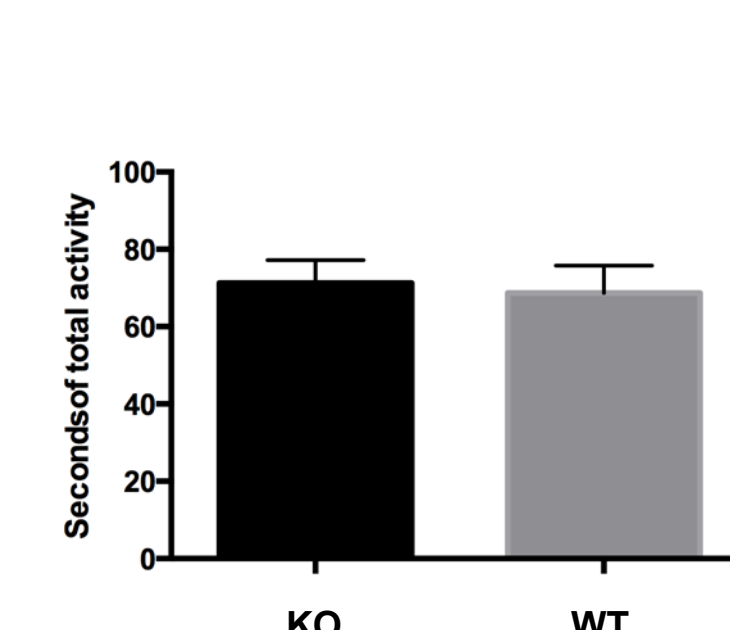


Figure 5. Comparison of Open Field Total Activity for Entire Sample. On average, knockout mice showed more overall activity than wild-type in the ten minutes, but this is not significant. p = 0.3894

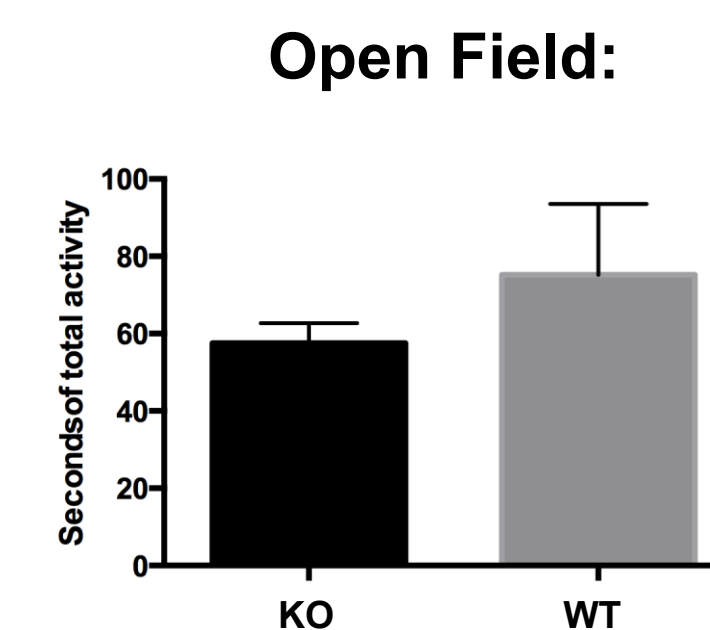


Figure 6. Comparison of Open Field Total Activity for Males. Male wild-type mice showed more overall activity than the knockout mice on average, but not significantly. p = 0.1256

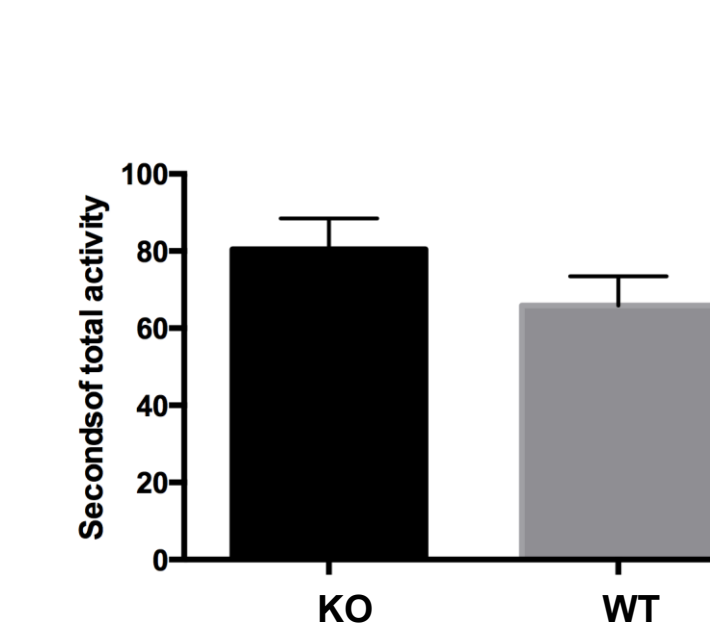


Figure 7. Comparison of Open Field Total Activity for Females. Female *Atg5* knockout mice showed more average activity than the wild-type mice, but not significantly. p = 0.1075

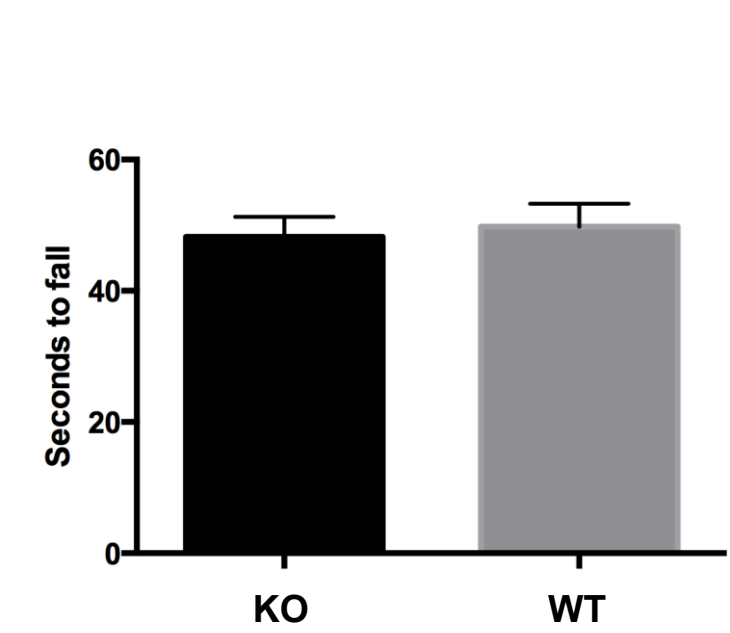


Figure 8. Comparison of Grip Strength, Represented by Average Time to Fall, for Entire Sample. Wild-type mice were able to hold on longer than knockouts on average, but this is not significant. p = 0.3747

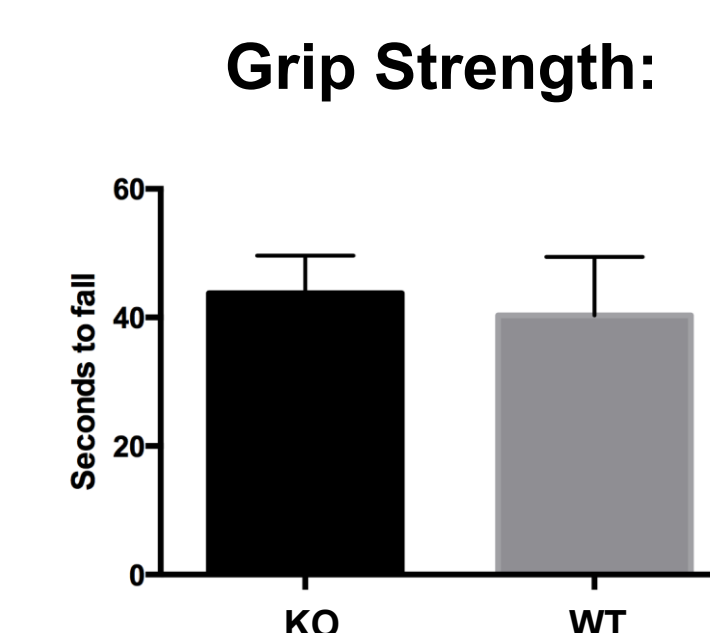


Figure 9. Comparison of Grip Strength for Males. On average, knockout males were able to hold on longer than wild-type males, but not significantly. p = 0.3728

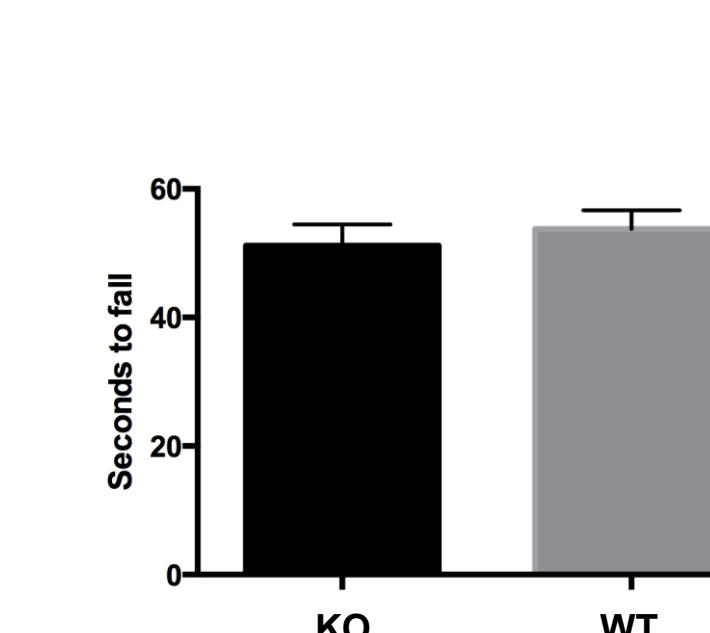


Figure 10. Comparison of Grip Strength for Females. Wild-type females were generally able to hold on longer than knockouts, but not significantly. p = 0.2843

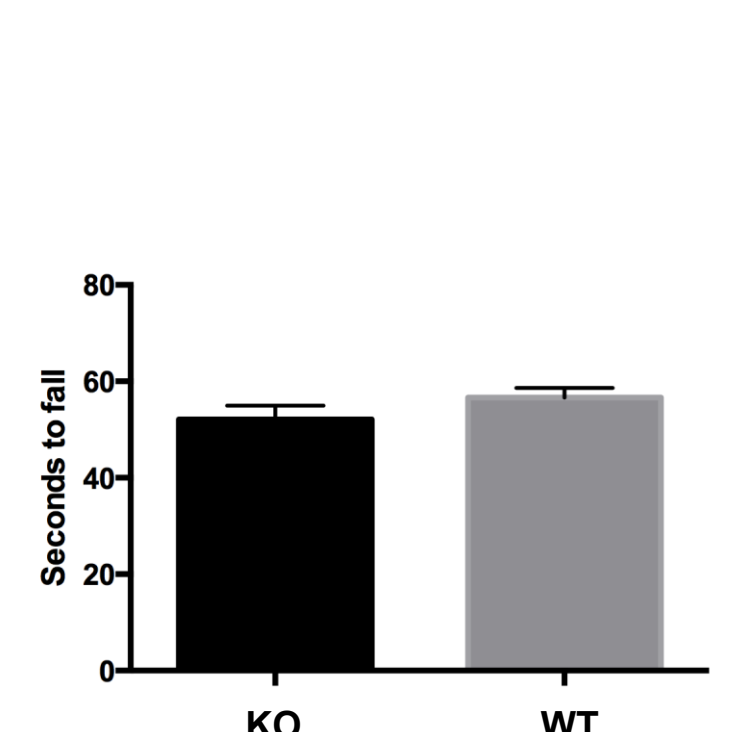


Figure 11. Comparison of Rotarod Performance for Entire Sample. On average, wild-type mice were able to stay on the Rotarod longer than knockouts, but not significantly. p = 0.1265

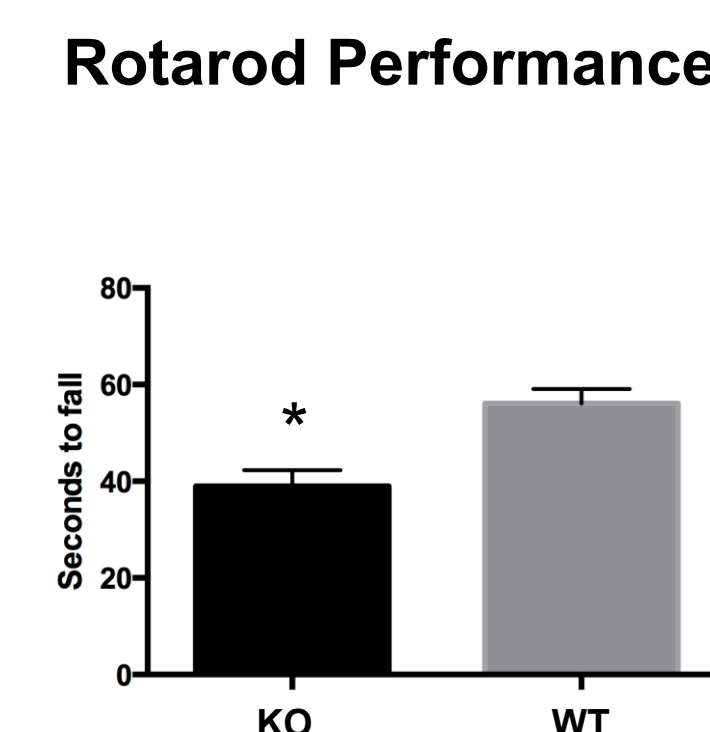


Figure 12. Comparison of Rotarod Performance of Males. Wild-type males were able to stay on the Rotarod significantly longer than knockouts. p = 0.0011

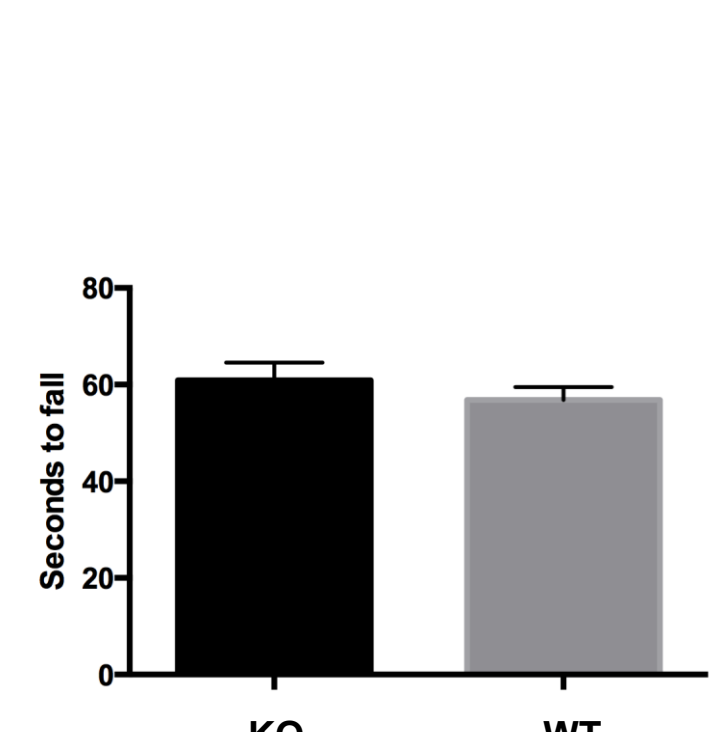


Figure 13. Comparison of Rotarod Performance of Females. Knockout females were able to stay on the Rotarod for an average time longer than the wild-type females. p = 0.198

Summary

Results:

- At 9 months post-induction a neurological phenotype emerged, with *Atg5* knockout animals demonstrating reduced ability to remain on the spinning RotaRod, reduced grip strength, abnormal gait, and abnormal clasping reflex.
- Open field activity and grip strength was not conclusively different between the knockouts and wild-types of either sex.
- There are observable sex differences between the male and female knockouts.
- Female knockout mice in this cohort exhibited a milder phenotype, with fewer exhibiting an abnormal clasping reflex, and on average staying on the Rotarod longer than the female wild-types. This is in contrast to a previous cohort, in which the knockout females were more severely affected than the males.
- Male mice in this group exhibited a more severe phenotype than the male wild-types, with significantly reduced ability to stay on the Rotarod, and a more severe clasping abnormality.
- These results further support the relationship between autophagy and affective disorders and the possibility that the sex differences in knockout mice contribute to varying neurodegeneration.

Conclusion: Deletion of the *Atg5* gene results in age-dependent development of behavioral and physical changes associated with the phenotype of "mania" and "neurodegeneration".

Acknowledgements and References

We would like to thank the University of Minnesota, College of Pharmacy for providing the GAP Grant, as well as the United States-Israel Binational Science Foundation for providing funding.

- Cleary et al., (2008) Antidepressive-like effects of rapamycin in animal models: implications for new targets for treatment of affective disorders. *Brain Research Bulletin* 76:469-473.
- Kara et al., (2013) Trehalose induced antidepressant-like effects and autophagy enhancement in mice. *Psychopharmacology*, 229(2): 367-75.

For further information
Please contact ander163@d.umn.edu