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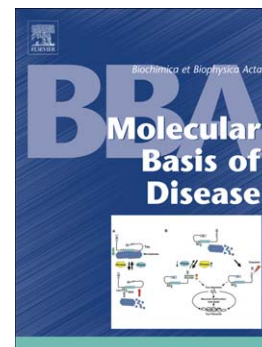
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Differential interaction between iron and mutant alpha-synuclein causes distinctive Parkinsonian phenotypes in *Drosophila*

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ABSTRACT

Alpha-synuclein aggregation is the central hallmark of both sporadic and familial Parkinson's disease (PD). Patients with different PD-causing genetic defects of alpha-synuclein usually show distinctive clinical features that are atypical to sporadic PD. Iron accumulation is invariably found in PD. Recent studies showed that mutant and wild-type alpha-synuclein may have differential interaction with iron and mutant alpha-synuclein toxicity could be preferentially exacerbated by iron. We hence hypothesized that iron overload could selectively influence mutant alpha-synuclein toxicity and disease phenotypes. To test the hypothesis, we investigated if *Drosophila melanogaster* over-expressing A53T, A30P, and wild-type (WT) alpha-synuclein have different responses to iron treatment. We showed that iron treatment induced similar reduction of survival rate in all flies but induced a more severe motor decline in A53T and A30P mutant alpha-synuclein expressing flies, suggesting interaction between mutant alpha-synuclein and iron. Although no significant difference in total head iron content was found among these flies, we demonstrated that iron treatment induced selective DA neuron loss in motor-related PPM3 cluster only in the flies that express A53T and A30P mutant alpha-synuclein. We provided the first *in vivo* evidence that iron overload could induce distinctive neuropathology and disease phenotypes in mutant but not WT alpha-synuclein expressing flies, providing insights to the cause of clinical features selectively exhibited by mutant alpha-synuclein carriers.

Key Words: Parkinson's disease; *Drosophila melanogaster*; iron; alpha-synuclein; A53T and A30P; dopaminergic neuron.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease and is clinically characterized by resting tremor, muscular rigidity, bradykinesia and postural instability [1, 2]. Although the etiology still remains unclear, it is widely accepted that the symptoms are mainly caused by the selective loss of dopaminergic (DA) neurons in the substantia nigra leading to depletion of dopamine in the striatum. Subsequent studies led to the identification of a number of central PD hallmarks in the substantia nigra, including the presence of Lewy bodies, mitochondrial dysfunction, iron accumulation and elevated oxidative stress [3-5]. Currently, there is still no cure for PD and only symptomatic treatments, such as L-DOPA medication and surgical deep brain stimulation, are available.

Alpha-synuclein, a natively unfolded protein with unclear function, has been found to be the principal component of Lewy bodies in both sporadic and familial forms of PD [3, 6-8]. The discovery of three alpha-synuclein gene (SNCA) missense mutations, including E46K, A53T and A30P, and gene locus multiplication that leads to elevated gene dosage in familial PD, suggests the critical role of alpha-synuclein in PD pathogenesis [9]. Differential clinical features have been found in patients with different genetic defects in SNCA. For example, PD patients carrying A53T mutation usually have a slightly earlier onset, faster disease progression, lower tremor prevalence and dementia when compared with sporadic PD patients [10]. In contrast, patients carrying A30P mutation resemble sporadic PD in most clinical features such as late age-at-onset and a mild but progressive disease development [8, 11, 12]. Patients carrying E46K usually show more severe parkinsonism, with an earlier age-at-onset accompanied with dementia [8]. In agreement with clinical findings, studies have also revealed significant differences between transgenic animal

models over-expressing different forms of alpha-synuclein. For example, *Drosophila* expressing A30P alpha-synuclein lose climbing ability significantly earlier than flies expressing A53T or wild-type (WT) alpha-synuclein [4]. Mice expressing A53T alpha-synuclein develop adult-onset neurodegenerative disease characterized by motor dysfunction while these phenotypes are not found in the A30P or WT variants [13].

The neuropathological mechanism through which different PD-causing genetic defects of alpha-synuclein confer differential clinical features remains an interesting and important question. Previous findings suggested that the toxicity of alpha-synuclein to DA neurons is related to its ability to aggregate into toxic oligomers or fibrils [3, 7, 8, 12, 14]. Although mutant alpha-synuclein tend to aggregate faster than wild-type (WT) alpha-synuclein (A53T>A30P>WT), their toxicity is not necessarily higher according to a number of *in vitro* studies [15, 16]. Other factors such as dopamine or other oxidative stress inducers might act in cooperation with mutant alpha-synuclein to induce selective and enhanced death of neurons, providing possible explanation to the development of distinctive clinical features [15, 16].

Iron accumulation has been constantly found in substantia nigra of PD patients [17-20]. Iron overload is known to be toxic due to the excessive synthesis of oxidative free radicals via the iron-dependent Fenton reaction. It has been reported that iron treatment or enhanced uptake induced loss of DA neurons and parkinsonian motor symptoms in rodents and *Drosophila*, indicating the potential pathogenic role of iron overload in PD [21, 22]. *In vitro* experiments suggested that iron can also bind to alpha-synuclein, facilitate alpha-synuclein aggregation and enhance toxicity of alpha-synuclein, in an order similar to the potency of intrinsic aggregation, i.e. A53T>A30P>WT [15]. Interestingly, it has recently been found that mutated forms of alpha-synuclein may have heightened ferrireductase

activity, which converts iron (III) ion (Fe^{3+}) to more toxic iron (II) ion (Fe^{2+}) that participates in the Fenton reaction [23, 24]. However, it remains unknown whether differential interaction of iron with different alpha-synuclein variants could induce distinctive neuropathology and hence differential disease phenotypes in mutant alpha-synuclein carriers.

Based on the above information, we hypothesized that iron overload could be a potent factor that could selectively alter mutant alpha-synuclein toxicity and disease phenotypes. To test the hypothesis, we examined if *Drosophila* over-expressing WT and mutant alpha-synuclein specifically in DA neurons, through the use of tyrosine hydroxylase (TH) driver, have different responses to iron treatment in terms of the lifespan of the animals, their motor activities and the integrity of the DA neurons.

2. Materials and methods

2.1. *Drosophila* and maintenance

All *Drosophila melanogaster* stocks were maintained in bottles containing standard cornmeal medium in a humidified incubator at 25°C under a 12 h light/dark cycle. *w¹¹¹⁸* (#3605), *UAS-synA53T* (#8148), *UAS-synA30P* (#8147), *UAS-synWT* (#8146) and *TH-gal4* (#8848) were purchased from Bloomington Stock Center.

2.2. Iron treatment

Flies were treated with dietary iron supplement of 15mM ammonium iron (III) citrate (FAC; Sigma) in 1% glucose (Sigma) or only 1% glucose as previously described [21, 25]. FAC was chosen for iron treatment because FAC is a commonly used dietary iron supplement

used in animal experiments including flies [26, 27]. Briefly, flies were collected within 24h after eclosion for experiments. Flies at 5 days post-eclosion were used for testing. They were first starved in empty vials for 3 h at 25°C and groups of 10 flies were placed in each vial containing filter paper (Bio-Rad Mini Trans-Blot) saturated with treatment solution for 5 days. The filter was changed every day.

2.3. Survival test

Survival test was performed according to the method described previously [28]. One hundred flies were used in the assay for each group. Log rank (Mantel-Cox) test was used to analyze survival curve difference.

2.4. Climbing assays

Negative geotaxis was performed to determine the motor ability of the flies according to the well-established protocol [4, 29] with some modifications. Briefly, 10 flies were anesthetized by CO₂ and placed in a plastic column (30 cm in length and 1.5 cm in diameter). After a 30 min recovery, the flies were gently tapped to the bottom of the column. After 10 second, the number of flies that were above the 8 cm-line was counted. The performance index is defined as Number_{top}/10. Three trials were performed blindly in each experiment at 1 min intervals, and six experiments were carried out for each genotype. Linear regression (slope comparison) was used to analyze decline rate of climbing ability.

2.5. Quantification of iron content in fly head extracts

Iron contents in fly heads were measured as described previously with little modifications [21, 30]. Briefly, 50 fly heads from different groups were weighed and homogenized in 20 mM HEPES buffer with pellet pestle. The homogenate was then mixed with an equal volume of ultra-pure nitric acid (Sigma) and incubated at 56°C for 48 h. Iron content was

measured using graphite furnace atomic absorption spectrometry (GFAAS; Hitachi, Z-2300, Japan). Iron standard curve was prepared for calculating iron concentration per unit wet weight.

2.6. Whole-mount immunostaining and confocal microscopy

Whole-mount immunostaining of fly brains was carried out as previously described [31]. Briefly, after dissection, the brains were fixed with 4% paraformaldehyde (PFA, Sigma) in 0.3% Triton X-100 and blocked with 5% heat-inactivated normal goat serum in 0.3% Triton X-100 at room temperature. The brains were then incubated with anti-tyrosine hydroxylase (TH) primary antibody (Millipore) at 1:500 dilution at 4°C for 48 h, followed by goat anti-rabbit Alexa568 secondary antibody (Invitrogen) at 4°C for another 48 h. Afterwards, the brains were mounted and fluorescent images were captured by Nikon C-1 confocal imaging system (Nikon, Japan). The brains were scanned through confocal Z-stacks and the number of TH-positive neurons of different clusters, including VUM, PPL1, PPL2, PPM1/2, PPM3, and PAL, in each hemisphere at each stack was counted manually with the use of ImageJ. 20 hemispheres were used for each group of flies. The number of TH-positive neurons was counted blindly by an observer.

2.7. Statistical analysis

All data were presented as mean \pm standard error (SEM). GraphPad Prism was used for generating graphs and SPSS 6.0 was used for statistical analysis. The differences between the means were all determined by ANOVA, unless otherwise stated. For example, Log rank (Mantel-Cox) test and linear regression for slope comparison were used to analyze survival curve difference. Linear regression was also used for comparing decline rate of climbing ability. $p < 0.05$ was used to indicate statistical significance.

3. Results

3.1. Iron treatment induced similar reduction of survival rate in flies

Previous studies have shown that both iron exposure and alpha-synuclein expression could reduce survival rate in flies [21, 29]. In the present study, we confirmed that alpha-synuclein expressing flies in general had a significantly lower survival rate than w1118 wild type flies after being fed with 1% glucose for 5 days (Figure 1A). There was no significant difference in the degree of survival rate reduction between A53T, A30P and WTalpha-synuclein (Figure 1A), implying similar level of toxicity exerted by different forms of alpha-synuclein. When they were fed with 15 mM FAC, we again observed significantly increased death rates of the flies with expression of alpha-synuclein and there were no differences among the alpha-synuclein flies, WT or mutants (Figure 1B). After we have further analyzed our data to compare the effects of glucose and FAC treatment on each fly line used, we found that, according to linear regression slope comparison of the survival curves, FAC treatment had a strong impact, significantly decreasing the survival rate in w1118 control flies (Figure 1C), the WT alpha synuclein (Figure 1D), the A30P alpha synuclein (Figure 1E) and the A53T alpha synuclein flies (Figure 1F).

3.2. Iron treatment induced a more severe motor decline in A53T and A30P mutant alpha-synuclein expressing flies

Apart from the effect on survival rate reduction, alpha-synuclein expression and iron exposure had also been found to induce age-dependent decline of motor ability in flies [21, 29]. In the present study, with the use of negative geotaxis, we investigated the effect of interaction between iron and different forms of alpha-synuclein in causing motor deficit in flies by comparing (1) difference between normal and alpha-synuclein-expressing flies and (2) difference between flies of the same genotype with or without iron treatment. As shown

in Figure 2A, compared with the w1118 flies, expression of WT alpha-synuclein did not impair the motor function. Although iron treatment tended to decrease the performance index, there was no significant difference in performance index between iron-treated WT alpha-synuclein-expressing flies and iron-treated w1118 flies, except day 3. Similarly, expression of A30P and A53T also did not significantly impair motor ability when compared with w1118 flies, except day 4 between A30P and w1118 flies (Figure 2B and 2C). However, upon iron treatment, a remarkable and highly significant impairment in motor ability was found in A30P and A53T flies when compared with w1118 flies, which was especially obvious after 5 days of iron treatment. Our results strongly implicated interaction between iron and mutant forms of alpha-synuclein in weakening the motor ability of flies.

3.3. Total head iron level was indistinguishable among flies under the same treatment

The apparent increased toxicity of iron in A30P and A53T alpha-synuclein flies might be due to enhanced accumulation of iron in the brains of these flies relative to control and WT alpha-synuclein flies. To gain further insight into the mechanism, we examined the level of iron in these different lines before and after 5 days of FAC treatment. By means of GFAAS, we found that total iron level in the heads of alpha-synuclein expressing flies was similar to that of w1118 flies and there was no significant difference between different alpha-synuclein expressing flies (Figure 3). Iron treatment for 5 days increased head iron level in w1118, A30P, A53T and WT flies by about 2-3 folds, but there was also no significant difference in head iron level between these flies (Figure 3), indicating that alpha-synuclein-induced toxicity and aggravated iron-induced toxicity in A53T and A30P flies might not be due to difference in total head iron level.

3.4. Iron treatment induced selective loss of PPM3 dopaminergic neurons in A53T

and A30P mutant alpha-synuclein expressing flies

Motor decline induced by alpha-synuclein expression or iron treatment in flies is known to be associated with loss of DA neurons [32-37]. We then examined the number of DA neurons with TH immunostaining to see if the more severe motor decline in mutant alpha-synuclein expressing flies induced by iron overloading was associated with DA neuron loss. Six DA neuron clusters, including VUM, PAL, PPL1, PPM1/2, PPL2 and PPM3, were assessed. The location of different DA clusters and the staining results are shown in Figure 4A and 4B. Our analysis showed that alpha-synuclein expression, regardless of the type of alpha-synuclein, selectively reduced the number of dopaminergic neurons in PPM1/2 cluster (Figure 5A) while iron treatment alone did not induce significant loss of dopaminergic neurons in the six DA clusters examined in w1118 flies (Figure 5B). However, upon iron treatment, the number of dopaminergic neurons in PPM3 cluster, which is highly related to motor ability, was significantly reduced only in A53T and A30P alpha-synuclein expressing flies (Figure 5C-F). Although DA neurons in PPM3 cluster of WT alpha-synuclein expressing flies became significantly lower than that of w1118 flies, no statistical difference was found in PPM3 DA neuron number between WT alpha-synuclein expressing flies with and without iron treatment (Figure 5F). On the whole, our results indicated that DA neurons in PPM1/2 were more sensitive to alpha-synuclein-induced toxicity while DA neurons in PPM3 cluster were more sensitive to the interaction of alpha-synuclein and iron, especially when the alpha-synuclein is in mutated form.

4. Discussion

Cellular toxicity induced by oligomerization and aggregation of alpha-synuclein has been regarded as a major pathophysiological mechanism underlying selective DA neuronal death in Parkinson's disease [38-41]. In addition, it is known that mutant alpha-synuclein,

especially A53T, is more prone to aggregate than WT alpha-synuclein [15]. On the other hand, there are studies suggesting that mutant alpha-synuclein might not necessarily enhance cell death unless other factors, such as dopamine or other oxidative stress inducers, are present [15, 16, 42]. In this study, we found that iron induced a more severe motor decline in A53T and A30P mutant alpha-synuclein expressing flies, an effect that was accompanied by selective loss of motor-related PPM3 DA cluster only in A53T and A30P alpha-synuclein expressing flies with iron treatment. These observations provide direct *in vivo* evidence that iron could be a critical factor in promoting the toxic effect of mutant alpha-synuclein. Our results are also in line with previous finding from *in vitro* studies that iron can enhance mutant alpha-synuclein toxicity, and in the order of A53T>A30P>WT, the same as the intrinsic aggregation order of these mutated proteins [15]. Our study is also the first to demonstrate selective susceptibility of motor-related PPM3 DA neurons to the toxic effect from the interaction between iron and mutant alpha-synuclein. However, it is worth to note that in our study, there were no clear differences between the A53T and A30P alpha-synuclein expressing flies with respect to their survival rates, the exaggerated motor impairment as well as selective DA neuronal loss in response to iron treatments. It is possible that as we only focused on TH-positive neuron number and negative geotaxis test, there might be additional impairments, motor or non-motor, that were undetected. On the other hand, we found that iron treatment itself did not induce any loss of DA neurons, which contradicts previous findings [21]. The discrepancy might be due to the different lines of flies or iron supplements used. One possibility is that Fe^{2+} from FeSO_4 is more readily absorbed in flies than Fe^{3+} from FAC, leading to reduced toxicity in our iron-treated flies.

It is widely accepted that the motor symptoms in PD are mainly caused by selective loss of DA neurons [43, 44]. There are 13 well-defined clusters of DA neurons in *Drosophila* [45,

46]. Among all DA clusters, although DA neurons in dorsomedial region, which include DA clusters PPM1/2 and PPM3, are frequently reported to be more sensitive to alpha-synuclein toxicity, loss of DA neurons in PPL1 has also been reported [29, 47]. Our results showed that DA neurons in PPM1/2 were selectively reduced in all alpha-synuclein expressing flies but remained unchanged upon iron treatment in all flies. Such findings indicated that PPM1/2 tend to be more sensitive to alpha-synuclein toxicity regardless of the types of alpha-synuclein, but not iron overload. TH loss in PPM1/2 in alpha-synuclein-expressing flies is consistent with previous studies [48]. However, loss of DA neurons in other clusters has also been reported and such discrepancy is thought to be related to differences in genetic background, culture environment or alpha-synuclein expression level [48]. Interestingly, DA neurons in PPM3 cluster were found to be selectively reduced in mutant alpha-synuclein expressing flies upon iron treatment. Such findings indicated that PPM3 is more sensitive to the toxic effects resulting from the interaction between mutant alpha-synuclein and iron overloading, but not alpha-synuclein or iron overload alone. In addition, loss of PPM3 cluster but not PPM1/2 was found to be associated with the more severe motor decline observed in mutant alpha-synuclein expressing flies. However, the reason behind selectively loss of DA neuron in certain cluster still remains largely unknown but definitely worth further investigation.

What is the basis of the differential interaction between mutant alpha-synuclein and iron *in vivo* observed in the present study? Although fully addressing this question is beyond the scope of the present study, we would like to provide a hypothetical scheme involving two possible pathways (Figure 6). Firstly, a recent study has provided strong evidence that alpha-synuclein is a cellular ferrireductase, responsible for reducing iron (III) to the more redox-active but toxic iron (II) and that there is a significant increase in ferrireductase activity in the lysates from cells over-expressing the E46K mutant [24]. It was suggested

that mutant alpha-synuclein might possess a higher ferrireductase activity and increased Fe^{2+} could induce oxidative stress via Fenton reaction [23, 24]. Alpha-synuclein mRNA stability [49] and hence expression [50] may also be increased after Fe^{2+} binds to IRE in the 5' UTR of the alpha-synuclein mRNA. On the other hand, there are also reports demonstrating binding of Fe^{2+} with alpha-synuclein C-terminus, which was found to be highly correlated with Fe^{2+} -induced alpha-synuclein aggregation [51]. Thus, it is possible that increased iron (II) level may increase iron-alpha-synuclein binding and hence alpha-synuclein aggregation. These scenarios are consistent with the findings in the present study in which we provided *in vivo* and phenotypic evidence of potentially synergistic/differential toxic effect due to interaction between iron and mutant alpha-synuclein. Whether the fact that mutant alpha-synuclein-expressing flies had synergistically increased motor deficits and TH loss is mediated by the proposed mechanisms is worth further investigation.

To conclude, we provided the first *in vivo* evidence that mutations in alpha-synuclein, but not WT alpha-synuclein, act in concert with iron overload in causing selective loss of motor-related DA neurons and exaggerated motor decline. Our findings suggest that iron accumulation, which is consistently found in substantia nigra of PD patients, may be a crucial factor contributing to the differential neuropathology and clinical features selectively exhibited by mutant alpha-synuclein carriers.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

Y. K. and Z.M.Q. conceived, organized and supervised the study; Z.J.Z. and K.C.W. performed the experiments; W.H.Y. contributed to data analysis; Y. K. and Z.M.Q. contributed to the analysis of data and wrote the manuscript.

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LEGENDS OF FIGURES

Figure 1. The effect of iron treatment on life span of alpha-synuclein expressing flies.

(A) A53T, A30P and WT alpha-synuclein expressing flies had a significantly lower survival rate than w1118 wild type flies after being fed with 1% glucose for 5 days. Data were expressed as percentages of total flies. (B) The survival rate of different flies treated with 15 mM FAC. Significantly increased death rates of the flies with expression of alpha-synuclein were observed. (C-F) The survival rate of different lines of flies treated with 1% glucose (control) or 15 mM FAC. Linear regression slope comparison of the survival curves revealed that FAC treatment decreased the survival rate in w1118 control flies (C), the WT (D), the A30P (E) and the A53T (F) alpha synuclein flies. ***, $p<0.001$

Figure 2. The effect of iron treatment on motor ability of alpha-synuclein expressing flies.

The motor performance of flies assessed by the negative geotaxis test for 5 days. (A) There was no difference in motor performance index of w1118 flies and those expressing WT alpha-synuclein, irrespective of treatment with FAC. (B) Although expression of A30P alpha-synuclein or FAC treatment in w1118 flies did not result in significantly reduced motor performance compared with w1118 flies, FAC treatment in the A30P alpha-synuclein flies caused a big and significant impairment in motor performance over the 5 days. (C) Similarly, FAC treatment in A53T alpha-synuclein flies strongly impaired the motor performance when compared with those of w1118 flies, FAC treatment or A53T alpha-synuclein expression alone, especially in day 5. Data were expressed as means \pm SEM. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$, compared with the respective WT or mutant alpha-synuclein without FAC treatment. ##, $p<0.01$ between A30P and w1118 group.

Figure 3. The effect of iron treatment on total head iron content of alpha-synuclein

expressing flies. GFAAS measurement of total head iron content in w1118 and different alpha-synuclein expressing flies treated with 1% glucose or 15 mM FAC. FAC treatment raised the head iron level significantly in all groups. However, no differences in iron content were found among the all groups with or without FAC treatment. Data were expressed as means \pm SEM. *, $p < 0.05$ vs. the corresponding control

Figure 4. The effect of iron treatment on dopaminergic neuron in alpha-synuclein expressing flies. (A) Schematic diagram of dopaminergic neuronal clusters in *Drosophila melanogaster*. (B) Low power (left) and higher magnification (right) confocal images of whole-mount tyrosine hydroxylase (TH) staining of 5 day alpha-synuclein over-expression and w1118 control *Drosophila* brain in 1% glucose or with added FAC treatment. PPM1/2 are labeled by arrows and PPM3 labeled by arrow head.

Figure 5. Quantification of the effect of iron treatment on dopaminergic neuron in alpha-synuclein expressing flies. The number of DA neurons in the six dopaminergic clusters of different flies lines under 1% glucose (A) and 15 mM FAC (B) treatment. * $p < 0.05$ compared with the corresponding w1118 group. (C-F) Comparison of the number of DA neurons in the six clusters under 1% glucose or 1% glucose with 15 mM FAC treatment. The number of dopaminergic neurons in PPM3 cluster was significantly reduced only in A53T (D) and A30P (E) alpha-synuclein expressing flies. Data were expressed as means \pm SEM. * $p < 0.05$ compared with the control (1% glucose) group.

Figure 6. A hypothetical scheme for the differential interaction between mutant alpha-synuclein and iron *in vivo*. It is well established that conversion of iron (III) to iron (II), a process catalyzed by ferrireductase activity, will lead to increased formation of ROS through Fenton reaction and increased alpha-synuclein mRNA stability [49] and hence

expression [50] after iron (II) binds to IRE in the 5' UTR of the alpha-synuclein mRNA. On the other hand, iron (II) level may promote aggregation of alpha-synuclein possibly by iron-alpha-synuclein binding, leading to Lewy body formation. Mutated alpha-synuclein may have increased ferrireductase activity, leading to enhanced conversion of iron (III) to iron (II). Lewy body aggregation may also be increased by enhanced binding between iron (II) and mutated alpha-synuclein. These actions of mutant alpha-synuclein (indicated by blue arrows) upregulated Lewy body formation and ROS level, which could explain the augmented DA neuron loss and thus motor deficits in alpha-synuclein mutant flies.

ABBREVIATIONS

WT	Wild-type
A53T	Ala53Thr
A30P	Ala30Pro
PD	Parkinson's disease
FAC	Ammonium iron (III) citrate
TH	Tyrosine hydroxylase

Figure 1

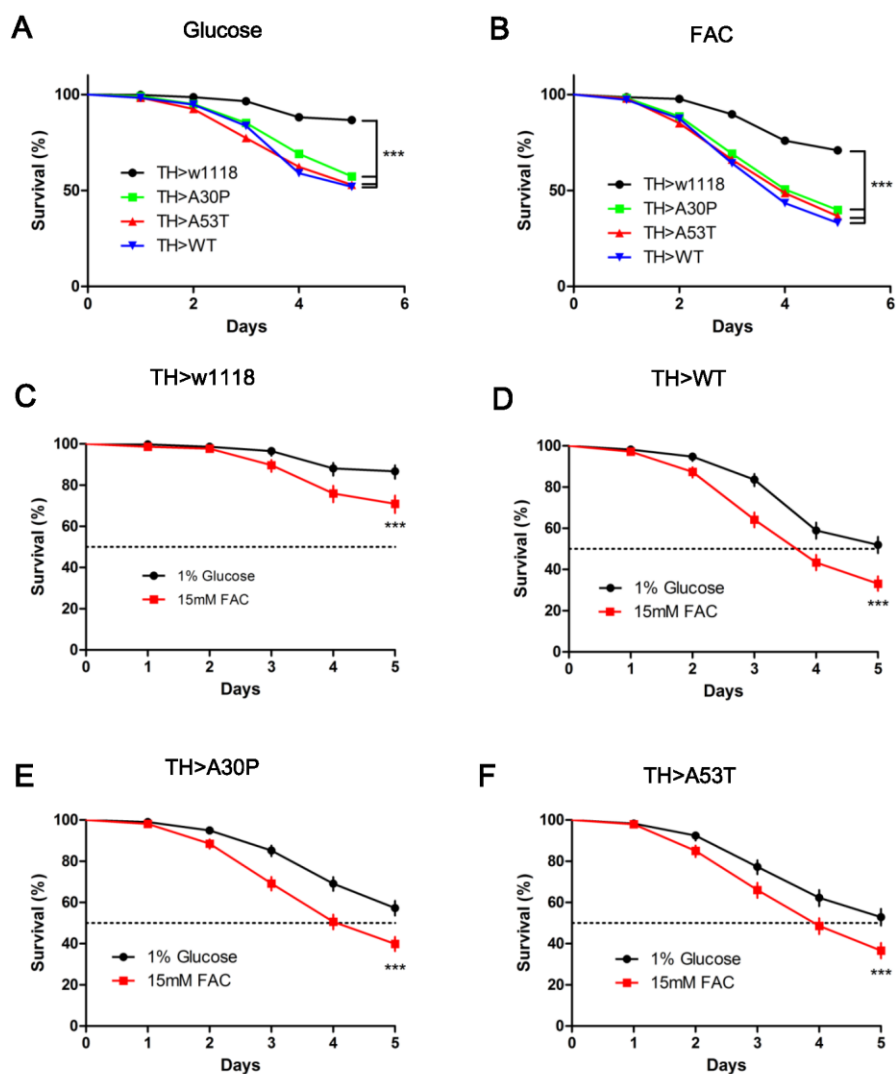


Figure 2

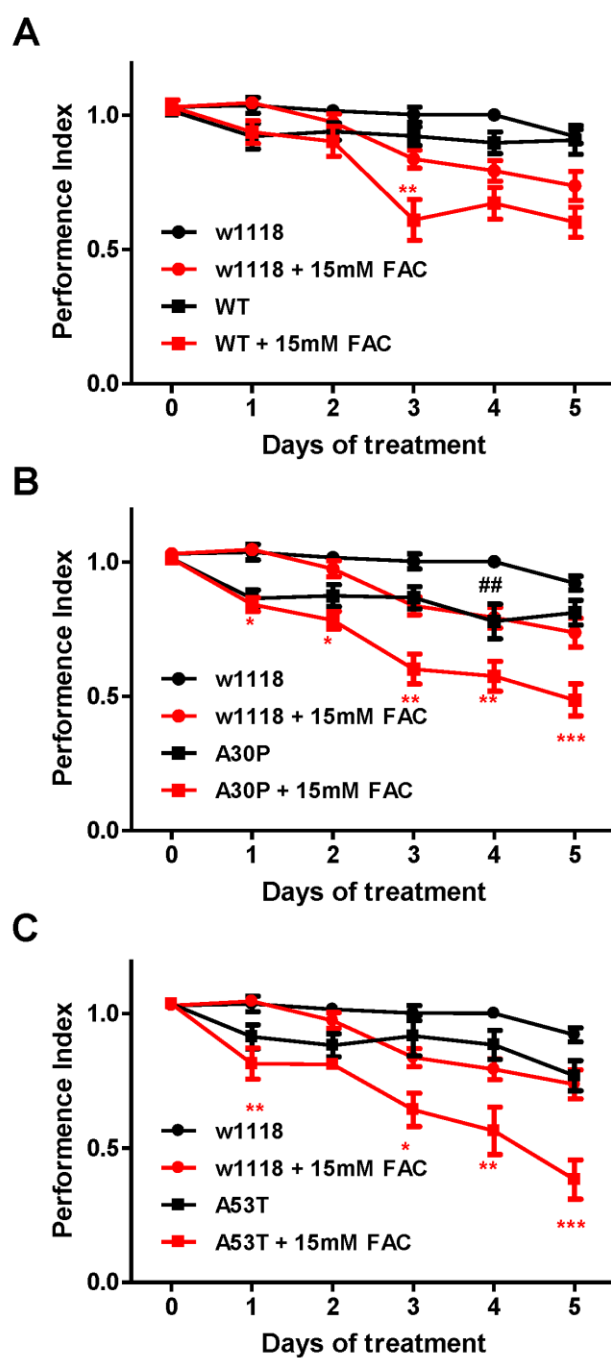


Figure 3

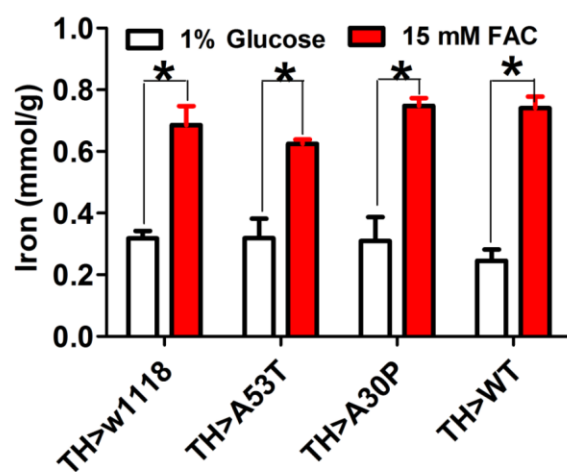


Figure 4

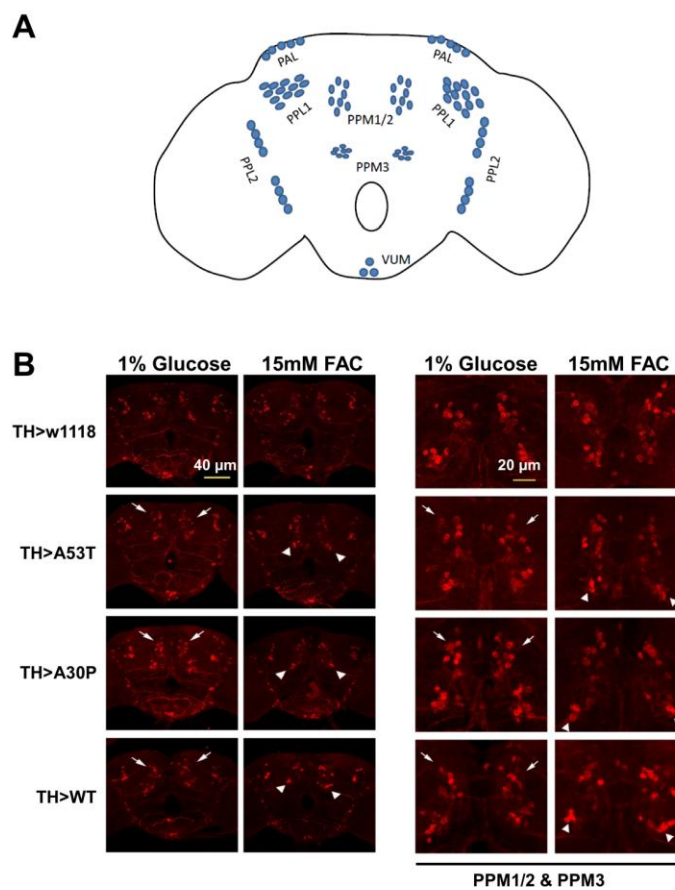


Figure 5

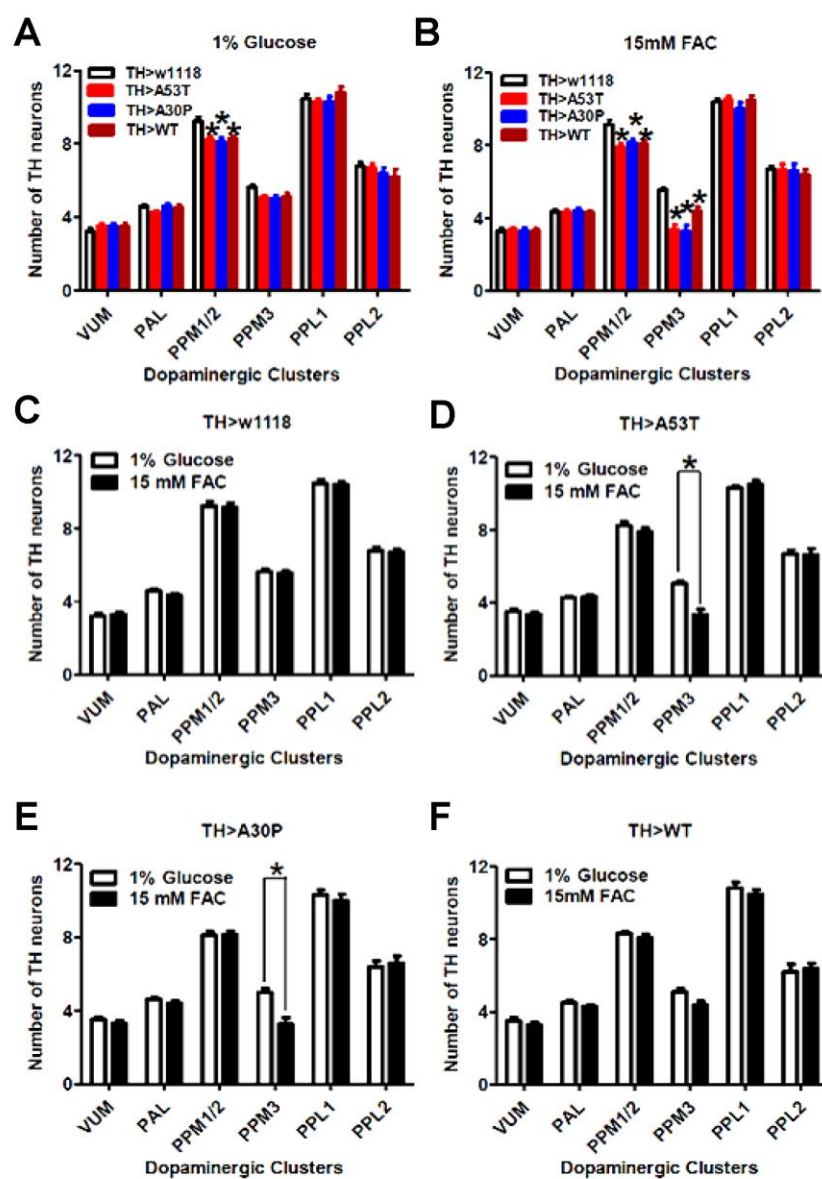
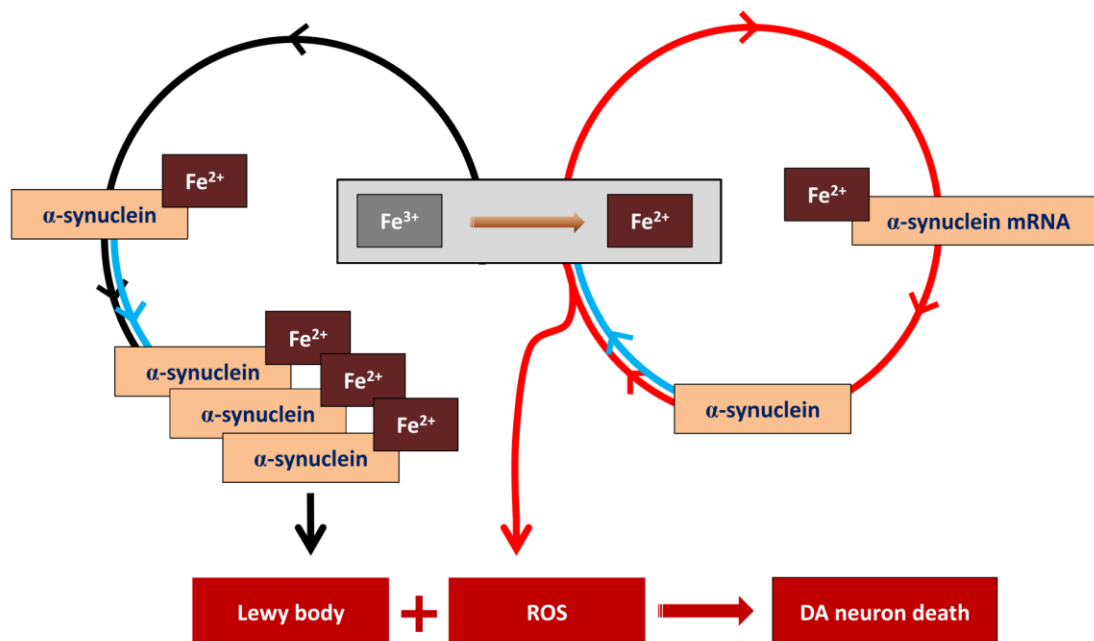


Figure 6



Highlights

- Iron reduces survival rate in flies expressing WT and mutant α -synuclein
- Iron induces more severe motor deficits in mutant α -synuclein expressing flies
- Iron induces selective neuron loss in PPM3 cluster in mutant α -synuclein expressing flies
- Interaction between iron and mutant α -synuclein underlies Parkinsonian neuropathology