

# Tryptophan-2,3-dioxygenase (TDO) inhibition ameliorates neurodegeneration by modulation of kynurenine pathway metabolites

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Edited by Solomon H. Snyder, The Johns Hopkins University School of Medicine, Baltimore, MD, and approved March 28, 2016 (received for review March 18, 2016)

**Metabolites of the kynurenine pathway (KP) of tryptophan (TRP) degradation have been closely linked to the pathogenesis of several neurodegenerative disorders. Recent work has highlighted the therapeutic potential of inhibiting two critical regulatory enzymes in this pathway—kynurenine-3-monooxygenase (KMO) and tryptophan-2,3-dioxygenase (TDO). Much evidence indicates that the efficacy of KMO inhibition arises from normalizing an imbalance between neurotoxic [3-hydroxykynurenine (3-HK); quinolinic acid (QUIN)] and neuroprotective [kynurenic acid (KYNA)] KP metabolites. However, it is not clear if TDO inhibition is protective via a similar mechanism or if this is instead due to increased levels of TRP—the substrate of TDO. Here, we find that increased levels of KYNA relative to 3-HK are likely central to the protection conferred by TDO inhibition in a fruit fly model of Huntington's disease and that TRP treatment strongly reduces neurodegeneration by shifting KP flux toward KYNA synthesis. In fly models of Alzheimer's and Parkinson's disease, we provide genetic evidence that inhibition of TDO or KMO improves locomotor performance and ameliorates shortened life span, as well as reducing neurodegeneration in Alzheimer's model flies. Critically, we find that treatment with a chemical TDO inhibitor is robustly protective in these models. Consequently, our work strongly supports targeting of the KP as a potential treatment strategy for several major neurodegenerative disorders and suggests that alterations in the levels of neuroactive KP metabolites could underlie several therapeutic benefits.**

neurodegeneration | KMO | TDO | Parkinson's disease | Alzheimer's disease

The kynurenine pathway (KP), the major catabolic route of tryptophan (TRP) metabolism in mammals (Fig. 1), has been closely linked to the pathogenesis of several brain disorders (1). This pathway contains several neuroactive metabolites, including 3-hydroxykynurenine (3-HK), quinolinic acid (QUIN) and kynurenic acid (KYNA) (2). QUIN is a well-characterized endogenous neurotoxin that specifically activates *N*-methyl-D-aspartate (NMDA) receptors, thereby inducing excitotoxicity (3, 4). The metabolites 3-HK and QUIN are also neurotoxic via the generation of free radicals and oxidative stress (5, 6). Conversely, KYNA—synthesized by kynurenine aminotransferases (KATs)—is neuroprotective through its antioxidant properties and antagonism of both the  $\alpha 7$  nicotinic acetylcholine receptor and the glycine coagonist site of the NMDA receptor (7–13). Levels of these metabolites are regulated at two critical points in the KP: (i) the initial, rate-limiting conversion of TRP into *N*-formylkynurenine by either tryptophan-2,3-dioxygenase (TDO) or indoleamine-2,3-dioxygenase 1 and 2 (IDO1 and IDO2); and (ii) synthesis of 3-HK from kynurenine by the flavoprotein kynurenine-3-monooxygenase (KMO) (1).

Alterations in levels of the KP metabolites have been observed in a broad range of brain disorders, including both neurodegenerative and psychiatric conditions (14). In neurodegenerative

diseases such as Huntington's (HD), Parkinson's (PD), and Alzheimer's (AD), a shift toward increased synthesis of the neurotoxic metabolites QUIN and 3-HK relative to KYNA may contribute to disease (1). Indeed, in patients with HD and HD model mice, 3-HK and QUIN levels are increased in the neostriatum and cortex (15, 16). Moreover, KYNA levels are reduced in the striatum of patients with HD (17). Several studies have also found perturbation in KP metabolites in the blood and cerebrospinal fluid of patients with AD, with decreased levels of KYNA correlating with reduced cognitive performance (18, 19). Similarly, in the basal ganglia of patients with PD, a reduction in KYNA levels combined with increased 3-HK has been observed (20, 21).

*Drosophila melanogaster* has provided a useful model for interrogation of the KP in both normal physiology and in neurodegenerative disease (22, 23). In fruit flies, TDO and KMO are encoded by *vermillion* (*v*) and *cinnabar* (*cn*), respectively, and both are implicated in *Drosophila* eye color pigmentation and brain plasticity (24, 25). In flies, TDO is the sole enzyme that catalyzes the initial step of the KP, as IDO1 and IDO2 are not present (Fig. 1), and so provides a distinctive model for examining the role of this critical step in the pathway. Moreover, we have previously found that downregulating *cn* and *v* gene expression significantly reduces neurodegeneration in flies expressing a mutant huntingtin (HTT) fragment—the central causative insult underlying HD (22). We also observed that pharmacological manipulations that reduced

## Significance

Neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD), and Huntington's (HD) present a significant and increasing burden on society. Perturbations in the kynurenine pathway (KP) of tryptophan degradation have been linked to the pathogenesis of these disorders, and thus manipulation of this pathway may have therapeutic relevance. Here we show that genetic inhibition of two KP enzymes—kynurenine-3-monooxygenase and tryptophan-2,3-dioxygenase (TDO)—improved neurodegeneration and other disease symptoms in fruit fly models of AD, PD, and HD, and that alterations in levels of neuroactive KP metabolites likely underlie the beneficial effects. Furthermore, we find that inhibition of TDO using a drug-like compound reverses several disease phenotypes, underscoring the therapeutic promise of targeting this pathway in neurodegenerative disease.

Author contributions: C.B., E.W.G., C.P.K., R.S., and F.G. designed research; C.B., K.V.S., S.S.I., F.M.N., J.G.E., G.G.L.M., and E.W.G. performed research; C.B., K.V.S., F.M.N., E.W.G., and F.G. analyzed data; and C.B., C.P.K., R.S., and F.G. wrote the paper.

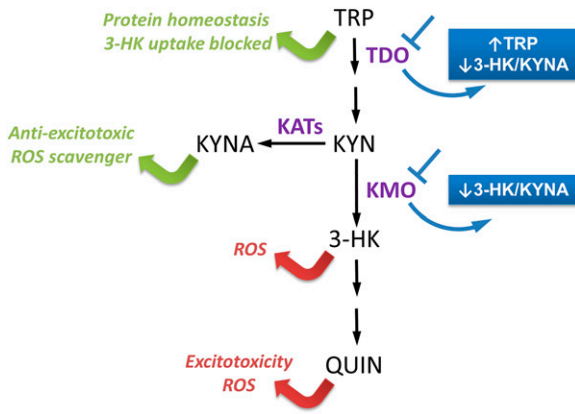
The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

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This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1604453113/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1604453113/-DCSupplemental).



**Fig. 1.** Consequences of KP manipulation. KP metabolites and enzymatic steps are indicated in black, whereas the key KP enzymes TDO, KMO, and KATs are indicated in purple. The metabolites 3-HK and QUIN are neurotoxic (as indicated by red arrows), whereas KYNA and TRP are neuroprotective (as indicated by green arrows). Inhibition of TDO results in increased TRP levels, and either TDO or KMO inhibition leads to a reduction in the 3-HK/KYNA ratio (highlighted in blue). The enzyme 3-hydroxyanthranilic acid dioxygenase is not present in flies, and thus QUIN is not synthesized.

the 3-HK/KYNA ratio were always associated with neuroprotection. Notably, reintroduction of physiological levels of 3-HK in HD flies that lacked this metabolite due to KMO inhibition was sufficient to abolish neuroprotection (22). Furthermore, in a *Caenorhabditis elegans* model of PD, genetic down-regulation of TDO ameliorates  $\alpha$ -synuclein (aSyn) toxicity (26). This effect appeared to be independent of changes in the levels of serotonin or KP metabolites but was correlated with increased TRP levels. Supplementing worms with TRP also suppressed aSyn-dependent phenotypes (26). The present study was designed to further define the mechanism(s) that underlies the neuroprotection conferred by TRP treatment and TDO inhibition and to extend our analyses of the neuroprotective potential of the KP to fruit fly models of AD and PD.

## Results

**TRP Is Neuroprotective in HD Flies via Modulation of Downstream KP Metabolites.** As work in *C. elegans* suggests that TDO inhibition and TRP treatment may confer protection against toxicity arising from misfolded proteins independent of KP metabolites (26), here we investigated whether alterations in KP metabolites were central to this protection in HD flies. These flies feature the panneuronal expression of a mutant HTT exon 1 encoding fragment (HTT93Q) under control of the *elavGAL4* panneuronal driver, and serve as a well-characterized model of HD (27). In particular, degeneration of photoreceptor neurons (rhabdomeres) in the eye serves as a robust and reproducible readout for neurodegeneration, which can easily be scored using the pseudopupil assay. HTT93Q flies were allowed to develop on media supplemented with various concentrations of TRP (from 0.4 to 10 mg/mL), and neurodegeneration was assessed at day 0 on newly emerged flies. TRP supplementation resulted in a dose-dependent amelioration of neurodegeneration compared with untreated controls, with 0.8 mg/mL being the minimum protective concentration ( $P < 0.001$ ), and the protection saturating at 3.5 mg/mL TRP ( $P < 0.001$ ; Fig. 2A). To assess whether TRP-induced neuroprotection was dependent upon changes in downstream neuroactive KP metabolites, we next determined the levels of the neurotoxic metabolite 3-HK and the neuroprotective metabolite KYNA. TRP treatment of HTT93Q flies substantially reduced levels of 3-HK relative to KYNA ( $P < 0.001$ ; Fig. 2B), predominantly driven by increased levels of KYNA (SI Appendix, Fig. S1A and B). Furthermore, we observed that the low level of emergence of adult HD flies from the pupal case (eclosion; SI Appendix, Fig. S1C) was significantly enhanced by

feeding of 3.5 mg/mL TRP ( $P < 0.001$ ; Fig. 2C). These data suggest that the neuroprotection conferred by TRP treatment is due—at least in part—to increased levels of KYNA in these flies.

We next explored the mechanism(s) by which TDO inhibition leads to neuroprotection. First, HTT93Q flies carrying a strong amorphic allele of *v* ( $v^{36}$ ) were used to assess the role of KYNA. These  $v^{-/-}$  HTT93Q flies exhibit a dramatic approximately eightfold increase in TRP levels compared with controls ( $P < 0.001$ ; Fig. 2D), as well as a significant  $\sim 80\%$  reduction in the 3-HK/KYNA ratio ( $P < 0.001$ ; Fig. 2E and SI Appendix, Fig. S1D and E). To reduce levels of KYNA in the HTT93Q  $v^{-/-}$  background, we used the nonspecific KAT inhibitor aminooxyacetic acid (AOAA), which effectively reduces KYNA synthesis in rodents in vitro and in vivo (28, 29). Animals administered 100  $\mu$ M of AOAA in their food exhibited a significant decrease in KYNA levels ( $P < 0.05$ ; Fig. 2F), which resulted in an increase in the 3-HK/KYNA ratio ( $P < 0.05$ ; Fig. 2G). Strikingly, these animals showed a complete reversal of the neuroprotection conferred by TDO inhibition ( $P < 0.001$ ; Fig. 2H). No changes were seen in levels of TRP or 3-HK (SI Appendix, Fig. S1F and G), so these findings strongly suggest that KYNA is central to the neuroprotection observed.

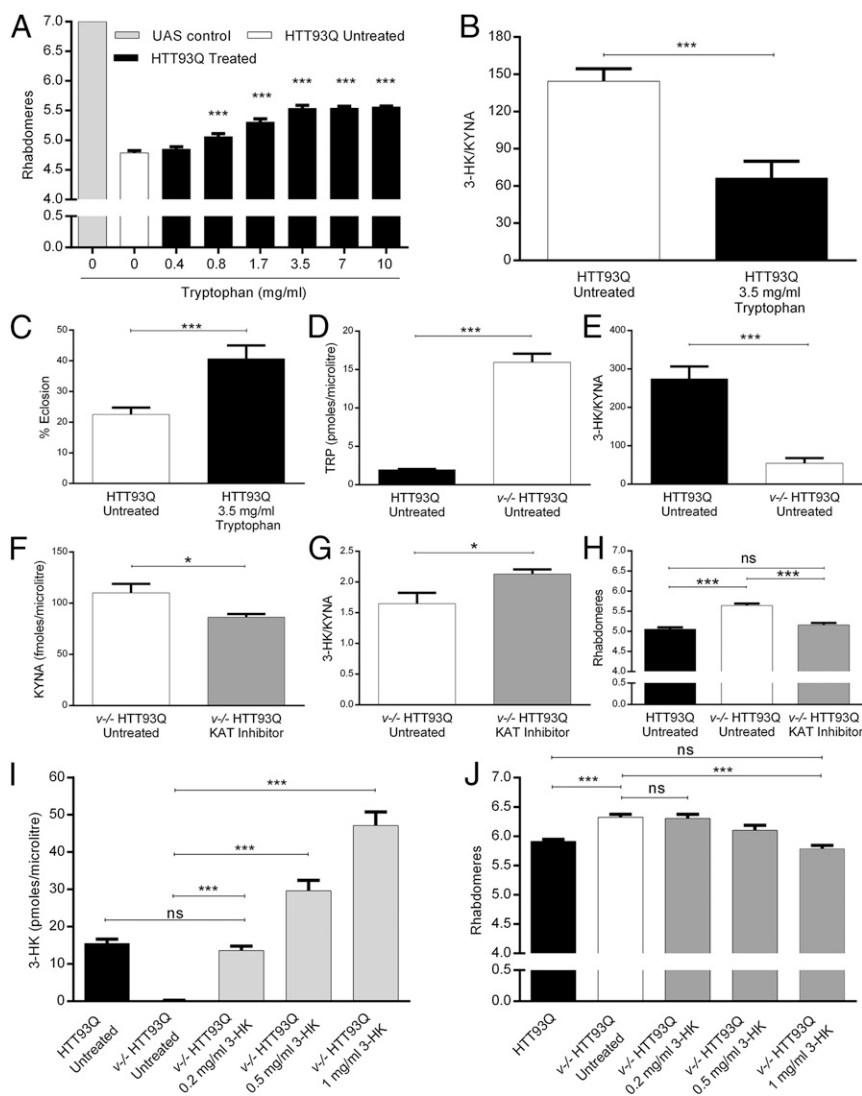
We next asked whether modulation in 3-HK levels also plays a role in the neuroprotection observed in TDO-deficient flies, which have greatly reduced 3-HK levels (22) (Fig. 2I). In line with our demonstration that reintroduction of 3-HK in KMO-deficient flies ( $kn^{-/-}$ ) is sufficient to restore neurodegeneration (22), we administered several concentrations of 3-HK (0.2–1 mg/mL) to the flies in the food (Fig. 2I and J). Surprisingly, we found that—unlike  $kn^{-/-}$  HTT93Q flies (22)—restoration of physiological levels of 3-HK in  $v^{-/-}$  HTT93Q flies (at the 0.2 mg/mL dose) did not reverse neuroprotection (Fig. 2J). Increasing 3-HK to hyper-physiological levels via administration of 1 mg/mL 3-HK enhanced neurodegeneration in  $v^{-/-}$  HTT93Q flies, thereby eliminating the neuroprotection normally observed ( $P < 0.001$ ; Fig. 2J). In all cases, we found that 3-HK treatment led to significant increases in the 3-HK/KYNA ratio (SI Appendix, Fig. S1H and I). Thus, restoration of physiological 3-HK levels is not sufficient to abrogate the neuroprotection conferred in TDO-deficient flies.

## The Excitotoxin QUIN Promotes Neurodegeneration in *Drosophila*.

*Drosophila* do not express the KP enzyme 3-hydroxyanthranilic acid dioxygenase, and thus fruit flies do not synthesize QUIN (30). Therefore, we fed *elavGAL4*-driven HTT93Q flies with increasing QUIN concentrations during development and assessed neurodegeneration by scoring the number of rhabdomeres at day 0. We first measured QUIN in wild-type (WT) and HD fly heads. As expected, QUIN was detected in flies fed 0.5 mg/mL QUIN, but not in the untreated group ( $P < 0.001$ ; Fig. 3A). Interestingly, we found that HTT93Q flies accumulate more QUIN than WT flies ( $P < 0.01$ ). Whereas QUIN feeding did not cause degeneration of rhabdomeres in WT flies (SI Appendix, Fig. S2A), QUIN treatment (0.2 and 0.5 mg/mL) enhanced neurodegeneration in HTT93Q flies in a dose-dependent manner (Fig. 3B). Notably, KMO inhibition did not protect against QUIN-induced neurotoxicity in HTT93Q flies carrying homozygous  $cn^3$  mutation, a strong amorphic *cn* allele (Fig. 3B).

## Endogenous Synthesis of KYNA in Fruit Flies Is Neuroprotective.

We next generated a *Drosophila* line carrying a transgene encoding hKAT (*UAS-hKAT*), the KP enzyme that converts kynurenine to KYNA (Fig. 1). In wild-type flies, panneuronal hKAT expression driven by *elavGAL4* caused a dramatic increase in KYNA levels at both day 1 and day 7 compared with controls ( $P < 0.001$ ; Fig. 3C), and in HD flies, dramatically reduced the 3-HK/KYNA ratio at day 1 and day 7 posteclosion ( $P < 0.01$ ; Fig. 3D and SI Appendix, Fig. S2B and C). This effect was associated with a significant amelioration of both rhabdomere neurodegeneration ( $P < 0.001$ ; Fig. 3E) and impaired eclosion in HTT93Q flies ( $P < 0.001$ ; Fig. 3F).



**Fig. 2.** TRP feeding ameliorates HTT93Q toxicity in fruit flies. (A) Rhabdomere quantification of HD flies treated with different concentrations of TRP during development. TRP concentrations higher than 0.4 mg/ml significantly ameliorate rhabdomere neurodegeneration.  $n = 6-13$  per condition,  $***P < 0.001$ . (B) The 3-HK/KYNA ratio is reduced in TRP-fed HTT93Q flies.  $n = 5-6$  per condition,  $***P < 0.001$ . (C) TRP feeding rescues HTT93Q-dependent eclosion defects. Untreated HD flies:  $n = 938$ ; TRP-treated HD flies:  $n = 728$ ,  $***P < 0.001$ . (D) TRP levels are significantly increased in  $v^{-/-}$  HTT93Q flies compared with HTT93Q flies.  $n = 5$  per condition,  $***P < 0.001$ . (E) 3-HK/KYNA levels are reduced in  $v^{-/-}$  HTT93Q compared with HTT93Q flies.  $n = 5$  flies per condition,  $***P < 0.001$ . (F) Treatment with the KAT inhibitor AOAA (100  $\mu$ M in the food) reduces the level of KYNA in  $v^{-/-}$  HTT93Q flies.  $n = 5$  per condition,  $*P < 0.05$ . (G) AOAA treatment leads to a reduction in the 3-HK/KYNA ratio.  $n = 5$  per condition,  $*P < 0.05$ . (H) KAT inhibition abrogates the neuroprotection conferred by the  $v$  mutation.  $n = 12-14$  per condition,  $***P < 0.001$ ; ns, not significant. (I) Feeding of 3-HK leads to increased levels of 3-HK in  $v^{-/-}$  HTT93Q flies.  $n = 5-6$  per treatment.  $***P < 0.001$ ; ns, not significant. (J) Supplementation of 3-HK in the food of  $v^{-/-}$  HTT93Q flies reduces neuroprotection compared with untreated HD flies.  $n = 8-12$  per condition,  $***P < 0.001$ ; ns, not significant. Data are the mean  $\pm$  SEM (one-way ANOVA with Newman-Keuls post hoc test).

### KMO and TDO Inhibition Ameliorates Disease Phenotypes in Fly Models of PD and AD.

As both KMO and TDO inhibition were robustly protective in HD model fruit flies, we tested the efficacy of these approaches in *Drosophila* models of PD and AD. For recapitulating these disorders, we used transgenic fly lines expressing human aSyn as a model of PD (31) and the human A $\beta_{42}$  peptide [either WT or the Arctic mutant form (E693G), which causes autosomal dominant AD, A $\beta_{42}$ Arc] as a model of AD (32, 33). We used RNA interference (RNAi) to down-regulate expression of the genes encoding either TDO ( $v$ ) or KMO ( $cn$ ) and found a dramatic reduction in the 3-HK/KYNA ratio, mainly due to increased synthesis of KYNA ( $P < 0.001$ ; Fig. 4H and SI Appendix, Fig. S3B and C). TRP levels were not significantly altered by these manipulations (SI Appendix, Fig. S3A), which reduce  $cn$  and  $v$  expression by  $\sim 80-95\%$  (22).

For both models, we first assessed larval crawling as an indication of behavioral impairments during early developmental stages (34). Expression of either aSyn or A $\beta_{42}$ Arc in motor neurons using the *c164GAL4* driver led to a reduction in the distance crawled by third instar larvae ( $P < 0.001$ ; Fig. 4A and B, respectively). The down-regulation of the genes encoding either TDO ( $v$ ) or KMO ( $cn$ ) by RNAi significantly enhanced crawling behavior in both models (aSyn,  $P < 0.01$ ; A $\beta_{42}$ Arc,  $P < 0.001$ ).

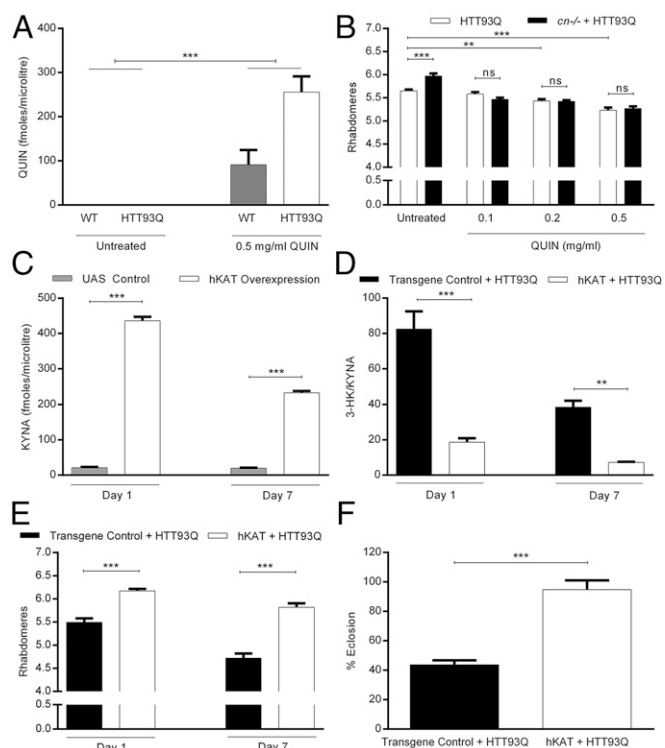
As the expression of aSyn shortens life span compared with control flies (35), we next assessed life span upon panneuronal expression of either aSyn or A $\beta_{42}$  constructs. We observed a small but significant improvement in the shortening of median life span in

aSyn flies in which either TDO ( $v$ ) or KMO ( $cn$ ) had been silenced ( $P < 0.001$ , Fig. 4C). Notably, silencing of either of the two enzymes also significantly ameliorated shortened median life span in A $\beta_{42}$  flies from 54 d to 66 d and 68 d, respectively ( $P < 0.001$ , Fig. 4D). Complementing this observation,  $cn$  down-regulation also significantly reversed shortened life span in the A $\beta_{42}$ Arc model ( $P < 0.001$ , SI Appendix, Fig. S4A).

Locomotor behavior in adult flies was assessed by examining negative geotaxis ability (climbing) as a measurement of motor impairment. *elavGAL4*-driven aSyn, A $\beta_{42}$ , and A $\beta_{42}$ Arc flies exhibited a reduction in climbing at all of the posteclosion ages tested (Fig. 4E and F and SI Appendix, Fig. S4B). Silencing of either TDO or KMO improved climbing ability in all these models (Fig. 4E and F and SI Appendix, Fig. S4B). Notably, scoring the number of rhabdomeres per ommatidium revealed that genetic knockdown of either enzyme also consistently reduced neurodegeneration in *elavGAL4*-driven A $\beta_{42}$ Arc flies at all ages tested ( $P < 0.01$ , Fig. 4G).

Finally, we interrogated the effect of pharmacological TDO inhibition in the three fly models of neurodegeneration, using the commercially available TDO inhibitor 680C91 (36). Feeding of 680C91 (100  $\mu$ M) to newly emerged HTT93Q flies resulted in dramatically reduced neurodegeneration 7 d posteclosion compared with flies fed vehicle alone (Fig. 5A,  $P < 0.001$ ). In PD and AD flies, pharmacological inhibition of TDO with 100  $\mu$ M 680C91 led to a significant amelioration in climbing performance compared with respective controls 10 d posteclosion (Fig. 5B,  $P < 0.001$ ).





**Fig. 3.** QUIN exacerbates neurodegeneration in HD flies and overexpression of hKAT is neuroprotective via increased KYNA levels. (A) QUIN levels in WT and HTT93Q-expressing flies. QUIN is detected in flies fed with 0.5 mg/mL of QUIN, but was not measurable in untreated flies.  $n = 3-5$  flies per treatment,  $***P < 0.001$ . (B) HTT93Q and  $cn^{-/-}$  HTT93Q flies fed QUIN exhibit increased rhabdomere degeneration compared with untreated flies. Neuroprotection conferred by the  $cn$  mutation is abolished by QUIN feeding.  $n = 11-12$  per treatment,  $**P < 0.01$ ,  $***P < 0.001$ . (C) Panneuronal overexpression of hKAT in a WT background causes an increase in KYNA production compared with controls at both posteclosion ages tested.  $n = 3-5$  per genotype,  $***P < 0.001$ . (D) HTT93Q flies with panneuronal overexpression of hKAT show a significant reduction in the 3-HK/KYNA ratio. The transgene control used in this experiment was a transgenic *Drosophila* line expressing an empty pJFRC2 vector.  $n = 4-5$  per condition,  $**P < 0.01$ ,  $***P < 0.001$ . (E) Overexpression of hKAT is neuroprotective in HTT93Q flies at both posteclosion ages tested.  $n = 9-13$  flies per condition,  $***P < 0.001$ . (F) Overexpression of hKAT ameliorates the eclosion phenotype observed in HTT93Q flies. Transgene control + HTT93Q flies:  $n = 1084$ ; hKAT + HTT93Q flies:  $n = 1,010$ ,  $***P < 0.001$ ; ns, not significant. Data are the mean  $\pm$  SEM (one-way ANOVA with Newman-Keuls post hoc test).

## Discussion

Impairments in KP metabolism have been linked to several neurodegenerative disorders, and in particular to the pathogenesis of HD (37). Notably, increased levels of 3-HK and QUIN have been measured in the neostriatum and cortex of patients with early stage HD (15), and these changes are associated with an up-regulation of *IDO1* transcription (38) and a reduction in the activity of KAT, which is critical for KYNA synthesis (17). These data in patients with HD are supported by observations in HD mice, which show increased cerebral KMO activity (39). We previously found that either genetic or pharmacological inhibition of KMO is protective in HD flies and leads to a corresponding increase in KYNA levels relative to 3-HK (22). Furthermore, we reported that KYNA treatment reduced neurodegeneration in these flies. Here, we have extended this work by generating transgenic flies that overexpress hKAT and thereby synthesize  $\sim 20$ -fold more KYNA than control flies. This increased formation of KYNA reduced neurodegeneration and eclosion defects in HD model flies. Furthermore, KMO inhibition by RNAi revealed beneficial effects in several behavioral and disease-relevant outcome

measures, including larval crawling, longevity, climbing, and rhabdomere degeneration, in AD and PD model flies. These results strongly support the notion that KMO inhibition has relevance as a treatment strategy in a broad range of neurodegenerative diseases. In addition, these data also suggest that the design of small molecules capable of increasing KAT activity could have therapeutic relevance for neurodegenerative disorders.

The present results, demonstrating that both genetic and pharmacological inhibition of TDO provides robust neuroprotection in fly models of AD and PD, also confirmed and extended the results of our previous study, which had identified TDO as a candidate drug target in HD flies (22). These protective effects are associated with a decrease in the 3-HK/KYNA ratio, i.e., a shift toward increased KYNA synthesis. Work in *C. elegans* has revealed that TDO inhibition is also protective in models of proteotoxicity, although amelioration of the phenotypes occurred independently of changes in the levels of KP metabolites and was instead associated with elevated TRP levels (26). Although the underlying mechanism remained unclear, the favorable effects of high TRP levels in the nematode were substantiated by the fact that TRP treatment conferred robust protection from disease-related phenotypes (Fig. 1). In the present study, too, TRP supplementation of the diet was effective, ameliorating rhabdomere degeneration and eclosion defects in HD flies. However, TRP feeding was also associated with a reduction in the 3-HK/KYNA ratio, suggesting that the protective effects of the amino acid may be linked to an increase in the production of the neuroprotective metabolite KYNA (Fig. 1). Indeed, partial inhibition of KYNA synthesis in TDO-deficient flies proved sufficient to completely reverse neuroprotection. In addition, restoration of physiological 3-HK levels in TDO-deficient HD flies did not reverse neuroprotection, in contrast to KMO-deficient flies (22). In primary neurons, 3-HK toxicity is dependent upon its uptake via neutral amino acid transporters, and coapplication of TRP can block this toxicity by competing for the same transporters (6). Thus, it is possible that the vast excess of TRP observed in the heads of HTT93Q  $v^{-/-}$  flies (approximately eightfold versus controls) competes with 3-HK for rhabdomere uptake, thereby requiring hyperphysiological levels of 3-HK to reverse TDO-dependent neuroprotection. A similar mechanism may also contribute to the neuroprotection observed with TRP treatment in general. Herein, we have also found that RNAi knockdown of either  $cn$  or  $v$  does not increase TRP levels, and thus the neuroprotection observed in the AD and PD flies strongly correlates with a decrease in the 3-HK/KYNA ratio. The mechanism causing TRP treatment to favor KYNA synthesis over the formation of 3-HK in *Drosophila*, as well as the unexpected qualitative differences in the effects of TDO inhibition and TRP administration on KP metabolism between fruit flies and nematodes, clearly requires further investigation.

Interestingly, we found that QUIN—which is not normally synthesized in fruit flies (30)—potentiated neurodegeneration in HD flies, and reversed the protective effects of KMO inhibition. As the same QUIN treatment did not cause neuron loss in wild-type flies, mutant HTT may potentiate vulnerability by enhancing NMDA receptor function (40, 41) and/or by increasing susceptibility to toxic free radicals (42), i.e., by augmenting the two major mechanisms known to be involved in QUIN-induced neurotoxicity (43). If verified in mammals, a reduction in brain QUIN levels—along with a decrease in 3-HK levels—relative to KYNA could therefore be especially promising in the treatment of HD (44). Our observation of increased levels of QUIN in HTT93Q versus WT flies is enigmatic, but may be due to altered feeding behavior, increased permeability of the blood-brain barrier (45, 46), or differences in KP metabolism, and would be interesting to explore in future studies.

In conclusion, the present set of experiments further validates the hypothesis that KP metabolism is causally linked to neuronal viability and that modulation of the KP constitutes a promising therapeutic strategy for a variety of major neurodegenerative disorders. Notably, we provide the first genetic evidence to our knowledge that KMO inhibition is protective in animal models of PD and AD and that pharmacological targeting of TDO is also



University of California, Irvine. *cn* and *v* RNAi lines are part of the phiC31 RNAi Library (KK) and were obtained from the Vienna *Drosophila* RNAi Center (53).

The gene encoding kynurenine aminotransferase (hKAT) was amplified from a human fetal cDNA library (54) and cloned into the pJRC2 vector (55)—a gift from Gerald Rubin (Addgene plasmid no. 26214)—by standard methods. The resulting construct was injected by BestGene into attP40 *Drosophila* strains (56).

Pseudopupil analysis, eclosion analysis, feeding experiments, measurement of KP metabolites, behavioral assays, longevity analysis, and statistical analyses are

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