


The Effect of Tryptophan 2,3-Dioxygenase Inhibition on Kynurenine Metabolism and Cognitive Function in the APP23 Mouse Model of Alzheimer's Disease

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International Journal of Tryptophan Research
Volume 13: 1–10
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DOI: 10.1177/1178646920972657



ABSTRACT: Alzheimer's disease (AD) is associated with progressive endogenous neurotoxicity and hampered inflammatory regulation. The kynurenine (Kyn) pathway, which is controlled by tryptophan 2,3-dioxygenase (TDO), produces neuroactive and anti-inflammatory metabolites. Age-related Kyn pathway activation might contribute to AD pathology in humans, and inhibition of TDO was found to reduce AD-related cellular toxicity and behavioral deficits in animal models. To further explore the effect of aging on the Kyn pathway in the context of AD, we analyzed Kyn metabolite profiles in serum and brain tissue of the APP23 amyloidosis mouse model. We found that aging had genotype-independent effects on Kyn metabolite profiles in serum, cortex, hippocampus and cerebellum, whereas serum concentrations of many Kyn metabolites were reduced in APP23 mice. Next, to further establish the role of TDO in AD-related behavioral deficits, we investigated the effect of long-term pharmacological TDO inhibition on cognitive performance in APP23 mice. Our results indicated that TDO inhibition reversed recognition memory deficits without producing measurable changes in cerebral Kyn metabolites. TDO inhibition did not affect spatial learning and memory or anxiety-related behavior. These data indicate that age-related Kyn pathway activation is not specific for humans and could represent a cross-species phenotype of aging. These data warrant further investigation on the role of peripheral Kyn pathway disturbances and cerebral TDO activity in AD pathophysiology.

KEYWORDS: Tryptophan, Kynurenine, Alzheimer, APP23, tryptophan 2,3-dioxygenase, TDO, aging

RECEIVED: July 4, 2020. **ACCEPTED:** October 18, 2020.

TYPE: Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Introduction

Late-onset Alzheimer's disease (AD) is the most prevalent cause of dementia and is projected to affect 131 million people worldwide by the year 2050.¹ Brains of affected individuals show typical neuropathological features, including gross atrophy of cortical and subcortical brain regions, such as the hippocampus, as well as neurofibrillary tangles consisting of hyperphosphorylated tau protein and amyloid beta (A β) plaques. Amongst the mechanisms involved in the pathogenesis of AD are increased activity of N-methyl-D-aspartate (NMDA) receptors, neuroinflammation, and disturbances of metabolic homeostasis.^{2–4} The kynurenine (Kyn) pathway, which is activated during aging, has been suggested to play a role in the pathophysiology of AD because it produces metabolites that affect NMDA receptors and is deeply embedded in immunological and metabolic functioning.^{5,6}

The Kyn pathway is the primary metabolic pathway of tryptophan (Trp) degradation and is a major source of *de novo* synthesis of nicotinamide adenine dinucleotide (NAD⁺).⁷ The production of Kyn is facilitated by the enzymes tryptophan

2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), which are regulated in a tissue-specific manner by pro-inflammatory cytokines and glucocorticoids.^{8,9} Metabolites of the Kyn pathway—*kynurenines*—have diverse biological functions including inflammatory control and metabolic regulation.¹⁰ Certain kynurenines have neuroactive properties mainly because they act on NMDA receptors. The production of these neuroactive kynurenines is cell-type specific.¹¹ For example, kynurenic acid (KA), which inhibits the NMDA receptor and is regarded a neuroprotectant, is produced by astrocytes, while quinolinic acid (QA), an NMDA receptor agonist which is generally considered an endogenous neurotoxin, is mainly produced by microglia or infiltrating macrophages.^{12–15} The production of KA and QA in the brain depends on cerebral TDO/IDO-dependent Trp metabolism and on uptake of Kyn from the blood.¹⁶ While aging is associated with increased production/accumulation of the neurotoxin QA in blood and brain,¹⁷ AD is additionally associated with reduced levels of the neuroprotectant KA in blood and brain^{18–22} and Kyn metabolism correlates with markers of disease progression.^{23–25} Kyn pathway



imbalances have therefore been suggested to be involved in AD pathophysiology.²⁶

The Kyn pathway could be a potential drug target in AD. Augmenting cerebral KA production by inhibition of Kyn metabolism in the blood (thereby increasing Kyn transport to the brain), prevented synaptic loss and improved memory function in a mouse model of AD.²⁷ In addition, Kyn pathway enzymes in the brain might be targeted directly. TDO is of particular interest in this regard as it is expressed in the hippocampus and is involved in the regulation of adult neurogenesis in mice.^{28,29} Neurogenesis within the hippocampus plays a crucial role in memory formation and is affected early in the course of AD.³⁰ Hippocampal TDO expression was found to be activated by A β oligomers³¹ and TDO expression was increased in hippocampal regions of AD patients.³² Inhibition of TDO prevented neurodegeneration-related phenotypes in *Caenorhabditis elegans* and *Drosophila melanogaster* models of AD.^{33,34} In *C. elegans*, this protection was independent of Kyn metabolites, while in *Drosophila* protection was established by increasing relative levels of KA. Additionally, in 3-month-old mice that express mouse/human amyloid precursor protein (APP)—which is cleaved to produce A β —and mutant human presenilin 1 (APP/PS1)—which is involved in cleavage of APP—a 4-week treatment with a TDO inhibitor prevented deficits in hippocampal-dependent memory function.³¹ Taken together, these data suggest that TDO could be an interesting drug target in AD.

In this study, we investigated the effect of aging on Kyn metabolite profiles in blood and brain of the APP23 amyloidosis mouse model. Our analyses reveal diverse disturbances with aging that show overlap with age-related kynurenine changes in humans.²² Next, we investigated the effect of long-term oral administration of a TDO inhibitor on Kyn metabolite profiles and cognitive functioning in APP23 mice. In our hands, TDO inhibition had minor effects on Kyn metabolite levels in blood and brain, but did improve recognition memory, while not affecting anxiety-related behavior and spatial learning and memory. These data suggests that age-related alterations of the Kyn pathway could be a cross-species phenotype of aging and warrants further investigation of TDO as a drug target in AD.

Methods

Animals

APP23 mice, which express mutated human APP (Swedish double mutation; K670N/M671L) under control of a murine Thy1 promoter, were obtained as previously described.³⁵ Colonies were maintained on a C57BL/6J background and backcrossed for at least 20 generations using PCR to establish genotypes. For the current study, we used male heterozygous APP23 and wild-type littermate mice group-housed in a conventional animal housing facility with a 12 hours/12 hours light/dark cycle (light on 8 AM), constant room temperature and humidity, and free access to food and water. The study

protocol was approved by the animal ethics committee of the University of Antwerp (reference number 2018-33) and was in compliance with the European Communities Council Directive on the protection of animals used for scientific purposes (2010/63/EU).

Materials

Dimethyl sulphoxide (DMSO) and the TDO inhibitor 680C91 were purchased from Sigma-Aldrich. Stock solutions of vehicle (VEH) and 680C91 (100 mM DMSO) were made and stored at -20°C for a maximum duration of 3 weeks. Directly prior to use, stock solutions were diluted in water (pH 3.2) with DMSO at a final concentration of 4.2%.

Experimental design

For the first part of the study focusing on HPLC/MS-MS analysis of kynurenine metabolites, untreated mice were sacrificed at 3 months (APP23 $n=4$; wild-type $n=5$), 6 months (APP23 $n=4$; wild-type $n=5$) and 12 months of age (APP23 $n=5$ and wild-type $n=5$).

For the second part of the study, 6-month-old wild-type and APP23 mice were treated with vehicle (VEH) or VEH + 680C91 (7.5 mg/kg, as previously described³¹) via oral gavage (7.5 ml/kg) for 6 days each week between 9:30 AM and 10:30 AM for a total period of 6 weeks (wild-type VEH $n=13$; wild-type VEH + 680C91 $n=12$; APP23 VEH $n=8$; APP23 VEH + 680C91 $n=8$). Behavioral assessment started in week 4 with the Morris water maze (MWM), followed by the novel object recognition (NOR) test. In week 5, the light/dark cycle was reversed (lights on 8 PM) and—after a 5-day adaptation period—the open field and elevated plus maze tests were performed on consecutive days. Mice were sacrificed 4 hours after the final treatment.

Behavioral experiments

Mice were allowed to adapt to the experimental room for at least 1 hour prior to the start of the experiments, which were performed by a single experimenter. All experiments were recorded and analyzed using a video-tracking system (Ethovision, Noldus, The Netherlands).

Morris water maze. The MWM was performed to assess hippocampus-dependent spatial learning and memory.³⁶ The setup consisted of a circular tank (diameter 150 cm, height 30 cm) filled with water that was opacified using non-toxic white paint and kept at 25°C . Invariable visual cues were placed around the pool. The MWM consisted of an acquisition phase and a probe trial. The acquisition phase was performed over the period of 4 days and consisted of 2 daily trial blocks (1 at 10:30 AM and 1 at 03:00 PM) of 4 trials with a 15-minute inter-trial interval. During the acquisition phase, a

round acrylic glass platform (diameter 15 cm) was placed 1 cm below the water surface on a fixed position in the center of one of the pool's quadrants. Mice were placed in the water facing the wall and were recorded while trying to find the hidden platform for a maximum duration of 120 seconds. The starting positions varied in a semi-random order. Mice that did not succeed to find the hidden platform within the given amount of time were guided to the platform where they had to remain for 10 seconds before being returned to their cage. The probe trial followed 4 days after the final acquisition trial. For this trial the platform was removed, mice were placed in the MWM at a fixed position and swimming trajectories were recorded during a period of 100 seconds. The total path length travelled to reach the platform, escape latency and swim speed were calculated for each trial block during the acquisition phase. The path length travelled to the target position (prior position of the platform) and the time spent in each quadrant (target, adjacent 1, adjacent 2 and opposite quadrant) and number of entries through target position were calculated for the probe trial.

Novel object recognition. The NOR test was performed to assess recognition memory and was performed during 4 consecutive days.³⁷ On the first 2 days of the protocol, mice were individually habituated to an empty arena (40 cm × 24 cm) during 10 minutes. On the third day (familiarization phase), 2 identical objects (brown-colored flasks) were placed 10 cm apart in the center of the arena and mice were allowed to freely explore the cage and objects for 5 minutes. On the fourth day (novel object phase) 1 object was replaced with a novel object (different color and shape, but similar in size). Mice were then placed in the arena and again allowed to explore for 5 minutes. Trajectories and nose-point locations were recorded. Exploration time was defined as the time during which the nose-point was directed toward 1 of the objects with a proximity of 3 cm. The recognition index (time spent exploring novel object divided by total time exploring both objects) was calculated as a measure of recognition.³⁸

Open field. Exploratory and anxiety-related behavior in the open field was measured for each mouse individually for 5 minutes during the dark phase of the animal's activity cycle in a brightly lit arena (50 cm × 50 cm). Mice always started from the same corner and were allowed 1 minutes of adaptation. Path length and location parameters, including the number of entries and time spent in the 7 cm × 7 cm corners and the center circle were recorded.

Elevated plus maze. The elevated plus maze was used to further assess anxiety-related behavior. The setup consisted of a cross-shaped maze with a central area giving access to 4 arms 30 cm in length and 5 cm in width. Two opposing arms were enclosed by 30 cm high walls, while the other opposing arms were not enclosed by walls. The maze was placed 60 cm above the floor.

Mice were placed in the central area facing the left enclosed arm. Trajectories were recorded during 5 minutes and the number of entries into the different arms and the time spent in the different arms were calculated.

HPLC/MS-MS analysis of kynurenine metabolites

Mice were anesthetized with a mixture of ketamine-xylazine (100 mg/kg and 20 mg/kg, respectively in a total volume of 10 ml/kg) administered intraperitoneally. Blood was drawn by retro-orbital puncture with a glass capillary and allowed to coagulate for at least 30 minutes at room temperature. Serum was separated by centrifuging at 1500 × *g* for 10 minutes and subsequently stored at -80°C. A thoracotomy was performed followed by whole body transcardial perfusion with PBS for 5 minutes. Brain regions (bilateral hippocampi, cortex and cerebellum) were dissected on ice and samples were immediately snap frozen in liquid nitrogen and stored at -80°C until further analysis. Samples were then homogenized in 1 ml ice-cold 0.1M acetic acid using bead homogenization at 4°C and centrifuged at 12000 *g* for 15 minutes. The supernatant was collected and stored at -80°C. Concentrations of Trp and Kyn pathway metabolites (Kyn, 3-Hk (3-hydroxykynurenine), AA (anthranilic acid), KA, XA (xanthurenic acid), QA, 3-HAA (3-hydroxyanthranilic acid), and PA (picolinic acid)) were measured using isotope dilution mass spectrometry as previously described.²²

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics 24 (IBM Corp, 2014) and JMP Pro 14. One- or 2-way ANOVAs were conducted to investigate the effect of age and genotype or treatment and genotype and their interaction on metabolite concentrations with Tukey post-hoc tests in the case of significant interaction. Planned contrasts were used to investigate whether metabolites followed a linear or quadratic trend with aging. Paired *t*-tests were used to investigate differences between the 2 days of novel object test and 2-way repeated measures ANOVA in combination with 2-way ANOVA was conducted to analyze Morris water maze data. The criterion α was set to .050 for all tests of significance.

Results

Peripheral kynurenine pathway activity is dysregulated in APP23 mice compared to wild-type but is similarly affected by aging

Age-related activation of the Kyn pathway—measured in blood—is common in humans and could contribute to Kyn pathway dysregulation in neurodegeneration. To characterize the effect of aging on Kyn pathway activity in mice in the context of neurodegeneration, we analyzed kynurenines in the serum of APP23 and wild-type mice at 3-, 6-, and 12-month-old (Figure 1b).

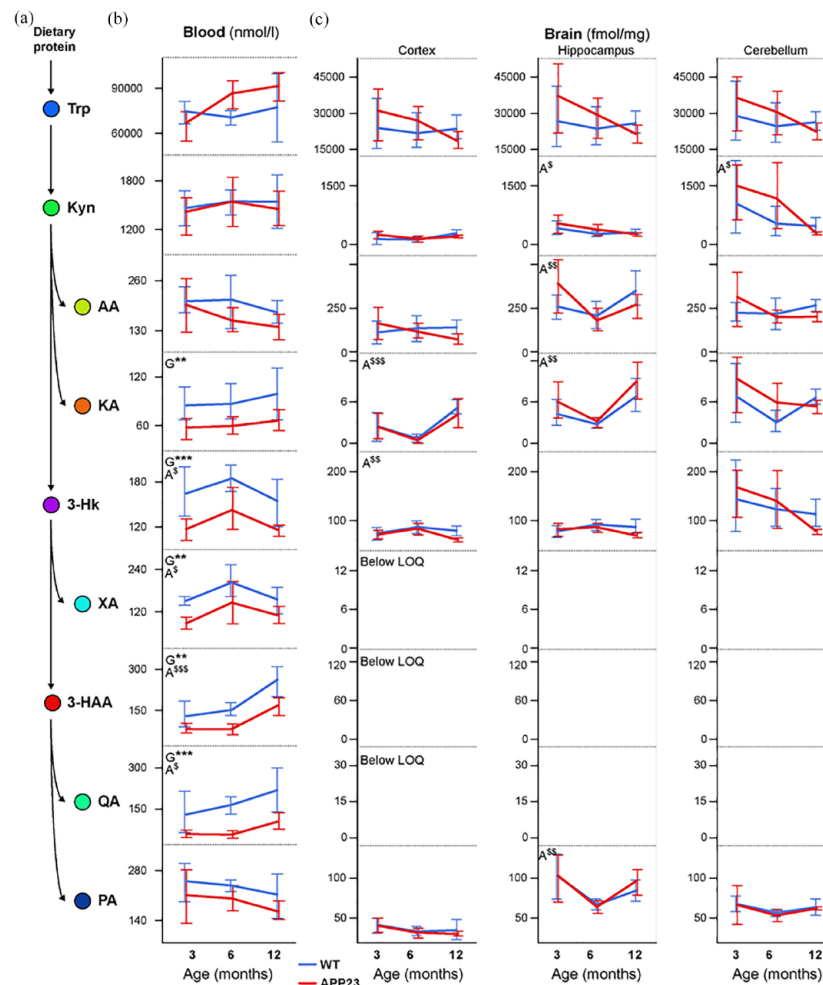


Figure 1. Reduction of kynurenine pathway metabolites in the blood in APP23 mice and genotype-independent kynurenine pathway alterations in blood and brain during ageing. (a) Simplified representation of Trp metabolism along the Kyn pathway. (b) Line plots showing median concentrations of metabolites in blood and (c) cortex, hippocampus and cerebellum from 3-, 6-, and 12-month-old wild-type (blue symbols) and APP23 (red symbols) mice ($n=4-5$). Error bars represent interquartile ranges. Significance of the main effect of genotype (G) or linear/quadratic effect of aging (A) in ANOVA are depicted using * and § respectively. */§ $P < .05$; **/§§ $P < .01$; ***/§§§ $P < .001$. Abbreviations: Trp, tryptophan; Kyn, kynurenine; AA, anthranilic acid; KA, kynurenic acid; 3-Hk, 3-hydroxykynurenine; XA, xanthurenic acid; 3-HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; PA, picolinic acid.

Our analyses showed that age did not interact with genotype to affect serum concentrations of Kyn metabolites. Regarding the effect of genotype, our analyses revealed reduced concentrations of KA ($F(1,28)=10.3$, $P=.004$), 3-Hk ($F(1,28)=17.3$, $P<.001$), XA ($F(1,27)=10.7$, $P=.004$), 3-HAA ($F(1,28)=14.5$, $P=.001$), and QA ($F(1,27)=22.3$, $P<.001$) in serum of APP23 mice compared to wild-type with differences ranging from 25% (KA) to 56% (QA).

Ageing was associated with changes in concentrations of XA ($F(2,27)=3.9$, $P=.037$), 3-HAA ($F(2,28)=15.1$, $P<.001$), and QA ($F(2,27)=3.8$, $P=.038$) in serum of wild-type and APP23 mice. Planned-contrast testing revealed differential trends between aging and these kynurenines with a quadratic trend peaking at 6 months for XA ($P=.012$), a trend with both a quadratic and linear component with a peak at 12 months for 3-HAA ($P<.001$ and $P=.037$, respectively) and a linear increase of QA ($P=.015$).

These results suggest dysregulated peripheral Kyn pathway activity in APP23 mice, which is characterized by reduced concentrations of several downstream Kyn metabolites. In addition, aging causes changes in peripheral Kyn pathway activity in mice that are similar in APP23 and wild-type.

Kynurenine pathway activity is not affected in the brain of APP23 mice and shows region-specific changes during aging

Because certain kynurenines can cross the blood-brain barrier, dysregulation of peripheral Kyn pathway activity can result in altered Kyn pathway activity in the brain.¹⁶ To analyze cerebral Kyn pathway activity, we analyzed Kyn metabolites in the cortex, hippocampus and cerebellum in 3-, 6-, and 12-month-old APP23 and wild-type mice (Figure 1c).

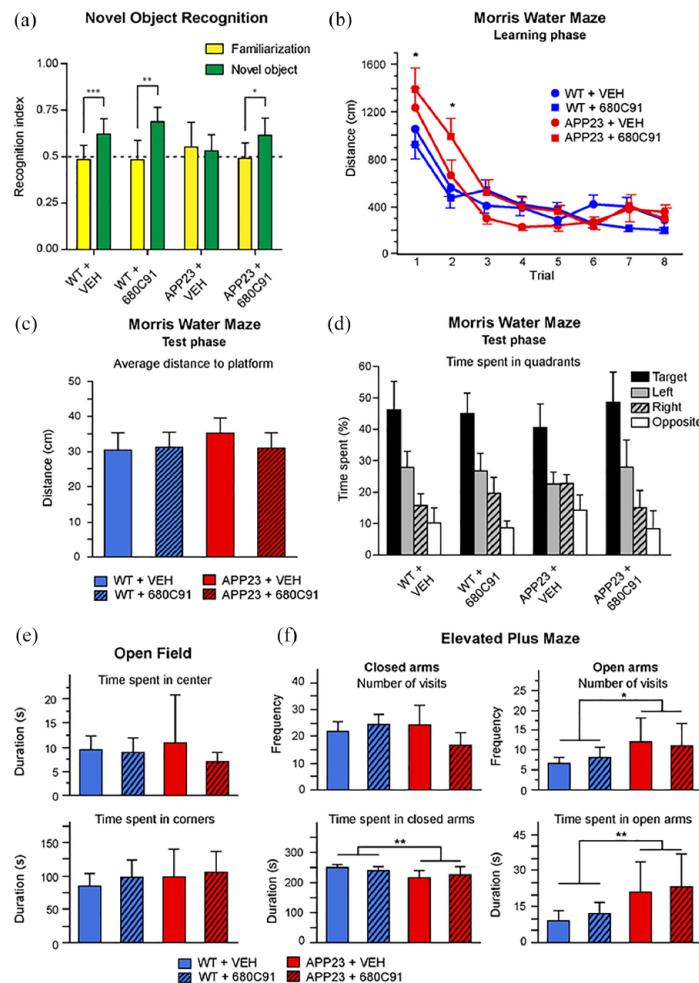


Figure 2. Inhibition of tryptophan 2,3-dioxygenase improves hippocampal-based recognition memory but does not influence measures of anxiety and spatial learning and memory in APP23 mice. (a) Graph showing recognition index during a 5-minute familiarization phase (time spent exploring familiar object 1/time spent exploring familiar objects 1 and 2) versus the recognition index during the novel object phase 24 hours later (time spent exploring novel object/time spent exploring both objects). Results from paired-sample *t* tests are shown. * $P < .05$; ** $P < .01$; *** $P < .001$. (b) Graph displaying the mean and standard errors of the total distance travelled during 8 consequent trials during the learning phase of the Morris water maze. Results from main effect of genotype by 2-way ANOVA are depicted. * $P < .05$. (c) Graph showing the average distance to the platform location during the Morris water maze test phase (probe trial). (d) Showing the percentage time spent in the different quadrants during the Morris water maze test phase (probe trial). (e) Line bars showing mean and 95% CI of the total time spent in the center and corners during the open field test paradigm in mice treated with vehicle (DMSO) or TDO inhibitor (680C91) through oral gavage ($n = 8-13$). (f) Line bars showing mean and 95% CI of the visiting frequency and the duration spent in the closed and open arms of the elevated plus maze. Results from 2-way ANOVA are depicted. * $P < .05$; ** $P < .01$.

Similar to our results in serum, interaction analyses indicated no interaction between age and genotype for Kyn metabolite concentrations in cortex, hippocampus or cerebellum. In addition, no differences in the concentrations of kynurenines were noted in any of the investigated brain regions between APP23 and wild-type mice.

Regarding the effect of age, our analyses showed age-related trends of Kyn pathway changes in the cortex, hippocampus, and cerebellum. Hippocampal Kyn pathway activity varied most strongly during aging with changes in levels of Kyn ($F(2,28) = 4.24$, $P = .028$), AA ($F(2,28) = 4.53$, $P = .023$), KA ($F(2,24) = 7.37$, $P = .005$), and PA ($F(2,24) = 7.37$, $P = .005$). Planned-contrast testing indicated a

linear decrease of Kyn ($P = .011$), quadratic trends with low concentrations at 6 months for AA and PA ($P = .007$ and $P = .001$, respectively) and a trend with both a quadratic and linear component peaking at 12 months for KA ($P = .034$ and $P = .009$, respectively). In the cortex, aging was associated with alterations of KA ($F(2,27) = 12.3$, $P < .001$) and 3-Hk ($F(2,28) = 4.3$, $P = .027$), which were both characterized by a quadratic trend ($P = .009$ and $P = .001$, respectively). In the cerebellum aging was associated with changes in Kyn ($F(2,28) = 3.79$, $P = .039$) with a linear decrease during aging ($P = .012$).

Finally, correlation analyses were performed to investigate the association between peripheral and central levels of Kyn

pathway metabolites. The results of these analyses were inconclusive due to small groups sizes (data not shown).

Taken together, these data provide evidence of Kyn pathway alterations during aging in the brain of APP23 and wild-type mice, which are most evident in the hippocampus. Surprisingly, although several kynurenines were reduced in the serum of APP23 mice, this was not paralleled by changes in cerebral metabolite concentrations.

Long-term TDO inhibition specifically improves recognition memory in APP23 mice

Studies in *C. elegans* and *Drosophila melanogaster* have shown that TDO inhibition can reduce A β toxicity.^{33,34} In addition, it was shown that oral administration of the TDO inhibitor 680C91 improved recognition memory in a mouse model of AD mice.³¹ To further establish the role of TDO in memory function in the context of AD, we studied the effect of long-term oral administration of 680C91 on learning and memory in 6-month old APP23 and wild-type mice.

To establish whether TDO inhibition could similarly improve recognition memory in APP23 mice, we conducted the NOR test. During the familiarization phase, all groups of mice spent an equal amount of time exploring both objects (data not shown). During the test phase, wild-type mice showed interest in the novel object which was not affected by treatment ($P < .001$ for vehicle and $P = .002$ for 680C91 for paired t -test). However, vehicle-treated APP23 mice showed impaired novel object recognition ($P = .787$ for paired t -test) which could be restored by 680C91 treatment ($P = .013$) (Figure 2a).

Next, to analyze whether TDO inhibition could also improve spatial learning and memory, APP23 and wild-type mice were trained during 8 trial blocks in the MWM. Repeated measures ANOVA indicated that mice were able to find the platform more easily with each consecutive trial block ($F(7,259) = 54.9$, $P < .001$ for the distance to the platform and $F(7,259) = 49.5$, $P < .001$ for escape latency) (Figure 2b). Although APP23 mice required a longer distance and time to find the platform during the first 2 trial blocks, all groups of mice performed similarly during the final trial blocks, suggesting no learning impairment in APP23 mice in this setup-up and no effect of 680C91 treatment. Swim speed was similar between groups during all trial blocks (ranging from 0.19 to 0.20 m/second between groups). A probe trial was conducted 4 days after the final trial block to assess spatial memory. The average distance to the specific target location during the probe trial was similar between the groups (Figure 2c). However, following up on a significant treatment-by-genotype effect ($F(1,40) = 8.0$, $P = .007$), post-hoc analyses indicated that APP23 mice treated with 680C91 spent less time in the quadrant on the right side of the target quadrant compared to wild-type mice ($P = .020$), suggesting a subtle difference in the search

patterns of these mice (Figure 2d). The number of entries through the target position (ranging from 4.8 to 5.4) was not affected by genotype ($F(1,40) < .1$, $P = .838$) or treatment ($F(1,40) < .15$, $P = .697$).

Taken together, impaired recognition memory in APP23 mice could be rescued by long-term oral administration of the TDO inhibitor 680C91 while spatial learning and memory were not impaired in APP23 mice and were not affected by 680C91 treatment in the current experimental set-up. These data suggest a role for TDO in specific types of memory in APP23 mice.

Long-term TDO inhibition does not affect anxiety in APP23 mice

Genetic inhibition of TDO was found to reduce anxiety-related behavior in mice.^{29,39} As anxiety could influence the performance of APP23 mice during memory testing, we next investigated the effect of long-term TDO inhibition on anxiety-related behavior in APP23 and wild-type mice.

Results from the open field test, which was performed to analyze general exploratory behavior, indicated that VEH- and 680C91-treated APP23 and wild-type mice spent a similar amount of time in the center circle and corners of the open field set-up (Figure 2e).

Next, to more specifically address anxiety-related behavior mice were tested in the elevated plus maze (Figure 2f). Following up on a significant treatment-by-genotype interaction effect ($F(1,40) = 5.9$, $P = .020$), post-hoc analyses indicated a trend toward reduced closed arm visits after 680C91 treatment in APP23 mice ($P = .060$), which was not found in wild-type mice ($P = .739$). Further analyses indicated that, irrespective of treatment, APP23 mice spent less time in the closed arms ($F(1,40) = 9.7$, $P = .004$) and more time in the open arms of the maze ($F(1,40) = 10.3$, $P = .003$), while also visiting the open arms more frequently than wild-type mice ($F(1,40) = 6.8$, $P = .013$).

Taken together, this data suggests that APP23 mice show reduced anxiety-related behavior which is not affected by long-term oral treatment with a TDO inhibitor.

Long-term TDO inhibition has a minor influence on kynurenines in serum of APP23 mice and does not affect brain kynurenines

Finally, to investigate whether TDO inhibition could have improved recognition memory in APP23 mice by modulating the Kyn pathway, we analyzed Kyn metabolites in serum and brain tissue of the VEH- and 680C91-treated APP23 and wild-type mice that had undergone behavioral tests.

The analyses indicated increased serum concentrations of Kyn in APP23 mice (genotype-effect: $F(1,40) = 10.3$, $P = .003$) (Figure 3a) while a trend was observed toward reduced Kyn in serum after treatment with 680C91

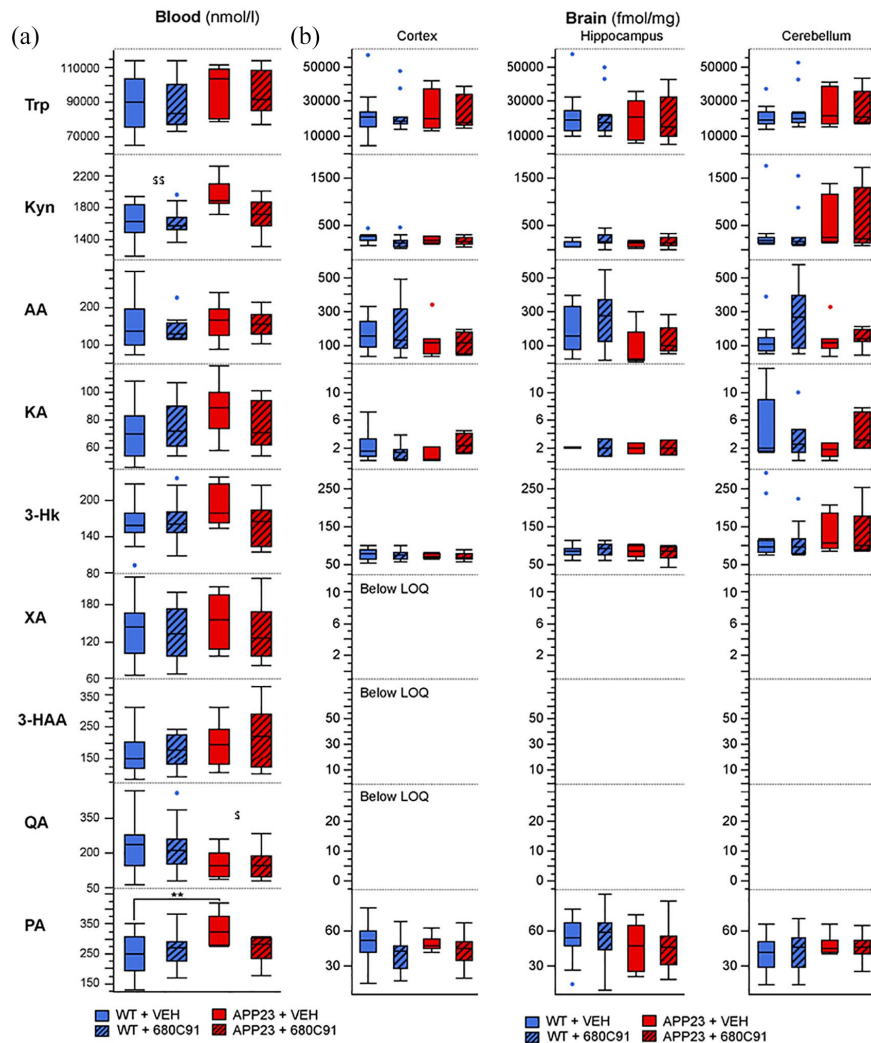


Figure 3. Inhibition of tryptophan 2,3-dioxygenase has minor effects on kynurenine metabolites in blood and does not affect kynurenine metabolites in the brains of APP23 mice. (a) Boxplots showing distributions of Kyn pathway metabolites in blood and (b) cortex, hippocampus and cerebellum from 6-month-old wildtype and APP23 mice after a 6-week treatment with vehicle (DMSO) or vehicle + 680C91 through oral gavage ($n=8-13$). Outliers are shown as red/blue dots. Significance of the main effect of genotype in two-way ANOVA is depicted with $^sP < .05$ or $^{s\$}P < .01$. Significance of Tukey post-hoc tests (in case of significant interaction) are depicted with $^*P < .05$ or $^{**}P < .01$. Abbreviations: Trp, tryptophan; Kyn, kynurenine; AA, anthranilic acid; KA, kynurenic acid; 3-Hk, 3-hydroxykynurenine; XA, xanthurenic acid; 3-HAA, 3-hydroxyanthranilic acid; QA, quinolinic acid; PA, picolinic acid.

(treatment-effect: $F(1,40) = 4.0$, $P = .052$). Concentrations of QA were reduced in APP23 mice ($F(1,40) = 5.2$, $P = .029$) and, following up on a significant genotype-by-treatment interaction effect ($F(1,40) = 4.67$, $P = .037$), post-hoc analyses indicated increased PA concentrations in VEH-treated APP23 mice compared to VEH-treated wild-type mice ($P = .024$), whereas concentrations did not differ between 680C91-treated APP23 or wild-type mice and VEH-treated wild-type mice ($P = .914$ and $P = .842$). This data suggests that long-term oral treatment with 680C91 specifically lowers PA concentrations while possibly reducing the increased Kyn concentrations in serum of APP23 mice.

680C91 treatment did not influence the concentrations of Kyn metabolites in the cortex, hippocampus or cerebellum (Figure 3b). In addition, there were no differences between APP23 and wild-type mice and no interaction effects.

Taken together, these results show that long-term treatment with the TDO inhibitor 680C91 has minor effects on Kyn metabolite profiles in blood of APP23 and wild-type mice and does not affect Kyn metabolite levels in the cortex, hippocampus and cerebellum.

Discussion

The Kyn pathway has been frequently linked to AD because of its involvement in aging, inflammation and neurotoxicity.⁶ Several studies have indicated that the Kyn pathway could provide biomarkers for AD^{18-20,22,40} and that Kyn pathway enzymes, including TDO, could potentially be targeted to prevent or delay amyloid-beta-induced cellular toxicity.^{33,34}

In accordance with studies in AD patients,^{18-20,22,41} we found that multiple Kyn metabolites downstream of Kyn were

reduced in serum of APP23 mice. The mechanisms underlying these changes are unknown, but could include increased metabolism or disturbances in the circadian rhythm—which have both been previously observed in APP23 mice.^{42,43} Such disturbances could affect Trp metabolism that shows a circadian pattern.^{44–47} As alterations in the circadian rhythm are common in AD patients and are closely related to metabolic dysfunction in AD pathology,⁴⁸ we speculate that reduced downstream Kyn pathway activity in AD patients or APP23 mice might reflect a state of metabolic and/or circadian dysregulation.

To our knowledge, this is the first report on the effect of aging on kynurenes in the blood in mice. In line with studies in humans,^{18,22,41} we found that QA accumulates during aging in blood in mice. Aging was recently shown to be associated with impaired metabolism of QA toward NAD⁺ in human macrophages, which significantly impacted their immune function.⁴⁹ Similarly, hampered QA-dependent de novo synthesis of NAD⁺ led to dysregulated cellular homeostasis in mouse models of hepatic and renal damage.^{50,51} We speculate that QA accumulation in the blood could represent a cross-species phenotype of aging indicative of maladaptive cellular responses to age-related damage. Differences between mice and men with regard to age-related changes include a lack of increase of serum Kyn in mice.^{17,52–58} This could be attributed to species-specific activity of Kyn pathway enzymes.⁵⁹

Our data indicated no alterations of Kyn metabolites in brain tissue of APP23 mice. This is in contrast with previous work on the Kyn pathway in AD mice^{27,32} and could result from the use of different AD mouse models and the extent of brain pathology. In line with evidence in AD patients,⁴¹ one study showed reduced KA concentrations in the cortex of 7-month-old J20 mice.²⁷ In contrast to APP23, expression of mutated APP is driven by a promoter that is expressed during embryonic development.⁶⁰ This could alter embryonic Kyn pathway activity and modulate KA concentrations in the brain in later life.⁶¹ Another study analyzed Kyn pathway activity in the brains of 3-, 6-, and 12-month-old triple transgenic mice (3xTg-AD)³² that overexpress mutant APP and MAPT (encoding tau protein) on a presenilin-1 (PS1) knock-in background.⁶² They demonstrated increased QA levels in the hippocampus of transgenic mice that progressed with aging. As we were unable to detect QA in brain samples, a similar increase of QA levels in our model seems unlikely. As QA is mainly produced by microglia in the brain,^{13,63} these results could be interpreted in line with evidence of age-dependent microglial activation in the 3xTg-AD model.⁶⁴ However, as QA concentrations are not increased in post-mortem brain tissue of AD patients,^{65–67} Kyn pathway disturbances in the 3xTg-AD model might not be related to AD pathology. The above considerations should be taken into account when studying the Kyn pathway in AD mouse models.

In line with studies showing that TDO inhibition can improve AD-related behavioral deficits,^{31,39} we demonstrated

that long-term oral administration of the TDO inhibitor 680C91 restored recognition memory deficits in APP23 mice. Several explanations could lie at the basis of this observation. Some of the Kyn pathway metabolites can cross the blood–brain barrier such as Trp, Kyn, 3-Hk, AA which are actively transported by the large neutral amino acid carrier system; and others via passive diffusion.^{16,68} As TDO is primarily expressed in the liver, TDO inhibition could change the cerebral uptake of Kyn metabolites by altering the composition of Kyn metabolites in the blood. However, our results gave no indication of clear changes of Kyn pathway activity, neither in the blood nor in the cortex, hippocampus, and cerebellum. This suggests that the effect of 680C91 on recognition memory is most likely not the result of peripheral TDO inhibition. Treatment with 680C91 was previously shown to inhibit cerebral TDO activity.⁶⁹ However, and although 680C91 affected Kyn levels in the serum, we did not observe a treatment effect on Kyn pathway activity in the brains of AD mice. We, therefore, speculate that TDO might have a role in brain physiology that is independent of its enzymatic function. Indeed, TDO inhibition reduced A β toxicity independent of Kyn metabolites in *C. elegans*.³³ Alternatively, as TDO expression is highly restricted to subregions in the brain, TDO inhibition could impact cellular function without causing measurable changes in metabolite concentrations at a regional level. Of interest in this regard, is the fact that TDO seems to be integrated in damage response mechanisms in the brain that are regulated by stress signals such as glucocorticoids and prostaglandins^{8,70,71} which might be activated by A β .³¹ Finally, 680C91 could elevate a global measure of brain extracellular (CSF) serotonin concentration⁷² and thereby influence cognitive function. To establish whether TDO inhibition indeed offers therapeutic potential in AD, mechanistic studies should address how TDO activation impacts neuronal functioning and how this might influence AD-related cognitive dysfunction.

We provided an extensive analysis of kynurenes in serum and brain tissue during aging in a mouse model of AD and a characterization of the effects of pharmacological TDO inhibition on the Kyn pathway and cognitive function. However, our results should be discussed in light of the study limitations. First of all, we observed differences in Kyn profiles between untreated and treated groups of mice (see Figures 1 and 2). These differences are possibly explained by differences in the time of the day at which mice were sacrificed. Secondly, contrary to previous reports from our group,⁷³ spatial learning and memory were not impaired in 6-month-old APP23 mice as assessed in the MWM. However, in the current study, mice were handled on a daily basis by a single experimenter during 3 weeks prior to the start of behavioral tests. Although chronic stress protocols can hamper mouse performance in the MWM,⁷⁴ daily handling of mice, and habituation to the experimenter can reduce stress-related behavior and improve cognitive function.^{75,76} The development of novel TDO inhibitors

that show higher solubility and stability will allow for the use of treatment routes such as food pellets or osmotic pumps that reduce such experimental biases.

In conclusion, this study revealed age-related and genotype-specific changes of the Kyn pathway in the APP23 mouse model of AD. As these changes were partially in accordance with studies in humans, the APP23 mouse model might be used to study the role of the Kyn pathway in AD. Furthermore, we demonstrated that long-term inhibition of TDO improved hippocampal-based recognition memory in APP23 mice without majorly influencing central measures of Kyn pathway activity. These data suggest that Kyn pathway activation could be a cross-species aging phenotype and warrants further investigation of the role of TDO in AD pathophysiology.

Acknowledgements

The authors would like to thank Jana Janssens, Jan van Erum, Elke Calus, and Tinne Koninckx for assisting in the breeding and sacrificing of the mice used in this study. This work was supported by the Research Foundation-Flanders (FWO), Special Research Fund UAntwerpen (Bijzonder Onderzoeksfonds, BOF, # 34423), agreement between Institute Born-Bunge and University of Antwerp, the Alzheimer Research Center Groningen (UMCG, The Netherlands), the Medical Research Foundation Antwerp, the Thomas Riellaerts research fund, and Neurosearch Antwerp.

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REFERENCES

- Prince M, Wimo A, Guerchet M, Gemma-Claire A, Wu Y-T, Prina M. World Alzheimer Report 2015: The Global Impact of Dementia - an analysis of prevalence, incidence, cost and trends. *Alzheimer's Dis Int.* 2015;84.
- Parsons MP, Raymond LA. Extrasynaptic NMDA receptor involvement in central nervous system disorders. *Neuron.* 2014;82(2):279-293. doi:10.1016/j.neuron.2014.03.030
- Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell.* 2010;140(6):918-934. doi:10.1016/j.cell.2010.02.016
- Jha SK, Jha NK, Kumar D, Ambasta RK, Kumar P. Linking mitochondrial dysfunction, metabolic syndrome and stress signaling in Neurodegeneration. *Biochim Biophys Acta - Mol Basis Dis.* 2017;1863(5):1132-1146. doi:10.1016/j.bbadis.2016.06.015
- Lovelace MD, Varney B, Sundaram G, et al. Recent evidence for an expanded role of the kynurenine pathway of tryptophan metabolism in neurological diseases. *Neuropharmacology.* 2017;112:373-388. doi:10.1016/j.neuropharm.2016.03.024
- Maddison DC, Giorgini F. The kynurenine pathway and neurodegenerative disease. *Semin Cell Dev Biol.* 2015;40:134-141. doi:10.1016/j.semdb.2015.03.002
- Liu L, Su X, Quinn WJ, et al. Quantitative analysis of NAD synthesis-breakdown fluxes. *Cell Metab.* 2018;27(5):1067-1080.e5. doi:10.1016/j.cmet.2018.03.018
- Dostal CR, Gamsby NS, Lawson MA, McCusker RH. Glia- and tissue-specific changes in the Kynurenine Pathway after treatment of mice with lipopolysaccharide and dexamethasone. *Brain Behav Immun.* 2018;69:321-335. doi:10.1016/j.bbi.2017.12.006
- Larkin PB, Sathyasaikumar KV, Notarangelo FM, et al. Tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase 1 make separate, tissue-specific contributions to basal and inflammation-induced kynurenine pathway metabolism in mice. *Biochim Biophys Acta - Gen Subj.* 2016;1860(11):2345-2354. doi:10.1016/j.bbagen.2016.07.002
- Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: tryptophan's metabolites in exercise, inflammation, and mental health. *Science.* 2017;357(6349):eaaf9794. doi:10.1126/science.aaf9794
- Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci.* 2012;13:465-477. doi:10.1038/nrn3257
- Guillemin GJ, Smythe G, Takikawa O, Brew BJ. Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. *Glia.* 2005;49(1):15-23. doi:10.1002/glia.20090
- Heyes MP, Achim CL, Wiley CA, Major EO, Saito K, Markey SP. Human microglia convert l-tryptophan into the neurotoxin quinolinic acid. *Biochem J.* 1996;320(Pt 2):595-597. doi:10.1042/bj3200595
- Guillemin GJ, Kerr SJ, Smythe GA, et al. Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection. *J Neurochem.* 2001;78(4):842-853. doi:10.1046/j.1471-4159.2001.00498.x
- Kiss C, Ceresoli-Borroni G, Guidetti P, Zielke CL, Zielke HR, Schwarcz R. Kynurenate production by cultured human astrocytes. *J Neural Transm.* 2003;110(1):1-14. doi:10.1007/s00702-002-0770-z
- Fukui S, Schwarcz R, Rapoport SI, Takada Y, Smith QR. Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. *J Neurochem.* 1991;56(6):2007-2017. doi:10.1111/j.1471-4159.1991.tb03460.x
- Theofylaktopoulos D, Middttun Ø, Ulvik A, et al. A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland Health Study. *Clin Exp Immunol.* 2013;173(1):121-130. doi:10.1111/cei.12092
- Giil LM, Middttun O, Refsum H, et al. Kynurenine pathway metabolites in Alzheimer's disease. *J Alzheimer's Dis.* 2017;60(2):495-504. doi:10.3233/JAD-170485
- Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenine and its metabolites in Alzheimer's disease patients. *Adv Med Sci.* 2010;55(2):204-211. doi:10.2478/v10039-010-0023-6
- Hartai Z, Juhász A, Rimanóczy Á, et al. Decreased serum and red blood cell kynurenine acid levels in Alzheimer's disease. *Neurochem Int.* 2007;50(2):308-313. doi:10.1016/j.neuint.2006.08.012
- Widner B, Leblhuber F, Walli J, Tilz GP, Demel U, Fuchs D. Tryptophan degradation and immune activation in Alzheimer's disease. *J Neural Transm.* 2000;107(3):343-353. doi:10.1007/s007020050029
- Sorgdrager FJH, Vermeiren Y, Van Faassen M, et al. Age- and disease-specific changes of the kynurenine pathway in Parkinson's and Alzheimer's disease. *J Neurochem.* 2019. doi:10.1111/jnc.14843
- Chatterjee P, Zetterberg H, Goozee K, et al. Plasma neurofilament light chain and amyloid- β are associated with the kynurenine pathway metabolites in pre-clinical Alzheimer's disease. *J Neuroinflammation.* 2019;16:186. doi:10.1186/s12974-019-1567-4
- Jacobs KR, Lim CK, Blennow K, et al. Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid- β and tau. *Neurobiol Aging.* 2019;80:11-20. doi:10.1016/j.neurobiolaging.2019.03.015
- Chatterjee P, Goozee K, Lim CK, et al. Alterations in serum kynurenine pathway metabolites in individuals with high neocortical amyloid- β load: a pilot study. *Sci Rep.* 2018;8:8008. doi:10.1038/s41598-018-25968-7
- Zádori D, Veres G, Szalárdy L, Klivényi P, Vécsei L. Alzheimer's disease: recent concepts on the relation of mitochondrial disturbances, excitotoxicity, neuroinflammation, and kynurenines. *J Alzheimer's Dis.* 2018;62(2):523-547. doi:10.3233/JAD-170929
- Zwilling D, Huang SY, Sathyasaikumar KV, et al. Kynurenine 3-monoxygenase inhibition in blood ameliorates neurodegeneration. *Cell.* 2011;145(6):863-874. doi:10.1016/j.cell.2011.05.020
- Ohira K, Hagihara H, Toyama K, et al. Expression of tryptophan 2,3-dioxygenase in mature granule cells of the adult mouse dentate gyrus. *Mol Brain.* 2010;3(1):1-14. doi:10.1186/1756-6606-3-26
- Kanai M, Funakoshi H, Takahashi H, et al. Tryptophan 2,3-dioxygenase is a key modulator of physiological neurogenesis and anxiety-related behavior in mice. *Mol Brain.* 2009;2:8. doi:10.1186/1756-6606-2-8
- Lazarov O, Hollands C. Hippocampal neurogenesis: learning to remember. *Prog Neurobiol.* 2016;138-140:1-18. doi:10.1016/j.pneurobio.2015.12.006
- Woodling NS, Colas D, Wang Q, et al. Cyclooxygenase inhibition targets neurons to prevent early behavioural decline in Alzheimer's disease model mice. *Brain.* 2016;139(7):2063-2081. doi:10.1093/brain/aww117
- Wu W, Nicolazzo JA, Wen L, et al. Expression of tryptophan 2,3-dioxygenase and production of kynurenine pathway metabolites in triple transgenic mice and human Alzheimer's disease brain. *PLoS One.* 2013;8(4):e59749. doi:10.1371/journal.pone.0059749
- van der Goot AT, Zhu W, Vazquez-Manrique RP, et al. Delaying aging and the aging-associated decline in protein homeostasis by inhibition of tryptophan

- degradation. *Proc Natl Acad Sci.* 2012;109(37):14912-14917. doi:10.1073/pnas.1203083109
34. Breda C, Sathyasaikumar KV, Sograte Idrissi S, et al. Tryptophan-2,3-dioxygenase (TDO) inhibition ameliorates neurodegeneration by modulation of kynurenine pathway metabolites. *Proc Natl Acad Sci.* 2016;113(19):5435-5440. doi:10.1073/pnas.1604453113
 35. Sturchler-Pierrat C, Abramowski D, Duke M, et al. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci.* 1997;94:13287-13292.
 36. D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev.* 2001;36(1):60-90. doi:10.1016/S0165-0173(01)00067-4
 37. Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats: 1. Behavioral data. *Behav Brain Res.* 1988;31(1):47-59. doi:10.1016/0166-4328(88)90157-X
 38. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process.* 2012;13(2):93-110. doi:10.1007/s10339-011-0430-z
 39. Too LK, Li KM, Suarna C, et al. Deletion of TDO2, IDO-1 and IDO-2 differentially affects mouse behavior and cognitive function. *Behav Brain Res.* 2016;312:102-117. doi:10.1016/j.bbr.2016.06.018
 40. Widner B, Leblhuber F, Walli J, Tiltz GP, Demel U, Fuchs D. Tryptophan degradation and immune activation in Alzheimer's disease. *J Neural Transm.* 2000;107(3):343-353. doi:10.1007/s007020050029
 41. Heyes MP, Saito K, Crowley JS, et al. Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain.* 1992;115(5):1249-1273. doi:10.1093/brain/115.5.1249
 42. Vloeberghs E, Van Dam D, Engelborghs S, Nagels G, Staufenbiel M, De Deyn PP. Altered circadian locomotor activity in APP23 mice: a model for BPSD disturbances. *Eur J Neurosci.* 2004;20(10):2757-2766. doi:10.1111/j.1460-9568.2004.03755.x
 43. Vloeberghs E, Van Dam D, Franck F, et al. Altered ingestive behavior, weight changes, and intact olfactory sense in an APP overexpression model. *Behav Neurosci.* 2008;122(3):491-497. doi:10.1037/0735-7044.122.3.491
 44. Rapoport MI, Beisel WR. Circadian periodicity of tryptophan metabolism. *J Clin Invest.* 1968;47(4):934-939. doi:10.1172/JCI105785
 45. Coggan SE, Smythe GA, Bilgin A, Grant RS. Age and circadian influences on picolinic acid concentrations in human cerebrospinal fluid. *J Neurochem.* 2009;108(5):1220-1225. doi:10.1111/j.1471-4159.2009.05868.x
 46. Carpenter LL, Anderson GM, Pelton GH, et al. Tryptophan depletion during continuous CSF sampling in healthy human subjects. *Neuropsychopharmacology.* 1998;19(1):26-35. doi:10.1016/S0893-133X(97)00198-X
 47. Kennedy JS, Gwirtsman HE, Schmidt DE, et al. Serial cerebrospinal fluid tryptophan and 5-hydroxy indoleacetic acid concentrations in healthy human subjects. *Life Sci.* 2002;71(14):1703-1715. doi:10.1016/S0024-3205(02)01899-4
 48. Mattis J, Sehgal A. Circadian rhythms, sleep, and disorders of aging. *Trends Endocrinol Metab.* 2016;27(4):192-203. doi:10.1016/j.tem.2016.02.003
 49. Minhas PS, Liu L, Moon PK, et al. Macrophage de novo NAD⁺ synthesis specifies immune function in aging and inflammation. *Nat Immunol.* 2019;20:50-63. doi:10.1038/s41590-018-0255-3
 50. Poyan Mehr A, Tran MT, Ralto KM, et al. De novo NAD⁺ biosynthetic impairment in acute kidney injury in humans. *Nat Med.* 2018;24(9):1351-1359. doi:10.1038/s41591-018-0138-z
 51. Katsyuba E, Mottis A, Zietak M, et al. De novo NAD⁺ synthesis enhances mitochondrial function and improves health. *Nature.* 2018;563(7731):354-359. doi:10.1038/s41586-018-0645-6
 52. Rist MJ, Roth A, Frommherz L, et al. Metabolite patterns predicting sex and age in participants of the Karlsruhe metabolomics and nutrition (KarMeN) study. *PLoS One.* 2017;12(8):1-21. doi:10.1371/journal.pone.0183228
 53. Collino S, Montoliu I, Martin F-PJ, et al. Metabolic signatures of extreme longevity in northern Italian centenarians reveal a complex remodeling of lipids, amino acids, and gut microbiota metabolism. *PLoS One.* 2013;8(3):e56564. doi:10.1371/journal.pone.0056564
 54. Yu Z, Zhai G, Singmann P, et al. Human serum metabolic profiles are age dependent. *Aging Cell.* 2012;11(6):960-967. doi:10.1111/j.1474-9726.2012.00865.x
 55. Capuron L, Schroecksnadel S, Féart C, et al. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms. *Biol Psychiatry.* 2011;70(2):175-182. doi:10.1016/j.biopsych.2010.12.006
 56. Niinistö P, Raitala A, Pertovaara M, et al. Indoleamine 2,3-dioxygenase activity associates with cardiovascular risk factors: the Health 2000 study. *Scand J Clin Lab Invest.* 2008;68(8):767-770. doi:10.1080/00365510802245685
 57. Pertovaara M, Raitala A, Lehtimäki T, et al. Indoleamine 2,3-dioxygenase activity in nonagenarians is markedly increased and predicts mortality. *Mech Ageing Dev.* 2006;127(5):497-499. doi:10.1016/J.MAD.2006.01.020
 58. Frick B, Schroecksnadel K, Neuraeter G, Leblhuber F, Fuchs D. Increasing production of homocysteine and neopterin and degradation of tryptophan with older age. *Clin Biochem.* 2004;37(8):684-687. doi:10.1016/J.CLINBIOCHEM.2004.02.007
 59. Fujigaki S, Saito K, Takemura M, et al. Species differences in L-tryptophan-kynurenine pathway metabolism: quantification of anthranilic acid and its related enzymes. *Arch Biochem Biophys.* 1998;358(2):329-335. doi:10.1006/abbi.1998.0861
 60. Mucke L, Masliah E, Yu GQ, et al. High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J Neurosci.* 2000;20(11):4050-4058. http://www.ncbi.nlm.nih.gov/pubmed/10818140
 61. Fushing ML, Bortz DM, Pociavsek A, et al. Elevated levels of kynurenic acid during gestation produce neurochemical, morphological, and cognitive deficits in adulthood: implications for schizophrenia. *Neuropharmacology.* 2015;90:33-41. doi:10.1016/j.neuropharm.2014.10.017
 62. Oddo S, Caccamo A, Shepherd JD, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular A β and synaptic dysfunction. *Neuron.* 2003;39(3):409-421. doi:10.1016/S0896-6273(03)00434-3
 63. Guillemain GJ, Smith DG, Smythe GA, Armati PJ, Brew BJ. Expression of the kynurenine pathway enzymes in human microglia and macrophages. *Adv Exp Med Biol.* 2003;527:105-112. Accessed January 9, 2019. http://www.ncbi.nlm.nih.gov/pubmed/15206722
 64. Belfiore R, Rodin A, Ferreira E, et al. Temporal and regional progression of Alzheimer's disease-like pathology in 3xTg-AD mice. *Aging Cell.* 2019;18(1):e12873. doi:10.1111/acel.12873
 65. Moroni F, Lombardi G, Robitaille Y, Etienne P. Senile dementia and Alzheimer's disease: lack of changes of the cortical content of quinolinic acid. *Neurobiol Aging.* 1986;7(4):249-253. doi:10.1016/0197-4580(86)90003-5
 66. Mouradian MM, Heyes MP, Pan JB, Heuser IJE, Markey SP, Chase TN. No changes in central quinolinic acid levels in Alzheimer's disease. *Neurosci Lett.* 1989;105(1-2):233-238. doi:10.1016/0304-3940(89)90043-8
 67. Sofic E, Halket J, Przyborowska A, et al. Brain quinolinic acid in Alzheimer's dementia. *Eur Arch Psychiatry Neurol Sci.* 1989;239(3):177-179. doi:10.1007/BF01739651
 68. Ruddick JP, Evans AK, Nutt DJ, Lightman SL, Rook GAW, Lowry CA. Tryptophan metabolism in the central nervous system: medical implications. *Expert Rev Mol Med.* 2006;8(20):1-27. doi:10.1017/S1462399406000068
 69. Cuartero MI, Ballesteros I, de la Parra J, et al. L-kynurenine/aryl hydrocarbon receptor pathway mediates brain damage after experimental stroke. *Circulation.* 2014;130(23):2040-2051. doi:10.1161/CIRCULATIONAHA.114.011394
 70. Brooks AK, Lawson MA, Smith RA, Janda TM, Kelley KW, McCusker RH. Interactions between inflammatory mediators and corticosteroids regulate transcription of genes within the kynurenine pathway in the mouse hippocampus. *J Neuroinflammation.* 2016;13(1):98. doi:10.1186/s12974-016-0563-1
 71. Ochs K, Ott M, Rauschenbach KJ, et al. Tryptophan-2,3-dioxygenase is regulated by prostaglandin E2 in malignant glioma via a positive signaling loop involving prostaglandin E receptor-4. *J Neurochem.* 2016;136(6):1142-1154. doi:10.1111/jnc.13503
 72. Salter M, Hazelwood R, Pogson CI, Iyer R, Madge DJ. The effects of a novel and selective inhibitor of tryptophan 2,3-dioxygenase on tryptophan and serotonin metabolism in the rat. *Biochem Pharmacol.* 1995;49(10):1435-1442. http://www.ncbi.nlm.nih.gov/pubmed/7539265.
 73. Van Dam D, D'Hooge R, Staufenbiel M, Van Ginneken C, Van Meir F, De Deyn PP. Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. *Eur J Neurosci.* 2003;17:388-396. doi:10.1046/j.1460-9568.2003.02444.x
 74. Moreira PS, Almeida PR, Leite-Almeida H, Sousa N, Costa P. Impact of chronic stress protocols in learning and memory in rodents: systematic review and meta-analysis. *PLoS One.* 2016;11(9):e0163245. doi:10.1371/journal.pone.0163245
 75. Neely C, Lane C, Torres J, Flinn J. The effect of gentle handling on depressive-like behavior in adult male mice: considerations for human and rodent interactions in the laboratory. *Behav Neurosci.* 2018;2018:1-7. doi:10.1155/2018/2976014
 76. Hurst JL, West RS. Taming anxiety in laboratory mice. *Nat Methods.* 2010;7(10):825-826. doi:10.1038/nmeth.1500