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Tyrosine Inhibits TyrRS-mediated DNA Repair and Induces Neuronal Oxidative DNA Damage

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Abstract

Human aging and neurodegenerative diseases accumulate oxidative DNA damage-associated mutations in neurons. Circadian-regulated tyrosine (Tyr) is increased during aging and in Alzheimer's Disease (AD). Tyr exacerbates the cognitive decline in the elderly and AD patients. Tyrosyl-tRNA synthetase (TyrRS) that activates Tyr for protein synthesis and participates in DNA repair is depleted in the affected brain regions of AD patients through an unknown mechanism. Here, we found that increased Tyr levels decrease the nuclear and neurite levels of TyrRS in neurons and cause oxidative DNA damage. Although Tyr inhibits protein synthesis at the elongation step, dopamine (DA)- a neurotransmitter derived from Tyr increases TyrRS levels. We previously showed that Tyr inhibits TyrRS-mediated activation of poly-ADP-ribose polymerase 1 (PARP1), a modulator of DNA repair. We now found that *trans*-resveratrol (*trans*-RSV) that binds to TyrRS mimicking 'Tyr conformation' decreases TyrRS, inhibits DNA repair and induces neurotoxicity. Conversely, *cis*-RSV binds to TyrRS mimicking a 'Tyr-free conformation,' increases TyrRS, facilitates DNA repair, and protects neurons against multiple neurotoxic agents in a TyrRS-dependent manner. Our results suggest that increased Tyr levels may have causal effects in human aging and neurocognitive disorders and offer a plausible explanation to divergent results obtained in clinical trials using RSV.

Introduction

TyrRS is a member of the evolutionarily conserved family of aminoacyl-tRNA synthetases (aaRSs) that activates the aromatic amino acid (AAA) Tyr for protein synthesis¹. TyrRS has a moonlighting function in DNA repair as well^{2,3} and is essential for cell survival⁴. In neurons, brain-derived neurotrophic factor (BDNF) stimulates the *de novo* synthesis of TyrRS⁵. Protein synthesis is required for long-term memory formation^{6,7}, and consistently, the brain protein level of TyrRS is depleted in the affected brain regions of AD patients⁸ through an unknown mechanism. Tyr is circadian-regulated with the highest serum levels in the morning and lowest at midnight (sleep time)^{9,10}. Sleep stimulates human brain protein synthesis¹¹, memory formation^{12,13}, and neuronal DNA repair¹⁴. Beyond feeding and protein synthesis¹⁵, Tyr level is modulated by the circadian activities of tyrosine hydroxylase (TH)¹⁶, tyrosine aminotransferases (TAT)^{17,18}, and gut microbiota¹⁹. However, serum levels of Tyr increase during human aging²⁰⁻²², and increased Tyr level is a biomarker for the development of type 2 diabetes (T2D) in humans²³, a major risk factor for dementia²⁴ and AD²⁵. Genetic mutations that increase the levels of Tyr (tyrosinemia)²⁶⁻²⁹ or its precursor L-phenylalanine (Phe, phenylketonuria [PKU])³⁰ cause multiple health problems, including cognitive deficits in children. Moreover, treatment with Tyr exacerbates the cognitive decline in the elderly³¹⁻³³ and AD patients³⁴, and dysregulated Tyr metabolism shortens lifespan in tyrosinemia patients³⁵ through unknown mechanisms.

We previously showed that Tyr inhibits TyrRS-dependent activation of PARP1³⁶, a major modulator of DNA repair^{37,38}. Here, we found that increased Tyr levels decrease the nuclear and neurite levels of TyrRS and cause neuronal oxidative DNA damage, potentially through the inhibition of protein synthesis at the elongation step. However, the neurotransmitter derived from

Tyr (dopamine) stimulates the *de novo* synthesis of neuronal TyrRS. Furthermore, consistent with our previous findings that *cis*-RSV evokes a ‘Tyr-free conformation’³⁹, and *trans*-RSV mimics a ‘Tyr conformation’³⁶ in TyrRS, we now show that *cis*- and *trans*-RSV have opposite effects on TyrRS levels and neuronal DNA repair. *cis*-RSV protects the neurons against Tyr and other neurotoxic agents-induced depletion of TyrRS and DNA damage. Therefore, our results provide a potential molecular basis for the age-associated accumulation of neuronal oxidative DNA damage⁴⁰⁻⁴³ and Tyr-induced cognitive impairments in elderly and AD patients³¹⁻³⁴.

Results

TyrRS is decreased in the hippocampal tissue samples of human AD patients.

AD decreases brain protein synthesis at the elongation step in humans⁴⁴⁻⁴⁷ through an unknown mechanism. Recently published human brain proteome showed decreased TyrRS and phenylalanyl-tRNA synthetase beta (PheRS β) levels in AD-affected brain regions⁸ (**Supplementary Fig. 1a** and **b**). We validated the depletion of TyrRS and PheRS β in the hippocampal region of AD patients. However, the level of PheRS α was not affected (**Fig. 1a** and **Supplementary Table 1**). Our re-analysis of a second brain proteome⁴⁸ showed that the protein levels of TyrRS and PheRS β correlate with cognitive performance in humans (**Supplementary Fig. 1c**)⁴⁸. Conversely, their decrease correlates with AD status and Braak stages (**Supplementary Fig. 1d**)⁴⁸. Intriguingly, a meta-analysis of human brain transcriptomic data from AD patients did not show any changes in the mRNA levels of TyrRS or PheRS β ^{49,50}. While PheRS β translation declines in an age-dependent manner (**Supplementary Fig. 1e**)⁵¹, TyrRS did not correlate with any known biomarkers of AD or other neurodegenerative diseases (**Supplementary Fig. 1f**)⁴⁸, indicating that TyrRS protein level might be modulated by hitherto unknown factors.

Tyrosine is increased during aging and in neurocognitive disorders in humans.

Aging is the highest risk factor for neurodegenerative diseases⁵² and intriguingly, the incidence of AD and other dementias is higher in women than in men⁵³. Tyr level increases during human aging²⁰⁻²² and consistently, Tyr levels are also increased in human AD brain tissues⁵⁴⁻⁵⁶. Although there is no sex difference in Tyr levels in children⁵⁷ or AD patients⁵⁸, interestingly, young women have lower serum Tyr levels than young men^{21,57,59}. However, menopause increases Tyr levels⁶⁰, resulting in a significant increase in Tyr levels in older women from ~60-70 yr²⁰ (**Supplementary Fig. 2a** and **Supplementary Table 2**). Beyond neurodegenerative diseases⁵², aging in humans is associated with increased incidents of other diseases as well⁶¹. Consistently, our literature analysis showed that Tyr and/or Phe are increased during delirium, heart failure (HF), Parkinson's Disease (PD), autism spectrum disorders (ASD), cardiovascular diseases (CVD), and other metabolic disorders in humans (**Supplementary Fig. 2b** and **Supplementary Table 3**). Interestingly, increased levels of Tyr and/or Phe inhibit aminoacyl-tRNA synthesis in the brain *ex vivo*^{62,63} and inhibit protein synthesis⁶⁴ and induce DNA damage^{65,66} and promote oxidative stress in brain⁶⁷ *in vivo* in rat. Because human AD brain has decreased levels of TyrRS (**Fig. 1a**), and AD decreases brain protein synthesis at the elongation step⁴⁴⁻⁴⁷, and aged neurons⁴⁰ and AD brain⁴² accumulate increased levels of oxidative DNA damage, and Tyr inhibits brain protein synthesis⁶⁴, we hypothesized that increased levels of Tyr negatively regulate the protein levels of TyrRS and might cause neuronal oxidative DNA damage.

Tyrosine decreases and dopamine increases neuronal TyrRS protein.

As expected, treatment with Tyr in rat cortical neurons (DIV 9/10) decreased TyrRS (**Fig. 1b**) with preferential effects in the nucleus and the neurites (**Fig. 1b**). Although Tyr depleted PheRS β , it did

not affect the levels of PheRS α (**Fig. 1c**). Similarly, Phe, 3,4-dihydroxy-L-phenylalanine (L-DOPA), and 6-hydroxydopamine (6-OHDA) also decreased the levels of TyrRS and PheRS β (**Fig. 1c** and **Supplementary Fig. 3a-c**). We previously showed that ~75% knockdown of TyrRS using siRNA (siRNA^{TyrRS}) does not affect cell viability³⁶. However, siRNA^{TyrRS} in cortical neurons (~50% knockdown) resulted in robust neurite degeneration (**Fig. 1d** and **Supplementary Fig. 3d**), indicating a critical role of TyrRS in maintaining neurite stability. Despite a significant decrease in the neurite levels of TyrRS, surprisingly, Tyr did not induce neurite degeneration (**Fig. 1b**). Moreover, the levels of TyrRS were restored in 16-24 hr (**Fig. 1e** and **Supplementary Fig. 3e**), suggesting that the effect of Tyr on TyrRS protein levels is reversible. The neurotransmitter dopamine (DA), which is decreased during aging⁶⁸, and in the affected brain regions of AD patients⁵⁴, is generated from Tyr (L-Tyr $\rightarrow$ L-DOPA $\rightarrow$ DA). DA dephosphorylates eukaryotic elongation factor 2 (eEF2)⁶⁹ and activates neuronal protein synthesis⁷⁰. Because BDNF activates eEF2⁷¹ and stimulates the *de novo* synthesis of neuronal TyrRS⁵ (**Supplementary Fig. 3f** and **g**), we tested if DA would also increase neuronal TyrRS levels. As expected, DA increased the protein levels of TyrRS (**Fig. 1f** and **Supplementary Fig. 3h**), and the effects of DA were abrogated by rapamycin (rapa) (**Fig. 1g**). Unlike DA⁶⁹, treatment with Tyr inhibited eEF2 (**Fig. 1h**). This data suggests that while Tyr decreases TyrRS protein levels, BDNF and DA increase it (**Fig. 1i**).

***cis*-RSV and *trans*-RSV have opposite effects on TyrRS levels in neurons.**

We previously showed that *trans*-RSV competes with Tyr to bind TyrRS³⁶. More importantly, we showed that low concentration *trans*-RSV adapts its *cis* conformation (*cis*-RSV) and increases the protein levels of TyrRS³⁶. Furthermore, we found that *cis*-RSV binding to TyrRS alters its normal function and mediates a PARP1-dependent stress response, whereas *trans*-RSV mimics Tyr

binding. Although *cis*-RSV and *trans*-RSV exist in mixtures^{72,73} and are stable for at least six weeks at 4°C^{72,73}, and in the cell for 24 hr⁷⁴, the sulfate metabolites of RSV that provide an intracellular pool⁷⁵ mainly generate *cis*-RSV⁷⁴. Clinical studies using a daily dose of 1,000 mg/day *trans*-RSV (>99% pure) reported a peak *trans*-RSV plasma concentration of 137μM⁷⁶ and 50-640μM *trans*-RSV accumulation in human tissues⁷⁵. Recent clinical studies using low-dose *trans*-RSV (75mg/dose) reported cognitive benefits in postmenopausal women⁷⁷⁻⁷⁹, who have higher Tyr levels⁶⁰. In contrast, clinical studies with 1,000 mg/dose *trans*-RSV resulted in brain volume loss in AD patients⁸⁰ through unknown mechanisms. Because the brain protein level of TyrRS correlates with cognitive performance in humans (**Supplementary Fig. 1c**)⁴⁸ and is essential for survival^{1,4}, we hypothesized that *cis*-RSV and *trans*-RSV would have differential effects on neuronal TyrRS levels. Consistent with the results in clinical trials, a low dose of *trans*-RSV (10μM) increased TyrRS and the high dose (50μM) decreased it, whereas *cis*-RSV (10-50μM) increased TyrRS protein levels (**Fig. 2a** and **Supplementary Fig. 4a**). *cis*-RSV rescued the effects of *trans*-RSV and Tyr-mediated decrease in TyrRS levels in a dose-dependent manner (**Fig. 2b** and **c**). High concentration *trans*-RSV decreased PheRSβ, not PheRSα levels, while low concentration *cis*-RSV increased their levels (**Fig. 2d**). Further, *cis*-RSV-mediated increase in TyrRS level was abrogated by adding cycloheximide (CHX) (**Fig. 2e**). Ser51 phosphorylation of eukaryotic initiation factor 2 alpha (p-eIF2α) and Thr56 phosphorylation of eEF2 (p-eEF2) inhibit protein synthesis at the initiation and elongation steps, respectively. While *cis*-RSV triggered a transient increase in the levels of p-eIF2α (**Supplementary Fig. 4b** and **c**), *trans*-RSV sustained the inhibition of eIF2α (**Supplementary Fig. 4b** and **c**). Consistent with a previous report⁸¹, *trans*-RSV inhibited eEF2 (**Fig. 2f**), but in contrast, *cis*-RSV activated eEF2 through dephosphorylation (**Fig. 2f**). Similarly, treatment with an eEF2K activator (nelfinavir)⁸² depleted neuronal TyrRS

which was rescued by *cis*-RSV (**Fig. 2g** and **Supplementary Fig. 4d**). We previously showed that genetic reduction of eEF2K attenuates age-related memory deficits in mice⁸³. Consistently, we found that TyrRS and PheRS β were increased in the brain tissue samples of eEF2K^{+/-} mice (**Fig. 2h**). To determine the mechanism of *cis*-RSV-mediated activation of eEF2, we tested if *cis*- and *trans*-RSV modulate the interaction of TyrRS with eEF2. We found that *cis*-RSV facilitated the interaction of eEF2 with TyrRS and protein phosphatase 2 (PP2A), whereas *trans*-RSV and Tyr decreased their interactions (**Fig. 2i**). These data suggest that *cis*-RSV and Tyr modulate the *de novo* synthesis of TyrRS at the elongation step, similar to BDNF⁷¹ and DA⁶⁹. Furthermore, similar to 6-OHDA (**Supplementary Fig. 3c**), other neurotoxic agents such as the N-Methyl-D-aspartate (NMDA), and the mitochondrial toxin 1-methyl-4-phenylpyridinium (MPP⁺) also decreased the levels of neuronal TyrRS after 4 hr of treatment (**Supplementary Fig. 4e** and **f**); *cis*-RSV suppressed this effect, whereas *trans*-RSV exacerbated it (**Supplementary Fig. 4e** and **f**), suggesting that neuronal TyrRS is a potential target of multiple neurotoxic agents.

Pharmacological activation of protein synthesis increases TyrRS levels in neurons.

Phosphorylation of eIF2 α modulates motor and cognitive function⁸⁴⁻⁸⁸, and neuronal survival⁸⁹. Pharmacological activation of mRNA translation using integrated stress response inhibitor (ISRIB) protects against age-related memory deficits⁹⁰. Consistently, treatment with ISRIB (5-50nM) increased the protein levels of TyrRS and PheRS β (**Supplementary Fig. 5a**) and rescued *trans*-RSV-mediated depletion of TyrRS (**Supplementary Fig. 5b**). Interestingly, ISRIB stimulated the dephosphorylation of eEF2 (**Supplementary Fig. 5a**), potentially mediated through increased levels of TyrRS. However, higher doses of ISRIB (250-500nM) decreased TyrRS levels (**Supplementary Fig. 5c**), consistent with some reported adverse effects of ISRIB^{91,92}. Because

circadian-regulated Tyr^{9,10} decreased neurite TyrRS (**Fig. 1b**), and sleep regulates synaptic protein synthesis⁹³, we wondered if the synaptic protein level of TyrRS is circadian-regulated. Our re-analysis of the mouse circadian proteomic⁹³ and metabolomic⁹⁴ data showed that synaptic protein level of only TyrRS (among the aaRSs) is circadian-regulated and is inversely correlated with Tyr levels (**Supplementary Fig. 5d**). Further, re-analysis of the human metabolome^{95,96} showed that sleep deprivation increases the serum levels of Tyr (**Supplementary Fig. S5e**). Collectively, these data suggest that Tyr is a potential endogenous modulator of synaptic and nuclear TyrRS.

Tyrosine induces oxidative DNA damage in neurons and *cis*-RSV protects against it.

Human aging and neurodegenerative diseases accumulate oxidative DNA damage-associated mutations in neurons through unknown mechanisms⁴⁰⁻⁴³. While sleep deprivation causes oxidative DNA damage^{97,98}, sleep stimulates neuronal DNA repair¹⁴ through an unknown mechanism. Because TyrRS has a moonlighting function in DNA repair^{2,3}, we hypothesized that decreased levels of Tyr in the night^{9,10} may switch the function of a fraction of TyrRS from protein synthesis to DNA repair, and conversely, aging and neurodegenerative diseases that increase Tyr levels (**Supplementary Fig. 2**) may inhibit TyrRS-mediated DNA repair. Therefore, increased Tyr levels and compounds that mimic ‘Tyr conformation’ in TyrRS may induce neuronal oxidative DNA damage. Conversely, compounds that mimic a ‘Tyr-free conformation’ in TyrRS may shift its function to facilitate neuronal DNA repair. To test this possibility, we treated neuronal cultures with *cis*-RSV, which evokes a ‘Tyr-free conformation’³⁹, or *trans*-RSV, which mimics a ‘Tyr conformation’ in TyrRS³⁶. Treatment with Tyr resulted in the accumulation of γ -H2AX foci (a marker of DNA damage) and 8-oxo-2'-deoxyguanosine (8-oxo-dG, a marker of oxidative DNA damage) (**Fig. 3a and b**). While *cis*-RSV rescued Tyr-induced accumulation of γ -H2AX, and 8-

oxo-dG, *trans*-RSV itself caused the accumulation of γ -H2AX and 8-oxo-dG (**Fig. 3a and b**). Tyr also decreased the levels of 8-oxoguanine-DNA glycosylase (OGG1) (**Fig. 3c**), analogous to the reported decrease in the levels of OGG1 in sleep-deprived humans⁹⁸ and in the AD brain tissues⁹⁹. *cis*-RSV rescued Tyr-mediated depletion of OGG1 (**Supplementary Fig. 6a**). 8-oxo-dG is highly mutagenic, driving a G-C $\rightarrow$ A-T transversion¹⁰⁰, and mutagenic frequency is reported to increase in aged neurons⁴⁰ and AD brain tissues⁴³. Consistently, γ -H2AX^{101,102} and 8-oxo-dG⁴² are accumulated in AD neurons. Further, D-Tyr, which does not get converted to DA¹⁰³ but is activated by TyrRS¹⁰⁴, induced neurotoxicity, whereas D-Phe and D-Trp had no effects (**Fig. 3d, and e and Supplementary Fig. 6b and c**). *cis*-RSV protected against D-Tyr-mediated neurotoxicity but not *trans*-RSV (**Fig. 3f**). Emerging works show a critical role of histone poly-ADP-ribosylation factor (HPF1) in DNA repair¹⁰⁵. Although *cis*-RSV did not affect the HPF1 levels, treatment with Tyr and *trans*-RSV decreased the levels of HPF1 (**Fig. 3g and Supplementary Fig. 6d and e**), indicating multiple mechanisms for Tyr-mediated accumulation of oxidative DNA damage in neurons. Consistently, we found that HPF1 is decreased in the hippocampal tissues from AD patients (**Fig. 3h**).

***cis*-RSV is neuroprotective and *trans*-RSV is neurotoxic.**

Because *cis*- and *trans*-RSV have opposite effects on neuronal oxidative DNA damage, we hypothesized that they would have differential effects on neuronal survival under stress conditions. We analyzed *cis*- and *trans*-RSV effects on the survival of rat primary cortical neurons exposed to different stress agents to test this hypothesis. As expected, the effect of *trans*-RSV on NMDA-mediated neurotoxicity showed a concentration-dependent dual response, where low concentrations of *trans*-RSV ($\leq 10\mu\text{M}$) evoked protective effects, but the higher concentrations

($\geq 25\mu\text{M}$) exacerbated the toxicity (**Fig. 4a**). In contrast, *cis*-RSV protected against NMDA-mediated toxicity in a concentration-dependent manner (**Fig. 4b**). Hence, the concentration-dependent dual response of *trans*-RSV on neuroprotection is consistent with *trans* to *cis* conversion at low concentrations^{36,74}, increasing TyrRS levels (**Fig. 2a**), and the retention of ‘*trans*/Tyr conformation’ at high concentrations^{36,39}, causing TyrRS depletion (**Fig. 2a**). We also found that *cis*-RSV (50 μM) suppressed the neurotoxicity induced by a DNA-damaging agent (etoposide, ETO) (**Supplementary Fig. 7a**), oxidative stress (H₂O₂) (**Supplementary Fig. 7b**), and mitochondrial inhibition (MPP⁺) (**Supplementary Fig. 7c**) but *trans*-RSV (50 μM) did not protect against these neurotoxic agents (**Supplementary Fig. 7a-c**). To test if the observed effects of *cis*- and *trans*-RSV on neurotoxicity are mediated via TyrRS, we carried out siRNA knockdown of TyrRS (siRNA^{TyrRS}) in rat cortical neurons. TyrRS knockdown (**Supplementary Fig. 3d**) blunted the neuroprotective effects of *cis*-RSV but did not diminish the toxicity of *trans*-RSV (50 μM) upon NMDA treatment (**Fig. 4c**). These results suggest that the neuroprotective effect of *cis*-RSV (and low-dose *trans*-RSV) is TyrRS dependent, but the neurotoxic effect of *trans*-RSV is TyrRS independent. Moreover, we found that *trans*-RSV (50 μM) by itself was neurotoxic in the rat primary cortical neuron cultures (**Fig. 4c**), whereas *cis*-RSV protected against the neurotoxicity induced by *trans*-RSV in a dose-dependent manner (**Fig. 4d**). Furthermore, a high concentration of *trans*-RSV and D-Tyr increased the levels of cleaved caspase-3 (a marker of apoptosis) in rat primary cortical neurons (**Supplementary Fig. 7d and e**). In contrast, *cis*-RSV decreased the levels of caspase-3 cleavage (**Supplementary Fig. 7d**). As expected, treatment with either ISRIB, eEF2K inhibitor or DA protected against neurotoxic effects of *trans*-RSV (**Supplementary Fig. 7f-h**), indicating a critical role of protein synthesis in neuronal survival.

***cis*-RSV and *trans*-RSV have opposite effects on the auto-PARylation of PARP1.**

We previously showed that Tyr inhibits the auto-poly-ADP-ribose(PAR)ylation of PARP1³⁶ while *cis*-RSV induced a ‘Tyr-free conformation’ in TyrRS³⁹ to stimulate the auto-PARylation³⁶. Consistent with the circadian regulation of Tyr (**Supplementary Fig. 5d**), auto-PARylation of PARP1 is also circadian-regulated¹⁰⁶. Because *trans*-RSV mimics the ‘Tyr conformation’ in TyrRS³⁶, we analyzed the effects of *cis*- and *trans*-RSV on the auto-PARylation of PARP1³⁶. As expected, a low concentration of *trans*-RSV (5μM), which converts to *cis*-RSV in solution^{36,74}, and *cis*-RSV stimulated the auto-PARylation of PARP1 (**Fig. 4e and f**) whereas higher concentrations of *trans*-RSV (≥25μM) inhibited the auto-PARylation of PARP1 (**Fig. 4f**). It is interesting to note that the apparent *K_i* value of *trans*-RSV-mediated inhibition of Tyr activation by TyrRS in an ATP-PPi exchange assay (Tyr + ATP → Tyr-AMP + PPi) was ~25μM³⁶ which is the lowest concentration of *trans*-RSV that significantly inhibits the auto-PARylation of PARP1 (**Fig. 4f**). We previously showed that Tyr inhibits auto-PARylation-dependent acetylation of proteins³⁶. Further, 500 mg/day dosing of *trans*-RSV (68.5μM peak plasma concentration) inhibited the acetylation of H3 at lysine 56 (Ac-K56-H3) in humans¹⁰⁷. Consistently, *trans*-RSV (≥25μM) inhibited H3 acetylation at lysines 9 and 56 (Ac-K9-H3 and Ac-K56-H3) (**Fig. 4e and f**), whereas *cis*-RSV increased the acetylation of H3 lysine 9 (Ac-K9-H3). Furthermore, TyrRS knockdown diminished the effects of low-concentration (5μM) *trans*-RSV and *cis*-RSV (25μM) on the auto-PARylation of PARP1 (**Fig. 4g and Supplementary Fig. 7i**).

***cis*-RSV stimulates the deADP-ribosylation of neuronal chromatin.**

DNA damage activates PARP1/HPF1-dependent serine ADP-ribosylation of histones¹⁰⁸, and removal of these ‘ADP-ribose chromatin scar’ is essential for neuronal survival^{109,110}. Importantly,

the brain samples of AD patients have increased levels of nuclear ADP-ribosylation¹¹¹, suggesting a potential role of ‘ADP-ribose chromatin scar’ in AD-related neurodegeneration. Although auto-PARYlation dissociates PARP1 from the chromatin¹¹², HPF1 inhibits the auto-PARYlation¹¹³, and increases *trans*-PARYlation of chromatin^{113,114}. Because *trans*-RSV inhibited the auto-PARYlation (**Fig. 4f**), we hypothesized that *trans*-RSV would increase PARP1-dependent *trans*-PARYlation of the chromatin. As expected, *trans*-RSV increased the association of PARP1 with chromatin and increased the levels of PARYlated proteins in the chromatin fraction (**Fig. 5a**). Further, low concentrations of *trans*-RSV ($\leq 10\mu\text{M}$) and *cis*-RSV prevented the interaction of PARP1 with histone H3 while the higher concentrations ($\geq 25\mu\text{M}$) of *trans*-RSV increased it (**Supplementary Fig. 8a**). Although *cis*-RSV-mediated auto-PARYlation of PARP1 resulted in its removal from the chromatin, unexpectedly, we found that *cis*-RSV activated the deADP-ribosylation of the chromatin fraction along with higher levels of TyrRS (**Fig. 5a** and **Supplementary Fig. 8b**). ADP-ribosyl-acceptor hydrolase 3 (ARH3) removes ‘ADP-ribose chromatin scar’^{109,110}, and as expected, *cis*- and *trans*-RSV had differential effects on the recruitment of ARH3 to the chromatin (**Fig. 5a** and **Supplementary Fig. 8c**). Despite having increased levels of nuclear ADP-ribosylation¹¹¹, the levels of ARH3 remained unchanged in the hippocampal region of human AD patients (**Supplementary Fig. 8d**), indicating that ARH3 may not be functional in human AD brain tissues in the absence of TyrRS. Consistently, we found that TyrRS interacted with ARH3 (**Fig. 5b**), suggesting a potential novel role of TyrRS in the removal of ‘ADP-ribose chromatin scar’ (**Fig. 5a**) that enhances neuronal DNA repair and survival.

‘Trapped’ PARP1 inhibits DNA repair and mediates the neurotoxic effects of *trans*-RSV.

Suicidal crosslinking of PARP1 to the damaged DNA¹¹⁵ causes cytotoxicity¹¹⁶, and therefore, cell survival depends on removing ‘trapped’ PARP1 on the broken DNA by either ablation^{38,117} or auto-PARylation^{112,118,119}. Because *trans*-RSV caused DNA damage (**Fig. 3a and b**) and inhibited the auto-PARylation of PARP1 (**Fig. 4f**), and ablation of PARP1 rescues ‘trapped’ PARP1-mediated neurotoxicity¹²⁰⁻¹²², we hypothesized that *trans*-RSV-mediated neurotoxicity is exerted through ‘trapped’ PARP1 on the damaged DNA. Consistently, small interfering RNA (siRNA) knockdown of PARP1 (siRNA^{PARP1}) protected against *trans*-RSV-mediated neurotoxicity but did not interfere with the effect of *cis*-RSV (**Fig. 5c**), indicating that ‘trapped’ PARP1 mediates the neurotoxic effects of *trans*-RSV. On the other hand, we found that *cis*-RSV facilitated the recruitment of DNA repair factors such as HPF1 and OGG1 to the chromatin (PARP2 and XRCC1 were not affected) while *trans*-RSV prevented their recruitment (**Supplementary Fig. 8e**). PARP1 inhibits flap endonuclease (FEN1)-dependent long patch base excision repair (LP-BER)¹²³, and consistently, *cis*-RSV decreased the recruitment of FEN1 (**Supplementary Fig. 8e**), indicating a potential role of TyrRS/ARH3-dependent deADP-ribosylation of PARP1 in limiting long patch BER. As further evidence of DNA repair, we assessed whether DNA synthesis occurred following *cis*- or *trans*-RSV treatment. The incorporation of nucleoside analogs into DNA from neurons was previously used as a readout of neuronal DNA repair^{37,102} and depletion of PARP1 or inhibitors of PARP1 increases the incorporation of nucleoside analogs³⁷. In agreement with the observation that ‘trapped’ PARP1 on the DNA impairs base excision repair (BER)^{124,125}, *trans*-RSV prevented the incorporation of the nucleoside analog CldU into DNA fibers isolated from neurons (**Fig. 5d** and **Supplementary Fig. 8f**). However, *cis*-RSV reduced the CldU incorporation (**Fig. 5d** and **Supplementary Fig. 8f**), suggesting the activation of PARP1-dependent short patch BER (SP-

BER)³⁷, potentially through the displacement of FEN1 (**Supplementary Fig. 8e**)¹²³ and/or increased recruitment of unmodified PARP1 to the chromatin (**Fig. 5a**)¹²⁵ that limits DNA resection³⁸. This decrease also indicates that *cis*-RSV might activate other DNA repair pathways that enhance discrimination against the incorporation of nucleoside analogs into the genome, which requires future explorations. Together these results show that *cis*- and *trans*-RSV may have opposite effects on TyrRS-regulated auto-PARylation^{36,39} and DNA repair^{2,3}.

Taken together, our results explain the opposite effects of low and high doses of *trans*-RSV in clinical trials³⁹. Our data suggest that the observed beneficial effects of RSV result from its *cis* conformation. The mechanism for opposite effects of *cis*- and *trans*-RSV on neuronal survival emerging from our studies are illustrated in **Figure 5e**. In this model, different forms of stress facilitate the interaction of TyrRS with PARP1 leading to its auto-PARylation³⁶ and subsequent removal from the damaged DNA allowing recruitment of DNA repair factors such as HPF1, ARH3, and OGG1 to repair the damage efficiently. *cis*-RSV-bound TyrRS facilitates removing auto-PARylated PARP1 from chromatin while activating ARH3-mediated removal of ‘ADP-ribose chromatin scar’ to achieve efficient neuronal DNA repair. In contrast, treatment with *trans*-RSV decreases TyrRS in the absence of which PARP1 gets ‘trapped’ on the damaged DNA and impedes DNA repair. This inefficient repair process results in the accumulation of ‘ADP-ribose chromatin scars’ which drives the subsequent induction of neurodegeneration.

Discussion

This study shows that circadian-regulated Tyr^{9,10} is a negative regulator of neuronal TyrRS, providing a potential molecular basis for circadian modulation of synaptic TyrRS

(Supplementary Fig. 5d)⁹³, accumulation of oxidative DNA damage in aged neurons and AD brain tissues⁴⁰⁻⁴³, Tyr-mediated cognitive impairments^{26-29,31-34}, and the decreased protein synthesis at the elongation step in human AD brain tissue samples⁴⁴⁻⁴⁷. Interestingly, mutant amino acid transporter that accumulates Tyr in *Neurospora crassa* is sensitive to Tyr¹²⁶, and Tyr detoxification is essential for the survival of hematophagous insects¹²⁷. Therefore, Tyr-mediated regulation of TyrRS might be an evolutionary conserved negative feedback regulatory mechanism of protein synthesis exploited by neurons to enhance plasticity. Interestingly, calorie restriction (CR) significantly lowers only Tyr levels among the AAAs^{128,129}, and improves memory^{130,131} in humans. Similarly, sleep that decreases Tyr levels stimulates human brain protein synthesis¹¹, memory formation^{12,13}, and neuronal DNA repair¹⁴, potentially through the circadian regulation of TyrRS-mediated auto-PARylation of PARP1^{36,106}. Interestingly, exercise stimulates the production of BDNF, and DA is also known to activate PARP1-dependent DNA repair¹³² and stimulates protein synthesis through activation of eEF2 in humans¹³³. Consistently, PARylation is required for long-term memory formation^{134,135} and contributes to spatial and fear memory acquisition^{136,137}. Not surprisingly, centenarians retain high levels of PARylation^{138,139} and nuclear PARP1 is decreased in the brains of PD¹⁴⁰ and AD^{141,142} patients. Moreover, inflammatory conditions trigger proteolytic cleavage of TyrRS¹⁴³ and inhibit PARP1-dependent DNA repair¹⁴⁴. Although aging is the single most important contributing factor to the development of AD⁵², intriguingly, AD does not occur naturally in naked mole-rats (NMR), which are the longest-lived of rodents resistant to AD^{145,146}. Because NMR maintains lower levels of Tyr¹⁴⁷ and higher levels of auto-PARylation of PARP1^{148,149}, whether decreased levels of Tyr in NMR contribute to its longevity and protection against AD through enhanced TyrRS/PARP1-dependent DNA repair^{148,149} need to be explored in the future. Although

increased levels of branched-chain amino acids (BCAAs) are associated with metabolic disorders^{23,150}, in this context, it is interesting to note that the levels of BCAAs are decreased in AD^{151,152} as well as in ASD¹⁵³⁻¹⁵⁵. However, an increased level of Tyr is associated with both neurocognitive and metabolic disorders (**Supplementary Fig. 2b** and **Supplementary Table 3**). Although there is no sex differences in Tyr levels in children⁵⁷, whether increased levels of Tyr in children with ASD^{156,157} or mutations of amino acid transporter (LAT1) that increase the levels of Tyr/Phe in the brain^{153,154} contribute to the increased incidence of mutations¹⁵⁸ and dysregulated protein synthesis in ASD¹⁵⁴ will be of future research interest. Therefore, Tyr-mediated induction of 8-oxo-dG and γ H2AX (**Fig. 3a-c**), and Tyr-mediated depletion of TyrRS shown here may have causal effects in human aging, motor, cognitive, and metabolic disorders (**Supplementary Fig. 9**).

Most recent clinical trials showed that low-dose *trans*-RSV (50mg/dose, 6 μ M peak plasma concentration) that gets converted to *cis*-RSV^{36,74} and induces a '*Tyr-free* conformation' in TyrRS³⁹ protected against human heart failure¹⁵⁹ where high Tyr levels negatively correlate with survival^{160,161}. In contrast, high-dose *trans*-RSV (500mg/dose, 68.5 μ M peak plasma concentration⁷⁶) that maintains '*trans/Tyr* conformation' in TyrRS and induces oxidative DNA damage (**Fig. 3a** and **b**) increased the CVD risk¹⁶². Consistently, oxidative DNA damage is elevated in CVD¹⁶³ and patients with heart failure¹⁶⁴. Therefore, in addition to a plausible explanation for the apparent benefits of low doses of *trans*-RSV⁷⁶, our study suggests that *cis*-RSV or compounds that use *cis*-RSV conformation as a pharmacophore may help in the chronotherapy of age-associated neurological disorders and potentially degenerative and metabolic diseases of other tissues.

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Figure 1

Figure 1. TyRS protein is decreased in the hippocampal region of AD patients, and tyrosine decreases nuclear and neurite levels of TyRS. **a.** Representative immunoblots and quantification for TyRS and PheRS α/β using anti-TyRS and PheRS α/β antibodies in the hippocampal region of AD patients (n=7) with age and sex-matched controls (n=7). **b.** Tyrosine preferentially decreases nuclear and neurite levels of TyRS. Spectral images (scale bar, 20 μ m), and quantitative IF analysis of TyRS in the nucleus, soma, and neurite of rat cortical neurons (DIV9) using anti-TyRS antibody after treatment with Tyr (250 μ M) for 4 hr. **c.** Tyrosine depletes TyRS and PheRS β , but not PheRS α . Primary cortical neurons were treated with Tyr (100-300 μ M) for 4 hr, and the levels of PheRS β and PheRS α were detected by WB analysis using their specific antibodies. **d.** TyRS knockdown using siRNA induces neurite degeneration. Representative images (scale bar, 20 μ m) for cortical neurons following siRNA^{TyRS} treatment for 72 hr (MAP2 – neurite marker, magenta, and DAPI – nuclear marker, blue). Neurons were

immunoassayed with anti-MAP2 antibody and quantified for neurite degeneration. **e.** *Tyr effect on TyrRS protein levels is reversible.* Primary cortical neurons were treated with Tyr (200 μ M) for up to 24 hr, and TyrRS was detected by WB analysis using anti-TyrRS antibody. **f.** *Dopamine (DA) increases neuronal TyrRS levels.* Representative immunoblots for TyrRS and PheRS α/β after treatment with DA (100-200 μ M) for up to 30 min in rat cortical neurons (DIV9). **g.** *Rapamycin prevents DA-induced increase in TyrRS levels.* Representative immunoblots for TyrRS and PheRS β after treatment with either DA (100 μ M) alone or in combination with rapamycin (100nM) in rat cortical neurons (DIV9). **h.** *Tyrosine induces the phosphorylation of eEF2.* Primary cortical neurons were treated with Tyr (100-500 μ M) for 8 hr and p-eEF2 was detected by WB analysis using anti-p-eEF2 antibody. The data represents mean $\pm$ SEM for n=3 experiments with significance measured using Student's paired t-test. **i.** *Schematic representation of the potential mechanism of regulation of the neuronal protein levels of TyrRS.*

Figure 2

Figure 2. Tyrosine and *cis*-RSV modulate the *de novo* synthesis of TyrRS at the elongation

step of protein synthesis. a. Low and high concentrations of *trans*-RSV (10 and 50 μ M) have

opposite effects on TyrRS, and low concentration *trans*-RSV mimics *cis*-RSV to increase the

protein levels of TyrRS. Representative immunoblots and quantification for TyrRS after

treatment with *cis*- and *trans*-RSV (10 and 50 μ M) for up to 16 hr in rat cortical neurons (DIV9).

b. *cis*-RSV protects neurons against *trans*-RSV-mediated decrease in TyrRS levels.

Representative immunoblots showing the protein levels of TyrRS after treatment with *trans*-RSV

(50 μ M) alone or in combination with different doses of *cis*-RSV for 16 hr in rat cortical neurons

(DIV9). **c.** *cis*-RSV protects neurons against Tyr-mediated depletion of TyrRS. Primary cortical

neurons were treated with Tyr (200 μ M) alone or in combination with *cis*-RSV for 8 hr, and

TyrRS was detected by WB using anti-TyrRS antibody. **d.** *cis*- and *trans*-RSV have opposite

effects on neuronal PheRS β . Representative immunoblots and quantification for PheRS α / β after

treatment with *cis*- and *trans*-RSV (5-50 μ M) for up to 16 hr in rat cortical neurons (DIV9). **e.** *cis*-RSV stimulates the *de novo* synthesis of neuronal TyrRS. Spectral images (scale bar, 20 μ m) and quantitative IF analysis using anti-TyrRS antibody. Primary cortical neurons were treated with *cis*-RSV (50 μ M) for 30 min either alone or in combination with CHX (100 μ M). **f.** *cis*- and *trans*-RSV have opposite effects on the phosphorylation of eEF2. Primary cortical neurons were treated with *cis*- and *trans*-RSV (5-50 μ M) for 8 hr and p-eEF2 was detected by WB analysis using anti-p-eEF2 antibody. **g.** *cis*-RSV protects neurons against nelfinavir-mediated depletion of TyrRS. Primary cortical neurons were treated with nelfinavir (20 μ M) alone or in combination with *cis*-RSV for 8 hr, and TyrRS and PheRS β were detected by WB analysis using their respective antibodies. **h.** *Genetic reduction of eEF2K increases TyrRS in vivo.* Quantitative western blots showing the protein levels of TyrRS and PheRS β using anti-TyrRS and PheRS β antibodies in the brain tissue samples from eEF2K^{+/-} mice. **i.** *cis*-RSV facilitates the interaction of PP2A with eEF2. Primary cortical neurons were treated with *cis*- and *trans*-RSV (50 μ M) or Tyr (200 μ M) either alone or in combination with *cis*-RSV. Immunoprecipitated eEF2 was probed for its interaction with PP2A and TyrRS using their specific antibodies. The data represents mean $\pm$ SEM for n=3 experiments with significance measured using Student's paired t-test.

Figure 3

Figure 3. Tyrosine induces oxidative DNA damage in neurons, and *cis*-RSV protects

neurons against it. a. *cis*-RSV and *trans*-RSV have opposite effects on Tyr-mediated

accumulation of γ-H2AX. Immunostaining images (scale bar, 10 μm) for DNA damage marker,

pSer139-H2AX foci (γ-H2AX, green; DAPI – nuclear marker, blue) in cortical neurons (DIV10)

after treatment with *cis*- and *trans*-RSV (50 μM) alone or in combination with Tyr (250 μM) for

24 hr. The graph represents the average number of γ-H2AX foci per n=30 neurons per treatment

condition for n = 4 experiments. **b. *cis*- and *trans*-RSV have opposite effects on Tyr-mediated**

induction of the oxidation level of neuronal DNA. Quantification of the levels of 8-oxo-2'-dG in

rat primary cortical neurons (DIV9/10) after treatment with Tyr (500 μM) either alone or in

combination with *cis* or *trans*-RSV (50 μM) for 16 hr. **c. Tyr decreases the protein levels of**

OGG1. Primary cortical neurons were treated with Tyr (100-300 μM) for 8 hr, and OGG1 was

detected by WB analysis using anti-OGG1 antibody. **d. D-Tyrosine and *trans*-RSV induce**

neurite degeneration. Representative images (scale bar, 20 μm) for cortical neurons following D-

Tyr or *cis*-RSV and *trans*-RSV (50 μ M) for 24 hr treatment (MAP2 – neurite marker, magenta and DAPI – nuclear marker, blue). Neurons were immunoassayed with anti-MAP2 antibody and quantified for neurite degeneration. **e.** *cis*-RSV protects neurons against D-Tyrosine-induced toxicity. Rat cortical neurons (DIV9) were treated with either D-Tyr alone or in combination with *cis* or *trans*-RSV (50 μ M) for 48 hr, and viability was assessed using MTT assay. **f.** *Tyrosine decreases the protein levels of HPF1.* Immunoblots showing the protein levels of HPF1 in rat cortical neurons (DIV9-10) after treatment with Tyr ($\leq$ 300 μ M) for up to 8 hr. **g.** *HPF1 is decreased in the hippocampal region of AD patients.* Representative immunoblots and quantification for HPF1 using anti-HPF1 antibody in the hippocampal region of AD patients (n=7) with age and sex-matched controls (n=7). The data represents mean $\pm$ SEM for n=3 experiments with significance measured using Student's paired t-test.

Figure 4

Figure 4. *cis*- and *trans*-RSV have opposite effects on neuronal survival under stress. a.

trans-RSV has opposite effects on neuronal survival under NMDA-mediated neurotoxicity. Rat cortical neurons (DIV9) were treated with NMDA (50μM for 5 min) and then with *trans*-RSV (5-50μM) for 24 hr. Cells were then exposed to NMDA (500μM for 5 min) and viability was assessed using MTT assay after 24 hr. **b.** *cis*-RSV provides dose-dependent neuroprotection against NMDA-mediated neurotoxicity. Rat cortical neurons (DIV9) were treated with NMDA (50μM for 5 min) and then with *cis*-RSV (5-50μM) for 24 hr. Cells were then exposed to NMDA (500μM for 5 min) and viability was assessed using MTT assay after 24 hr. **c.** *TyrRS* knockdown blunts the neuroprotective effects of *cis*-RSV and exacerbates the neurotoxicity of *trans*-RSV. Rat cortical neurons (DIV7) were transfected with *TyrRS* or control siRNA (75nM) and then treated with *cis*-RSV (50μM) or *trans*-RSV (5, 10, 50μM) for 24 hr. Neurons were then exposed to excitotoxic NMDA (500μM for 5 min), and viability was assessed using MTT assay after 24 hr.

d. *cis*-RSV protects from *trans*-RSV-mediated neurotoxicity. Rat cortical neurons (DIV 8) were treated with *trans*-RSV alone or combined with different doses of *cis*-RSV (10-50 μ M) for 48 hr, and viability was measured using MTT assay. **e, f.** *cis*-RSV and *trans*-RSV have opposite effects on the auto-PARylation of PARP1. Representative immunoblot images and quantification using specific antibodies for PARylation, PARP1, Ac-K9-H3, Ac-K56-H3 levels after treatment of cortical neurons (DIV9) with *cis*- and *trans*-RSV for 15 min (* indicates $p \leq 0.01$). **g.** *cis*-RSV and low dose *trans*-RSV-dependent auto-PARylation of PARP1 is TyrRS dependent. Rat cortical neurons (DIV7) were transfected with control and TyrRS siRNA followed by treatment with *cis*- (25 μ M) and *trans*-RSV (5 μ M) for 15 min and immunoblotting and quantification using the specific antibodies for PARylation, PARP1 and TyrRS. The data represents mean $\pm$ SEM for n=3 experiments with significance measured using Student's paired t-test.

Figure 5

Figure 5. *cis*- and *trans*-RSV have opposite effects on auto-PARylation of PARP1 and neuronal DNA repair. a. *cis*-RSV removes auto-PARylated PARP1 from chromatin and *trans*-RSV 'traps' PARP1 onto the chromatin. Representative immunoblots and quantification from chromatin fraction of cortical neurons (DIV9) depicting PARP1 and PAR, ARH3, TyrRS after treatment with *cis*- and *trans*-RSV (50 μ M) for 1 hr. **b.** *TyrRS* interacts with *ARH3*. Immunoprecipitated (IP) *TyrRS* from cortical neurons (DIV9) was immunoblotted (IB) using anti-*TyrRS* and anti-*ARH3* antibodies to detect the interaction of *TyrRS* with *ARH3*. Total *TyrRS* and *ARH3* were detected by WB. **c.** *siRNA* knockdown of *PARP1* rescues *trans*-RSV-mediated neurotoxicity. Rat cortical neurons (DIV7) were transfected with siRNA against *PARP1* (siRNA^{PARP1}) or control siRNA (75nM) and then treated with *cis*-RSV (50 μ M) or *trans*-RSV (50 μ M) for 72 hr. Neuronal viability was assessed and quantified using an MTT assay. The knockdown was verified using immunoblot and quantified using specific antibodies for *PARP1*.

d. *trans*-RSV inhibits nucleoside incorporation in a DNA fiber assay. Cortical neurons (DIV9/10) were treated with *cis*-or *trans*-RSV (50 μ M) for 8 hr followed by a 30 min pulse labeling using 50 μ M of nucleoside analog, CldU (5-chloro-2'-deoxyuridine). DNA fiber assay was performed according to the published protocol followed by immunostaining for single-stranded (ss) DNA (red) and CldU (green). The incorporation of CldU in DNA during the repair process was assessed using ImageJ by counting the number of CldU positive DNA fibers for a total of 300 fibers per condition. All graphs represent mean $\pm$ SEM with statistical significance calculated using Student's paired t-test. **e.** *Proposed mechanism of cis*-RSV-mediated neuroprotection and *trans*-RSV-mediated neurotoxicity.

Methods

Postmortem hippocampal tissue samples

All human postmortem tissues were obtained from the University of Washington School of Medicine BioRepository and Integrated Neuropathology (BRaIN) Laboratory and Precision Neuropathology Core. AD diagnosis was based on cognitive assessments of dementia and neuropathological verification of AD neuropathologic change using Braak staging (AD stages V–VI) stage, and Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) scores^{165,166}. Studies were performed using flash frozen hippocampal tissue from male and female patients clinically diagnosed with AD ($n = 7$) and age-matched controls ($n = 7$). Donor information is presented in Supplementary Table 1. The mean age of death was 89.6 years. Postmortem interval (PMI) ranged between 2 and 11 hr, with a mean of 5.3 hr.

Cell Culture

Primary cortical neurons were dissected from E18 Sprague Dawley rats pups in Hibernate E (BrainBits) and dissociated using the Neural Tissue Dissociation kit (Miltenyi Biotec). Minced cortices were incubated in a pre-warmed enzyme mix at 37 °C for 15 min; tissues were then triturated and strained using a 40 μ m cell strainer. After washing and centrifugation, neurons were seeded in 50 μ g/ml poly-D-Lysine (Sigma Aldrich) coated tissue culture plates. NBActive-1 medium (BrainBits) supplemented with 100 U/ml of Penicillin-Streptomycin (Life Technologies), 2 mM L-Glutamine (Life Technologies), and 1 X N21 supplement (R&D Systems) was used as culture medium. Control (non-targeting), TyrRS, and PARP1 siRNAs were obtained from Invitrogen (# AM4635, s443, and s130207, respectively). Rat cortical neurons at 5 DIV were transfected with 75 nM control or TyrRS siRNA using Dharmafect 3

Transfection Reagent. A second transfection was done two days later using 75nM of TyrRS siRNA, followed by cell collection or assays after another 48 hrs. For PARP1 siRNA, neurons at 7 DIV were transfected with 75nM siRNA for both control and PARP1 siRNA.

Western blotting

Cultured neurons (DIV 9-10) were washed once with cold 1× PBS and lysed in cell lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na₃VO₄, 1 µg/ml leupeptin supplemented with protease inhibitor). The lysates were centrifuged at 10,000 RPM for 15 mins at 4°C to separate the chromatin-bound and soluble fractions. Lysates were quantified using Bio-Rad Protein Assay, and equal amounts of protein were loaded onto a 4 to 12% gradient gel (NuPAGE-Invitrogen). Protein was transferred from the gel to 0.2 µm NC membranes at 25 V for 10 mins using transfer stacks (iBlot 2- Invitrogen) and blocked with 5% non-fat milk in TBST (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.01% Tween-20) for 1 hr before application of primary antibodies. Primary and secondary antibodies were incubated overnight at 4 °C and for 1 hr at room temperature, respectively. Immobilon ECL Ultra Western HRP Substrate (WBULS0500, Millipore) and a luminescent image analyzer (ChemiDoc Imaging System, Bio-Rad) were used to detect proteins. Quantification of western blots was done using ImageJ (Version 1.53c).

List of antibodies used for western blotting

Antibody	Company	Catalog No.	Dilution
Acetyl-Histone H3 (Lys56)	Cell Signaling Technology	4243	1:1000 (WB)

Acetyl-Histone H3 (Lys9)	Cell Signaling Technology	9649	1:1000 (WB)
ARH3	Proteintech	16504-1-AP	1:1000 (WB)
eEF2	Cell Signaling Technology	2332	1:1000 (WB)
eIF2 α	Cell Signaling Technology	5324	1:1000 (WB)
Fen1	Proteintech	14768-1-AP	1:1000 (WB)
GAPDH	Cell Signaling Technology	2118	1:2000 (WB)
H3	Proteintech	17168-1-AP	1:1000 (WB)
HPF1	Novus Biologicals	NBP1-93973	1:1000 (WB)
OGG1	Proteintech	15125-1-AP	1:1000 (WB)
PARP1	Proteintech	66520-1-Ig	1:1000 (WB)
PARP2	Abcam	ab177529	1:500 (WB)
PheRS α	Proteintech	18121-1-AP	1:1000 (WB)
PheRS β	Proteintech	16341-1-AP	1:1000 (WB)
Phospho-eEF2 (Thr56)	Cell Signaling Technology	2331	1:1000 (WB)
Phospho-eIF2 α (Ser51)	Cell Signaling Technology	3398	1:1000 (WB)
Poly (ADP-Ribose) Polymer	Abcam	ab14459	1:1000 (WB)
PP2A C	Cell Signaling Technology	2038	1:1000 (WB)
TyrRS	Abcam	ab50961	1:1000 (WB)
XRCC1	Millipore	ABE559	1:1000 (WB)

DNA Fiber Analysis

Cultured cortical neurons (DIV9) were treated with *cis*- and *trans*-RSV (50 μ M) for 8 hr, followed by the addition of thymidine analog, 50 μ M CldU (5-Chloro-2'-deoxyuridine) for 30 mins. DNA

fiber analysis was done according to established protocols^{167,168}. Briefly, cells were isolated by trypsinization, embedded in agarose plugs, and subjected to proteinase K (0.5% SDS, 0.1 M EDTA, 1 mg/ml Proteinase K) digestion at 50°C for 16 hr. Plugs were dissolved with agarose (Fisher [NEB], 50-811-726) for 16 hrs. Molecular combing was performed using the FiberComb[®] Molecular Combing System (Genomic Vision) with a constant stretching factor of 2 kb/μm using vinylsilane coverslips (20 × 20 mm; Genomic Vision), according to the manufacturer's instructions. Combed coverslips were incubated at 60°C for 2 hrs in a pre-warmed hybridization oven to minimize photo-breaking, followed by denaturation of the DNA fibers (0.5M NaOH + 1M NaCl) for 8 mins. The coverslips were then washed with PBS, followed by serial ethanol dehydration (70–100%). Following two 1x PBS washes, the coverslips were blocked in 3% BSA/1x PBS for 30 mins followed by incubation with α-BrdU (for CldU) (BD [347,580]) (1:100) and ssDNA antibody (Millipore MAB3034) (1:100), for 2 hr at 37°C. After three PBST washes, secondary antibody incubation was done using α-mouse AlexaFluor 594 and α-rat AlexaFluor 488 (1:500) for 1 hr at 37°C. Coverslips were washed three times with 1x PBST, dehydrated and mounted on slides with mounting media. The stained DNA fibers were visualized using a fluorescence microscope (EVOS FL, ThermoFisher Scientific). Analysis was performed in ImageJ by counting the total ssDNA (red) and the CldU labeled fibers (green). For each treatment condition, 300 fibers were counted, and the average ratio of CldU incorporation for ssDNA fibers per condition was used for final representation.

Immunofluorescence (IF)

Cultured cortical neurons (DIV 9-10) were fixed in 4% formaldehyde for 15 mins, followed by permeabilization and blocking for 30 mins in 5% BSA (PBS) and 0.1% (Tween20) at room

temperature. Incubation with primary antibodies was done at 4°C overnight. The names and dilutions of the primary antibodies used for IF are described below. Secondary antibodies were incubated for 1 hr at room temperature. Secondary antibodies used were: Alexa Fluor 647 (anti-chicken), Alexa Fluor 555 (anti-mouse), Alexa Fluor 488 (anti-rabbit) from Invitrogen at 1:1000 dilution. Coverslips were then mounted using DAPI (4',6-diamidino-2-phenylindole)-supplemented mounting medium, Prolong Gold Antifade (Invitrogen) and imaged with Leica DMI6000 epifluorescent microscope using oil immersion 63x/NA 1.4 objective. The quantification for total protein levels in neurons was done using ImageJ (Version 1.53c), and imaging parameters were matched for exposure, gain, and offset.

List of antibodies used for IF

Antibody	Company	Catalog No.	Dilution
MAP2	Abcam	Ab5392	1:500
phospho-histone H2AX (Ser139)	Cell Signaling Technology	9178	1:400
TyrRS	Novus Biologicals	NBP1-32551	1:200
8-hydroxy-2'-deoxyguanosine	Abcam	ab48508	1:200

Drug Treatments

All drugs/inhibitors stock solutions (1000x) were prepared in DMSO or ethanol and diluted in culture media for final concentration. *cis*-RSV was purchased from Cayman Chemicals (Item No. 10004235, $\geq 98\%$ purity) and *trans*-RSV was purchased from Millipore-Sigma (catalog No. 34092, $\geq 99\%$ purity) and the stocks were prepared in ethanol. The various compounds used for treatments and their stock concentrations are listed below.

Compound	Catalog	Stock Concentration	Final Concentration	Solvent
Rapamycin	53123, Alfa Aesar	100 μ M	5-50nM	DMSO
ISRIB	5284, Tocris	250 μ M	5-500nM	DMSO
NMDA	0114, Tocris	50mM	50 μ M	PBS
MPP+ Iodide	D048, Sigma Aldrich	100mM	50 μ M	DMSO
A484594	324516, Millipore	100 μ M	100nM	DMSO
Nelfinavir mesylate hydrate	N0986, TCI	100 μ M	20-40 μ M	PBS
L-Tyr	194759, MP Biomedicals	100mM	0.1-0.5mM	PBS
Dopamine HCl	H60255, Sigma Aldrich	100mM	0.1-0.5mM	PBS
L-DOPA	A11311, Alfa Aesar	100mM	0.1-0.5mM	PBS
L-Phe	A13238, Alfa Aesar	100mM	0.1-0.5mM	PBS
D-Tyr	143865, BTC	100mM	0.5-2mM	PBS
D-Trp	215145, BTC	100mM	0.1-1mM	PBS
D-Phe	225200, BTC	100mM	0.5-2mM	PBS
6-OHDA (hydrobromide)	25330, Cayman Chemical	100 mM	0.1 - 0.3 mM	PBS
BDNF	B3795-5UG, Sigma Aldrich	100 μ g/ml	100 ng/ml	PBS

Neurite Degeneration Index

The neurite degeneration index was calculated as described previously^{169,170}. Samples were imaged using ImageXpress Micro 4 at a magnification of 10x to capture the entire field of interest. The samples analyzed for neurite degeneration were stained using the standard immunofluorescence procedure with MAP2 (Alexa fluor 647) for neurites and DAPI staining for the nucleus. Neurite degeneration was quantified using 5-6 regions of interest of equal sizes from each treatment condition. The analysis of neurite degeneration was done using ImageJ. The fluorescent images for MAP2 staining were binarized, so that pixel intensity of regions corresponding to neurite staining was converted to black, and all other regions were converted to white. Healthy intact neurites show a continuous tract, whereas degenerated axons have a particulate structure due to fragmentation or beading. To detect degenerated neurites, we used

the particle analyzer module of ImageJ. We calculated the percentage of the area of the small fragments or particles (size = 3–10 μm^2) to the intact neurites (size > 25 μm^2) with information derived from the binary images. A degeneration index (DI) was calculated as the fragmented neurite area ratio over the intact neurite area.

Cell Viability Assays

Rat cortical neurons (DIV 10-11) were exposed to different treatments (NMDA, ETO, H_2O_2 , MPP^+) after seeding 20,000 cells/well in 96-well plates. Cell viability was then assessed at 48 hrs after the initial exposure to NMDA. 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assays were used to assess change in cell viability. Rat cortical neurons (DIV 9) were exposed to 5 μM etoposide (ETO, 28435 Chem Implex), 400 μM H_2O_2 (H1009, Sigma Aldrich) or 10 μM MPP^+ (D048, Sigma Aldrich) for 24 hrs after pre-treatment with *cis*-RSV or *trans*-RSV (50 μM) for 16 hr. Cultured rat cortical neurons were incubated with MTT (0.5 mg/mL). In the MTT assay, after 2 hr incubation, the insoluble purple product formazan resulting from the reduction of MTT by NAD(P)H-dependent oxidoreductases present in cells with viable mitochondria was solubilized in dimethyl sulfoxide at room temperature, under agitation, and protected from light. The percentage of MTT reduced as measured by the difference between the absorbances at 570 nm read in a spectrophotometer (Spectramax 190R Molecular Devices, UK). Results are presented as a percentage of control (wells incubated with the vehicle).

Use of Publicly Available Proteomics Data for the Analysis of TyrRS Levels in Human Brain

The proteomic data for TyrRS in human brain samples were obtained from the public databases as mentioned below. The graphical representation for biweight midcorrelation (BICOR) score of

TyrRS protein level in the brain was created by retrieving and analyzing data from a large-scale proteomic database associated with a previously published work in *Nat Med* **26**, 769–780 (2020)⁴⁸. The published proteomic analysis⁴⁸ used label-free mass spectrometry to quantitate the protein levels in the clinical samples of dorsolateral prefrontal cortex (DLPFC) regions of patients with or without AD. The parameters used were: disease status, scored as AD = 2, Asymptomatic AD = 1, Control = 0 (n=419), tau neurofibrillary tangle burden (Braak stage, I-VI according to increasing severity, n = 419) and cognitive performance assessed by the Cognitive Abilities Screening Instrument (CASI) score (n = 56). Differences in protein levels were assessed by two-sided Welch's t-test and corrected for multiple comparisons to obtain *p* values. Z-score was measured in terms of standard deviations from the mean.

The region-specific information about TyrRS protein levels was retrieved from a recent public brain proteomic database associated with a previously published work in *Commun Biol* **2**, 43, doi:10.1038/s42003-018-0254-9 (2019)⁸. The log fold change in TyrRS protein levels from six distinct regions from human post-mortem brain of AD cases versus asymptomatic controls, namely, entorhinal cortex (ERC), hippocampus (Hip), cingulate gyrus (CG), sensory cortex (SCx), the motor cortex (MCx) and cerebellum (CER) were identified using mass spectrometry from donors (n = 9 AD cases, n = 9 asymptomatic controls). Statistical significance was determined using a global false discovery rate (FDR) threshold of 5%, i.e., the largest set of proteins with an average local FDR $\leq 5\%$ were deemed significant.

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Author contributions.

M.J. designed and performed most of the experiments, and analyzed the data, and cowrote the manuscript. X.Z. and T.M. and C.D.K. provided tissue samples. C.N.R. helped with some of the analysis of the data. J.S. performed the DNA fiber assay and helped M.J. with the analysis. M.S. supervised the research, conceived the idea and designed experiments and analyzed the data and wrote the manuscript. All authors reviewed, edited and approved the final draft of the manuscript.

Competing interest

The authors declare no competing financial interest associated with this study.

Data availability.

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors. Correspondence and requests for materials should be addressed to Mathew Sajish, Email: mathew2@cop.sc.edu

Supplementary Figure 1

Supplementary Figure 1. Human brain protein levels of TyrRS and PheRSβ correlate with cognitive performance and AD severity, but TyrRS levels do not correlate with known molecular markers of neurodegeneration. **a** and **b**. *TyrRS* (**a**) and *PheRSβ* (**b**) are decreased in disease-affected brain regions of AD patients. Graph created by the re-analysis of published data from Xu *et al.* (2019)⁸. Changes in protein levels of TyrRS from six functionally distinct regions of human post-mortem brain of AD cases versus asymptomatic controls: entorhinal cortex (ERC), hippocampus (Hip), cingulate gyrus (CG), sensory cortex (SCx), motor cortex (MCx) and cerebellum (CER) identified using mass spectrometry. Donors ($n = 9$ AD cases, $n = 9$ asymptomatic controls) were well matched for age and post-mortem delay. Proteins with an average local false discovery rate ($FDR \leq 5\%$) were deemed significant. **c** and **d**. *Brain protein levels of TyrRS and PheRSβ correlate with human cognitive performance and dementia.* Biweight midcorrelation (BICOR) coefficient of TyrRS and PheRSβ were created using data published by Johnson *et al.* (2020)⁴⁸. Increased brain TyrRS and PheRSβ levels show a positive

correlation with human cognitive function (**c**), and its decrease correlates with AD case status and disease severity (**d**). **e.** *Mouse aging correlates with decreased ribosome occupancy of mRNA of PheRS β , not TyrRS.* Graph created by the re-analysis of published data from Anisimova, A. S. *et al.* (2020)⁵¹. Mouse livers representing six age groups (1-, 3-, 11-, 20-, 26-, and 32-month old) were used. The dynamics of age-related changes in ribosomal coverage of mRNAs were assessed by obtaining a list of nonoverlapping segments with stable footprint coverage and normalized to the mean coverage at three month. **f.** *Brian TyrRS levels do not correlate with known neurodegenerative disease markers.* BICOR coefficient of only PheRS β , not TyrRS shows a significant correlation with known molecular markers of neurodegenerative diseases.

Supplementary Figure 2

Supplementary Figure 2. Tyrosine is lower in young women but increased during human aging, and in patients with neurocognitive and metabolic disorders. a. *A model of age-dependent increase in serum tyrosine concentrations.* The model graph was built using data obtained from different longitudinal metabolomic studies assessing the effect of aging on metabolic profiles along with their study demographics mentioned in Supplementary Table 2. **b.** Illustration of various neurocognitive and metabolic disorders with reported increased in serum Tyr and/or Phe as reported in works mentioned in Supplementary Table 3.

Supplementary Figure 3

Supplementary Figure 3. BDNF stimulates the *de novo* synthesis of TyrRS in neurons. **a.**

Phenylalanine depletes TyrRS and PheRS α/β . Primary cortical neurons were treated with Phe (100-300 μ M) for 8 hr, and the levels of TyrRS, and PheRS α/β were detected by WB analysis using their specific antibodies. **b.** *L-DOPA decreases neuronal TyrRS.* Representative immunoblots showing TyrRS, PheRS α/β levels after treatment with increasing concentrations of L-DOPA (100-300 μ M) for 8hr using their specific antibodies. **c.** *6-OHDA decreases the levels of TyrRS.* Representative immunoblots showing TyrRS, PheRS β levels after treatment with increasing concentrations of 6-OHDA (100 and 200 μ M) for 8hr using their specific antibodies. **d.** *TyrRS knockdown using siRNA in rat cortical neurons.* Rat cortical neurons (DIV7) were transfected with siRNA against TyrRS or control siRNA (75nM) for 72 hr, and the levels of TyrRS were quantified using anti-TyrRS antibody. **e** and **f.** *BDNF activates the *de novo* protein synthesis of TyrRS.* Primary cortical neurons were treated with BDNF (50nM) for 1hr either alone (**e**) or in combination with rapamycin (rapa, 100nM) (**f**), and subjected to either IF (**e**) or

WB (f) analysis using anti-TyrRS antibody to detect changes in the protein levels of TyrRS. All experiments were repeated 3 independent times and quantified for statistical significance.

Supplementary Figure 4

Supplementary Figure 4. Neurotoxic agents decrease TyrRS levels in neurons. **a.** *cis*- and *trans*-RSV have opposite effects on neuronal TyrRS protein. Representative spectral images (scale bar, 20μm) the protein level of neuronal TyrRS in rat cortical neurons (DIV10) following treatment with either *cis*-RSV or *trans*-RSV for 16 hr (MAP2 – neurite marker, magenta; DAPI – nuclear marker, blue; TyrRS – red-yellow spectral image). **b.** *cis*- and *trans*-RSV have differential effects on the phosphorylation of eIF2α. Primary cortical neurons were treated with *cis*- and *trans*-RSV (5-50μM) for 2 hr and p-eIF2α were detected by WB analysis using anti-p-eIF2α antibody. **c.** *trans*-RSV sustains the levels of p-eIF2α while the effect of *cis*-RSV is transient. Primary cortical neurons were treated with *cis*- and *trans*-RSV (25μM) for up to 8 hr and changes p-eIF2α were detected by WB analysis using anti-p-eIF2α antibody. The effect of p-eIF2α was also measured using the expression levels of ATF4. **d.** *Nelfinavir* (*eEF2K* activator) depletes neuronal TyrRS. Primary cortical neurons were treated with nelfinavir (20-

40 μ M) for 8 hr and changes in the levels of TyrRS, PheRS β and p-eEF2 were detected by WB analysis using their corresponding antibodies. **e** and **f**. *cis*-RSV prevents, and *trans*-RSV exacerbates the decrease in the levels of TyrRS by neurotoxic agents. Representative spectral images (scale bar, 20 μ m) and quantification for neuronal TyrRS after treatment with neurotoxic agents (50 μ M NMDA or 100 μ M MPP⁺) for 4 hr in combination with *cis*- and *trans*-RSV (50 μ M) in rat cortical neurons (DIV9). The graphical representation is for mean $\pm$ SEM TyrRS protein levels with statistical significance calculated using Student's paired t-test.

Supplementary Figure 5

Supplementary Figure 5. Synaptic TyrRS is circadian-regulated, and sleep deprivation

increases serum tyrosine levels in humans. a. ISRIB increases TyrRS levels in neurons.

Primary cortical neurons were treated with ISRIB (≤ 50 nM) for 8 hr and changes in the levels of TyrRS, PheRS β , p-eEF2, eEF2, p-eIF2 α , and eIF2 α were detected and quantified by WB analysis using their respective antibodies. **b. ISRIB protects neurons against trans-RSV-mediated depletion of TyrRS.** Primary cortical neurons were treated with trans-RSV (25 μ M) alone or in combination with ISRIB (10 nM) for 8 hr and changes in the levels of TyrRS were detected by WB. **c. High concentrations of ISRIB decrease the levels of TyrRS in neurons.** Primary cortical neurons were treated with ISRIB (100-500 nM) for 8 hr and changes in the levels of TyrRS, and PheRS β were detected by WB analysis using their respective antibodies. **d. Synaptic TyrRS protein is circadian-regulated.** Graph depicting the circadian protein levels of synaptic TyrRS and circadian levels of serum tyrosine were generated using data from the re-analysis of the

mouse circadian proteomic (Noya, S. B. *et al.* (2019))⁹³ and metabolomic (Eckel-Mahan, K. L. *et al.* (2012))⁹⁴ data. **e.** *Human sleep deprivation disrupts circadian regulation of tyrosine.*

Representative graph depicting the circadian levels of serum tyrosine of an individual was generated using data from the re-analysis of the human circadian metabolomic data (Grant, L. K. *et al.* (2019), and (Kervezee, L. *et al.* (2019))^{95,96}.

Supplementary Figure 6

Supplementary Figure 6. D-tyrosine is neurotoxic and *trans*-RSV decreases HPF1 levels in neurons. **a.** *cis*-RSV rescues Tyr-mediated depletion of OGG1 protein. Primary cortical neurons were treated with either Tyr (200 μ M) alone or in combination with *cis*-RSV (50 μ M) for 8 hr, and the levels of OGG1 was detected by WB analysis using anti-OGG1 antibody. **b.** *D*-Tyr decreases both TyrRS and PheRS β proteins. Primary cortical neurons were treated with D-Tyr (2.5 and 5mM) for 8 hr, and the levels of TyrRS, and PheRS β were detected by WB analysis using their specific antibodies. **c.** *D*-Tyrosine induces neurotoxicity. Rat cortical neurons (DIV9) were treated with either D-Tyr or D-Phe or D-Trp (2mM) for 48 hr, and viability was assessed using MTT assay. **d.** *trans*-RSV decreases HPF1 levels. Immunoblots showing the protein levels of HPF1 in rat cortical neurons (DIV9/10) after treatment with *cis*- and *trans*-RSV for up to 16

hr. Graphs represents mean $\pm$ SEM from n = 4 experiments with statistical significance calculated using Student's paired t-test. **e.** *Quantification of Tyr-mediated decrease in the levels of HPF1 as shown in Fig. 3f.*

Supplementary Figure 7

Supplementary Figure 7. Pharmacological activation of protein synthesis protects neurons against *trans*-RSV-mediated toxicity. **a-c.** Only *cis*-RSV protects neurons against etoposide (**a**), H_2O_2 (**b**) and MPP^+ (**c**) toxicity. Rat cortical neurons (DIV9) were exposed to 5 μ M etoposide (ETO), 400 μ M H_2O_2 or 10 μ M MPP^+ for 24 hr after pre-treatment with *cis*-RSV or *trans*-RSV (50 μ M) for 16 hr. Cell viability was assessed using MTT assay. **d.** *trans*-RSV induces neuronal apoptosis. Immunoblots showing the levels of cleaved caspase-3 in rat cortical neurons (DIV9/10) after the treatment with *cis*- and *trans*-RSV (25 and 50 μ M) for 24 hr. **e.** D-Tyr induces neuronal apoptosis. Immunoblots showing the levels of cleaved caspase-3 in rat cortical neurons (DIV 9/10) after the treatment with D-Tyr (2mM) for 16 hr. **f.** ISRIB protects neurons from *trans*-RSV-mediated neurotoxicity. Rat cortical neurons (DIV8) were treated with *trans*-RSV alone or in combination with ISRIB (10nM) for 48 hrs and viability was measured using MTT assay. **g.** A-

484954 (eEF2K inhibitor) protects neurons from trans-RSV-mediated toxicity. Rat cortical neurons (DIV8) were treated with *trans*-RSV alone or in combination with A484954 (100nM) for 48 hr and viability was measured using MTT assay. **h.** *DA protects neurons from trans-RSV-mediated toxicity.* Rat cortical neurons (DIV8) were treated with *trans*-RSV alone or in combination with DA (100μM) for 72 hr and viability was measured using MTT assay. **i.** *Quantification of TyrRS protein levels in Fig. 4g.*

Supplementary Figure 8

Supplementary Figure 8. *cis*- and *trans*-RSV have opposite effects on the recruitment of DNA repair factors to the chromatin. **a.** *cis*-RSV prevents and *trans*-RSV increases the interaction of PARP1 with histone H3. Representative immunoblots and quantification of the interaction of H3 with PARP1. Cortical neurons (DIV9) were treated with *cis*- and *trans*-RSV (5-50 μ M) for 30 min and PARP1 was immunoprecipitated (IP) using antibody against PARP1. The interaction of PARP1 with H3 was determined using anti-H3 antibody. **b.** Quantification of TyrRS in the chromatin fraction in Fig. 5a. **c.** Quantification of ARH3 in the chromatin fraction in Fig. 5A. **d.** ARH3 levels are not affected in the hippocampal tissues of human AD patients. Representative immunoblots and quantification for ARH3 using anti-ARH3 antibody in the hippocampal region of AD patients (n=7) with age and sex-matched controls (n=7). **e.** *cis*-RSV and *trans*-RSV have differential effects on chromatin recruitment of DNA repair proteins. Representative immunoblots using specific antibodies, and quantification from chromatin fraction of cortical neurons (DIV9)

depicting HPF1, OGG1, FEN1, XRCC1, and PARP2 after treatment with *cis*- and *trans*-RSV (50 μ M) for 1 hr. **f.** *Quantification of neuronal DNA fibers for fig. 5d.*

Extended Data Figure 9

Supplementary Figure 9. Potential mechanism of Tyr-mediated induction of age-associated neurocognitive disorders, DNA damage, and genomic instability.

Supplementary Table 1

Patient demographics

Diagnosis	Sex	Age	PMI (hr)	APOE	Braak Stage	CERAD Score
No dementia	F	92	06:00	3/3	III	Sparse
No dementia	M	86	03:15	3/3	III	Absent
No dementia	F	95	03:05	3/4	III	Absent
No dementia	F	92	06:00	3/3	III	Sparse
No dementia	M	78	06:00	3/3	II	Sparse
No dementia	M	86	03:15	3/3	III	Absent
No dementia	F	91	11:00	4/3	III	Absent
AD	M	88	04:40	4/3	VI	Moderate
AD	M	91	07:00	3/4	V	Moderate
AD	F	96	04:45	3/3	VI	Frequent
AD	F	98	02:30	3/3	IV	Frequent
AD	M	88	04:40	4/3	VI	Moderate
AD	M	91	07:00	3/4	V	Moderate
AD	F	90	03:51	3/3	V	Frequent

PMI: post-mortem interval; *APOE*: Apolipoprotein E allele status; M: Male; F: Female

Supplementary Table 2

Plasma Tyrosine concentration	Age (mean)	Reference
64.1 ± 15.1 µM 59.8 ± 16.8 µM	46.2 ± 6.4 (n = 174, M) 43.6 ± 4.8 (n = 1150, F)	Andraos, <i>et al.</i> , 2021 ¹
61 (54-70) µM 52 (47-61) µM 63 (55-69) µM 59 (50-75) µM	26 ± 4 (n = 68, M) 25 ± 5 (n = 72, F) 71 ± 7 (n = 32, M) 72 ± 8 (n = 42, F)	Caballero, <i>et al.</i> , 1991 ²
66.48 ± 17.29 µM 78.4 ± 15.24 µM 72.59 ± 17.04 µM 81.7 ± 17.69 µM	62.71 (55-74, n = 317, F) 69.71 (62-81, n = 317, F) 62.69 (55-74, n = 273, M) 69.69 (62-81, n = 273, M)	Chak, <i>et al.</i> , 2019 ³

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Supplementary Table 3

Condition/Disease	Elevated Amino Acids	References
Aging	Tyr , Val, Gly, Leu, Ile, Gln	Chak, C.M, <i>et al.</i> 2019 ¹ Darst BF, <i>et al.</i> 2019 ² Hwangbo, N. <i>et al.</i> 2021 ³ Andraos, <i>et al.</i> 2021 ⁴ van de Rest, O., <i>et al.</i> 2017 ⁵ Ravaglia, G. <i>et al.</i> 2002 ⁶ Kochhar, S. <i>et al.</i> 2006 ⁷ Kouchiwa, T. <i>et al.</i> 2012 ⁸ Rist, M. J. <i>et al.</i> 2017 ⁹
Menopause	Tyr , Val, Glu, Ile, Gly	Auro, K. <i>et al.</i> 2014 ¹⁰
Sleep Deprivation	Tyr, Phe , Trp, Ile, Thr, Pro, Lys	Grant, L. K. <i>et al.</i> 2019 ¹¹ Gehrman, P. <i>et al.</i> 2018 ¹²
Delirium	Tyr , Trp Tyr, Phe , Trp	Pandharipande, P. P. <i>et al.</i> 2009 ¹³ Watne, L. O. <i>et al.</i> 2016 ¹⁴
Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI)	Tyr, Phe , Trp, Asp, Thr, Glu, Gln, Met, His, Pro, Val and Ala (elevated in brain of AD patients) Tyr, Phe (plasma for MCI)	Storga, D., <i>et al.</i> 1996 ¹⁵ Fekkes, D. <i>et al.</i> 1998 ¹⁶ Kim, Y. H. <i>et al.</i> 2019 ¹⁷ Huo, Z. <i>et al.</i> 2020 ¹⁸ Ravaglia, G. <i>et al.</i> 2004 ¹⁹ Ravaglia, G. <i>et al.</i> 2002 ⁶
Parkinson's Disease (PD)	Tyr, Phe	Jimenez-Jimenez, F. J. <i>et al.</i> 2020 ²⁰ Vascellari, S. <i>et al.</i> 2020 ²¹ Zhao, H. <i>et al.</i> 2018 ²² Wu, J.F. <i>et al.</i> 2016 ²³ T. Hatano, <i>et al.</i> 2016 ²⁴
Autism Spectrum Disorders (ASD)	Tyr, Phe , Glu, Asn, Ala, Lys, Arg, Cys, His, Met, Ser, Val	Skalny, A. V. <i>et al.</i> 2020 ²⁵ Zou, M. Y. <i>et al.</i> 2020 ²⁶ Aldred, S. <i>et al.</i> 2003 ²⁷
Traumatic brain injury (TBI)	Tyr, Phe	Vuille-Dit-Bille, <i>et al.</i> 2012 ²⁸
Tyrosinemia I	Tyr	Bendadi, F. <i>et al.</i> 2014 ²⁹
Tyrosinemia II	Tyr	R. Huhn, H. <i>et al.</i> 1998 ³⁰
Tyrosinemia III	Tyr	Ellaway, C. J. <i>et al.</i> 2001 ³¹
Phenylketonuria (PKU)	Phe	Blau, N., <i>et al.</i> 2010 ³²
Diabetes, Obesity, and Insulin Resistance	Tyr, Phe , Leu, Ile, Val	Felig, P., <i>et al.</i> 1969 ³³ Newgard, C. B., <i>et al.</i> 2009 ³⁴ Wang, T. J. <i>et al.</i> 2011 ³⁵ R. Yang, <i>et al.</i> 2014 ³⁶ P. Wurtz, <i>et al.</i> 2014 ³⁷ Hellmuth <i>et al.</i> , 2016 ³⁸
Cardiovascular Disease (CVD)	Tyr, Phe	Wurtz, P. <i>et al.</i> 2015 ³⁹ P. Welsh, <i>et al.</i> 2018 ⁴⁰
Hypertension	Tyr, Phe , Leu, Ile, Val	Altorf-van der Kuil, W. <i>et al.</i> 2013 ⁴¹ F. Teymoori, <i>et al.</i> 2018 ⁴²

Myocardial Ischemia-Reperfusion injury	Tyr, Phe	A. Surendran, <i>et al.</i> 2019 ⁴³
Heart failure	Tyr, Phe Met, His	Stryeck, S. <i>et al.</i> 2019 ⁴⁴ Murashige, D. <i>et al.</i> 2020 ⁴⁵ Hakuno, D., <i>et al.</i> 2015 ⁴⁶ Cheng, M. L. <i>et al.</i> 2015 ⁴⁷
Stroke	Phe , His	Vojinovic, D. <i>et al.</i> 2020 ⁴⁸
Liver Diseases (Cirrhosis Hepatitis)	Tyr, Phe , Trp	S. J. O'Keefe, <i>et al.</i> 1981 ⁴⁹ C. H. Dejong, <i>et al.</i> 2007 ⁵⁰ M. Y. Morgan, <i>et al.</i> 1982 ⁵¹ A. J. McCullough, <i>et al.</i> 1981 ⁵²
Auto-Immune Disorders	Tyr, Phe	D. Blackmore, L. Li, <i>et al.</i> 2020 ⁵³
Sepsis	Tyr, Phe , Cys, Met, Ala, Asp, Glu, Pro	H. R. Freund, <i>et al.</i> 1978 ⁵⁴ H. Freund, <i>et al.</i> 1979 ⁵⁵

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