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Vitamin E is essential for Purkinje neuron integrity

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Abstract

Alpha-tocopherol (vitamin E) is an essential dietary antioxidant with important neuroprotective functions. Alpha-tocopherol deficiency manifests primarily in neurological pathologies, notably cerebellar dysfunctions such as spinocerebellar ataxia. To study the roles of α -tocopherol in the cerebellum, we used the *Ttpa*^{-/-} mice which lack the tocopherol transfer protein (TTP) and are a faithful model of vitamin E deficiency and oxidative stress. When fed vitamin E deficient diet, *Ttpa*^{-/-} mice had un-detectable levels of α -tocopherol in plasma and several brain regions. Dietary supplementation with α -tocopherol normalized plasma levels of the vitamin, but only modestly increased its levels in the cerebellum and prefrontal cortex, indicating a critical function of brain TTP. Vitamin E deficiency caused an increase in cerebellar oxidative stress evidenced by increased protein nitrosylation, which was prevented by dietary supplementation with the vitamin. Concomitantly, vitamin E deficiency precipitated cellular atrophy and diminished dendritic branching of Purkinje neurons, the predominant output regulator of the cerebellar cortex. The anatomic decline induced by vitamin E deficiency was paralleled by behavioral deficits in motor coordination and cognitive functions that were normalized upon vitamin E supplementation. These observations underscore the essential role of vitamin E and TTP in maintaining CNS function, and support the notion that α -tocopherol supplementation may comprise an effective intervention in oxidative stress-related neurological disorders.

Keywords

vitamin E; tocopherol; Purkinje neuron; cerebellum; ataxia; oxidative stress

The term vitamin E refers to a family of plant-derived neutral lipids that function as lipid-soluble antioxidants by scavenging lipid peroxyl radicals generated in the cellular membranes. Of all naturally occurring members of the vitamin E family (tocopherols and tocotrienols), α -tocopherol is selectively enriched in blood and tissues of most vertebrates, whereas other ‘vitamers’ are excreted or catabolized (O’Byrne et al., 2000). This

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biochemical discrimination is achieved by the actions of the hepatic alpha tocopherol transfer protein (TTP; (Hosomi et al., 1997)), responsible for the selective retention of α -tocopherol, and the vitamin E ω -hydroxylase CYP4F2, which initiates catabolism of other vitamin E forms (Sontag and Parker, 2002, 2007). Adequate α -tocopherol status is considered essential for human health, and appropriate intake levels of the vitamin have been formalized by the Institutes of Medicine (Ford and Sowell, 1999, Ford et al., 2006). Despite the natural abundance of vitamin E in plant-based foods, a very significant number of individuals do not consume the recommended intake levels (Fulgoni et al., 2011a, Fulgoni et al., 2011b).

As a major lipid-soluble antioxidant, α -tocopherol is essential for all cells. However, the vitamin appears to be especially critical for CNS function. Thus, vitamin E deficiency presents primarily as neurological and neuromuscular disorders, specifically spinocerebellar ataxia (Sokol, 1988, 1990, Gohil et al., 2010, Muller, 2010). Of special interest is familial ataxia with vitamin E deficiency (AVED, OMIM #277460; (Cavalier et al., 1998)), which results from loss-of-function mutations in the tocopherol transfer protein gene, *TTPA* in humans and in the *Ttpa*^{-/-} knock-out mouse model (Terasawa et al., 2000, Yokota et al., 2001). On the anatomic levels, prevailing lesions are characterized by axonopathy and neuromuscular injuries, with appearance of axonal swellings and reduced myelination (Southam et al., 1991). Massive deposition of lipid peroxidation products and lipofuscin aggregates are indicative of CNS oxidative stress. On the functional level, affected neurons display attenuated axonal transport, and altered mitochondrial respiratory control (Thomas et al., 1993, Cuddihy et al., 2008). Electrophysiological studies in vitamin E deficient rodents indicated compromised somatosensory- and visual-evoked potentials (Goss-Sampson et al., 1988, Goss-Sampson et al., 1990, Yokota et al., 2001). In accordance with the ataxic hallmark of vitamin E deficiency, mild cerebellar atrophy was reported in vitamin E deficient patients (Sokol, 1988), and in one case, mild focal loss of cerebellar Purkinje neurons was observed (Yokota et al., 2000). Manifestation of the ataxic phenotype likely involves injury to cerebellar Purkinje neurons, since these cells are critical mediators of motor output that originates in the cerebellar cortex (Eccles et al., 1966a, d, Altman, 1972, Optican, 1998). In light of these observations, vitamin E supplementation has been utilized in a number of oxidative stress-related neurological disorders. Such intervention has proven noteworthy in human patients suffering from Alzheimer's disease, Parkinson's disease and Down's syndrome (Sano et al., 1997, Buhmann et al., 2004, Sung et al., 2004, Liu et al., 2007, Perrone et al., 2007). Similarly, in a mouse model of Alzheimer disease, vitamin E delayed disease progression (Nishida et al., 2006), possibly by attenuating lipid peroxide-induced inhibition of beta amyloid clearance (Nishida et al., 2009).

Although the critical importance of vitamin E in neurological health has been recognized for over 50 years, little is known regarding the specific roles of α -tocopherol in the CNS, or the mechanisms by which it elicits its neuroprotective effects. To begin to address this issue, we characterized the neurological manifestation of vitamin E deficiency in the CNS of the *Ttpa*^{-/-} mice, with specific emphasis on properties that can be prevented by high-level supplementation with α -tocopherol.

1. Experimental Procedures

1.1 Mice and tissues

All animal work was performed according to the Institutional Animal Care and Use Committee (IACUC)-approved protocols at Case Western Reserve University. The *Ttpa*^{-/-} (B6.129S4-Ttpa^{tm1Far/J}) mouse model was generated by targeted disruption in exons 1 and 2 of the *Ttpa* gene and was described earlier (Terasawa et al., 2000). For breeding, *Ttpa*^{+/-} female mice were crossed with *Ttpa*^{-/-} males and offspring genotype determined by PCR. At

four weeks of age, *Ttpa*^{+/+} mice were maintained on normal chow (34 mg α -tocopheryl acetate/kg diet), whereas, *Ttpa*^{-/-} mice were placed on a vitamin E deficient (no tocopherol) or vitamin E supplemented diet (600 mg α -tocopheryl acetate / kg diet). Animals were sacrificed at 17 months of age, brain tissue excised, and either fixed in 3.7% paraformaldehyde for 24 hours and paraffin-embedded, or flash frozen for use in biochemical analyses.

1.2 Golgi-Cox staining

Freshly-harvested sagittal-cut half brains were rinsed with PBS, processed according to the Rapid GolgiStain protocol (FD Neuro Technologies, Columbia, MD) and embedded in Tissue Freezing Media (Fisher). 150 μ m-thick sections were cut using a vibratome (VT1000, Leica, Buffalo Grove, IL), mounted onto gelatin-subbed slides (Fisher) and stained according to the kit protocol. Purkinje neurons that were spatially matched between the experimental groups at random cerebellar locations were chosen for analysis, and cells exhibiting obvious structural injury from sectioning were excluded. Purkinje neurons were visualized at 40 \times magnification using a Zeiss Axioplan microscope and traced utilizing NeuroLucida neuron tracing software version 5 (mbf Bioscience, Williston, VT, USA). For each experimental group, a total of 12 neurons were assessed in three different animals.

1.3 Lipid analysis

Lipids were extracted by a two-step separation process followed by silylation, as described in a previous report (Sontag and Parker, 2007). Extracted lipids were analyzed by GC-MS on a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5872 mass selective detector operated in selected ion mode. The GC-MS settings for analysis were as described in a previous report for long-chain metabolites (Sontag and Parker, 2007). α -Tocopherol and unesterified cholesterol concentrations were determined using internal deuterated standards.

1.4 Measurement of total protein-bound 3-nitrotyrosine (3-NT)

The cerebellar protein-bound 3-NT was determined immunochemically as previously described (Sultana and Butterfield, 2008). Briefly, cerebella samples were homogenized in Laemmli buffer (0.125 M Trizma base, pH 6.8, 4% sodium dodecyl sulfate, 20% glycerol) and blotted onto nitrocellulose membrane using a vacuum-manifold apparatus. The membranes were incubated with 1:1000 dilution of rabbit anti-nitrotyrosine (Sigma) antibody followed by alkaline phosphatase-conjugated goat anti-rabbit secondary antibody and visualization using Sigma fast tablet (BCIP/NBT; Sigma-Aldrich). Quantification of each sample was performed using Scion Image software.

1.5 Morphological assessment of Purkinje neurons

Paraffin-embedded sagittal-cut brains were sectioned 25, 50, and 100 μ m from the midline and stained with hematoxylin and eosin (H & E). The Purkinje neuron cell body area and number were determined in matched 500 μ m regions of lobules III, V and VIII by tracing a region of interest (ROI) around the individual somas and calculating the area using ImageJ software. Histogram distribution were fit to a Gaussian function and used to quantitatively compare the frequency of the cell body area between the experimental groups. Three animals from each experimental group were analyzed.

1.6 Rotarod

To assess motor coordination and balance mice were subjected to a Rotarod test. Each mouse performed in 4 trials on each of 4 consecutive days on a suspended accelerating Rotarod (Rotamex-5, Columbus Instruments). For each run the time to falling off the rod

(latency) and speed of the accelerating rod was recorded. Data from the final day of the testing paradigm are presented.

1.7 Fear conditioning

To assess hippocampal and amygdala function in associative learning, mice were challenged with a fear conditioning test, as previously described (Greco et al., 2010). Briefly, the testing paradigm included an initial habituation period on day 1 followed by contextual, altered context and cued testing on day 2 using a standardized fear-conditioning testing chamber equipped for sensing and recording freeze events (Med Associates, Burlington, VT). A white noise of 80 dB for 30 seconds constitutes the Conditioned Stimulus (CS) that is followed by a 0.5 mA electric shock (Unconditioned Stimulus; US). The training day included repetitions of CS and US alterations followed by the animals return to the home cage. On day 2 of the test, the number of contextual freeze events is recorded for a 5 minute test period without stimuli. Once the testing chamber is 'redecorated' with different walls, floors and odor cues the mice are again recorded for freezing events in the absence of stimuli for 5 minutes. This arm of the test measures the animals altered context fear response. During the final 3 minutes of the test, the number of freezes in the altered chamber in combination with the CS was determined to ascertain the cue-dependent fear conditioning.

1.8 Statistics

Statistical analyses and graphing were done using GraphPad Prism6 (LaJolla, CA). Student's t-test analysis was used to compare the dendritic branching in *Ttpa*^{+/+} vs. *Ttpa*^{-/-} on vitamin E deficient diets (Figure 4). One-way ANOVA was used to test for mean differences in each experiment comparing *Ttpa*^{+/+}, *Ttpa*^{-/-} -VE (vitamin E deficient), and *Ttpa*^{-/-} +VE (vitamin E supplemented). The p value and F value are presented for each ANOVA in the figure legends (Figures 1, 2, 3 and 5). Post-hoc comparisons using Tukey's procedure was used to determine significance between all three-group contrasts (*Ttpa*^{+/+} vs. *Ttpa*^{-/-} -VE, *Ttpa*^{+/+} vs. *Ttpa*^{-/-} +VE, *Ttpa*^{-/-} -VE vs. *Ttpa*^{-/-} +VE). In these analyses, the mean difference, 95% confidence intervals, and adjusted p values are provided in the figure legends.

2. Results

2.1 Vitamin E levels are diminished in *Ttpa*-null mice

Figure 1 shows the levels of α -tocopherol in plasma and cerebellar extracts from wild-type and TTP-null littermate mice. To eliminate variations resulting from changes in lipid profiles, we normalized vitamin E levels to unesterified cholesterol, and present these as mole ratios. As expected and reported earlier (Terasawa et al., 2000, Yokota et al 2001) *Ttpa*^{-/-} animals fed a supplemented diet exhibited the highest levels of circulating α -tocopherol, whereas vitamin E levels of *Ttpa*^{-/-} animals fed vitamin E deficient diet were below our limit of quantification (< 0.2 μ M, Figure 1A). Thus dietary supplementation with vitamin E completely normalizes plasma α -tocopherol levels of *Ttpa*^{-/-} mice. In the cerebellum and prefrontal cortex, however, dietary supplementation with vitamin E increased α -tocopherol levels only modestly (Figure 1B), despite the very high vitamin content of the diet (600 mg α -tocopherol / Kg diet in normal rodent chow). These findings indicate that TTP functionally is essential for equilibrating vitamin E levels in the central nervous system.

2.2 Vitamin E deficiency increases oxidative stress

In line with α -tocopherol's established antioxidant activity (Burton et al., 1983, Burton and Traber, 1990, Traber and Atkinson, 2007), vitamin E deficiency in *Ttpa*^{-/-} mice is

accompanied by significant increases in the levels of oxidative stress markers, such as TBARS (Yokota et al., 2001), F2-isoprostanes (Terasawa et al., 2000) and tHODEs (Yoshida et al., 2010). To complement these signatures of oxidative stress, we investigated the levels of 3-nitrotyrosine (3-NT). Modification of proteins by 3-NT is the outcome of nitrogen-generated reactive radical and has been shown to be elevated in AD patients and models (Butterfield et al., 2011). Indeed, data in Figure 2 show that *Ttpa*^{-/-} mice presented with a 3-fold increase in cerebellar 3-nitrotyrosine (3-NT), indicating elevation of free radical levels under conditions of vitamin E deficiency. Supplementation of *Ttpa*^{-/-} mice with vitamin E prevents the increase in 3-NT levels, albeit not completely; this indicates that brain-TTP is necessary to maintain α -tocopherol levels, and in turn control the accumulation of free radicals in the central nervous system.

2.3 Vitamin E status affects the integrity of cerebellar Purkinje neurons

The cerebellar origin of vitamin E-deficiency-induced ataxia (Cavalier et al., 1998), the critical role of Purkinje neurons in projecting cerebellar cortex output (Eccles et al., 1966e, Optican, 1998) and the known sensitivity of cerebellar Purkinje neurons to oxidative stress (Chen et al., 2003, Kern and Jones, 2006) raise the possibility that integrity of Purkinje neurons is compromised in *Ttpa*^{-/-} mice. To examine this issue, we undertook a comprehensive morphological analysis of the size, number and connectivity of cerebellar Purkinje neurons. First, we used computer-aided image analysis to assess the cell body size of individual Purkinje neurons in defined regions of the cerebellum (see Experimental Procedures). Although the absolute number of Purkinje neurons were not different between the experimental groups (data not shown), individual cell body area were drastically different (Figure 3). Accordingly, the average cell body size of the *Ttpa*^{-/-} deplete animals was markedly reduced by 25% compared to the *Ttpa*^{+/+} mice. Long-term supplementation with vitamin E completely prevented this atrophy (mean cell body size: *Ttpa*^{+/+}: 803 \pm 268 μ m²; *Ttpa*^{-/-} vitamin E deficient: 619 \pm 189 μ m²; *Ttpa*^{-/-} vitamin E supplemented: 852 \pm 282 μ m²; ANOVA: [F (2, 1405) = 106, p < 0.0001]. Tukey's post-hoc analysis *Ttpa*^{+/+} vs -VE (Mean difference = 183, 95% CI [144, 223], adjusted p < 0.0001), *Ttpa*^{+/+} vs +VE (Mean difference = 51.3, 95% CI [13.5, 89.0], adjusted p = 0.0042), -VE vs +VE (Mean difference = 235, 95% CI [195, 274], adjusted p < 0.0001) (Figure 3C). These findings indicate that vitamin E deficiency contributes to marked atrophy of the Purkinje neurons, and that this fate was prevented by high-dose, long-term supplementation with α -tocopherol. Apparently, the physiological benefit of supplementation was achieved despite the modest increase in cerebellar α -tocopherol levels (Figure 1B) and minor improvement of oxidative stress (Figure 2).

Next we examined the arborization of Purkinje neurons in the various experimental groups. Visual inspection of the Golgi-stained cerebellar sections indicated that dendritic branching of Purkinje neurons was diminished in the vitamin E deficient *Ttpa*^{-/-} animals compared to the *Ttpa*^{+/+} mice (Figures 4A, 4B). The variation in arborization between animals from the different experimental groups was confirmed by dedicated computer-aided tracing software (NeuroLucida) which analyzes the dendritic branching of Purkinje neurons in three dimensions, (as shown in Figures 4C and 4D). Sholl analysis (Figure 4E), the gold standard (Sholl, 1953) for quantitative evaluation of neuritic arborization, showed that dendrites in the vitamin E deficient *Ttpa*^{-/-} animals presented with less intersections (mean branching distance from the cell body was \sim 70 μ m and \sim 130 μ m for the vitamin E-deficient and wild-type mice, respectively), and a narrower distribution profile (area under the curve 1391 and 2756 for the vitamin E-deficient *Ttpa*^{-/-} and wild-type mice, respectively). These anatomic differences suggested that vitamin E deficiency reduced the ability of Purkinje neurons to synapse with climbing fibers of the inferior olive and the parallel fibers of the granule cells (Eccles et al., 1966c, d, e, Optican, 1998). Moreover, the absolute number of intersections in

Ttpa^{-/-} vitamin E deficient mice was significantly reduced (30%) compared to the control animals (Figure 4F). Collectively, our results suggest that vitamin E is essential for maintaining the morphological and functional integrity of the cells that are critical regulators of motor coordination.

2.4 Altered behavior in Vitamin E deficient mice

To evaluate the functional consequences of the morphological changes described above, we assessed the motor coordination ability of mice in the different experimental groups. The ability of a trained mouse to remain on a rotating rod at increasing speeds is an accepted measure of cerebellar-controlled motor coordination (Dunham and Miya, 1957, Jones and Roberts, 1968). We found that the vitamin E deficiency severely compromised performance in this assay. Specifically, the average time to fall (latency) for the vitamin E depleted *Ttpa*^{-/-} mice was approximately 3-fold shorter than that exhibited by the wild-type animals (Figure 5A). Importantly, dietary supplementation with α -tocopherol completely prevented the motor coordination deficit of the *Ttpa*^{-/-} mice. This indicated that the modest increase in cerebellar α -tocopherol content (30% of the levels in the wild-type animals; Figure 5B) was sufficient for providing protection against some functional deficits. Taken together, these observations suggest that adequate vitamin E status is essential for anatomic integrity and physiological function of the Purkinje neurons, essential regulators of motor coordination.

Although, vitamin E deficiency manifests primarily in cerebellar disorders, α -tocopherol is maintained at considerable levels in other regions of the CNS (Vatassery et al., 2006, Vatassery et al., 2007, Gohil et al., 2008). To investigate the possible roles of vitamin E in other brain regions, we subjected mice from the different experimental groups to the fear-conditioning tests, generally accepted to report on functionality of the hippocampus and amygdala (Buccafusco, 2001). We did not observe any significant differences among the experimental groups in the context-dependent or altered-context dependent results (data not shown), suggesting that hippocampal functioning is not affected by our experimental treatments. However, the vitamin E depleted *Ttpa*^{-/-} animals exhibited a profound (ca. 4-fold) deficit in the cue-dependent fear conditioning test (Figure 5B). Dietary supplementation with α -tocopherol prevented the cognitive deficit. These results support the notion that adequate vitamin E status is important in proper amygdala function and emotive learning.

3. Discussion

It has been recognized for more than fifty years that adequate levels of vitamin E are critical for maintaining neurological health. The most striking evidence for this requirement is the pathological manifestations of the familial syndrome ataxia with vitamin E deficiency (AVED) in affected humans (Ouahchi et al., 1995, Cavalier et al., 1998). These individuals present with low or non-detectable levels of plasma α -tocopherol and progressive spinocerebellar ataxia, sometimes accompanied by retinitis pigmentosa, proprioception and areflexia (Harding, 1987, Goss-Sampson et al., 1988, Sokol, 1990, Cavalier et al., 1998). Importantly, pathologic progression can be halted in affected individuals by high-dose supplementation with α -tocopherol (Harding et al., 1985, Matsuya et al., 1994, Amiel et al., 1995, Yokota et al., 1996, Cavalier et al., 1998). Similar findings were established in genetic and dietary models of vitamin E deficiency in mice (Yokota et al., 2001), rats (Einarson, 1953, Goss-Sampson et al., 1988), monkeys (Dinning and Day, 1957) and horses (Mayhew et al., 1987, Aleman, 2011).

Vitamin E deficiency-induced neurodegeneration is characterized by axonopathy, demyelination, spheroid formation and defective retrograde transport, prominently affecting the sensory neurons and more specifically the dorsal root ganglion cells (Goss-Sampson et

al., 1988, Southam et al., 1991). Importantly, our understanding of the cellular and molecular origins of the cerebellar defects observed in vitamin E deficient animals and humans has been extremely limited. In line with vitamin E's established function as an antioxidant, increased levels of oxidative stress markers have been described in the vitamin E depleted *Ttpa*^{-/-} mice. Specifically, altered levels of TBARS, 4-HNE adducts, t-IsoPGF_{2α} and t-HODE and glutathione were demonstrated in the cerebellum, cortex, and spinal cord of these mice as compared to vitamin E sufficient animals (Yokota et al., 2001, Gohil et al., 2010, Yoshida et al., 2010). These changes are thought to cause specific cellular dysfunctions such as defective retrograde axonal transport (Southam et al., 1991) and impaired mitochondrial function in affected neurons (Thomas et al., 1993, Pillai et al., 1994), which eventually lead to axonal degeneration (Koeppen, 2005). However, the specific nature and site(s) of neurological injury have not been reported. We therefore examined the anatomic and functional integrity of the cerebellum during vitamin E deficiency. As a model system we utilized a genetic mouse model of AVED in which expression of the TTP protein is disrupted (Terasawa et al., 2000, Jishage et al., 2001). These animals display systemic vitamin E deficiency, and present late-onset ataxic symptoms similar to affected humans (Yokota et al., 2001). To correlate the biochemical, anatomical and functional deficits that accompany vitamin E deficiency, we terminated our experiments at the age of 17 months, when overt ataxic symptoms were obvious.

Multiple neurodegenerative diseases are characterized by deterioration of Purkinje neurons and ataxia, such as ataxia-telangiectasia (A-T) Paula-Barbosa et al., 1983, Gatti and Vinters, 1985), Friedreich ataxia (Simon et al., 2004) and Niemann Pick Type C disease (NPC) (Yu and Lieberman, 2013). Our findings demonstrate that prolonged vitamin E deficiency causes marked damage to cerebellar Purkinje neurons. Specifically, we observed atrophy of the cell bodies (Figure 3) and diminished dendritic branching (Figure 4) of these cells. To our knowledge, this is the first detailed illustration of morphological aberrations observed in the Purkinje neurons during vitamin E deficiency. Since Purkinje neurons are key regulators of motor coordination of the cerebellar cortex (Eccles et al., 1966a, d, b, e, Optican, 1998, Womack and Khodakhah, 2003), the anatomic deterioration of these cells likely contributes to the ataxic manifestations of vitamin E deficiency. Since our studies focused on only on cerebellar Purkinje neurons, we cannot rule out the possibility that the primary injury occurred at other site(s), rendering pathological consequences described here to be secondary in nature. Also, we cannot conclusively determine the involvement of other cell-types in vitamin E deficiency-induced cerebellar damage. For example, our findings may reflect the outcome of demyelination or sensory neuropathy that cause compromise Purkinje neuron integrity. Multiple studies demonstrated that vitamin E deficiency negatively affects the composition, transcriptome profile and mitochondrial function in myelin (Enrione et al., 1999, Yokota et al., 2001, Podratz et al., 2004, Cuddihy et al., 2008, Gohil et al., 2004, Hyland et al., 2006). Similarly, oligodendrocytes dysfunction is known to contribute to Purkinje neuron degeneration in NPC disease and multiple sclerosis (Ko et al., 2005, Yu and Lieberman, 2013). Lastly, axonal dystrophy in sensory tracts has been reported in AVED patients (Sung et al., 1981, Harding, 1982, Harding et al., 1985), in cystic fibrosis patients, in secondary vitamin E deficiency in humans (Geller et al., 1977), and in rodent models of vitamin E deficiency (Sung et al., 1981, Southam et al., 1991, Yokota et al., 2001). Thus, despite the data that link vitamin E deficiency to Purkinje neuron degeneration, the detailed process that underlies disease etiology awaits further investigation.

We note that structural and functional damage to the cerebellum was evident at a relatively late stage of deficiency (17 months) as compared to published reports of the onset of oxidative stress in the same deficiency model. Reduction in cerebellar glutathione was reported to occur after 5 months (Gohil et al., 2008) and increased plasma lipid peroxidation was obvious at 6 months (Yoshida et al., 2010). These observations indicate that oxidative

stress preceded the pathological changes in Purkinje neurons and the ataxic phenotype, and support a cause-and-effect relationship between oxidative stress and cerebellar damage. Moreover, our findings suggest a modest increase in vitamin E in the cerebellum can sufficiently decrease levels of free radical generation enough to elicit beneficial outcomes.

Inherited ataxias, such as Friedreich's ataxia and SCA-6 (Koeppen, 2005, 2011) share with vitamin E deficiency not only deterioration of Purkinje neurons, but also a dramatic elevation in the levels of oxidative stress markers (Pandolfo, 2008) and low-rate of correct diagnosis. The efficacy of vitamin E supplementation in preventing neurological decline reported here (Figures 2 and 4) and elsewhere (Ren et al., 2006, Gohil et al., 2008, Nishida et al., 2009, Gohil et al., 2010) indicates that supplementation with α -tocopherol may compromise a valuable therapeutic approach. In support of this notion a limited trial reported that intervention with vitamin E and coenzyme Q led to significant improvements in standardized ataxia scores in Friedreich's ataxia patients (Hart et al., 2005). Careful examination of similar interventions in the treatment of other oxidative stress-related neuropathologies will provide critically valuable information.

It is interesting to note that although the dose of α -tocopherol used here normalized plasma levels of the vitamin (Figure 1A), it had only a modest effect on concentrations of the vitamin in the cerebellum and prefrontal cortex (<30% of the levels in un-supplemented wild-type animals, Figure 1B). This observation indicates that TTP is essential for 'communicating' between two separate pools of vitamin E: one in the circulation and the other in the CNS. Indeed, in cases of the heritable mutations in *TTPA*, extremely high doses of vitamin E (up to 2 g α -tocopherol/day) are necessary to achieve symptomatic effect (Gohil et al., 2010). An additional important conclusion from these results is that in cases of TTP dysfunction (e.g. in AVED patients or in carriers of common polymorphic variants in the TTP promoter; (Wright et al., 2009, Ulatowski et al., 2012), plasma concentrations of α -tocopherol levels *do not* reflect vitamin E status in the CNS. It is therefore critically important to examine the utility of CNS-localized markers for diagnostic assessment, such as concentrations of α -tocopherol or oxidative stress markers in the cerebrospinal fluid (Hensley et al., 2011).

In addition to the established deficits in motor coordination that accompany vitamin E deficiency, we observed that *Ttpa*^{-/-} mice exhibit a cognitive deficit, manifesting in reduced response to the fear-conditioning test, specifically in the altered environment cue-dependent challenge (Figure 5B). These results indicate that vitamin E deficiency caused a functional compromise of the amygdala region of the brain, thought to control memory formation and emotive responses. Since accurate measurements of α -tocopherol levels in the amygdala are technically challenging, we measure the vitamin's level in the prefrontal cortex, which modulates amygdalar emotive stimuli. Levels of α -tocopherol in this region in deficient *Ttpa*^{-/-} mice were below our detection level (Figure 1B).

A number of groups reported that the levels of vitamin E vary among different regions of the brain (Vatassery et al., 2006, Vatassery et al., 2007, Gohil et al., 2008). These observations indicate that selected micro-environments in the CNS regulate α -tocopherol's level independently of the cerebellar vitamin pool. These isolated pools of the antioxidant may be meaningful in disease conditions, and underscore the need to understand the function and transport of vitamin E and TTP throughout the CNS.

4. Conclusion

Taken together, our findings extend previous observations regarding the critical roles of α -tocopherol in the CNS, and provide new insights into the requirement for vitamin E in

supporting the functions of cerebellar Purkinje neurons and the amygdala. Ongoing work on the transport of vitamin E within the CNS will reveal the molecular mechanisms that underlie these phenomena.

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References

- Aleman M. Miscellaneous neurologic or neuromuscular disorders in horses. *The Veterinary clinics of North America Equine practice*. 2011; 27:481–506. [PubMed: 22100041]
- Altman J. Postnatal development of the cerebellar cortex in the rat. II. Phases in the maturation of Purkinje cells and of the molecular layer. *J Comp Neurol*. 1972; 145:399–463. [PubMed: 5044254]
- Amiel J, Maziere JC, Beucler I, Koenig M, Reutenauer L, Loux N, Bonnefont D, Feo C, Landrieu P. Familial isolated vitamin E deficiency. Extensive study of a large family with a 5-year therapeutic follow-up. *J Inher Metab Dis*. 1995; 18:333–340. [PubMed: 7474901]
- Buccafusco, JJ. *Methods of behavior analysis in neuroscience*. Boca Raton: CRC Press; 2001.
- Buhmann C, Arlt S, Kontush A, Moller-Bertram T, Sperber S, Oechsner M, Stuerenburg HJ, Beisiegel U. Plasma and CSF markers of oxidative stress are increased in Parkinson's disease and influenced by antiparkinsonian medication. *Neurobiol Dis*. 2004; 15:160–170. [PubMed: 14751781]
- Burton GW, Cheeseman KH, Doba T, Ingold KU, Slater TF. Vitamin E as an antioxidant in vitro and in vivo. *Ciba Found Symp*. 1983; 101:4–18. [PubMed: 6557906]
- Burton GW, Traber MG. Vitamin E: antioxidant activity, biokinetics, and bioavailability. *Annu Rev Nutr*. 1990; 10:357–382. [PubMed: 2200468]
- Butterfield DA, Reed T, Sultana R. Roles of 3-nitrotyrosine- and 4-hydroxynonenal-modified brain proteins in the progression and pathogenesis of Alzheimer's disease. *Free Radic Res*. 2011; 45:59–72. [PubMed: 20942567]
- Cavalier L, Ouahchi K, Kayden HJ, Di Donato S, Reutenauer L, Mandel JL, Koenig M. Ataxia with isolated vitamin E deficiency: heterogeneity of mutations and phenotypic variability in a large number of families. *Am J Hum Genet*. 1998; 62:301–310. [PubMed: 9463307]
- Chen P, Peng C, Luff J, Spring K, Watters D, Bottle S, Furuya S, Lavin MF. Oxidative stress is responsible for deficient survival and dendritogenesis in purkinje neurons from ataxia-telangiectasia mutated mutant mice. *J Neurosci*. 2003; 23:11453–11460. [PubMed: 14673010]
- Cuddihy SL, Ali SS, Musiek ES, Lucero J, Kopp SJ, Morrow JD, Dugan LL. Prolonged alpha-tocopherol deficiency decreases oxidative stress and unmasks alpha-tocopherol-dependent regulation of mitochondrial function in the brain. *J Biol Chem*. 2008; 283:6915–6924. [PubMed: 18180306]
- Dinning JS, Day PL. Vitamin E deficiency in the monkey. I. Muscular dystrophy, hematologic changes, and the excretion of urinary nitrogenous constituents. *J Exp Med*. 1957; 105:395–402. [PubMed: 13428910]
- Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. *Journal of the American Pharmaceutical Association American Pharmaceutical Association*. 1957; 46:208–209. [PubMed: 13502156]
- Eccles JC, Llinas R, Sasaki K. The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. *J Physiol*. 1966a; 182:268–296. [PubMed: 5944665]
- Eccles JC, Llinas R, Sasaki K. The inhibitory interneurons within the cerebellar cortex. *Exp Brain Res*. 1966b; 1:1–16. [PubMed: 5910941]
- Eccles JC, Llinas R, Sasaki K. Intracellularly recorded responses of the cerebellar Purkinje cells. *Exp Brain Res*. 1966c; 1:161–183. [PubMed: 5943696]

- Eccles JC, Llinas R, Sasaki K. The mossy fibre-granule cell relay of the cerebellum and its inhibitory control by Golgi cells. *Exp Brain Res.* 1966d; 1:82–101. [PubMed: 5910945]
- Eccles JC, Llinas R, Sasaki K. Parallel fibre stimulation and the responses induced thereby in the Purkinje cells of the cerebellum. *Exp Brain Res.* 1966e; 1:17–39. [PubMed: 5910940]
- Einarson L. Deposits of fluorescent acid-fast products in the nervous system and skeletal muscles of adult rats with chronic vitamin-E deficiency. *J Neurol Neurosurg Psychiatry.* 1953; 16:98–109. [PubMed: 13053230]
- Enrione EB, Weeks OI, Kranz S, Shen J. A vitamin E-deficient diet affects nerve regeneration in rats. *Nutrition.* 1999; 15:140–144. [PubMed: 9990579]
- Ford ES, Schleicher RL, Mokdad AH, Ajani UA, Liu S. Distribution of serum concentrations of alpha-tocopherol and gamma-tocopherol in the US population. *Am J Clin Nutr.* 2006; 84:375–383. [PubMed: 16895886]
- Ford ES, Sowell A. Serum alpha-tocopherol status in the United States population: findings from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol.* 1999; 150:290–300. [PubMed: 10430234]
- Fulgoni VL 3rd, Keast DR, Auestad N, Quann EE. Nutrients from dairy foods are difficult to replace in diets of Americans: food pattern modeling and an analyses of the National Health and Nutrition Examination Survey 2003-2006. *Nutrition research.* 2011a; 31:759–765. [PubMed: 22074800]
- Fulgoni VL 3rd, Keast DR, Bailey RL, Dwyer J. Foods, fortificants, and supplements: Where do Americans get their nutrients? *J Nutr.* 2011b; 141:1847–1854. [PubMed: 21865568]
- Gatti RA, Vinters HV. Cerebellar pathology in ataxia-telangiectasia: the significance of basket cells. *Kroc Foundation series.* 1985; 19:225–232. [PubMed: 3864937]
- Geller A, Gilles F, Shwachman H. Degeneration of fasciculus gracilis in cystic fibrosis. *Neurology.* 1977; 27:185–187. [PubMed: 556835]
- Gohil K, Godzdzanker R, O'Roark E, Schock BC, Kaini RR, Packer L, Cross CE, Traber MG. Alpha-tocopherol transfer protein deficiency in mice causes multi-organ deregulation of gene networks and behavioral deficits with age. *Ann N Y Acad Sci.* 2004; 1031:109–126. [PubMed: 15753139]
- Gohil K, Oommen S, Quach HT, Vasu VT, Aung HH, Schock B, Cross CE, Vatassery GT. Mice lacking alpha-tocopherol transfer protein gene have severe alpha-tocopherol deficiency in multiple regions of the central nervous system. *Brain Res.* 2008; 1201:167–176. [PubMed: 18299118]
- Gohil K, Vasu VT, Cross CE. Dietary alpha-tocopherol and neuromuscular health: search for optimal dose and molecular mechanisms continues! *Mol Nutr Food Res.* 2010; 54:693–709. [PubMed: 20187127]
- Goss-Sampson MA, Kriss A, Muller DP. A longitudinal study of somatosensory, brainstem auditory and peripheral sensory-motor conduction during vitamin E deficiency in the rat. *J Neurol Sci.* 1990; 100:79–84. [PubMed: 2089143]
- Goss-Sampson MA, MacEvilly CJ, Muller DP. Longitudinal studies of the neurobiology of vitamin E and other antioxidant systems, and neurological function in the vitamin E deficient rat. *J Neurol Sci.* 1988; 87:25–35. [PubMed: 2461431]
- Greco SJ, Bryan KJ, Sarkar S, Zhu X, Smith MA, Ashford JW, Johnston JM, Tezapsidis N, Casadesus G. Leptin reduces pathology and improves memory in a transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis.* 2010; 19:1155–1167. [PubMed: 20308782]
- Harding AE. The clinical features and classification of the late onset autosomal dominant cerebellar ataxias. A study of 11 families, including descendants of the 'the Drew family of Walworth'. *Brain.* 1982; 105:1–28. [PubMed: 7066668]
- Harding AE. Vitamin E and the nervous system. *Critical reviews in neurobiology.* 1987; 3:89–103. [PubMed: 3552452]
- Harding AE, Matthews S, Jones S, Ellis CJ, Booth IW, Muller DP. Spinocerebellar degeneration associated with a selective defect of vitamin E absorption. *N Engl J Med.* 1985; 313:32–35. [PubMed: 4000224]
- Hart PE, Lodi R, Rajagopalan B, Bradley JL, Crilley JG, Turner C, Blamire AM, Manners D, Styles P, Schapira AH, Cooper JM. Antioxidant treatment of patients with Friedreich ataxia: four-year follow-up. *Arch Neurol.* 2005; 62:621–626. [PubMed: 15824263]

- Hensley K, Barnes LL, Christov A, Tangney C, Honer WG, Schneider JA, Bennett DA, Morris MC. Analysis of postmortem ventricular cerebrospinal fluid from patients with and without dementia indicates association of vitamin E with neuritic plaques and specific measures of cognitive performance. *J Alzheimers Dis.* 2011; 24:767–774. [PubMed: 21321395]
- Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H, Inoue K. Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Lett.* 1997; 409:105–108. [PubMed: 9199513]
- Hyland S, Muller D, Hayton S, Stoecklin E, Barella L. Cortical gene expression in the vitamin E-deficient rat: possible mechanisms for the electrophysiological abnormalities of visual and neural function. *Ann Nutr Metab.* 2006; 50:433–441. [PubMed: 16847396]
- Jishage K, Arita M, Igarashi K, Iwata T, Watanabe M, Ogawa M, Ueda O, Kamada N, Inoue K, Arai H, Suzuki H. Alpha-tocopherol transfer protein is important for the normal development of placental labyrinthine trophoblasts in mice. *J Biol Chem.* 2001; 276:1669–1672. [PubMed: 11076932]
- Jones BJ, Roberts DJ. The quantitative measurement of motor inco-ordination in naive mice using an accelerating rotarod. *J Pharm Pharmacol.* 1968; 20:302–304. [PubMed: 4384609]
- Kern JK, Jones AM. Evidence of toxicity, oxidative stress, and neuronal insult in autism. *J Toxicol Environ Health B Crit Rev.* 2006; 9:485–499. [PubMed: 17090484]
- Ko DC, Milenkovic L, Beier SM, Manuel H, Buchanan J, Scott MP. Cell-autonomous death of cerebellar purkinje neurons with autophagy in Niemann-Pick type C disease. *PLoS genetics.* 2005; 1:81–95. [PubMed: 16103921]
- Koeppe AH. The pathogenesis of spinocerebellar ataxia. *Cerebellum.* 2005; 4:62–73. [PubMed: 15895563]
- Koeppe AH. Friedreich's ataxia: pathology, pathogenesis, and molecular genetics. *J Neurol Sci.* 2011; 303:1–12. [PubMed: 21315377]
- Liu Q, Xie F, Rolston R, Moreira PI, Nunomura A, Zhu X, Smith MA, Perry G. Prevention and treatment of Alzheimer disease and aging: antioxidants. *Mini Rev Med Chem.* 2007; 7:171–180. [PubMed: 17305591]
- Matsuya M, Matsumoto H, Chiba S, Kashiwagi M, Kasahara M. A sporadic case of essential vitamin E deficiency manifested by sensory-dominant polyneuropathy and retinitis pigmentosa. *No To Shinkei.* 1994; 46:989–994. [PubMed: 7826714]
- Mayhew IG, Brown CM, Stowe HD, Trapp AL, Derksen FJ, Clement SF. Equine degenerative myeloencephalopathy: a vitamin E deficiency that may be familial. *J Vet Intern Med.* 1987; 1:45–50. [PubMed: 3506620]
- Muller DP. Vitamin E and neurological function. *Mol Nutr Food Res.* 2010; 54:710–718. [PubMed: 20183831]
- Nishida Y, Ito S, Ohtsuki S, Yamamoto N, Takahashi T, Iwata N, Jishage K, Yamada H, Sasaguri H, Yokota S, Piao W, Tomimitsu H, Saido TC, Yanagisawa K, Terasaki T, Mizusawa H, Yokota T. Depletion of vitamin E increases amyloid beta accumulation by decreasing its clearances from brain and blood in a mouse model of Alzheimer disease. *J Biol Chem.* 2009; 284:33400–33408. [PubMed: 19679659]
- Nishida Y, Yokota T, Takahashi T, Uchihara T, Jishage K, Mizusawa H. Deletion of vitamin E enhances phenotype of Alzheimer disease model mouse. *Biochem Biophys Res Commun.* 2006; 350:530–536. [PubMed: 17026966]
- O'Byrne D, Grundy S, Packer L, Devaraj S, Baldenius K, Hoppe PP, Kraemer K, Jialal I, Traber MG. Studies of LDL oxidation following alpha-, gamma-, or delta-tocotrienyl acetate supplementation of hypercholesterolemic humans. *Free Radic Biol Med.* 2000; 29:834–845. [PubMed: 11063909]
- Optican LM. Low rates yield big returns. *Nat Neurosci.* 1998; 1:8–9. [PubMed: 10195099]
- Ouahchi K, Arita M, Kayden H, Hentati F, Ben Hamida M, Sokol R, Arai H, Inoue K, Mandel JL, Koenig M. Ataxia with isolated vitamin E deficiency is caused by mutations in the alpha-tocopherol transfer protein. *Nat Genet.* 1995; 9:141–145. [PubMed: 7719340]
- Pandolfo M. Friedreich ataxia. *Arch Neurol.* 2008; 65:1296–1303. [PubMed: 18852343]
- Paula-Barbosa MM, Ruela C, Tavares MA, Pontes C, Saraiva A, Cruz C. Cerebellar cortex ultrastructure in ataxia-telangiectasia. *Ann Neurol.* 1983; 13:297–302. [PubMed: 6847143]

- Perrone S, Longini M, Bellieni CV, Centini G, Kenanidis A, De Marco L, Petraglia F, Buonocore G. Early oxidative stress in amniotic fluid of pregnancies with Down syndrome. *Clin Biochem.* 2007; 40:177–180. [PubMed: 17208212]
- Pillai SR, Traber MG, Kayden HJ, Cox NR, Toivio-Kinnucan M, Wright JC, Braund KG, Whitley RD, Gilger BC, Steiss JE. Concomitant brainstem axonal dystrophy and necrotizing myopathy in vitamin E-deficient rats. *J Neurol Sci.* 1994; 123:64–73. [PubMed: 8064324]
- Podratz JL, Rodriguez EH, Windebank AJ. Antioxidants are necessary for myelination of dorsal root ganglion neurons, in vitro. *Glia.* 2004; 45:54–58. [PubMed: 14648545]
- Ren YR, Nishida Y, Yoshimi K, Yasuda T, Jishage K, Uchihara T, Yokota T, Mizuno Y, Mochizuki H. Genetic vitamin E deficiency does not affect MPTP susceptibility in the mouse brain. *J Neurochem.* 2006; 98:1810–1816. [PubMed: 16787402]
- Sano M, Ernesto C, Thomas RG, Klauber MR, Schafer K, Grundman M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS, Thal LJ. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N Engl J Med.* 1997; 336:1216–1222. [PubMed: 9110909]
- Sholl DA. Dendritic organization in the neurons of the visual and motor cortices of the cat. *J Anat.* 1953; 87:387–406. [PubMed: 13117757]
- Simon D, Seznec H, Gansmuller A, Carelle N, Weber P, Metzger D, Rustin P, Koenig M, Puccio H. Friedreich ataxia mouse models with progressive cerebellar and sensory ataxia reveal autophagic neurodegeneration in dorsal root ganglia. *J Neurosci.* 2004; 24:1987–1995. [PubMed: 14985441]
- Sokol RJ. Vitamin E deficiency and neurologic disease. *Annu Rev Nutr.* 1988; 8:351–373. [PubMed: 3060170]
- Sokol RJ. Vitamin E and neurologic deficits. *Adv Pediatr.* 1990; 37:119–148. [PubMed: 2176058]
- Sontag TJ, Parker RS. Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status. *J Biol Chem.* 2002; 277:25290–25296. [PubMed: 11997390]
- Sontag TJ, Parker RS. Influence of major structural features of tocopherols and tocotrienols on their omega-oxidation by tocopherol-omega-hydroxylase. *J Lipid Res.* 2007; 48:1090–1098. [PubMed: 17284776]
- Southam E, Thomas PK, King RH, Goss-Sampson MA, Muller DP. Experimental vitamin E deficiency in rats. Morphological and functional evidence of abnormal axonal transport secondary to free radical damage. *Brain.* 1991; 114(Pt 2):915–936. [PubMed: 1710528]
- Sultana R, Butterfield DA. Slot-blot analysis of 3-nitrotyrosine-modified brain proteins. *Methods Enzymol.* 2008; 440:309–316. [PubMed: 18423227]
- Sung JH, Mastri AR, Park SH. Axonal dystrophy in the gracile nucleus in children and young adults. Reappraisal of the incidence and associated diseases. *J Neuropathol Exp Neurol.* 1981; 40:37–45. [PubMed: 7205326]
- Sung S, Yao Y, Uryu K, Yang H, Lee VM, Trojanowski JQ, Pratico D. Early vitamin E supplementation in young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer's disease. *FASEB J.* 2004; 18:323–325. [PubMed: 14656990]
- Terasawa Y, Ladha Z, Leonard SW, Morrow JD, Newland D, Sanan D, Packer L, Traber MG, Farese RV Jr. Increased atherosclerosis in hyperlipidemic mice deficient in alpha-tocopherol transfer protein and vitamin E. *Proc Natl Acad Sci U S A.* 2000; 97:13830–13834. [PubMed: 11095717]
- Thomas PK, Cooper JM, King RH, Workman JM, Schapira AH, Goss-Sampson MA, Muller DP. Myopathy in vitamin E deficient rats: muscle fibre necrosis associated with disturbances of mitochondrial function. *J Anat.* 1993; 183(Pt 3):451–461. [PubMed: 8300427]
- Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. *Free Radic Biol Med.* 2007; 43:4–15. [PubMed: 17561088]
- Ulatowski L, Dreussi C, Noy N, Barnholtz-Sloan J, Klein E, Manor D. Expression of the alpha-tocopherol transfer protein gene is regulated by oxidative stress and common single-nucleotide polymorphisms. *Free Radic Biol Med.* 2012; 53:2318–2326. [PubMed: 23079030]
- Vatassery GT, Lam C, Smith WE, Quach HT. Apolipoprotein E exerts selective and differential control over vitamin E concentrations in different areas of mammalian brain. *J Neurosci Res.* 2006; 84:1335–1342. [PubMed: 16941498]

- Vatassery GT, Quach HT, Smith WE, Santacruz KS, Roy S. Apolipoprotein e deficiency leads to altered brain uptake of alpha tocopherol injected into lateral cerebral ventricles. *Biochim Biophys Acta*. 2007; 1772:797–803. [PubMed: 17560088]
- Womack MD, Khodakhah K. Somatic and dendritic small-conductance calcium-activated potassium channels regulate the output of cerebellar Purkinje neurons. *J Neurosci*. 2003; 23:2600–2607. [PubMed: 12684445]
- Wright ME, Peters U, Gunter MJ, Moore SC, Lawson KA, Yeager M, Weinstein SJ, Snyder K, Virtamo J, Albanes D. Association of variants in two vitamin e transport genes with circulating vitamin e concentrations and prostate cancer risk. *Cancer Res*. 2009; 69:1429–1438. [PubMed: 19190344]
- Yokota T, Igarashi K, Uchihara T, Jishage K, Tomita H, Inaba A, Li Y, Arita M, Suzuki H, Mizusawa H, Arai H. Delayed-onset ataxia in mice lacking alpha -tocopherol transfer protein: model for neuronal degeneration caused by chronic oxidative stress. *Proc Natl Acad Sci U S A*. 2001; 98:15185–15190. [PubMed: 11752462]
- Yokota T, Shiojiri T, Gotoda T, Arai H. Retinitis pigmentosa and ataxia caused by a mutation in the gene for the alpha-tocopherol-transfer protein. *N Engl J Med*. 1996; 335:1770–1771. [PubMed: 8965888]
- Yokota T, Uchihara T, Kumagai J, Shiojiri T, Pang JJ, Arita M, Arai H, Hayashi M, Kiyosawa M, Okeda R, Mizusawa H. Postmortem study of ataxia with retinitis pigmentosa by mutation of the alpha-tocopherol transfer protein gene. *J Neurol Neurosurg Psychiatry*. 2000; 68:521–525. [PubMed: 10727494]
- Yoshida Y, Itoh N, Hayakawa M, Habuchi Y, Saito Y, Tsukamoto Y, Cynshi O, Jishage K, Arai H, Niki E. The role of alpha-tocopherol in motor hypofunction with aging in alpha-tocopherol transfer protein knockout mice as assessed by oxidative stress biomarkers. *J Nutr Biochem*. 2010; 21:66–76. [PubMed: 19157826]
- Yu T, Lieberman AP. Npc1 acting in neurons and glia is essential for the formation and maintenance of CNS myelin. *PLoS genetics*. 2013; 9:e1003462. [PubMed: 23593041]

Abbreviations

<i>TTPA</i> and <i>Ttpa</i> for the human and murine versions respectively	α-tocopherol transfer protein gene
TTP	α -tocopherol transfer protein
CYP4F2	cytochrome P450 family 4, subfamily F, polypeptide 2
AD	Alzheimer's disease
PD	Parkinson's disease
DS	Down's syndrome
AVED	ataxia with vitamin E deficiency
AUC	area under the curve
SNPs	single nucleotide polymorphisms
4-HNE	4-hydroxy-2-nonenal
MDA	malondialdehyde
t-HODE	total hydroxyoctadecadienoic acid
t-IsoPGF_{2α}	total iso-prostaglandin F _{2α}
ROS	reactive oxygen species
IOM	Institute of Medicine

SCA	spinocerebellar ataxia
3-NT	3-nitrotyrosine
NPC	Niemann Pick disease type C
MS	Multiple Sclerosis

Highlights

1. Vitamin E deficiency results in elevated oxidative stress in the cerebellum
2. Vitamin E deficiency results in impaired motor and cognitive functions
3. Vitamin E deficiency results in deterioration of Purkinje neurons.
4. Vitamin E supplementation prevents anatomic and functional damage.
5. Adequate vitamin E status is essential for optimal neurological health.

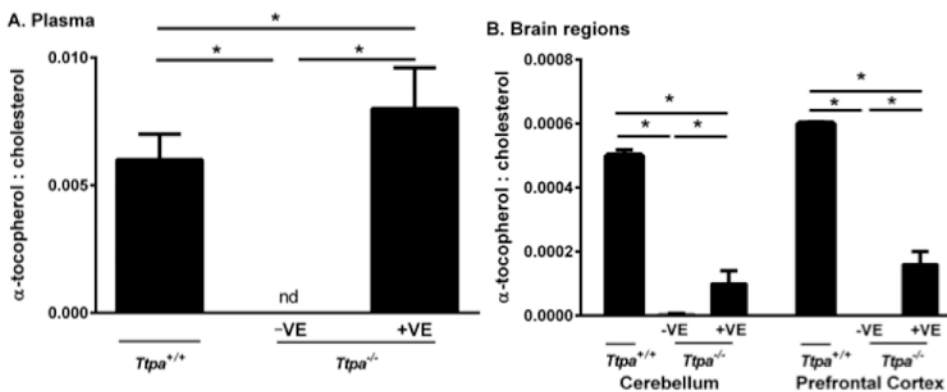


Figure 1. α -tocopherol status in plasma (A) and brain regions (B) of experimental models
 α -tocopherol was assayed by GC-MS and normalized to unesterified cholesterol content of the same sample -VE: vitamin E deficient diet; +VE: vitamin E supplemented diet; CX- prefrontal cortex and CB- cerebellum; *nd* indicates 'non-detectable'. Error bars reflect the standard deviation of the mean. $n=4-5$ animals per group. Asterisks indicate statistically significant differences of $p < 0.05$ per one-way ANOVA and Tukey's post-hoc analysis. Plasma ANOVA: [F (2, 8) = 53.07, $p = 0.000024$]. CX ANOVA: [F (2, 12) = 885.1, $p = < 0.0001$]. CB ANOVA: [F (2, 12) = 537, $p = < 0.0001$]. Tukey's post-hoc: Plasma: *Ttpa*^{+/+} vs -VE (Mean difference= 0.0054, 95% CI [0.0033, 0.0075], adjusted $p=0.0002$), *Ttpa*^{+/+} vs +VE (Mean difference= -0.0025, 95% CI [-0.0048, -0.0002], adjusted $p=0.03$), -VE vs +VE (Mean difference= -0.0079, 95% CI [-0.0101, -0.0056], adjusted $p < 0.0001$); CX: *Ttpa*^{+/+} vs -VE (Mean difference= 0.0006, 95% CI [0.00056, 0.00064], adjusted $p < 0.0001$), *Ttpa*^{+/+} vs +VE (Mean difference= 0.00044, 95% CI [0.00040, 0.00048], adjusted $p < 0.0001$), -VE vs +VE (Mean difference= -0.00016, 95% CI [-0.000199, -0.000121], adjusted $p < 0.0001$); CB: *Ttpa*^{+/+} vs -VE (Mean difference= 0.000495, 95% CI [0.000452, 0.000538], adjusted $p < 0.0001$), *Ttpa*^{+/+} vs +VE (Mean difference= -0.0004, 95% CI [0.00036, 0.00044], adjusted $p < 0.0001$), -VE vs +VE (Mean difference= -0.000095, 95% CI [-0.000138, -0.000052], adjusted $p = 0.0002$).

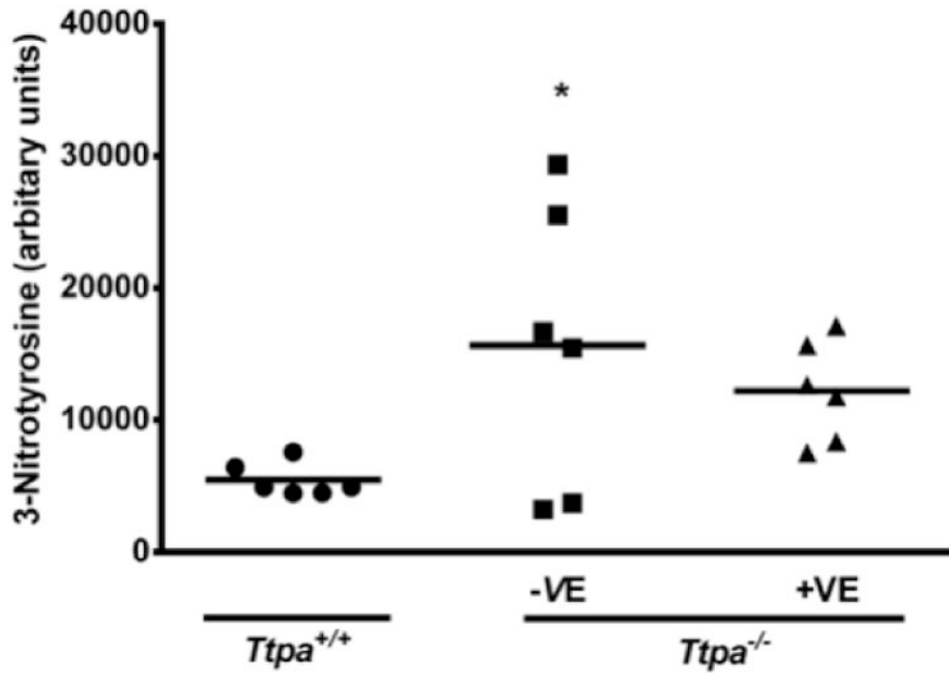


Figure 2. Oxidative stress is prevented with vitamin E supplementation

3-nitrotyrosine (3-NT) levels in cerebella lysates from each experimental group (*Ttpa*^{+/+}; *Ttpa*^{-/-} -VE: vitamin E deficient diet; *Ttpa*^{-/-} +VE: vitamin E supplemented diet) were measured by slot blot analysis method and quantified as described in Experimental procedures. Data are represented in a scatter plot, in which the horizontal line designates the mean value. Asterisks indicate a statistically significant difference between the *Ttpa*^{-/-} mice and wild-type littermates, as determined by one-way ANOVA [F (2, 15) = 3.68, p= 0.0499] and Tukey's multiple comparisons post-hoc analysis *Ttpa*^{+/+} vs -VE (Mean difference= -10228, 95% CI [-20175, -280.1], adjusted p=0.04); n=6 animals.

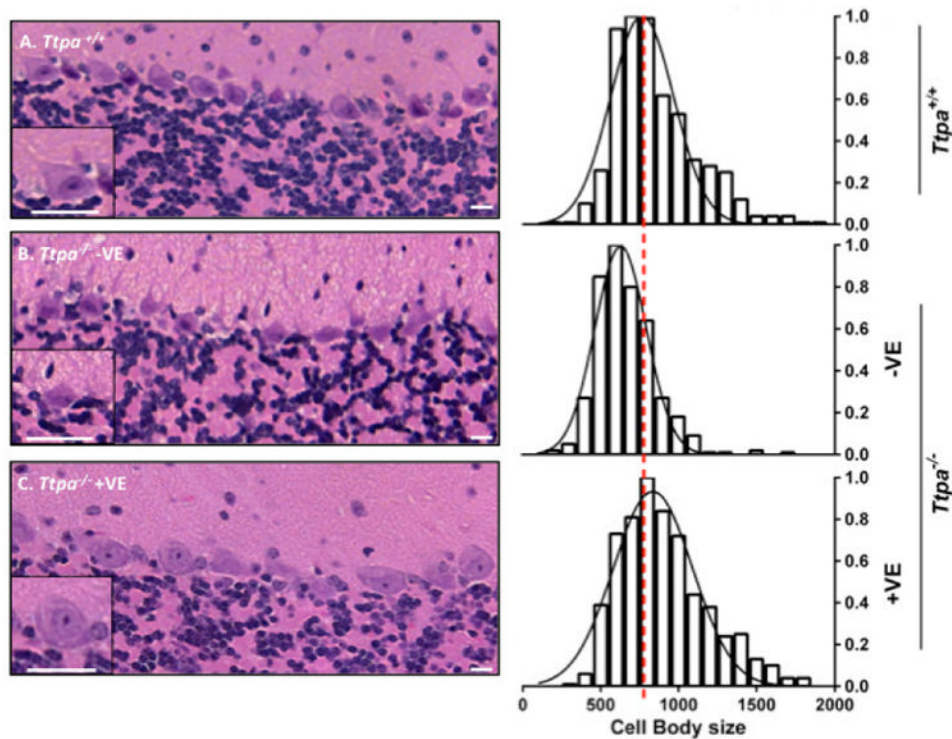


Figure 3. Vitamin E is required for Purkinje neuron integrity

Cerebella of the indicated experimental groups ($Ttpa^{+/+}$; $Ttpa^{-/-}$ -VE: vitamin E deficient diet; $Ttpa^{-/-}$ +VE: vitamin E supplemented diet) were fixed and embedded as described in Experimental procedures. Thin sections (5 μm) were stained with hematoxylin and eosin and microscopic images of the Purkinje neuron layer were obtained. **Left panels:** representative images from sections of each of the indicated experimental groups (representative Purkinje neuron cell body is shown in the inset for each image; scale bar = 50 μm). **Right panels:** frequency distribution histograms of Purkinje neuron cell body size in each genotype, measured using the ImageJ software. Size analysis was performed on 425 Purkinje neurons from three different animals for each experimental group. Solid line shows fit of the experimental data (bars) to a Gaussian distribution. Red-dotted line compares the amplitude for each experimental group.

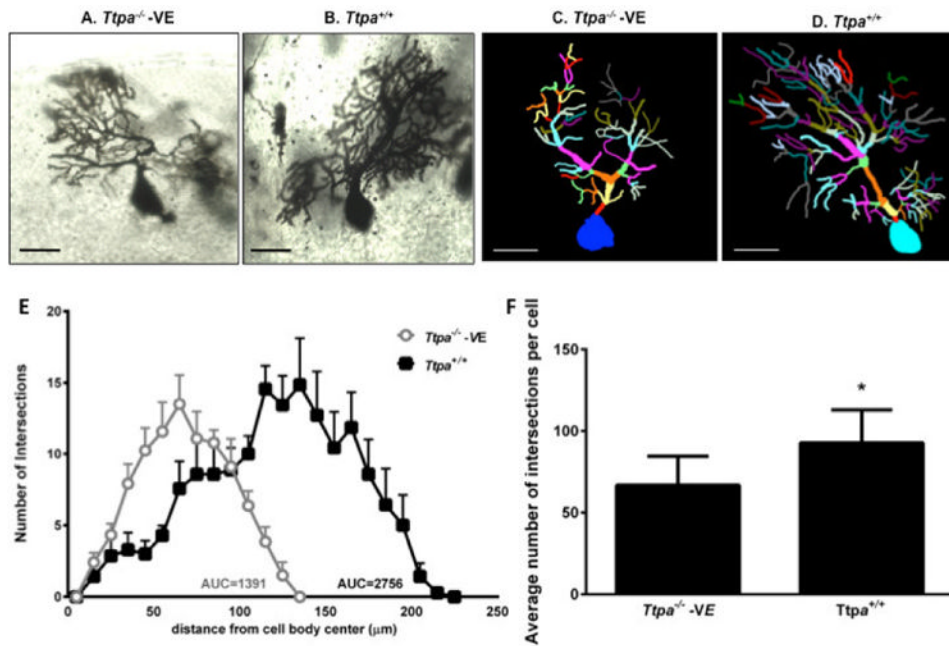


Figure 4. Vitamin E deficiency disrupts dendritic branching of Purkinje neurons

Cerebella from the indicated experimental groups (*Ttpa*^{+/+}; *Ttpa*^{-/-}-VE: vitamin E deficient diet) were excised and stained with Golgi-Cox reagents. Micrographs of representative Purkinje neurons from vitamin E deficient *Ttpa*^{-/-} and wild-type mice are shown in panels (A) and (B), respectively. Panels (C) and (D) show computer-generated (NeuroLucida software) three-dimensional tracings of Golgi-Cox stained Purkinje neurons from *Ttpa*^{-/-} vitamin E deficient and *Ttpa*^{+/+} mice, respectively. (E) Scholl profile of NeuroLucida-generated data, showing the number of intersections in each 5 μm concentric circle from the middle of the Purkinje neuron cell body. AUC: area under curve. (F) The average number of intersections per Purkinje neuron in *Ttpa*^{-/-} vitamin E deficient and *Ttpa*^{+/+} mice. Twelve Purkinje neurons were analyzed from 3 animals of each genotype. All scale bars= 50 μm . In panels E and F the error bars reflect the standard deviation of the mean. Asterisks indicate statistical significance as determined by a Student's t-test; $p < 0.05$ compared to the wild-type mice.

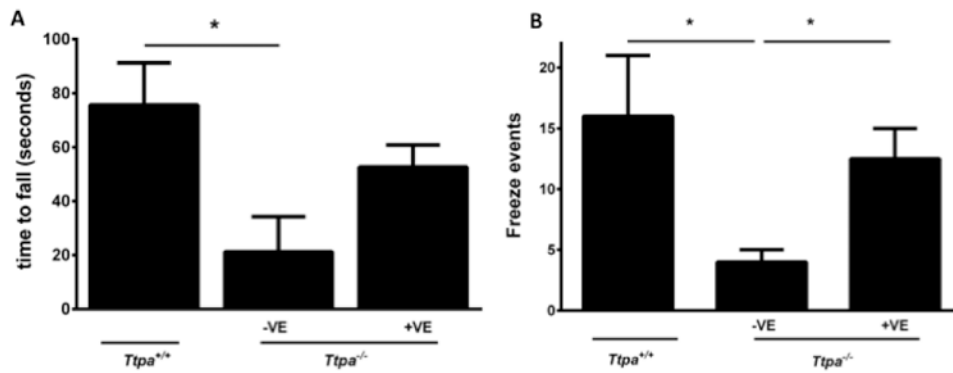


Figure 5. Vitamin E is essential for motor coordination and for cognitive function
Mice from each experimental group (*Ttpa*^{+/+}; *Ttpa*^{-/-} -VE: vitamin E deficient diet; *Ttpa*^{-/-} +VE: vitamin E supplemented diet) were subjected to behavioral testing. **(A)** Rotarod testing was carried out after 3 days of training runs. Shorter time to latency reflects diminished motor coordination. Asterisks indicate a statistically significant difference between the *Ttpa*^{-/-} mice and wild-type littermates, as determined by one-way ANOVA [F (2, 7) = 4.77, p<0.049] and Tukey's multiple comparisons post-hoc analysis (Mean difference= 54.4, 95% CI [2.27, 106.5], adjusted p=0.04) **(B)** Cue-dependent fear conditioning was performed after 24 hours period of habituation and training. Decrease in freeze events reflects compromised memory formation. Asterisks indicate statistically significant differences of p< 0.05 per one-way ANOVA and Tukey's post-hoc analysis between the vitamin E deficient animals and the other experimental groups. ANOVA: [F (2, 6) = 10.63, p= 0.011]. Tukey's post-hoc: *Ttpa*^{+/+} vs -VE (Mean difference= 12, 95% CI [3.79, 20.2], adjusted p=0.0099), -VE vs +VE (Mean difference= -8.5, 95% CI [-16.71, -0.286], adjusted p =0.044). Error bars reflect the standard deviation of the mean. Analyses of 3-5 animals per experimental group.