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Laboratory Study

Sodium selenate specifically activates PP2A phosphatase, dephosphorylates tau and reverses memory deficits in an Alzheimer's disease model

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ABSTRACT

Neurofibrillary tangles composed of abnormally hyperphosphorylated tau protein are a hallmark of Alzheimer's disease (AD) and related tauopathies. Tau hyperphosphorylation is thought to promote aggregation with subsequent tangle formation. Reducing tau phosphorylation by boosting the activity of the key phosphatase/s that mediate dephosphorylation of tau could be a viable clinical strategy in AD. One of the key phosphatases implicated in regulating tau protein phosphorylation is the serine–threonine phosphatase PP2A. We have determined that sodium selenate can act as a specific agonist for PP2A, significantly boosting phosphatase activity. Acute treatment of either neuroblastoma cells or normal aged mice with sodium selenate rapidly reduced tau protein phosphorylation. Sodium selenate-treated transgenic TAU441 mice had significantly lower levels of phospho- and total tau levels in the hippocampus and amygdala compared with controls and exhibited significantly improved spatial learning and memory on the Morris Water Maze task. Sodium selenate is a specific activator of PP2A with excellent oral bioavailability, and favourable central nervous system penetrating properties. Clinical studies in patients with AD are envisaged in the near future.

1. Introduction

Neurofibrillary tangles composed of abnormally hyperphosphorylated tau are a hallmark of Alzheimer's disease (AD) and related tauopathies.[1–3] The role of tau protein hyperphosphorylation and subsequent aggregation in the aetiology of AD has attracted increasing attention. A growing body of work places tau at a signaling crux, possibly receiving cues from extra-neuronal amyloid-beta (Aβ) species, orchestrating the eventual demise of selected neuronal populations.[4]

The two defining pathological features of AD are: (i) the neuritic amyloid plaques largely composed of the Aβ peptide;[5] and (ii) numerous neurofibrillary tangles in the neuronal cell body and dystrophic neurites composed of the abnormally hyperphosphorylated microtubule-associated protein tau.[2,6] The neurofibrillary tangles, although commonly associated with AD, are not unique to it, with several related disorders, such as frontotemporal dementia, supranuclear palsy and dementia pugilistica, among others, also exhibiting neurofibrillary degeneration. All of these neurodegenerative disorders, collectively termed "tauopathies", are characterised clinically by progressive dementia.[7] Abnormalities in the tau gene alone are unequivocally linked to dementia, with inherited cases of frontotemporal dementia with Parkinsonism linked to chromosome-17 (FTDP-17) all carrying missense mutations in the tau gene.[8–10] These mutant tau proteins, like the tau protein in neurofibrillary tangles, exhibit pronounced hyperphosphorylation, suggesting the phosphorylation status of tau may be associated with neurodegeneration and subsequent dementia.[11]

Although many efforts by the pharmaceutical industry have been directed to discover and develop agents that interfere with β-amyloid deposition, comparatively little has been done to identify "druggable" candidates that would reduce tau protein phosphorylation and/or levels.

Tau protein phosphorylation could be reduced either by inhibiting the key kinase/s responsible for the Alzheimer's signature phospho-tau epitopes or by boosting the activity of the key phosphatase/s that mediate dephosphorylation of those same

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phospho-epitopes. One key phosphatase implicated in regulating tau protein phosphorylation is the serine–threonine phosphatase PP2A, which is found colocalised with tau and microtubules in the brain[12] and is the most active phosphatase in mediating dephosphorylation of abnormal phospho-tau.[13,14] PP2A hypofunction is also associated with AD brain specimens.[15–17] Aside from its key role in regulating tau phosphorylation, PP2A is also a key mediator of cellular proliferation and subsequent malignant transformation. We have previously identified that sodium selenate specifically boosts the activity of the protein phosphatase PP2A and leads to dephosphorylation of the key survival protein Akt in human cancer (manuscript in preparation). We here demonstrate that the small, water-soluble compound, sodium selenate, is a specific and potent activator of PP2A heterotrimers composed of the PR55 B subunit form of PP2A and that treatment with sodium selenate dephosphorylates tau and reverses memory deficits in an AD model.

2. Materials and methods

2.1. Cell culture

Human neuroblastoma SH-SY5Y and BE2M17 cell lines were obtained from Janetta Culvenor (Department of Pathology, University of Melbourne, Vic). The human prostate cancer cell line PC3 was obtained from American Type Culture Collection (Manassas, VA, USA). SH-SY5Y and PC3 cells were routinely cultured in RPMI 1640 medium (Invitrogen; Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS: Invitrogen), 1% non-essential amino acids (Sigma-Aldrich; St Louis, MO, USA), 10 mM HEPES (Invitrogen), 1 mM sodium pyruvate (Invitrogen) and 1% antibiotic/antimycotic mixture (Invitrogen). BE2M17 cells were cultured in Opti-MEM I reduced serum medium (Invitrogen) supplemented with 10% FBS, 1% non-essential amino acids, 1 mM sodium pyruvate, and 1% antibiotic/antimycotic mixture. Cells were cultured at 37 °C in 5% CO_2.

PC3 prostate carcinoma cells (2×10^6) were plated into 100 mm dishes, allowed attach for 8 hours, serum starved overnight and lysed for Akt immunoprecipitation. SH-SY5Y or BE2M17 cells (2×10^5) were plated in each well in a 6-well plate, either on uncoated surfaces or coated with 0.5 μg/mL fibronectin (Sigma-Aldrich), and were allowed to attach overnight at 5% CO_2/37 °C in the complete growth medium. The medium was replaced with fresh complete growth medium containing 100 μM concentration of sodium selenate and the cells were cultured for 3 hours.

2.2. Selenium compounds

Sodium selenate and all other organic (selenocystamine dihydrochloride, selenocystine, selenomethionine, selenium-(methyl) selenocysteine hydrochloride) and inorganic (selenious acid, sodium selenite, selenium dioxide) forms of selenium used were obtained from Sigma-Aldrich.

2.3. Phosphatase assays

PP2A (0.05 U) was mixed with 5 μL of 40 mM $NiCl_2$ and 5 μL of bovine serum albumin solution (5 mg/mL) in a microcentrifuge tube and various treatments were added as indicated, and the volume adjusted to 80 μL with para-nitrophenyl phosphate (pNPP) assay buffer. Samples were pre-incubated at 37 °C for 15 minutes in the presence of either sodium selenate (5 mM) or okadaic acid (500 nM) (Calbiochem, San Diego, CA, USA). To start the phosphatase reaction, 120 μL of pNPP substrate (1.5 mg/mL pNPP in 50 mM Tris-HCl, pH 7.0) was added, and samples incubated for a further 15 minutes at 37 °C. Absorbance of each sample was measured in duplicate at 405 nm with 590 nm as a reference. PP2A activity (A) was calculated using the following equation:

$$A = VL \times A_{405}/(1.78 \times 10^4\ M^{-1}\ cm^{-1} \times 0.25\ cm \times 15\ min \times 0.05\ U\ enzyme)$$

where V is the sample volume and $1.78 \times 10^4\ M^{-1}\ cm^{-1}$ is the extinction coefficient.

In an alternative assay using a synthetic phosphopeptide as a substrate, 0.01–0.05 U purified human PP2A-AC dimer was incubated with 100 μM of phosphothreonine peptide at 37 °C for 15 minutes in the presence of various forms of selenium at a concentration of 50 μM. Free phosphate was measured by the addition of malachite green, with absorbance measured at 590 nm.

2.4. Lysate preparation and Immunoblotting

Cells were washed twice in cold PBS and were lysed in 150 μL of cold radioimmunoprecipitation assay (RIPA) buffer (containing 10% glycerol; 20 mM Tris; 137 mM NaCl; 0.1% sodium dodecyl sulfate [SDS]; 0.05% Igepal (Sigma); 1% Triton X-100, 2 mM ethylenediaminetetra-acetic acid; 10% NaV, 2% NaF and 1× Complete Protease Inhibitor (Roche; Indianapolis, IN, USA)). Similarly, brain tissues were homogenised on dry ice using a mortar and pestle and lysed in RIPA buffer. Samples were incubated on ice for 30 minutes, followed by centrifugation at 13,000 revolutions per minute for 10 minutes at 4 °C. The supernatant was collected and the protein concentration was determined using the bicinchoninic acid (BCA) assay (Sigma-Aldrich). Protein (50 μg) was loaded into each lane of a 10% SDS–polyacrylamide gel. The proteins were transferred onto polyvinylidene difluoride membranes (Millipore; Bedford, MA, USA), which were blocked with 5% skim milk for 2 hours. Primary antibodies were incubated overnight at 4 °C in 5% skim milk, followed by a 1 hour incubation with polyclonal goat anti-mouse (Dako; Glostrup, Denmark) coupled to horseradish peroxidase (HRP) at room temperature. Following washing, antibody binding was detected using Amersham enhanced chemiluminescent (ECL) western blotting detection reagent (GE Healthcare Life Sciences, Piscataway, NJ, USA). Membranes were stripped using 2% SDS/β-mercaptoethanol stripping buffer and reprobed for tubulin to confirm protein loading.

2.5. Akt Immunoprecipitation

Cells were then lysed and total Akt immunoprecipitated from 500 μg of lysate with 1:100 dilution of pan-Akt monoclonal antibody and 30 μL protein A-sepharose Fast Flow beads (Sigma). Negative controls (blank) had the immunoprecipitating antibody omitted. Following repeated washing the beads were boiled in 3× SDS protein loading buffer for 5 minutes, centrifuged at high speed, and the resulting supernatant resolved by gel electrophoresis. Whole cell lysate (100 μg) was run out on the same gel for comparison, and the resulting membranes probed with antibodies that specifically recognise members of either B or B′ families of subunits. Successful pulldown of Akt was confirmed by probing the same blots with an antibody that recognises pan-Akt.

2.6. Antibodies

The following antibodies were obtained from Pierce Endogen (Rockford, IL, USA) unless otherwise stated: anti-human paired helical filament (PHF)-tau monoclonal (clone AT100; AT180; AT270), and anti-human tau monoclonal (clone HT7); polyclonal goat anti-mouse immunoglobulins/HRP (Dako; Glostrup, Denmark),

anti-tubulin (G712A: Promega; Madison, WI, USA), anti-Akt (Cell Signaling Technology; Irvine, CA, USA) and anti-PP2A B- and B′-subunits (Millipore).

2.7. Acute treatment in vivo studies

Fourteen-week-old Balb/C nude male mice were obtained from Animal Resources Centre (Perth, WA, Australia). Mice were subcutaneously injected with sodium selenate at 1.5 μg/μL in PBS with 200 μL per injection. Brain tissues were collected at 2 hours and 6 hours after sodium selenate injection.

2.8. Organotypic hippocampal slice cultures

Wistar rat pups (8–11 days old) were decapitated and the hippocampus rapidly dissected into ice-cold Gey's balanced salt solution (GBSS; Sigma-Aldrich) supplemented with 4.5 mg/mL glucose. Transverse sections (400 μm) were cut on a McIlwain tissue chopper and placed back into ice-cold GBSS. Slices were separated and plated onto Millicell CM culture inserts (4 per well) and maintained at 37 °C/5% CO_2 for 14 days. Maintenance medium consisted of 25% heat-inactivated horse serum, 25% Hank's balanced salt solution (HBSS) and 50% minimum essential medium (MEM) with added Earle's salts supplemented with 1 mM glutamine and 4.5 mg/mL glucose. The medium was changed every 3–4 days.

2.9. Neurotoxicity assay

A neurotoxicity screen was carried out using selenium species as indicated, to determine exposure levels that would cause toxicity on hippocampal slice cultures. Each compound was made to 5 mM in serum-free medium (SFM), and then used at 1 μL/mL, 5 μL/mL and 20 μL/mL, to give final concentrations of 5 μM, 25 μM and 100 μM. Damage was measured after 24 hours and 48 hours. Briefly, 14-day cultures were transferred to SFM (75% MEM, 25% HBSS supplemented with 1 mM glutamine and 4.5 mg/mL glucose) containing 5 μg/mL of the fluorescent exclusion dye propidium iodide (PI). Cultures were allowed to equilibrate in SFM for 60 minutes prior to imaging. PI fluorescence was detected using a Leica DM IL inverted microscope (Leica Microsystems; Wetzlar, Germany) fitted with a rhodamine filter set. Neuronal damage was assessed with the aid of ImageJ software (http://rsb.info.nih.gov/ij/). Images were captured using a monochrome charge coupled device (CCD) camera and saved for offline analysis. Light transmission images were captured prior to the addition of drugs, and PI fluorescence images recorded at the end of the 24-hour recovery period. The area of the CA1 region was then determined from the transmission image. The area of PI fluorescence in the CA1 was measured using the threshold function on ImageJ, and neuronal damage expressed as the percentage of the CA1 in which PI fluorescence was detected above background levels.

2.10. Transgenic model

Transgenic TMHT mice (C57BL/6 background) over-expressing the human TAU441 gene with two mutations, V337M and R406W, under the control of a brain-specific murine Thy-1 promoter were used for behavioural studies and immunohistochemistry. Mice were kept according to the Institutional Standard Operating Procedures, to international standards of animal care and welfare. Animals were housed under a constant light-cycle (12 hours light/dark). Dried, pelleted standard rodent chow (Altromin®; Altromin, Lage, Germany) and normal tap water were available ad libitum. Groups of 15, 5-month-old transgenic animals were randomly assigned to a 12-week sodium selenate or vehicle (water) treatment. A corresponding untreated group of mice was analysed already at baseline.

2.11. Immunohistochemical determination of tau pathology

Tau depositions were determined using the monoclonal tau-antibodies AT180, HT7, AT8, AT270. Thick (5 μm) coronal paraffin sections from each of the five different layers were stained with the above-described monoclonal mouse anti-human tau-antibodies and visualised using a secondary anti-mouse Cy3 (The Jackson Laboratory; Bar Harbor, ME, USA). Tiled images were recorded using a Pixel Fly camera (PCO Computer Optics; Ketheim, Bavaria, Germany) mounted on a Nikon E800 microscope (Nikon; Tokyo, Japan). Images were evaluated with ImageProPlus (version 4.5.1.29) image analysis software (Media Cybernetics; Silver Spring, MD, USA). We assessed whether sodium selenate treatment could reduce levels of PHF phospho tau (AT180) epitopes or total tau (HT7) levels in the hippocampus and amygdala compared to vehicle and baseline animals ($n = 3$ per group). To adjust for minimal differences in size, we quantified tau load data, related to the individual region size in the slice.

2.12. Behavioural testing

Behavioural testing (Rota Rod, Open Field test, Probe trial, Nose Poke Curiosity and Activity Test and the Morris Water Maze task) was performed as detailed in the *Supplementary Methods* section.

2.13. Statistics

Means, standard deviation (SD) or standard errors of the mean (SEM) were calculated for all measured variables. Tests for normal distribution (Kolmogorov Smirnov and Shapiro Wilk *W*-test) were performed on all histological variables. Analysis of variance (ANOVA) with associated post-hoc tests were performed for histological measures, with significance assumed at $p < 0.05$. Group differences were calculated by a parametric ANOVA using a Newman–Keuls Multiple Comparison post-hoc test, if normally distributed, otherwise the non-parametric Kruskall–Wallis ANOVA was used followed by Dunnett's post-hoc test. Differences between exclusively treated groups were calculated using a 2-tailed, unpaired *t*-test.

3. Results

3.1. In vitro PP2A activation analysis

The effect of sodium selenate on PP2A phosphate activity compared to the phosphatase inhibitor okadaic acid and other organic and inorganic forms of selenium is shown in Fig. 1a and b respectively, indicating a clear agonistic effect. The dose-response relationship between selenate and PP2A phosphatase activity is shown in Fig. 1c. Pulldown of Akt from PC3 cells indicated that the regulatory subunit of PP2A associated with Akt was a member of the PR55 B subunit family (Fig. 1d).

3.2. Effect of sodium selenate on tau phosphorylation

Sodium selenate produced a consistent decline in levels of the PHF phospho-tau protein in both BE2M17 (Fig. 2a) and SH-SY5Y (Fig. 2b) human neuroblastoma cells. Surprisingly, we also noted a decrease in total tau levels as assessed by the tau monoclonal HT7 (Fig. 2a, b). Similarly, we readily detected decreased PHF phospho-tau proteins in the CNS of normal aged mice, whilst however total tau levels remained unchanged (Fig. 2c).

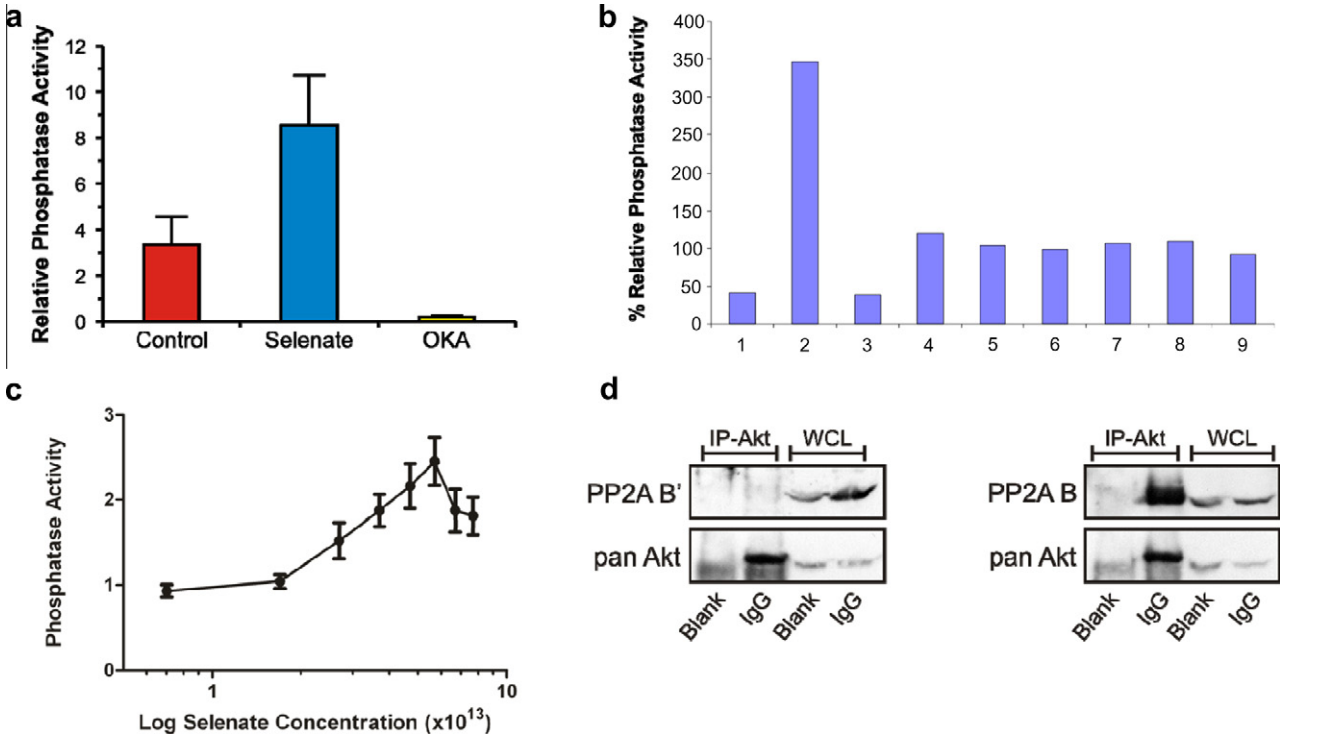

Fig. 1. (a) Relative phosphatase activity of purified protein phosphatase PP2A core dimer (measured as hydrolysis of para-nitrophenyl phosphate) incubated with or without sodium selenate or okadaic acid (OKA) showing that selenate specifically boosts the activity of PP2A. (b) Percentage relative PP2A increase in activity over control core dimer after incubation of PP2A dimer with phospho-threonine peptide with different compounds showing the superior stimulatory effects of selenate: 1, sodium sulfate; 2, sodium selenate; 3, selenocystamine dihydrochloride; 4, selenious acid; 5, sodium selenite; 6, selenium dioxide; 7, selenocystine; 8, selenomethionine; and 9, selenium-(methyl) selenocysteine hydrochloride. (c) Relative phosphatase activity of PP2A core dimer when incubated with increasing concentrations of sodium selenate showing a clear dose-response relationship. (d) Immunoprecipitation of Akt from human PC3 cells showing that the regulatory B subunit associated with Akt was a member of the PR55 family. WCL = whole cell lysate.

3.3. Neurotoxicity assay

We determined the neurotoxicity of sodium selenate and the closely related inorganic form sodium seleinite, as well as the major organic form selenomethionine in primary hippocampal slice cultures. Strikingly, selenate was completely non-toxic even at 100 μM, whereas selenomethionine and, in particular selenite, were very toxic at this concentration and even at substantially lower concentrations (Fig. 3).

3.4. Tau assessment in selenate treated transgenic mice

The area of IHC stained regions were highly constant throughout all investigated brains (Supplementary Fig. 1), indicating that selenate treatment did not induce CNS atrophy. A significant reduction in the percentage of the relative AT180-tau staining area in the hippocampus in animals treated with selenate (1.2 mg/mL drinking water) compared to vehicle-treated controls (*t*-test of treated groups: $p = 0.04$, Fig. 4a, c) was observed. This effect on PHF phospho-tau was even more pronounced in the amygdala where selenate treatment also significantly decreased the relative AT180-tau area compared to the vehicle group (ANOVA: $p < 0.05$, *t*-test of treated groups: $p = 0.0045$, Fig. 4a, c) but not compared to the untreated baseline group.

The relative HT7-total tau staining area in the hippocampus was significantly reduced in animals treated with selenate (1.2 mg/mL) compared to vehicle (ANOVA: $p < 0.05$, *t*-test of treated group: $p = 0.04$; Fig. 4b, d) and the untreated baseline group (ANOVA: $p < 0.05$; Fig. 4b, d). Again selenate decreased the relative HT7-total tau staining area in the amygdala in a more pronounced manner compared to both the untreated baseline (ANOVA: $p < 0.01$), as well as the vehicle-treated groups (ANOVA: $p < 0.05$; *t*-test for treated only: $p = 0.0018$, Fig. 4b, d).

3.5. Behavioural testing

A series of standard behavioural tests were initially applied to assess whether selenate treatment had any deleterious effects on generalised activity. The TMHT transgenic mice do not exhibit any motor deficits and treatment with selenate did not induce any variation in motor capabilities as assessed by the Rota Rod test (Fig. 5a, b).

Spontaneous psychomotoric activation was investigated in the Open Field (OF) apparatus. Generalised activity parameters (activity, hyperactivity, and rearing behaviour) were not influenced by selenate treatment (Supplementary Figs. 2, 3). Whilst selenate treated mice showed a slight trend to disturbed thigmotactic behaviour during the first OF session, by the end of treatment in the second session thigmotactic behaviour normalised to the level of the vehicle controls (Supplementary Fig. 3). Interestingly, selenate treatment significantly reduced fecal boli rates compared to baseline rates (ANOVA: $p = 0.018$, $p < 0.05$; Supplementary Fig. 2). Curiosity behaviour remained unchanged from the sodium selenate treatment (Supplementary Fig. 4).

We then investigated spatial learning and memory in the TMHT transgenic mice using the Morris Water Maze (MWM) navigation task. Expression of FTD-17 mutant tau lesions in distinct brain regions like hippocampus, striatum, basal forebrain, cerebellum and cerebral cortex have been shown to impair MWM accomplishment, and at 8 months of age TMHT transgenic mice have significantly worse MWM results compared to 5-month-old baseline TMHT transgenic or non-transgenic littermates (unpublished data).

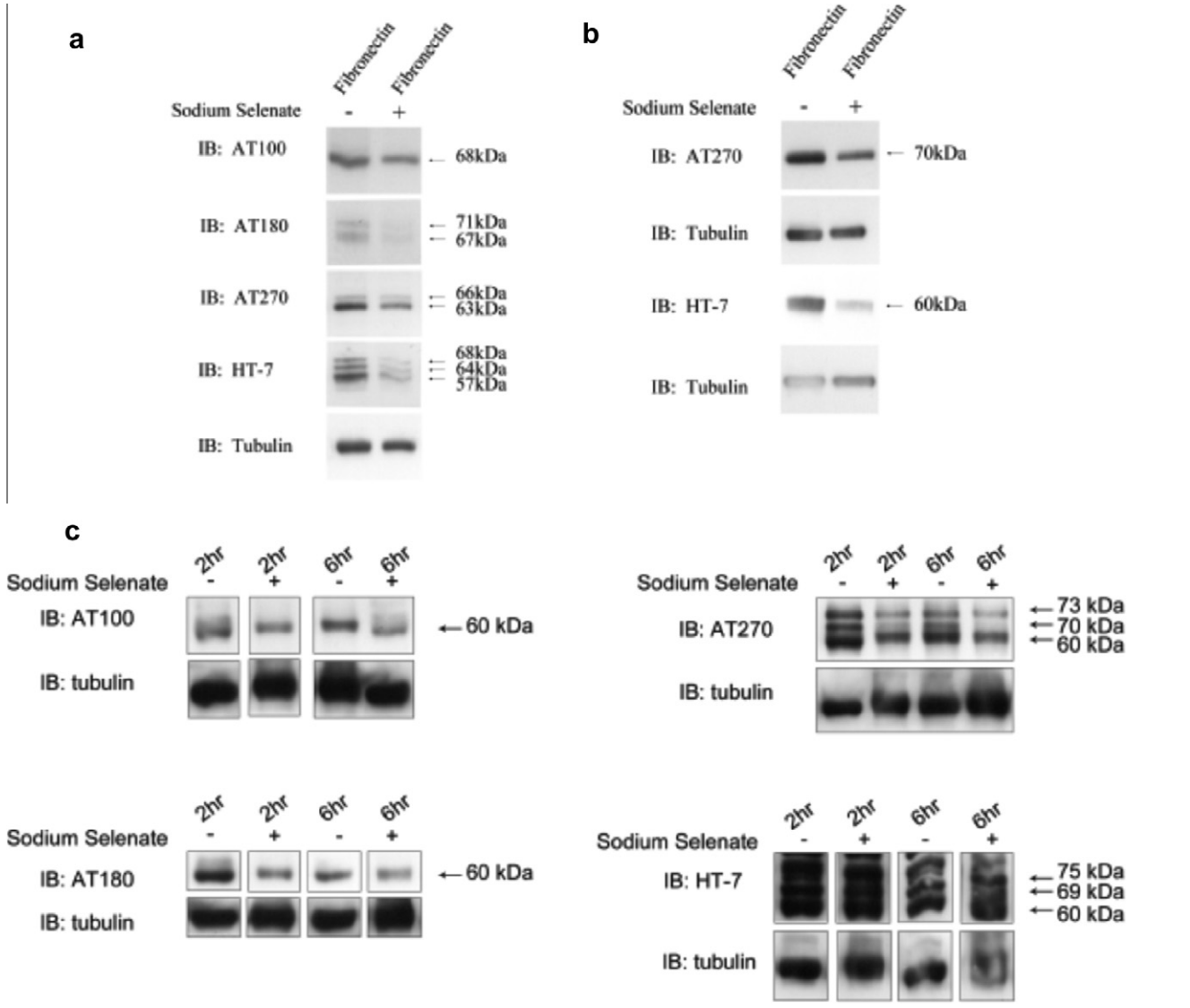

Fig. 2. Immunoblots of cell lines with anti-human PHF-tau antibodies (AT100 and HT-**5**; anti-tubulin lane indicates protein loading) in the presence (+) or absence (–) of sodium selenate (a) human neuroblastoma BE2M17 cell line plated with (+) or without (–) fibronectin coating; and (b) human neuroblastoma SH-SY5Y cell line with (+) or without (–) fibronectin coating, showing a consistent decline in PHF phospho-tau protein and total tau levels. (c) Immunoblot of total brain lysates from aged Balb/C Nu Nu male mice with anti-human PHF-tau antibodies (as indicated) either with (+) or without (–) sodium selenate showing decreased PHF phospho-tau proteins in the central nervous system of mice while total tau levels remained unchanged. PHF = paired helical filament.

We observed a pronounced and significant improvement in spatial memory of selenate-treated TMHT transgenic mice with escape latency significantly shorter in comparison to the vehicle group and even compared to the 3-months younger baseline group (Fig. 5c). Selenate-treated animals found the platform on day 1, 2 and 3 faster ($p \leqslant 0.01$) than animals from the vehicle group and on day 1 (acquisition day), also faster than the 3-month younger animals from the baseline group (Fig. 5c). We also observed pronounced improvements in escape latency compared to baseline, with a clear trend for selenate-treated TMHT transgenic mice to have shorter escape latencies (Fig. 5c; Table 1), strongly suggesting that selenate treatment has not only impeded the rate of spatial memory function decline but reversed it. There was also a trend for selenate-treated animals to have shorter swim path lengths (Fig. 5d; Table 2).

4. Discussion

Reduced phosphatase activity has been implicated in the formation of hyperphosphorylated tau tangles in AD as well as other neurodegenerative conditions. *In vitro*, several phosphatases have been implicated in inducing dephosphorylation of tau, including PP1, PP2A, PP2B and PP5. However, PP2A accounts for some 71% of the total tau phosphatase activity of the human brain and PP2A activity is decreased in AD brain specimens,[13] strongly implicating PP2A as the predominant tau phosphatase in the human brain.

The B alpha (PR55Balpha) regulatory subunit form of the PP2A heterotrimer complex binds to and regulates tau phosphorylation.[18,19] Levels of the same PP2A subunit are significantly reduced in AD in affected brain regions and tangle-bearing neurons.[19] PP2A mRNA expression is also decreased in the AD hippocampus.[17]

An agent which could specifically boost the activity of this hetero-trimer could be useful for the treatment of these diseases. This agent would also need to be able to penetrate the blood-brain barrier, exhibit minimal neurotoxicity, and preferably be orally bio-available.

We have shown here that sodium selenate can specifically boost the activity of PP2A containing the PR55 subunit and this can

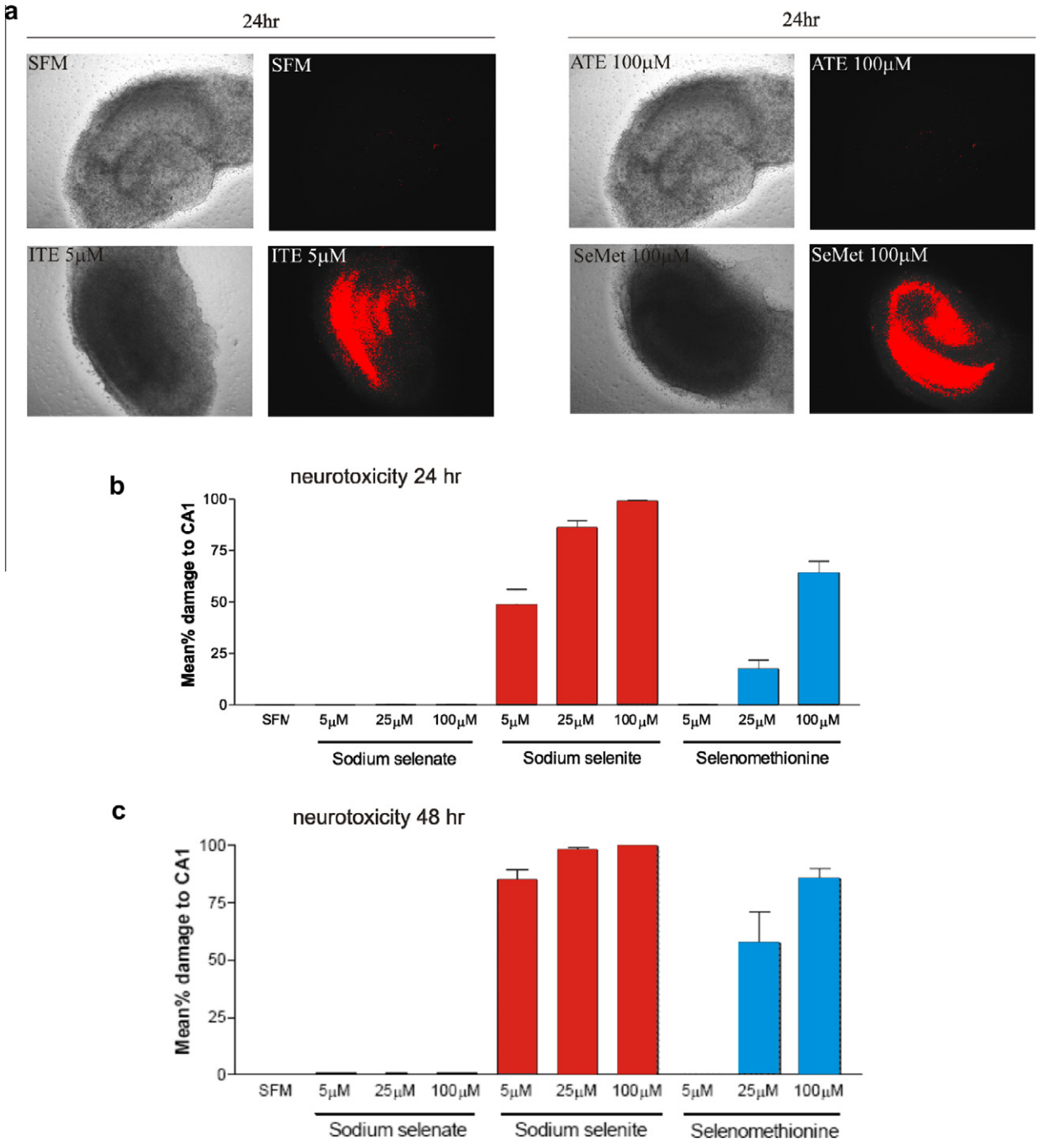

Fig. 3. Neurotoxicity of selenium compounds on primary rat cultured hippocampal slices. (a) Transmission (left) and propridium iodide (PI) fluorescence (right) images of hippocampal slice cultures after treatment with either sodium selenate (ATE), sodium selenite (ITE) or selenomethionine (SeMet) for 24 hours. (b, c) The mean percentage damage to the hippocampal CA1 region as assessed by uptake of the fluorescent cell permeability marker PI at (b) 24 hours and (c) 48 hours after exposure to the indicated selenium compounds showing that sodium selenate was non-toxic compared to selenite and selenomethionine. SFM = serum free medium.

induce therapeutically relevant dephosphorylation of tau in cell culture. Given that selenium compounds have been reported to be toxic,[20] any potential neurotoxicity would counteract any neuroprotective effects of treatment. We therefore tested sodium selenate in a neurotoxicity assay using primary rat hippocampal slice cultures and clearly demonstrated that selenate is non-toxic to neurons even at high concentrations, whilst the closely related inorganic form sodium selenite and the predominant organic form selenomethionine induced significant cell death.

Multiple pathogenic tau gene mutations in many different families with Parkinsonism linked to chromosome 17 (FTDP-17) have unequivocally shown that tau abnormalities cause neurodegenerative disease. Different tau transgenic vertebrate and invertebrate models which reproduce aspects of AD and FTD have further underlined the role of tau in neurodegenerative disorders.[4] Several FTDP-17 missense *tau* mutations, including V337M and R406W have been demonstrated to reduce the ability of bacterially expressed recombinant tau protein to bind to and promote the assembly of microtubules.[21,22]

We utilised transgenic mice, TMHT, over-expressing human tau441 bearing the missense mutations V337M and R406W under the control of the brain specific murine Thy-1 promoter on a

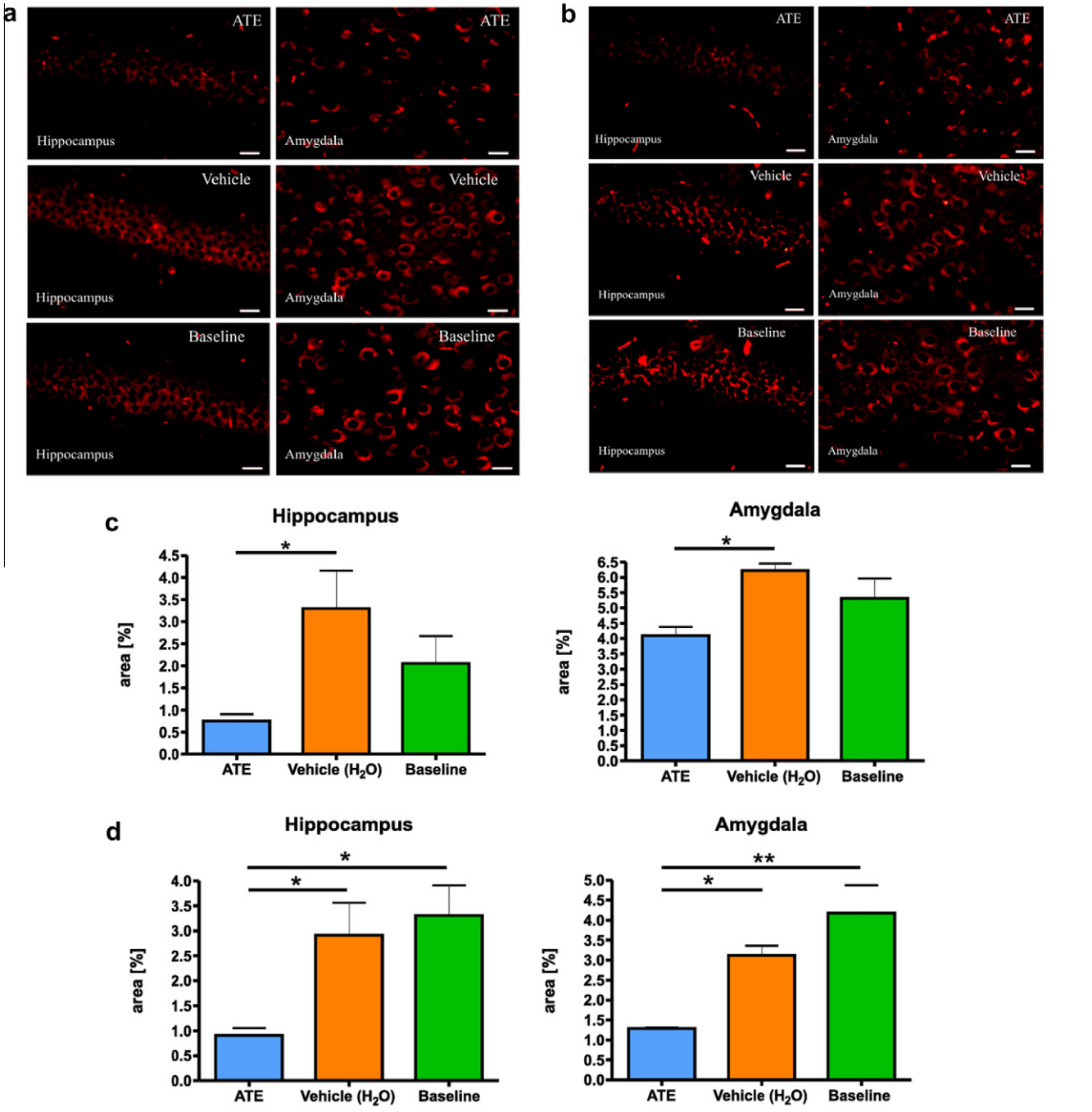

Fig. 4. AT180 (a, c) and HT7 (b, d) immunohistochemistry of TMHT mice compared to transgenic hTAU441 TMHT mice treated with selenate (ATE). (a, b) Representative immunohistochemically stained slices in the neuronal layer of the CA1 region of the hippocampus and the amygdala of TMHT mice after ATE treatment showing reduced PHF tau load as well as a reduction of tau-positive cells (scale bars: 25 μm). (c, d) Mean (± standard errors of the mean) of the effect of treatment on tau load determined by (c) AT180 and (d) HT7 immunohistochemistry in hTAU441 transgenic TMHT mice. Graphs represent tau load measured by the relative (c) tau-AT180 or (d) tau-HT7 immunopositive area, $^{*}p \leqslant 0.05$. PHF = paired helical filament.

C57BL/6 background as a model for neurodegenerative disease. This human mutated tau isoform is expressed at high levels, resulting in an age-dependent increase of tau pathology. The THMT mice develop tau pathology at an early age, starting at 3–5 months. Severity of the brain pathology correlates with increasing behavioural deficits. We found that chronic treatment with sodium selenate for 3 months from 5 months of age lowered phospho-tau levels compared with both age-matched controls and even baseline 5-month-old tau transgenic mice, which was associated with a generalised improvement in memory function as assessed by the Morris Water Maze in the same two sets of animals. These findings indicate that sodium selenate may not only halt tau-related pathology and cognitive impairment, but may actually reverse it.

Sodium selenate exhibits remarkable specificity towards the PP2A phosphatase, being unable to enhance phosphatase activity of other phosphatases including PP1 whose catalytic domain is most closely related to that of PP2A (manuscript in preparation). This property does not, however, appear to be mediated by the presence of selenium *per se*, as other selenium compounds are unable to clearly boost PP2A activity.

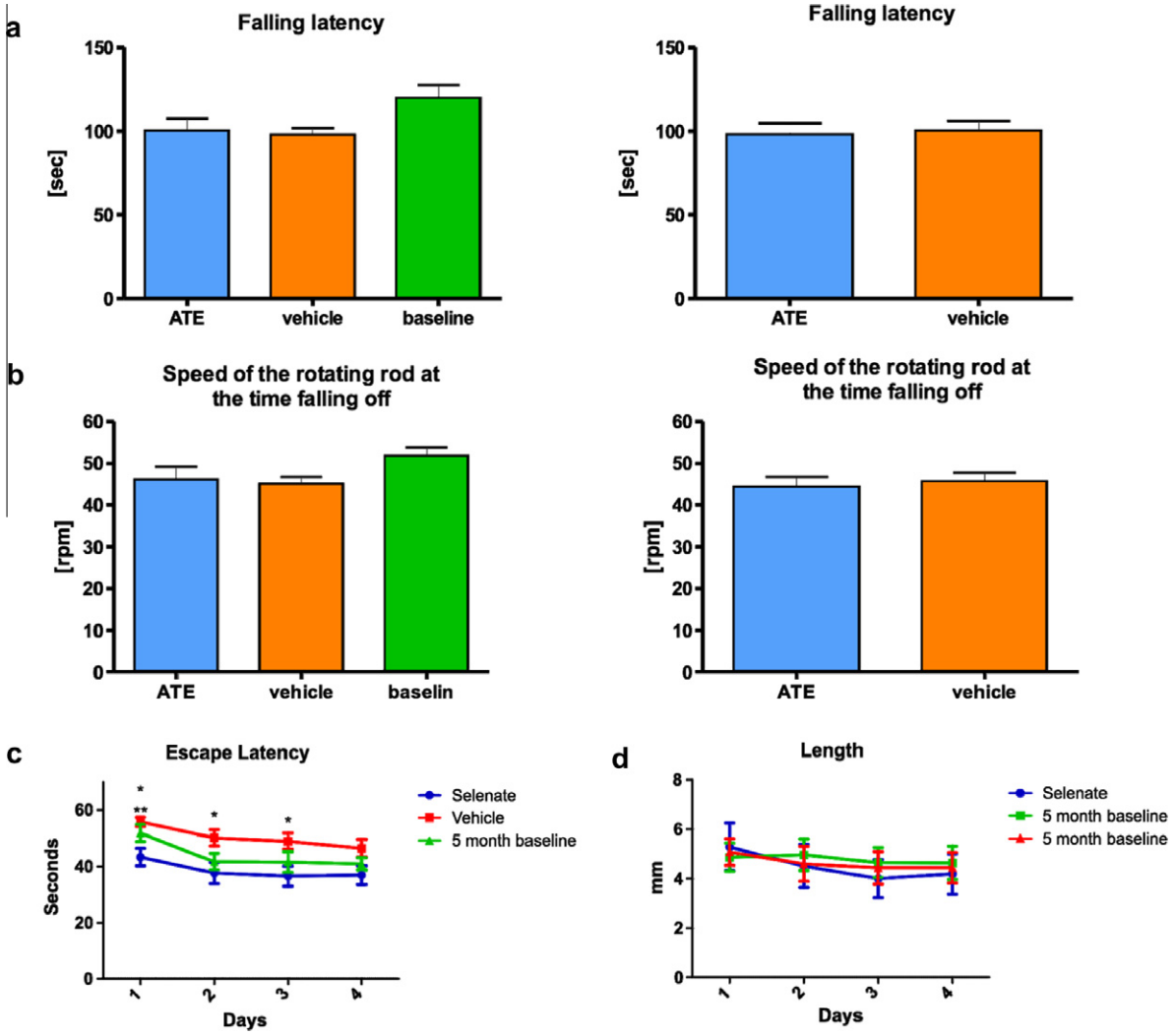

Fig. 5. Treatment of TMHT transgenic mice with sodium selenate (ATE). (a, b) Rota Rod test results of motor abilities after 1.5 months of treatment (turn 1), 3 months of treatment (turn 2) and untreated 5-month-old animals (baseline) showing no effect of a treatment with ATE: (a) falling latency (b) speed (revolutions per minute [rpm] of the speed of the rotating rod at the time of falling off). (c, d) Morris Water Maze (MWM) task results of learning and memory after treatment with ATE compared to vehicle and baseline: (c) time (s) of escape latency showing shorter escape latency compared to vehicle-treated and baseline mice; and (d) length of swimming path (m) to reach the platform in the MWM, showing a trend towards shorter paths in ATE-treated mice. Each point represents the means of all three trials of all animals of a group/day. See also Table 1 and 2 for further details. ($^{*}p \leqslant 0.05$ vs. vehicle; $^{**}p \leqslant 0.05$ vs baseline).

Table 1
Comparison of escape latencies of TMHT transgenic mice in the Morris Water Maze task. See Fig. 5C.

TIME			
Day no.	*n*	*n*	*p* values
ATE *vs.* vehicle			
1	15	16	0.002*
2	15	16	0.008*
3	15	16	0.027*
4	15	16	0.102
ATE *vs.* baseline			
1	15	15	0.029*
2	15	15	0.683
3	15	15	0.367
4	15	15	0.513
Vehicle *vs.* baseline			
1	16	15	0.519
2	16	15	0.066
3	16	15	0.119
4	16	15	0.175

ATE = sodium selenate treatment; vehicle = water, *vs.* = *versus*, baseline = 5-month-old untreated mice.
* $p < 0.05$.

Table 2
Comparison of swimming path lengths of TMHT transgenic mice in the Morris Water Maze task. See Fig. 5D.

LENGTH			
Day no.	*n*	*n*	*p* values
ATE *vs.* vehicle			
1	15	16	0.495
2	15	16	0.471
3	15	16	0.682
4	15	16	0.519
ATE *vs.* baseline			
1	15	15	0.624
2	15	15	0.806
3	15	15	1.000
4	15	15	0.935
Vehicle *vs.* baseline			
1	16	15	0.626
2	16	15	0.771
3	16	15	0.711
4	16	15	0.626

ATE = sodium selenate treatment; vehicle = water, *vs.* = *versus*, baseline = 5-month-old untreated mice.

In summary, sodium selenate is a specific activator of PP2A with excellent oral bioavailability, favourable CNS penetrating properties and in contrast to other selenium compounds, exhibits minimal neurotoxicity. These findings now provide a strong rationale for early stage clinical studies in mild cognitive impairment (MCI)/mild AD patients in the foreseeable future.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jocn.2010.04.020.

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