Acta Neuropsychiatrica

cambridge.org/neu

Anti-HIV drugs promote β-amyloid deposition and impair learning and memory in BALB/c mice

S.S. Zulu[1], O. Abboussi[2], N. Simola[3], M.V. Mabandla[1] and W.M.U. Daniels[4]

[1]School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Westville Campus, Durban, South Africa; [2]Division of Neuroscience, School of Medicine, Ninewells Hospital, University of Dundee, Dundee, UK; [3]Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy and [4]School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Original Article

Cite this article: Zulu SS, Abboussi O, Simola N, Mabandla MV, and Daniels WMU. (2020) Anti-HIV drugs promote β-amyloid deposition and impair learning and memory in BALB/c mice. *Acta Neuropsychiatrica* **32:**257–264. doi: 10.1017/neu.2020.19

Received: 1 September 2019
Revised: 24 April 2020
Accepted: 30 April 2020
First published online: 7 May 2020

Key words:
HIV-associated cognitive motor complex; reverse transcriptase inhibitors; oxidative stress; beta-secretase; amyloid

Author for correspondence:
S.S. Zulu,
Emails: zuluss86@gmail.com or 207506590@stu.ukzn.ac.za

Abstract

Objectives: Growing evidence suggested that antiretroviral (ARV) drugs may promote amyloid beta (Aβ) accumulation in HIV-1-infected brain and the persistence of HIV-associated neurocognitive disorders (HANDs). It has also been shown that lipid peroxidation upregulates β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) expression and subsequently promotes Aβ peptide production. In the present study, we examined whether chronic exposure to the anti-HIV drugs tenofovir disoproxil fumarate (TDF) and nevirapine induces lipid peroxidation thereby promoting BACE1 and Aβ generation and consequently impair cognitive function in mice. *Methods:* TDF or nevirapine was orally administered to female BALB/c mice once a day for 8 weeks. On the 7th week of treatment, spatial learning and memory were assessed using the Morris water maze test. The levels of lipid peroxidation, BACE1, amyloid β 1-42 (Aβ1-42) and Aβ deposits were measured in the hippocampal tissue upon completion of treatment. *Results:* Chronic administration of nevirapine induced spatial learning and memory impairment in the Morris water maze test, whereas TDF did not have an effect. TDF and nevirapine administration increased hippocampal lipid peroxidation and Aβ1-42 concentration. Nevirapine further upregulated BACE1 expression and Aβ deposits. *Conclusion:* Our results suggest that chronic exposure to TDF and nevirapine contributes to hippocampal lipid peroxidation and Aβ accumulation, respectively, as well as spatial learning and memory deficits in mice even in the absence of HIV infection. These findings further support a possible link between ARV drug toxicity, Aβ accumulation and the persistence of HANDs.

Significant outcomes

- Chronic exposure to the ARV drug nevirapine impairs spatial learning and memory in mice.
- TDF and nevirapine increase level of lipid peroxidation and Aβ1-42 concentration.
- Nevirapine upregulates BACE1 and Aβ deposition in hippocampus.

Limitations

- ARV drugs treatments were (TDF and nevirapine) administered to naive mice; therefore, the synergistic effects of HIV and ARV treatment on cognitive function are lacking.
- Although doses of ARV used in this study were comparable to human therapeutic doses, they do not account for differences in drug metabolism rates.
- We only conducted a cognitive assessment on the Morris water maze. More behavioural assessments are needed in future studies to test other cognitive domains.

Introduction

Among HIV comorbidities, HIV-associated neurocognitive disorder (HAND) is commonly observed in persons living with HIV (McArthur *et al.*, 2010). Recent clinical observations during the antiretroviral therapy (ART) era have indicated that HAND may occur in 15–50% of HIV-infected patients (Bryant *et al.*, 2015). The introduction of combination ART (cART) has improved the well-being of HIV patients, and the prevalence of severe HIV-associated dementia has decreased significantly (Sacktor *et al.*, 2001). Despite this promising outcome, the prevalence of milder forms of HAND which includes mild dysfunctions in attention, learning/memory, working memory, executive function and fine motor skills (Woods *et al.*, 2009) has increased significantly (Gannon, 2014; Shah *et al.*, 2016). However, it remains unclear whether the

increased prevalence of mild forms of HAND is due to the intrinsic risk of developing dementia with age or other direct or indirect factors of the condition itself such as the prolonged exposure to antiretroviral (ARV) drugs (Ciccarelli *et al.*, 2011; Giunta *et al.*, 2011). Studies investigating the potential adverse effects of ARVs in the central nervous system (CNS) remains surprisingly few, and even less in females, despite the emerging evidence linking certain ARVs to adverse neuropsychiatry outcomes. For instance, Ciccarelli *et al.* (2011) found that 47% patients receiving efavirenz and tenofovir disoproxil fumarate (TDF) had cognitive impairments. A recent study found that efavirenz treatment in adolescents cause fronto-striatal dysfunction indicated by blunts proactive inhibitory behavioural responses (Du Plessis *et al.*, 2019). This finding suggests that ARV may contribute to the persistence of HAND. Therefore, is a need to understand the molecular mechanisms underlying these neuropsychiatric dysfunctions for the proper management of HIV-infected individuals.

There are at least six commonly prescribed classes of drugs available for the treatment of HIV infection: (1) nucleoside reverse transcriptase inhibitors (NRTIs), (2) non-nucleoside reverse transcriptase inhibitors (NNRTI), (3) protease inhibitors (PI), (4) maturation inhibitors, (5) integrase inhibitors and (6) entry inhibitors (Ene *et al.*, 2011; Davies & Taylor, 2013). NRTIs and NNRTIs are widely used and are considered the backbone of ART. TDF is a commonly used NRTI and part of the regime of first-line drugs. The most common side effect of TDF is nephrotoxicity (Kohler *et al.*, 2009; Lebrecht *et al.*, 2009; Cote *et al.*, 2006). Further, it was reported some adults experience adverse neuropsychiatric events after switching from a regimen of efavirenz without TDF to a regimen of EFV with TDF (Allavena *et al.*, 2006). Nevirapine, on the other hand, is one of the first NNRTI's to be introduced and has been the key drug to prevent mother to child transmission of HIV. Nonetheless, nevirapine has also been associated with adverse effects such as gastric complications, hepatotoxicity, and neuropsychiatric complications including cognitive impairments and hallucinations in HIV-infected individuals (Wise *et al.*, 2002). Further, an *in vivo* study showed that nevirapine treatment impair recognition (Romão *et al.*, 2011). These studies suggest that ARV drug contribute to the neuropathogenesis of HAND. However, molecular mechanism remains undefined.

There growing evidence that HIV infected individuals may present intraneuronal amyloid beta (Aβ) accumulation or perivascular diffuse Aβ depositions, whereas extracellular amyloid plaques are predominant features in Alzheimer's disease (Andras & Toborek, 2013; Ortega & Ances, 2014). Although the molecular underlying is unknown, *in vitro* studies have shown that ARV expose increase reactive oxygen species (ROS) production initiating endoplasmic reticulum stress in culture cells (Akay *et al.*, 2014b; Nooka & Ghorpade, 2017). The increased ROS production triggers a cascade of events including increased lipid peroxidation and β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) (Tamagno *et al.*, 2008, 2012). The BACE1 is a key enzyme involved in the breakdown of the membrane bound amyloid-β precursor protein (AβPP) to generate Aβ. Also, Cerebral Aβ deposition-induced impairment of cognitive behaviour has previously been reported to be greater in female than in male mice (Wang *et al.*, 2003; Howlett *et al.*, 2004).

Aim of this study

The present study, therefore, investigated whether chronic administration of ARV drugs, TDF and nevirapine to naïve BALB/c mice induce molecules changes linked to cognitive impairments. We hypothesised that chronic administration of these drugs would cause lipid peroxidation and upregulate BACE1 to process AβPP and increase Aβ production in the hippocampus.

Materials and methods

Experimental approach

To investigate the effects TDF and nevirapine on cognitive function, lipid peroxidation and Aβ generation, we performed an *in vivo* experiment using BALB/c mice. A total of 30 BALB/c female mice were assigned randomly by allocation into 3 groups ($n = 10$ per group) in this order: (1) Control, (2) TDF and (3) nevirapine. Animals were administered their respective treatments for a period of 8 weeks. On week 7 of treatment, they were subjected to behavioural assessment. After 8 weeks animals were sacrificed, hippocampal tissue ($n = 6$ per group) were used for biochemical investigations. Lipid peroxidation was assessed by 4-hydroxynonenal (4-HNE) and Aβ1-42 measurement by ELISA, BACE1 expression was assessed by Western Blotting. Immunohistochemistry ($n = 4$ per group) was used to measure Aβ deposition in the hippocampus.

Animals

The 30 female BALB/c mice (8–12 weeks old) used in this study were obtained from the Biomedical Resource Unit (BRU) at the University of KwaZulu-Natal. Female BALB/c mice were chosen firstly because preclinical studies investigating the long term effects of ART in this gender, are limited, in spite of this gender being more susceptible to drug toxicity than males (Zopf *et al.*, 2008; Amacher, 2014) and secondly, because previous studies have shown that the formation of Aβ aggregates can be induced in this mouse strain (Boelen *et al.*, 2007; Little *et al.*, 2014). Animals were housed under standard laboratory conditions with a 12-h light-dark cycle, 23–25 °C room temperature, humidity of 55 ± 5, food and water available *ad libitum*. Animal's oestrus cycle was synchronised 7 days before experiments were initiated (Schank & McClintock, 1992; Zuena *et al.*, 2013). All experimental protocols and care of animals were approved by the University of KwaZulu-Natal Ethics Committee (Protocol reference number AREC/075/015D).

Drugs and treatment

ARV drugs were purchased from Pharmed Pharmaceutical (Pty) Ltd, South Africa. Mice were administered dose of 5 mg/kg/day TDF or 3.3 mg/kg/day Nevirapine for 8 weeks. These drugs (TDF and nevirapine) have been approved by the FDA as therapy for HIV infected humans and are administered orally by patients. Therefore, to remain clinically relevant, animal dosing was performed daily (morning) via oral gavage. There is limited information in the literature regarding ARV drug metabolism and clearance in animal models, hence a dose equivalent to human therapeutic dose was used (dose calculation-human TDF daily dose: 300 mg/60 kg human = 5 mg/kg; 30 g mouse = 0.150 mg/day). Treatments included TDF (0.150 mg/day), Nevirapine (0.1 mg/day), or vehicle (distilled water). This treatment regime also allowed comparison to previous studies which evaluated ARV toxicity mouse models (Kohler *et al.*, 2009; Romão *et al.*, 2011; de Oliveira *et al.*, 2014). Drugs treatments were freshly prepared daily by dissolving

https://doi.org/10.1017/neu.2020.19 Published online by Cambridge University Press

tablets (TDF and nevirapine) in distilled water and then administered to the animals via oral gavage. The control group received distilled water also via oral gavage. The weight of the animals was recorded daily, and drug volumes were adjusted accordingly (10 ml/kg).

Morris water maze

On week 7 of treatment, all mice were subjected to the Morris water maze which is an established test used to assess spatial learning and memory in rodents (Vorhees & Williams, 2006). Our Morris water maze consisted of a circular pool with a 120 cm diameter and a wall height of 85 cm. It had a hidden platform of 10 × 10 cm located in one of the quadrants. The pool was filled with water to a depth of 30 cm (22 ± 1 °C) that was 1 cm above the level of the platform. Visual cues were attached to the walls of the pool and to the walls of the testing room to orientate the animals to the location of the platform. Each mouse was positioned with its face towards the wall and released from each of the four different quadrants of the maze to find the hidden platform. A time interval of 60 s was allowed for the animal to find the platform. If the animal failed to locate the platform, it was guided to the platform and allowed to remain on the platform for 15 s to orientate itself. This training session was repeated for 5 consecutive days. Time to reach the hidden platform was recorded and is reported as escape latency. On the sixth day, the platform was removed, and memory retention of the platform location was assessed. The time spent in the target quadrant was therefore recorded. Behavioural assessments were manually scored during the test by an experience investigator.

Tissue collection for respective assays

On the final day of drug administration 6 hours after the final drug dosage, animals to be used for ELISA analysis were euthanised and immediately decapitated, dorsal hippocampal tissue was harvested, snap frozen in liquid nitrogen and stored in a bio-freezer at −80 °C for subsequent biochemical analyses. For the immunohistochemistry procedure, whole brains were collected following transcardial perfusion.

Mouse 4-HNE ELISA

4-HNE levels were quantified using an ELISA kit (Elabscience Biotechnology Co., China). Hippocampal tissue was homogenised in phosphate-buffered saline (0.01 M, pH = 7.4). The tissue homogenate was centrifuged for 5 min at 5000 g. The supernatant (100 µl) of each sample or HNE standards were added to each well of a 96-well plate and incubated for 10 min at 37 °C. After incubation, wells were washed and 100 µl of biotinylated antibody was added to each well and incubated at 37 °C for a further 1 h on an orbital shaker. After incubation, wells were washed after which a 100 µl of horseradish peroxidase (HRP) conjugated working solution was added to each well. The plate was then incubated for 30 min at 37 °C. The wells were once again washed, and the substrate solution was added to each well. The optical density (OD) of each well was measured using a micro-plate reader set to 450 nm as per the manufacturer's protocol.

Western blotting

Dorsal hippocampal tissue was lysed in RIPA lysis buffer (w/v) (Sigma, USA). The homogenates were centrifuged at 3578*g* for 10 min at 4 °C. Thereafter, supernatants were extracted and their protein concentration determined using the Bradford method (Kruger, 2009). Samples were then denatured for 5 min at 95 °C. Equal amounts of protein (20 µg) were resolved by electrophoresis on a 10% sodium dodecyl-sulfate polyacrylamide gel in running buffer (Bio-Rad, South Africa), at 200 V for 1 h. Proteins were transferred onto nitrocellulose membrane (Bio-Rad, South Africa) using a transfer buffer (Bio-Rad, South Africa) at 100 V for 1 h. The membrane was blocked with blocking buffer (Li-COR, Germany) for 2 h and thereafter incubated with primary monoclonal BACE rabbit antibody (Cell Signaling, USA) and monoclonal β-actin mouse antibody (Cell Signaling, USA) (1:1000 dilution in blocking buffer) overnight. After completion of incubation with primary antibodies, the membrane was washed three times with 0.1 M PBS-Tween for 10 min. The secondary antibodies IRDye 800CW goat anti-rabbit and IRDye 680RD goat anti-mouse (LI-COR, Germany) (1:10,000 dilution in blocking buffer) were then added to the membrane and incubated for 2 h at room temperature. Afterwards, the membrane was washed three times with 0.1 M PBS-Tween and washed once with PBS. Finally, the membrane was viewed on an Odyssey CLx LI-COR infrared fluorescence imaging system (Biosciences, Germany). The intensities of signals on the blots were normalised to β-actin in a single channel using the LI-COR Odyssey Image Studio software (LI-COR Biosciences).

Mouse Aβ1-42 ELISA

The concentration levels of Aβ1-42 were quantified using an ELISA kit (Elabscience Biotechnology Co., China). Dorsal hippocampal tissue was homogenised in phosphate-buffered saline (0.01 M, pH = 7.4). The tissue homogenate was centrifuged for 5 min at 5000 g. A volume of 100 µl of supernatant or Aβ1-42 standards were added to each well and then incubated for 90 min at 37 °C. After incubation, the liquid was removed and 100 µl of biotinylated antibody was added to each well and incubated at 37 °C for 1 h on an orbital shaker. After 1-h incubation, wells were washed three times with wash buffer and 100 µl of HRP-conjugated working solution was added to each well. The plate was then incubated for 30 min at 37 °C. The liquid was aspirated and wells were washed. Substrate solution was added to each well and then finally stop solution was added. Optical density of each well was measured using a micro-plate reader set to 450 nm as per the manufacturer's protocol.

Immunohistochemistry

Mice were anaesthetised with an intraperitoneal injection of pentobarbital (100 mg/kg) and then transcardially perfused with physiological saline (0.9%) containing protease inhibitors followed by 4% paraformaldehyde before removal of brains. Whole brains were post-fixed in 4% paraformaldehyde for 24 h. All brains were then cryopreserved in 15% (w/v) and 30% (w/v) sucrose in PBS for 24 h at 4 °C. Brains were embedded in optimal cutting temperature compound and then frozen in liquid nitrogen. Coronal sections (30 µm) were cut using a cryostat (Leica, Germany). Frozen sections were air-dried at room temperature for 15 min and covered with 4% paraformaldehyde for 10 min. The sections were then rinsed with PBST (0.25% Triton-X100 in PBS) and blocked for 30 min at room temperature in 5% goat serum. After incubation with monoclonal mouse APP/β-amyloid antibody (NAB228) (Cell Signaling, USA) diluted in PBS, for 24 h at 4 °C, sections were washed with PBS and incubated with anti-mouse HRP-linked secondary antibody (Cell Signaling, USA) for 1 h at room temperature. The DAB substrate kit (Cell Signaling, USA) was used for

https://doi.org/10.1017/neu.2020.19 Published online by Cambridge University Press

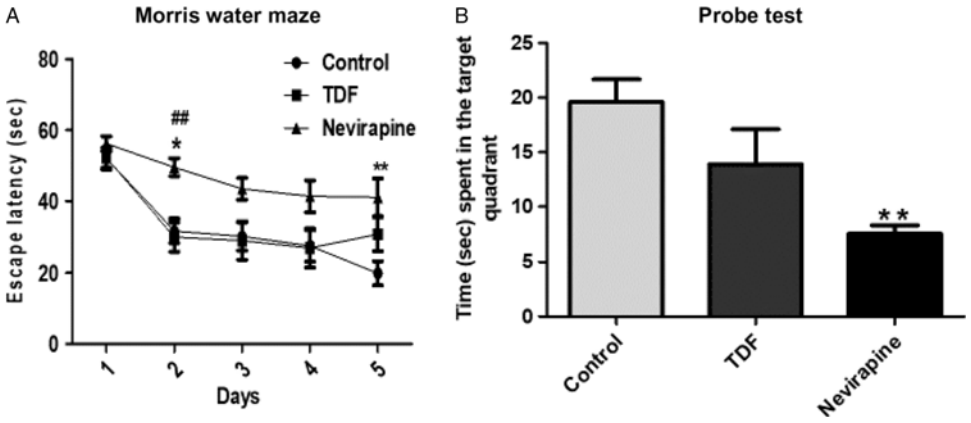

Fig. 1. The effect of chronic administration of TDF and nevirapine on spatial learning and memory in mice. (A) Learning in the Morris water maze, that is, time to locate the hidden platform. Nevirapine-treated mice showed significantly greater escape latencies in the hidden platform test compared to control mice ($^{*}p < 0.05$; $^{**}p < 0.01$) or TDF ($^{\#\#}p < 0.01$). (B) Probe trials to assess spatial memory retrieval, that is, time spent in the target quadrant in the Morris water maze. Nevirapine-treated mice spent significantly less time in the target quadrant compared to control group ($^{**}p < 0.01$). Data are presented as means ± SEM ($n = 10$ per group).

detection. Sections were washed again and mounted. The images were captured using a microscope (Leica, Germany) and analysed using ImageJ software (National Institutes of Health, USA). A measure of amyloid load was determined as follows: Aβ deposits in each of five representative sections of the dorsal hippocampus of each of the four animals per group were counted, and an average Aβ deposits number per animal was recorded. This number of Aβ deposits occupying in the investigated dorsal hippocampal area was taken as a measure of hippocampal amyloid deposit load per mouse.

Statistical analysis

Data were analysed using GraphPad Prism version 5 software. The Shapiro–Wilk test was used to test the data for normality. The results are presented as mean ± standard error of the mean. The escape latencies in the Morris water maze were analysed with two-way repeated measures ANOVA with drug treatment as main factor and days as repeated measure, followed by Bonferroni *post hoc* comparisons to determine differences between groups. All other data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test to determine differences between groups. Differences were considered statistically significant when $p < 0.05$.

Results

Morris water maze

We assessed spatial learning by analysing the time taken to find the hidden platform (escape latency) and subsequently looked at memory retention as indicated by the time spent in the target quadrant during the probe test. Repeated measures ANOVA showed a main effect of treatment on spatial learning [$F(2, 108) = 8.834$, $p = 0.01$]. Bonferroni *post hoc* comparisons showed no significant differences in escape latency between groups on day 1; however, on day 2, the escape latency of the nevirapine-treated group ($n = 10$) was significantly increased compared to control group ($n = 10$) ($p < 0.05$) or TDF ($n = 10$) ($p < 0.01$, Fig. 1A). On day 3 and 4, the latency was not significantly different between the groups; however, there is a trend towards an increased latency in mice treated with nevirapine. On day 5, the latency to escape of the nevirapine-treated group was again significantly increased compared to the control group ($p < 0.01$).

In the probe test, one-way ANOVA data analysis showed a significant impairment of memory retention [$F(2, 27) = 7.123$, $p = 0.0033$]. Bonferroni *post hoc* test showed no significant differences were observed for the TDF-treated group compared to the control group ($p > 0.05$); however, a significant decrease in time spent by the nevirapine-treated group in the target quadrant compared to the control group ($p < 0.01$, Fig. 1B).

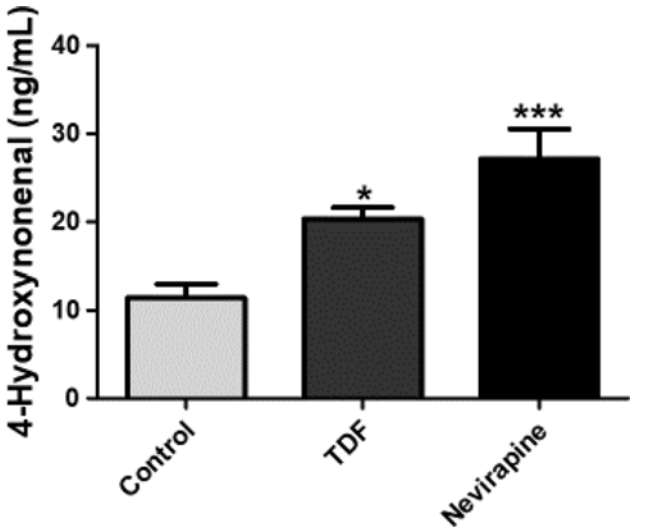

Fig. 2. Long-term administration of antiretroviral drugs, TDF and nevirapine, increased the concentration of 4-hydroxynonenal in the hippocampus. The TDF group ($^{*}p < 0.05$) and the nevirapine group ($^{***}p < 0.001$) had significantly higher levels than the control group. Data are presented as means ± SEM ($n = 6$ per group).

Lipid peroxidation (4-HNE) concentration

4-HNE is a cytotoxic lipid-derived aldehyde often used as an indicator of lipid peroxidation (Ayala *et al.*, 2014). 4-HNE increase in the brain is also associated with increased Aβ production and is used as a key indicator of neurodegenerative pathologies (Di Domenico *et al.*, 2017). HNE concentration in hippocampal tissue was measured in all groups after 8 weeks of drug administration. One-way ANOVA showed a main effect of treatment on HNE concentrations [$F(2, 15) = 11.83$, $p = 0.0008$]. Bonferroni *post hoc* test showed a significantly higher concentration of HNE in the TDF-treated group ($p < 0.05$) and nevirapine-treated group ($p < 0.001$) compared to the control group (Fig. 2).

BACE1 expression

Hippocampus BACE1 expression was measured using Western blotting in all groups. One-way ANOVA revealed a significant

https://doi.org/10.1017/neu.2020.19 Published online by Cambridge University Press

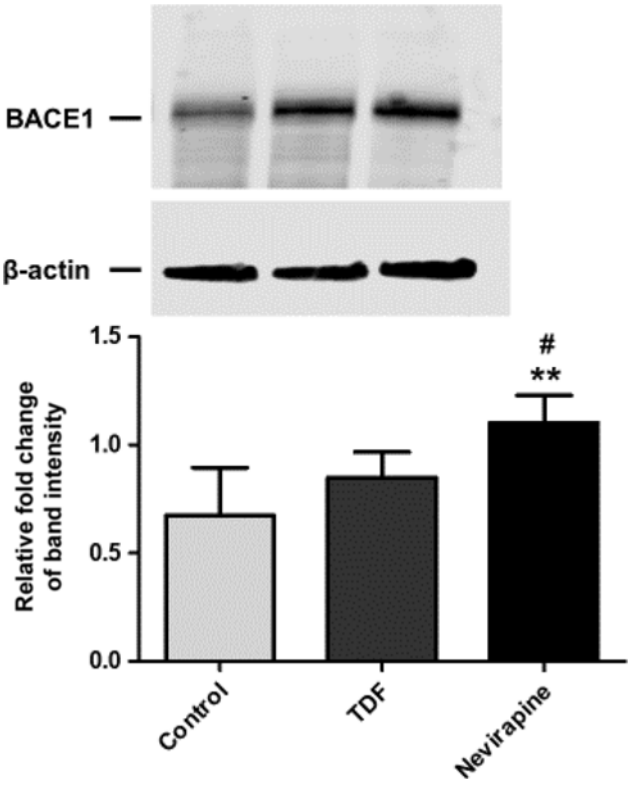

Fig. 3. Effect of TDF and nevirapine treatment on BACE1 expression in the hippocampus of mice. BACE1 expression was measured by Western blot and band intensity was normalized against β-actin. Results revealed a significant increase in BACE1 expression in the nevirapine-treated group when compared to the control group (**$p < 0.01$) and TDF (#$p < 0.05$) groups. Data are presented as means ± SEM ($n = 6$ per group).

difference in BACE1 expression in the hippocampus [F (2, 15) = 10.66, $p = 0.0013$]. Bonferroni *post hoc* test showed a significant increase in the expression of BACE1 in the nevirapine-treated group compared to control ($p < 0.01$) and TDF groups ($p < 0.05$, Fig. 3).

Aβ1-42 concentration

The Aβ1-42 peptide is considered to be more neurotoxic than Aβ1-40 (Tamagno *et al.*, 2012); therefore, we thought that measuring the longer version of the peptide would be more relevant to our current study. One-way ANOVA showed a significant difference [F (2, 15) = 10.56, $p = 0.0014$] in the Aβ1-42 concentrations of the various groups of animals. Bonferroni *post hoc* test showed a significantly high Aβ1-42 concentration in the TDF ($p < 0.05$) and nevirapine-treated ($p < 0.01$) group compared to controls, Fig. 4.

Aβ deposition

The last objective of the present study was to determine, through immunohistochemical analysis, whether ARV drugs affect Aβ deposition in the hippocampus. One-way ANOVA showed a main effect of treatment on the number of Aβ deposits in the hippocampus [F (2, 09) = 5.957, $p = 0.0255$]. Bonferroni *post hoc* test showed a significant increase in the number of Aβ deposits in the nevirapine group compared to control mice ($p < 0.05$, Fig. 5).

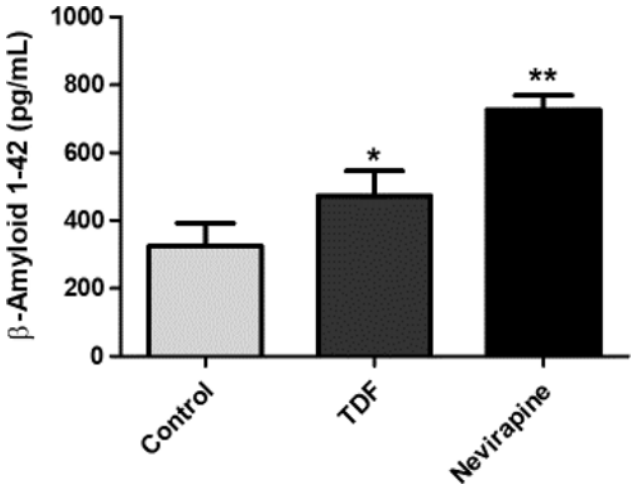

Fig. 4. The effects of TDF and nevirapine treatment on amyloid-β 1-42 peptide concentration in the hippocampus. Data showed significantly higher levels of Aβ1-42 in the hippocampus of TDF-treated group compared to the control (*$p < 0.05$) and nevirapine group (**$p < 0.01$). Data are presented as means ± SEM ($n = 6$ per group).

Discussion

Understanding the aetiology of HAND is critical for better management of HIV. Whether ARV drugs contribute to the development of HAND has not yet been clarified. This study subsequently set out to investigate whether chronic exposure to anti-HIV drugs (TDF and nevirapine) per se can negatively impact cognitive function. Since Aβ accumulation is among the neuropathologies observed in post-mortem brains of patients that were infected with HIV (Turner *et al.*, 2016; Solomon *et al.*, 2017), the present study tested the hypothesis that ARV drug treatment promote of Aβ and cognitive impairment. We further proposed that the accumulation of the Aβ is associated with drug-induced lipid peroxidation and the upregulation of the enzyme BACE1.

In the Morris water maze test, our findings showed that chronic exposure to nevirapine impaired spatial learning and memory. This finding is in line with a previous study by Romão *et al.* (2011) which showed nevirapine treatment impaired recognition memory formation evaluated in the object recognition test in male CF-1 mice aged 60 days. These impairments were associated with lipid peroxidation (4-HNE), upregulation of BACE1 and increased Aβ in the hippocampus. Similarly, TDF treatment impaired learning and induced lipid peroxidation, but the effects were not as profound as nevirapine. Further, TDF did not affect BACE1expression and in Aβ deposition, despite an increase in Aβ. In overall, animals that were treated with TDF showed milder neurochemical changes than nevirapine-treated mice. These findings are consistent with previous *in vitro* studies showing that neuronal cell exposed to ARV had an increase Aβ production (Giunta *et al.*, 2011; Brown *et al.*, 2014b; Hui *et al.*, 2019). Although the mechanisms by which ART promote Aβ are still unclear, a recent study by Hui *et al.* (2019) found that nevirapine and TDF cause de-acidification endolysosomes and inhibited clearance of Aβ. Our findings are corroborate previous studies which showed that ARV treatment induce oxidative damage in mice (Zuena *et al.*, 2013; de Oliveira *et al.*, 2014). We speculate that differences in effect between the two drugs can be attributed to (1) metabolisation of these drugs and (2) weak inhibition of DNA polymerase by TDF, as well as TDF previously reported limited penetration into the central nervous system (Birkus *et al.*, 2002; Ferrer & Rakhmanina, 2013).

https://doi.org/10.1017/neu.2020.19 Published online by Cambridge University Press

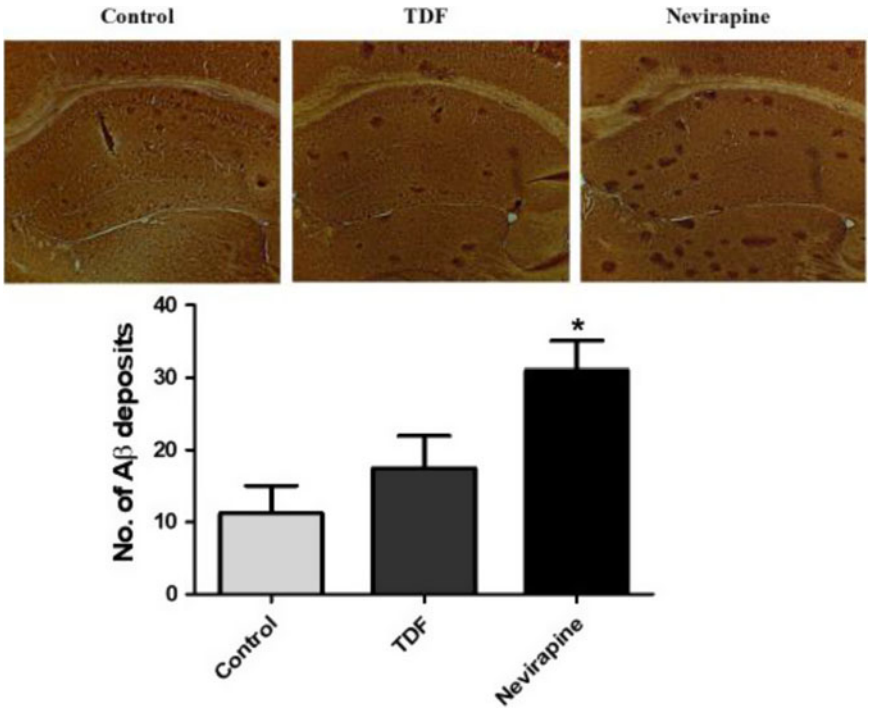

Fig. 5. Effect of chronic administration of antiretroviral drugs, TDF and nevirapine, on Aβ deposition in the dorsal hippocampus (scale bar = 200 μm). Immunohistochemical staining for Aβ showed no significant increase in Aβ deposit number in the TDF-treated group compared to control animals. A significant increase in the number of Aβ deposition was observed in the nevirapine-treated group when compared to the control group (*$p < 0.05$). Data are presented as means ± SEM ($n = 4$ per group).

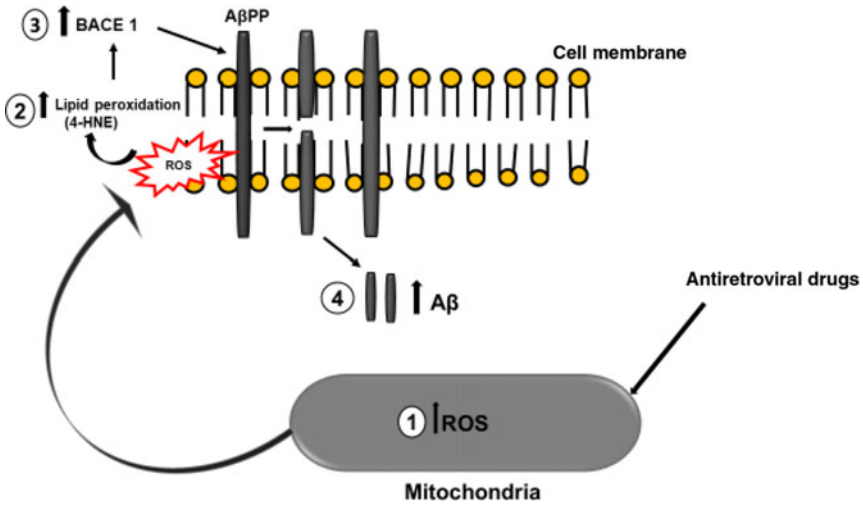

Fig. 6. Diagram representing the sequence of events on antiretroviral drugs promoting Aβ production. (1) Antiretroviral promote reactive oxygen species (ROS). (2) ROS induce lipid peroxidation. (3) BACE1 expression and activity increased by lipid peroxidation. (4) Amyloid peptide (Aβ) generated following the sequential cleavage of its precursor, the amyloid-β precursor protein (AβPP) by BACE1.

On the other hand, the considerable deleterious effect of nevirapine on spatial learning and memory may be related to its ability to produce greater lipid peroxidation in the hippocampus. Lipid peroxidation indicated by 4-HNE in the present study signify a greater intensity of oxidative stress in the hippocampus leading to pathological conditions that may include cognitive dysfunction. This reasoning is plausible as several studies have associated with oxidative stress with brain ageing and neurodegenerative processes (Mariani *et al.*, 2005; Reddy *et al.*, 2011; Montgomery *et al.*, 2012).

Studies suggest that NRTIs inhibit mitochondrial DNA polymerase-γ causing the depletion of mitochondrial DNA. This aberration in DNA quantity leads to excessive production of mitochondrial ROS which in turn may be responsible for oxidative damage to lipid, amino acid and DNA components of the cell (Bienstock & Copeland, 2004; Apostolova *et al.*, 2011). Similarly, NNRTIs have shown to mediate cellular toxicity by inhibition of creatine kinase and cytochrome C complex IV activity in the brain (Apostolova *et al.*, 2010; Streck *et al.*, 2011). Preclinical and clinical studies have linked NRTIs and NNRTIs exposure with cognitive dysfunction in HIV-1-infected individuals through mechanism involving oxidative stress (Akay *et al.*, 2014a; Brown *et al.*, 2014a; Sharma, 2014). It seems however that alternative downstream molecular mechanisms may be recruited by the different classes of ARVs resulting in the one drug (NNRTIs) showing greater iatrogenic damage than the other (NRTIs).

The aetiology of Alzheimer's disease has been closely linked to oxidative stress and Aβ neurotoxicity (Hardy & Selkoe, 2002). BACE1 is regarded as a key enzyme in Aβ peptide synthesis (Vassar *et al.*, 1999), and cleavage of APP by BACE1 is the initial step towards Aβ production. Different studies have shown that 4-HNE upregulates BACE1 in neuroblastoma cell lines and

https://doi.org/10.1017/neu.2020.19 Published online by Cambridge University Press

primary cortical cultures (Tamagno *et al.*, 2005, 2008; de O'Leary *et al.*, 2012), a finding supported by another study that showed elevated Aβ in wild-type mice following intracerebral infusion of 4-HNE (Arimon *et al.*, 2015). The link between lipid peroxidation, BACE1 expression and Aβ production has previously been established (Mouton-Liger *et al.*, 2012; Muche *et al.*, 2017) (Fig. 6).

Further support for our postulate comes from histopathological studies that identified Aβ accumulation in the central nervous system of HIV-positive patients on ART (Esiri *et al.*, 1998; Brew *et al.*, 2005; Green *et al.*, 2005). Our results, therefore, support evidence that ART may contribute to the formation of Aβ in the brains of HIV-infected persons and that the observed Aβ deposits may partially stem from ARV treatment. We have also previously shown that TDF and nevirapine upregulate neuroinflammatory cytokine and astrogliosis *in vivo* (Zulu *et al.*, 2018). As such our findings point to a possibility that ART may be one of the aetiological factors of HAND and suggest that it may be worthwhile to investigate whether the supplementation of ARVs with agents that reduce oxidative stress hold any benefit to HIV-infected individuals on ART with respect to the possible development of HAND. In conclusion, this study demonstrated that ARV drugs, TDF and nevirapine, induce lipid peroxidation. Also, nevirapine promotes Aβ by upregulating BACE1 and AβPP production in the hippocampus and subsequently cause spatial learning and memory impairments. Therefore, consideration should be given to HIV medication since it might be among the factors that contribute to the persistence of neurocognitive disorders in HIV-infected persons on cART.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/neu.2020.19

Authors contributions. SSZ, WMUD and MVM conceived of the presented idea.
SSZ and OA carried out the experiments.
SSZ wrote the manuscript with support from WMUD, OA and NS
NS, WMUD contributed to the interpretation of the results
Critical revision of the article was done by MVM and OA.
All authors discussed the results and contributed to the final manuscript.

Financial support. The authors wish to thank the National Research Foundation of South Africa and the College of Health Sciences from the University of KwaZulu-Natal for their financial support. NS wishes to thank the Italian Ministry of Foreign Affairs (MAE) for financial support.

Statement of interest. Authors declare no competing interests.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

Akay C, Cooper M, Odeleye A, Jensen BK, White MG, Vassoler F, Gannon PJ, Mankowski J, Dorsey JL and Buch AM (2014a) Antiretroviral drugs induce oxidative stress and neuronal damage in the central nervous system. *Journal of Neurovirology* **20**, 39–53.

Akay C, Cooper M, Odeleye A, Jensen BK, White MG, Vassoler F, Gannon PJ, Mankowski J, Dorsey JL, Buch AM, Cross SA, Cook DR, Pena MM, Andersen ES, Christofidou-Solomidou M, Lindl KA, Zink MC, Clements J, Pierce RC, Kolson DL and Jordan-Sciutto KL (2014b) Antiretroviral drugs induce oxidative stress and neuronal damage in the central nervous system. *Journal of Neurovirology* **20**, 39–53.

Allavena C, Le Moal G, Michau C, Chiffoleau A and Raffi F (2006) Neuropsychiatric adverse events after switching from an antiretroviral regimen containing efavirenz without tenofovir to an efavirenz regimen containing tenofovir: a report of nine cases. *Antiviral Therapy* **11**, 263–265.

Amacher DE (2014) Female gender as a susceptibility factor for drug-induced liver injury. *Human & Experimental Toxicology* **33**, 928–939.

Andras IE and Toborek M (2013) Amyloid beta accumulation in HIV-1-infected brain: the role of the blood brain barrier. *IUBMB Life* **65**, 43–49.

Apostolova N, Blas-Garcia A and Esplugues JV (2011) Mitochondrial interference by anti-HIV drugs: mechanisms beyond Pol-gamma inhibition. *Trends in Pharmacological Sciences* **32**, 715–725.

Apostolova N, Gomez-Sucerquia LJ, Moran A, Alvarez A, Blas-Garcia A and Esplugues JV (2010) Enhanced oxidative stress and increased mitochondrial mass during Efavirenz-induced apoptosis in human hepatic cells. *British Journal of Pharmacology* **160**, 2069–2084.

Arimon M, Takeda S, Post KL, Svirsky S, Hyman BT and Berezovska O (2015) Oxidative stress and lipid peroxidation are upstream of amyloid pathology. *Neurobiology of Disease* **84**, 109–119.

Ayala A, Muñoz MF and Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Medicine and Cellular Longevity* **2014**, 360438.

Bienstock RJ and Copeland WC (2004) Molecular insights into NRTI inhibition and mitochondrial toxicity revealed from a structural model of the human mitochondrial DNA polymerase. *Mitochondrion* **4**, 203–213.

Birkus G, Hajek M, Kramata P, Votruba I, Holy A and Otova B (2002) Tenofovir diphosphate is a poor substrate and a weak inhibitor of rat DNA polymerases alpha, delta, and epsilon*. *Antimicrob Agents Chemother* **46**, 1610–1613.

Boelen E, Stassen FR, van der Ven AJ, Lemmens MA, Steinbusch HP, Bruggeman CA, Schmitz C and Steinbusch HW (2007) Detection of amyloid beta aggregates in the brain of BALB/c mice after Chlamydia pneumoniae infection. *Acta Neuropathologica* **114**, 255–261.

Brew BJ, Pemberton L, Blennow K, Wallin A and Hagberg L (2005) CSF amyloid beta42 and tau levels correlate with AIDS dementia complex. *Neurology* **65**, 1490–1492.

Brown LA, Jin J, Ferrell D, Sadic E, Obregon D, Smith AJ, Tan J and Giunta B (2014a) Efavirenz promotes β-Secretase expression and increased Aβ 1-40, 42 via oxidative stress and reduced microglial phagocytosis: implications for HIV associated neurocognitive disorders (HAND). PLoS One 9, e95500.

Brown LAM., Jin J, Ferrell D, Sadic E, Obregon D, Smith AJ., Tan J and Giunta B (2014b) Efavirenz promotes β-Secretase expression and increased $A\beta_{1\text{-}40,42}$ via oxidative stress and reduced microglial phagocytosis: implications for HIV associated neurocognitive disorders (HAND). *PLoS One* **9**, e95500.

Bryant AK, Ellis RJ, Umlauf A, Gouaux B, Soontornniyomkij V, Letendre SL, Achim CL, Masliah E, Grant I and Moore DJ (2015) Antiretroviral therapy reduces neurodegeneration in HIV infection. *AIDS* **29**, 323–330.

Ciccarelli N, Fabbiani M, di Giambenedetto S, Fanti I, Baldonero E, Bracciale L, Tamburrini E, Cauda R, de Luca A and Silveri MC (2011) Efavirenz associated with cognitive disorders in otherwise asymptomatic HIV-infected patients. *Neurology* **76**, 1403–1409.

Cote HC, Magil AB, Harris M, Scarth BJ, Gadawski I, Wang N, Yu E, Yip B, Zalunardo N, Werb R, Hogg R, Harrigan PR and Montaner JS (2006) Exploring mitochondrial nephrotoxicity as a potential mechanism of kidney dysfunction among HIV-infected patients on highly active antiretroviral therapy. *Antiviral Therapy* **11**, 79–86.

Davies S and Taylor S (2013) Antiretroviral pharmacology. *Medicine* **41**, 474–478.

de O'Leary JC, Obregon D, Fernandez F, Tan J and Giunta B (2012) The impact of HAART on advanced brain aging: implications for mitochondrial dysfunction and APP processing. *Journal of Antivirals and Antiretrovirals* **10**, 2.

de Oliveira HM, Damiani AP, Dias Rde O, Romao PR and Andrade VM (2014) Effect of antiretroviral drugs on the DNA damage in mice. *Environmental Toxicology and Pharmacology* **37**, 390–395.

di Domenico F, Tramutola A and Butterfield DA (2017) Role of 4-hydroxy-2-nonenal (HNE) in the pathogenesis of alzheimer disease and other selected age-related neurodegenerative disorders. *Free Radical Biology and Medicine* **111**, 253–261.

du Plessis S, Perez A, Fouche JP, Phillips N, Joska JA, Vink M, Myer L, Zar HJ, Stein DJ and Hoare J (2019) Efavirenz is associated with altered

fronto-striatal function in HIV+ adolescents. *Journal of Neurovirology* **25**, 783–791.

Ene L, Duiculescu D and Ruta SM (2011) How much do antiretroviral drugs penetrate into the central nervous system?. *Journal of Medicine and Life* **4**, 432–439.

Esiri MM, Biddolph SC and Morris CS (1998) Prevalence of Alzheimer plaques in AIDS. *Journal of Neurology, Neurosurgery & Psychiatry* **65**, 29–33.

Ferrer K and Rakhmanina N (2013) Neuropsychiatric effects of tenofovir in comparison with other antiretroviral drugs. *Neurobehavioral HIV Medicine* **5**, 1–10.

Gannon PJ (2014) Assessing the Contribution Antiretroviral Therapy to Neuronal Damage and Death as a Mediator of Cognitive Decline in HIV-Associated Neurocognitive Disorders. *Publicly Accessible Penn Dissertations*, 1280.

Giunta B, Ehrhart J, Obregon DF, Lam L, Le L, Jin J, Fernandez F, Tan J and Shytle RD (2011) Antiretroviral medications disrupt microglial phagocytosis of beta-amyloid and increase its production by neurons: implications for HIV-associated neurocognitive disorders. *Molecular Brain* **4**, 23.

Green DA, Masliah E, Vinters HV, Beizai P, Moore DJ and Achim CL (2005) Brain deposition of beta-amyloid is a common pathologic feature in HIV positive patients. *AIDS* **19**, 407–411.

Hardy J and Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353–356.

Howlett DR, Richardson JC, Austin A, Parsons AA, Bate ST, Davies DC and Gonzalez MI (2004) Cognitive correlates of Aβ deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. *Brain Research* **1017**, 130–136.

Hui L, Ye Y, Soliman ML, Lakpa KL, Miller NM, Afghah Z, Geiger JD and Chen X (2019) Antiretroviral drugs promote amyloidogenesis by Deacidifying endolysosomes. *Journal of Neuroimmune Pharmacology*, 1–10.

Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, Johnson DM, Russ R, Tran D, Raper CM, Santoianni R and Lewis W (2009) Tenofovir renal toxicity targets mitochondria of renal proximal tubules. *Laboratory Investigation* **89**, 513–519.

Kruger NJ (2009) The Bradford method for protein quantitation. *The Protein Protocols Handbook* **15**, 17–24.

Lebrecht D, Venhoff AC, Kirschner J, Wiech T, Venhoff N and Walker UA (2009) Mitochondrial tubulopathy in tenofovir disoproxil fumarate-treated rats. *Journal of Acquired Immune Deficiency Syndromes* **51**, 258–263.

Little CS, Joyce TA, Hammond CJ, Matta H, Cahn D, Appelt DM and Balin BJ (2014) Detection of bacterial antigens and Alzheimer's disease-like pathology in the central nervous system of BALB/c mice following intranasal infection with a laboratory isolate of Chlamydia pneumoniae. *Frontiers in Aging Neuroscience* **6**, 304.

Mariani E, Polidori M, Cherubini A and Mecocci P (2005) Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview. *Journal of Chromatography B* **827**, 65–75.

Mcarthur JC, Steiner J, Sacktor N and Nath A (2010) Human immunodeficiency virus-associated neurocognitive disorders: mind the gap. *Annals of Neurology* **67**, 699–714.

Montgomery MK, Buttemer WA and Hulbert AJ (2012) Does the oxidative stress theory of aging explain longevity differences in birds? II. Antioxidant systems and oxidative damage. *Experimental Gerontology* **47**, 211–222.

Mouton-Liger F, Paquet C, Dumurgier J, Bouras C, Pradier L, Gray F and Hugon J (2012) Oxidative stress increases BACE1 protein levels through activation of the PKR-eIF2alpha pathway. *Biochimica et Biophysica Acta* **1822**, 885–896.

Muche A, Arendt T and Schliebs R (2017) Oxidative stress affects processing of amyloid precursor protein in vascular endothelial cells. *PLoS One* **12**, e0178127.

Nooka S and Ghorpade A (2017) HIV-1-associated inflammation and antiretroviral therapy regulate astrocyte endoplasmic reticulum stress responses. *Cell Death Discovery* **3**, 17061–17061.

Ortega M and Ances BM (2014) Role of HIV in amyloid metabolism. *Journal of Neuroimmune Pharmacology* **9**, 483–491.

Reddy PH, Reddy TP, Manczak M, Calkins MJ, Shirendeb U and Mao P (2011) Dynamin-related protein 1 and mitochondrial fragmentation in neurodegenerative diseases. *Brain Research Reviews* **67**, 103–118.

Romão PRT, Lemos JC, Moreira J, de Chaves G, Moretti M, Castro AA, Andrade VM, Boeck CR, Quevedo J and Gavioli EC (2011) Anti-HIV drugs nevirapine and efavirenz affect anxiety-related behavior and cognitive performance in mice. *Neurotoxicity Research* **19**, 73–80.

Sacktor N, Lyles RH, Skolasky R, Kleeberger C, Selnes OA, Miller EN, Becker JT, Cohen B and Mcarthur JC (2001) HIV-associated neurologic disease incidence changes: multicenter AIDS cohort study, 1990–1998. *Neurology* **56**, 257–260.

Schank JC and Mcclintock MK (1992) A coupled-oscillator model of ovarian-cycle synchrony among female rats. *Journal of Theoretical Biology* **157**, 317–362.

Shah A, Gangwani MR, Chaudhari NS, Glazyrin A, Bhat HK and Kumar A (2016) Neurotoxicity in the post-HAART Era: caution for the antiretroviral therapeutics. *Neurotoxicity Research* **30**, 677–697.

Sharma B (2014) Oxidative stress in HIV patients receiving antiretroviral therapy. *Current HIV Research* **12**, 13–21.

Solomon IH, de Girolami U, Chettimada S, Misra V, Singer EJ and Gabuzda D (2017) Brain and liver pathology, amyloid deposition, and interferon responses among older HIV-positive patients in the late HAART era. *BMC Infectious Diseases* **17**, 151.

Streck EL, Ferreira GK, Scaini G, Rezin GT, Goncalves CL, Jeremias IC, Zugno AI, Ferreira GC, Moreira J, Fochesato CM and Romao PR (2011) Non-nucleoside reverse transcriptase inhibitors efavirenz and nevirapine inhibit cytochrome C oxidase in mouse brain regions. *Neurochemical Research* **36**, 962–966.

Tamagno E, Guglielmotto M, Aragno M, Borghi R, Autelli R, Giliberto L, Muraca G, Danni O, Zhu X, Smith MA, Perry G, Jo DG, Mattson MP and Tabaton M (2008) Oxidative stress activates a positive feedback between the gamma- and beta-secretase cleavages of the beta-amyloid precursor protein. *Journal of Neurochemistry* **104**, 683–695.

Tamagno E, Guglielmotto M, Monteleone D and Tabaton M (2012) Amyloid-β production: major link between oxidative stress and BACE1. *Neurotoxicity Research* **22**, 208–219.

Tamagno E, Parola M, Bardini P, Piccini A, Borghi R, Guglielmotto M, Santoro G, Davit A, Danni O, Smith MA, Perry G and Tabaton M (2005) Beta-site APP cleaving enzyme up-regulation induced by 4-hydroxynonenal is mediated by stress-activated protein kinases pathways. *Journal of Neurochemistry* **92**, 628–636.

Turner RS, Chadwick M, Horton WA, Simon GL, Jiang X and Esposito G (2016) An individual with human immunodeficiency virus, dementia, and central nervous system amyloid deposition. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* **4**, 1–5.

Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G and Citron M (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286**, 735–741.

Vorhees CV and Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols* **1**, 848–858.

Wang J, Tanila H, Puoliväli J, Kadish I and Van Groen T (2003) Gender differences in the amount and deposition of amyloidβ in APPswe and PS1 double transgenic mice. *Neurobiology of Disease* **14**, 318–327.

Wise ME, Mistry K and Reid S (2002) Drug points: neuropsychiatric complications of nevirapine treatment. *Bmj* **324**, 879.

Woods SP, Moore DJ, Weber E and Grant I (2009) Cognitive neuropsychology of HIV-associated neurocognitive disorders. *Neuropsychology Review* **19**, 152–168.

Zopf Y, Rabe C, Neubert A, Gaßmann KG, Rascher W, Hahn EG, Brune K and Dormann H (2008) Women encounter ADRs more often than do men. *European Journal of Clinical Pharmacology* **64**, 999.

Zuena AR, Giuli C, Venerosi Pesciolini A, Tramutola A, Ajmone-Cat MA, Cinque C, Alema GS, Giovine A, Peluso G, Minghetti L, Nicolai R, Calamandrei G and Casolini P (2013) Transplacental exposure to AZT induces adverse neurochemical and behavioral effects in a mouse model: protection by L-acetylcarnitine. *PLoS One* **8**, e55753.

Zulu SS, Simola N, Mabandla MV and Daniels WMU. (2018) Effect of long-term administration of antiretroviral drugs (Tenofovir and Nevirapine) on neuroinflammation and neuroplasticity in mouse hippocampi. *Journal of Chemical Neuroanatomy* **94**, 86–92.

https://doi.org/10.1017/neu.2020.19 Published online by Cambridge University Press