ELSEVIER

Alzheimer's & Dementia ■ (2017) 1-10

Alzheimer's & Dementia

Featured Article

Mechanical stress increases brain amyloid β, tau, and α-synuclein concentrations in wild-type mice

Marcel Levy Nogueira[a,b,c,e,*], Minoo Hamraz[d,e], Mohammad Abolhassani[d,e], Erwan Bigan[c,e], Olivier Lafitte[d,e], Jean-Marc Steyaert[c,e], Bruno Dubois[a,b,e,f], Laurent Schwartz[c,e]

[a]*Institut de la Mémoire et de la Maladie d'Alzheimer (IM2A), Département de Neurologie, Hôpital de la Pitié-Salpêtrière, AP-HP, Paris, France*
[b]*Institut de Recherche Translationnelle en Neurosciences (IHU-A-ICM), Institut du Cerveau et de la Moelle Epinière (ICM), Paris, France*
[c]*Laboratoire d'informatique (LIX), UMR 7161, École Polytechnique, Université Paris-Saclay, Palaiseau, France*
[d]*Nosco Pharmaceuticals, Paris, France*
[e]*LAGA, UMR 7539, Université Paris 13, Sorbonne Paris Cité, Villetaneuse, France*
[f]*INSERM, CNRS, UMR-S975, Institut du Cerveau et de la Moelle Epinière (ICM), Paris, France*

Abstract

Introduction: Exposure to traumatic brain injury is a core risk factor that predisposes an individual to sporadic neurodegenerative diseases. We provide evidence that mechanical stress increases brain levels of hallmark proteins associated with neurodegeneration.

Methods: Wild-type mice were exposed to multiple regimens of repetitive mild traumatic brain injury, generating a range of combinations of impact energies, frequencies, and durations of exposure. Brain concentrations of amyloid β 1–42 ($A\beta_{1-42}$), total tau, and α-synuclein were measured by sandwich enzyme-linked immunosorbent assay.

Results: There was a highly significant main effect of impact energy, frequency, and duration of exposure on $A\beta_{1-42}$, tau, and α-synuclein levels ($P < .001$), and a significant interaction between impact energy and duration of exposure for $A\beta_{1-42}$ and tau ($P < .001$), but not for α-synuclein.

Discussion: Dose-dependent and cumulative influence of repetitive mild traumatic brain injury–induced mechanical stress may trigger and/or accelerate neurodegeneration by pushing protein concentration over the disease threshold.

Keywords: Alzheimer's disease; Parkinson's disease; Animal models; Mechanical stress; Repetitive mild traumatic brain injury; Amyloid; Tau; α-synuclein

1. Background

While aging is the greatest risk factor for dementia, several pieces of evidence suggest that exposure to traumatic brain injury (TBI) increases the likelihood of developing neurodegenerative diseases later in life, including Alzheimer's disease and Parkinson's disease [1,2]. A common denominator among these neurodegenerative disorders is the abnormal accumulation of misfolding proteins, such as 42 amino acid-long form of the amyloid β peptide ($A\beta_{1-42}$) and tau protein, hallmark proteinopathies of Alzheimer's disease [3], and α-synuclein, a key neurodegenerative biomarker in Parkinson's disease. First described nearly a century ago in boxers as "punch drunk" or "dementia pugilistica" [4], chronic traumatic encephalopathy is a progressive neurodegeneration characterized by a widespread brain deposition of Aβ, tau, and α-synuclein [5–7]. The frequent association found between chronic traumatic encephalopathy and other neurodegenerative disorders suggests that repetitive mild TBI (rmTBI), the most common form of head injury in humans, can promote the accumulation of multiple proteins

The authors declare no conflict of interest.

*Corresponding author. Tel.: +33 1 42 16 75 67; Fax: +33 6 42 16 75 41.

E-mail address: marcel.levy@psl.aphp.fr

https://doi.org/10.1016/j.jalz.2017.11.003
1552-5260/

and trigger the development of TBI-induced neurodegenerative disease [8].

As it remains unclear how abnormal protein accumulation after TBI relates to the reported increased risk of Alzheimer's disease and Parkinson's disease, we speculate on the potential relevance of mechanical stress triggering neurodegeneration as a direct consequence of cascades initiated at the time of impact, reflected by initial changes in Aβ, tau, and α-synuclein concentrations in brain tissues [9]. Preclinical rmTBI studies using transgenic mice models of amyloidosis or tauopathy produce elevated brain $A\beta_{1-42}$ or tau levels, respectively, with increased protein deposition [10,11]. To further elucidate how mechanical stress triggers neurodegeneration, we propose an rmTBI-induced mechanical stress model that can significantly increase brain levels of multiple proteins associated with the development of neurodegenerative diseases. To this end, we expose wild-type BALB/c mice to multiple paradigms of rmTBI using a weight-drop mechanism [12] and long-term exposure to mechanical stress. Post-injury brain levels of $A\beta_{1-42}$, tau, and α-synuclein were measured by sandwich enzyme-linked immunosorbent assay (ELISA).

2. Methods

2.1. Animal care and maintenance

The subjects of these experiments were 5 to 6-week-old male BALB/c mice, weighing 19–24 g (n = 156; Nosco Pharmaceuticals, Paris, France). Animal handling and experimentation were performed in accordance with the European Community's guidelines regarding the care and use of laboratory animals. Mice were housed in a vivarium (10 per cage) under a 12 h light/12 h dark cycle and given access to pellet food and water *ad libitum*. Mice were allowed to adapt to the vivarium for at least 1 week before the experimental procedures. They were randomized into injured and sham mice groups (six mice per group). After the injury, animals were rapidly returned to their home cages for recovery.

2.2. Mechanical stress model of rmTBI

To assess the impact of mechanical stress on changes in brain levels of Aβ, tau, and α-synuclein, a mice model of human rmTBI was used as previously described [13,14]. Animal models of rmTBI approximate the conditions associated with repeated concussion encountered in contact sports [14]. Major modifications were implemented to test multiple injury paradigms in mice [12] without causing skull fracture, intracranial bleeding, or seizures [14] after long-term exposure to injury. The essential components and overall arrangement of the rmTBI apparatus consist of a simple weight-drop device illustrated in Fig. 1. Animals were placed into 50-mL conical polypropylene tubes, 30 mm in diameter and 115 mm in length, and featuring an opening of ~1 × 1 cm, large enough to allow for ventilation and exposure of the cranial scalp. The head was positioned at the cone end [15], subtending a narrow angle of 60° that restrained head mobility. At the caudal end of the tube, a flat-top screw cap restrained the mouse from moving, and a hole kept the tail out of the tube. The head and body were thus carefully stabilized, obviating the need for anesthesia [16]. The tube was held by a frame and placed at an angle, so that the midline of the scalp was perpendicular to a vertical hollow guide tube 17 mm in caliber placed right above it. The falling weights consisted of stainless steel marbles weighing 13.5 or 20 g that were dropped vertically through the hollow guide tube, 20 or 40 cm in height, delivering the impact to the dorsal aspect of the skull.

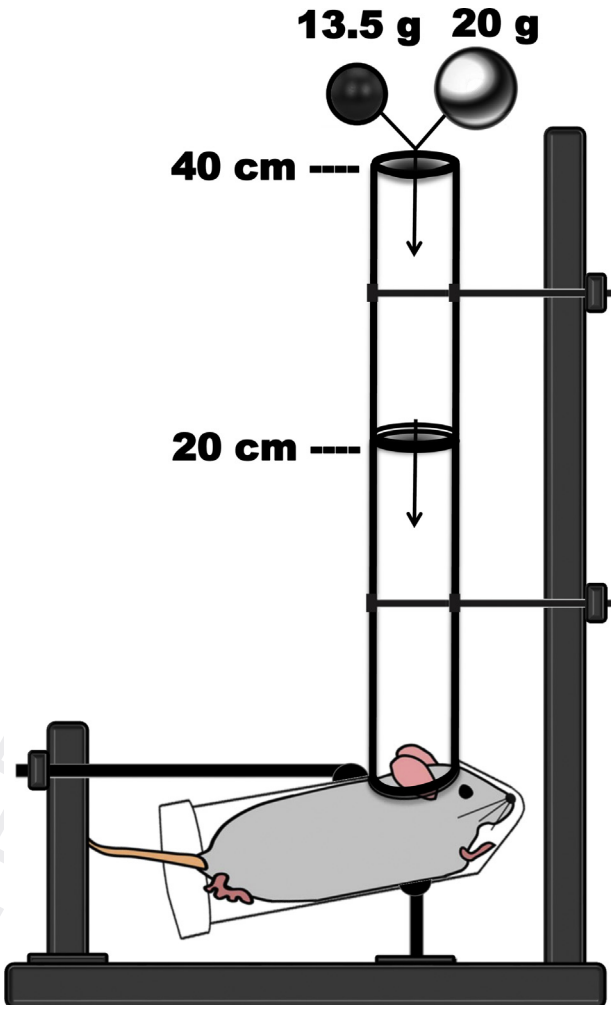

Fig. 1. Schematic illustration of the weight-drop device showing the essential components of the apparatus that comprised a vertical guide tube for the dropped weight situated above the mouse stage. Mice were restrained in a 50-mL conical polypropylene tube of 30 mm in diameter and 115 mm in length equipped with an ~1 × 1 cm opening large enough to expose the cranial scalp. The falcon tube was held by a frame and oriented at an angle, so that the scalp midline was perpendicularly oriented under a vertical hollow guide tube. Stainless steel marbles of 13.5 or 20 g drop vertically through the path of the hollow guide tube of 20 or 40 cm in height delivering the impact to the dorsal aspect of the skull.

2.3. Injury regimens and schedules

Animals were randomized into 24 groups of injured mice and two sham groups (n = 156; six mice per group), enabling us to explore different rmTBI regimens in a comparative fashion (Fig. 2). Mice randomized to injury underwent one of the 24 injury regimens that generate a range of combinations of impact intensity (dropping of 13.5 or 20-g weights, from 20 and 40-cm heights), frequency (once, twice, or 4×/day), and duration of exposure (10 or 20 weeks). Mice were tested during a regular animal house visit performed at 8 am, 12 am, 4 pm, and 7:30 pm for 4×/day schedules, at 8 am and 4 am for 2×/day schedules, and at 12 am for 1×/day schedule.

Impact intensity was quantified by the amount of kinetic energy that the falling weight possesses due to its motion at the time of impact. Assuming that air resistance is negligible, energy input was calculated using classical mechanics formulae, $E = (m \cdot g \cdot h)$, where E is the mechanical energy expressed in Joules (J), m is the mass (13.5 or 20 g), g is the acceleration due to gravity (9.8 m/s^2), and h is the drop height (20 or 40 cm). The weights and drop heights were estimated below the threshold intensity of previous models that involved zero mortality (40 g) and minimal observable neurological and behavioral effects in subsequent repeated injury regimens [12–14]. The calculated low-energy impacts generated were of 0.03 J (13.5 g − 20 cm), 0.04 J (20 g − 20 cm), 0.05 J (13 g − 40 cm), and 0.08 J (20 g − 40 cm).

Mice showed no post-traumatic neurological abnormalities, such as stereotyped muscle spasticity or irregular limb movements, following head impacts. Respiratory distress, including postimpact gasping/heaving, was also not observed. However, after final injury, signs of lethargy, tendency of social isolation, or increased aggression could be recognized in approximately 15% of mice that sustained impacts of 0.08 J. Assessment of the systematic neurological and behavioral outcome was outside the scope of the present study, taking into account the subconcussive nature of head impacts in this model. The animals were euthanized at day 10 after final injury, and brain tissues were processed as reported in the next section. Control animals had the same age as experimental animals at the time of sacrifice and underwent the same procedures except the weight drop.

2.4. ELISA quantitation of brain $A\beta_{1-42}$ peptides, total tau, and α-synuclein proteins

Forebrains were rapidly frozen after dissection and stored at −20°C. They were solubilized with T-PER (Pierce) in the presence of protease inhibitor (Roche) and phosphatase inhibitor mixtures (Sigma-Aldrich). The homogenates were centrifuged at 10,000 rpm for 200 min at 4°C. The supernatants were collected and stored at −80°C. The pellets were resuspended in 70% formic acid and centrifuged as in the previous step. The supernatants were collected and stored at −80°C. Formic acid fractions were measured for insoluble fragments. Soluble and insoluble levels of $A\beta_{1-42}$ and total tau (phosphorylated and nonphosphorylated forms) as well as soluble levels of α-synuclein were quantitatively assessed in the whole brain by a sandwich ELISA (Invitrogen; Camarillo, CA, USA and Millipore; Billerica, MA, USA) as per the manufacturer's directions. Data were expressed in picograms and per milliliter of homogenate. For α-synuclein, loading samples were diluted twice to obtain a final concentration over the entire range of the assay. ELISA analysis was performed on samples of tissue homogenates from two mouse brains, generating three samples per group (as n = 6 mice/group) and a total of 78 samples for the 26 rmTBI groups.

2.5. Statistical analyses

Concentrations of $A\beta_{1-42}$, total tau, and α-synuclein (n = 78) were analyzed as a function of weight-drop parameters (impact energy, frequency, and duration of exposure). Fold change in protein concentrations was calculated based on median values by comparison with sham mice. Statistical intergroup differences were calculated using the Kruskal–Wallis test. Differences between 0.03 J (13.5 g – 20 cm) rmTBI groups versus sham were evaluated by Mann-Whitney-Wilcoxon test. Furthermore, a two-way weighted analysis of variance, assuming that the homogeneity of variances was violated,

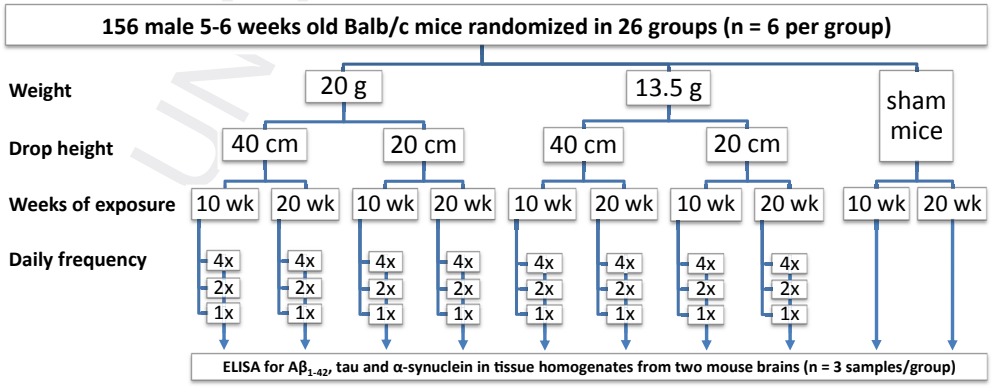

Fig. 2. Study flow diagram. Abbreviations: $A\beta_{1-42}$, amyloid β_{1-42}; ELISA, enzyme-linked immunosorbent assay.

Table 1
$A\beta_{1-42}$, total tau, and α-synuclein brain concentrations (pg/mL) stratified by rmTBI parameter

		Impact energy					Frequency/day				Duration of exposition		
	Sham	0.03 J 13.5 g 20 cm	0.04 J 20 g 20 cm	0.05 J 13 g 40 cm	0.08 J 20 g 40 cm	*P**	1×	2×	4×	*P**	10 weeks	20 weeks	*P**
N	6	18	18	18	18		24	24	24		36	36	
$A\beta_{1-42}$													
Median (Q1, Q3)	18.8 (18.2, 20.1)	28.6 (23.6, 36.1)‡	39.5 (31.0, 57.2)	39.3 (30.4, 44.5)	46.1 (33.6, 69.7)	<.001	32.2 (25.4, 44.5)	37.4 (32.7, 50.5)	38.9 (31.8, 50.5)	.001	29.9 (23.8, 33.2)	46.0 (39.4, 59.2)	<.001
Fold-change†	-	1.52	2.10	2.09	2.09		1.71	1.99	2.07		1.47	2.50	
Total Tau													
Median (Q1, Q3)	1082 (1061, 1113)	1309 (1208, 1393)‡	1633 (1262, 2072)	1467 (1224, 1857)	1692.0 (1274, 2841)	.004	1313 (1205, 1754)	1403 (1261, 1969)	1500 (1304, 2245)	.004	1230 (1139, 1314)	1930 (1526, 2215)	<.001
Fold-change†	-	1.21	1.51	1.37	1.6		1.21	1.30	1.39		1.14	1.76	
A-synuclein													
Median (Q1, Q3)	8.0 (7.1, 8.7)	64.2 (35.0, 99.7)‡	64.9 (41.3, 109)	84.0 (51.4, 157)	117.8 (59.2, 165)	<.001	58.6 (38.6, 103)	80.1 (44.7, 135)	87.3 (53.9, 162)	<.001	44.8 (35.4, 53.6)	150.4 (100, 164)	<.001
Fold-change†	-	8.04	8.12	10.50	14.7		7.34	10.0	10.9		6.4	17.6	

Abbreviations: rmTBI, repetitive mild traumatic brain injury; J, Joules; Q1, first quartile; Q3, third quartile.
*P values indicate statistically significant group differences ($P < .05$) calculated using Kruskal–Wallis test corrected from multiplicity of tests using Bonferroni procedure.
†Fold change represents the ratio between injured and sham protein levels (median).
‡The minimum amount of impact energy required to achieve significant differences between rmTBI and sham groups (Mann-Whitney-Wilcoxon test; $A\beta_{1-42}$, $P = .001$, tau; $P = .003$; and α-synuclein, $P \leq .001$).

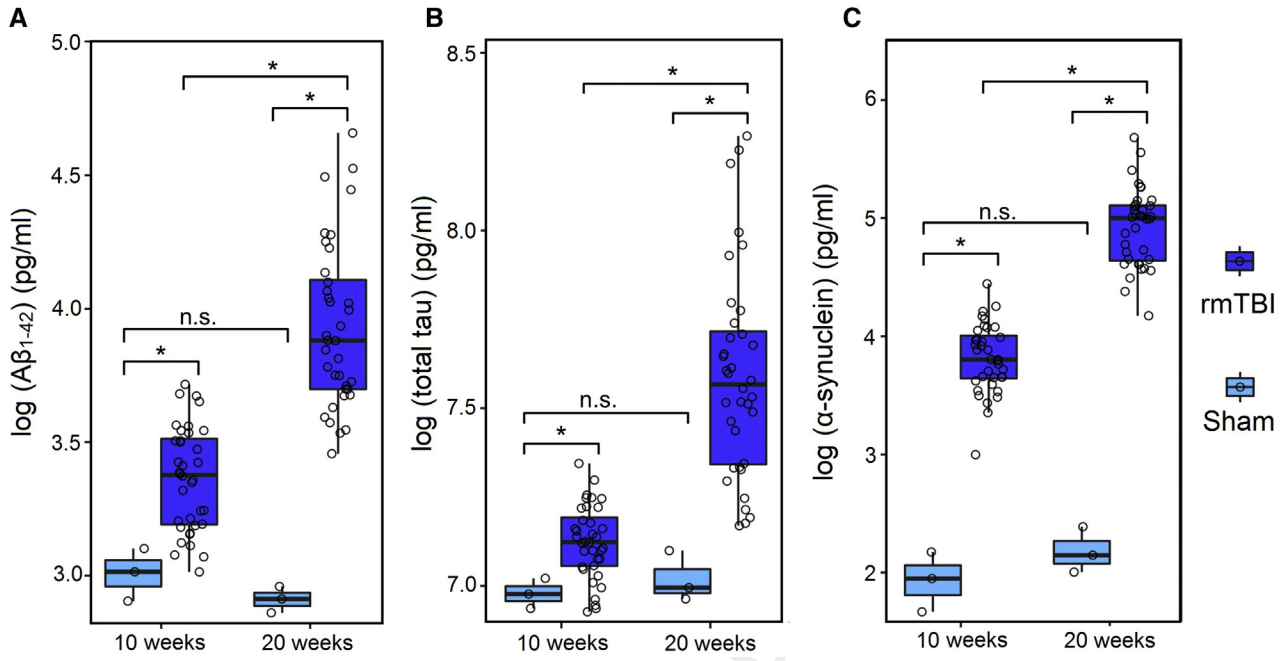

Fig. 3. Enzyme-linked immunosorbent assay testing for (A) $A\beta_{1–42}$, (B) total tau, and (C) α-synuclein in brain homogenates 10 days after final injury. In sham mice, there was no difference in brain levels of $A\beta_{1–42}$ ($P = .464$), total tau ($P = .841$), or α-synuclein ($P = .572$) in 10- versus 20-week groups. After 10 and 20 weeks of sustaining rmTBI, injured mice had increased $A\beta_{1–42}$, tau, and α-synuclein (n = 36, $P < .001$) than sham mice. Compared with the 10-week group, animals subjected to 20 weeks of rmTBI have significantly more $A\beta_{1–42}$, total tau, and α-synuclein ($P < .001$). Abbreviations: $A\beta_{1–42}$, amyloid-$\beta_{1–42}$; rmTBI, repetitive mild traumatic brain injury.

followed by Tukey's post-hoc comparison tests were performed to assess the effect of exposure to rmTBI versus sham, the effect of duration of exposure in experimental 10- and 20-week groups, and their interaction. Given the high variability in outcome data between injured groups (n = 72) compared with sham (n = 6), a logarithmic transformation was employed to conduct the analysis on protein levels. Hence, a three-way analysis of variance was performed among experimental rmTBI groups to test main and interaction effects for impact energy, frequency, and duration of exposure. Effect sizes were calculated using the omega squared estimate. All tests were corrected for multiplicity using Bonferroni method. Statistical analysis was performed using R 3.3.2 software.

3. Results

3.1. $A\beta_{1–42}$, total tau, and α-synuclein levels as a function of rmTBI parameters

Mice were exposed to repeated subconcussive head impacts using a 13.5 or 20-g weight dropped from 20 to 40 cm to impart different forces of impact ranging from

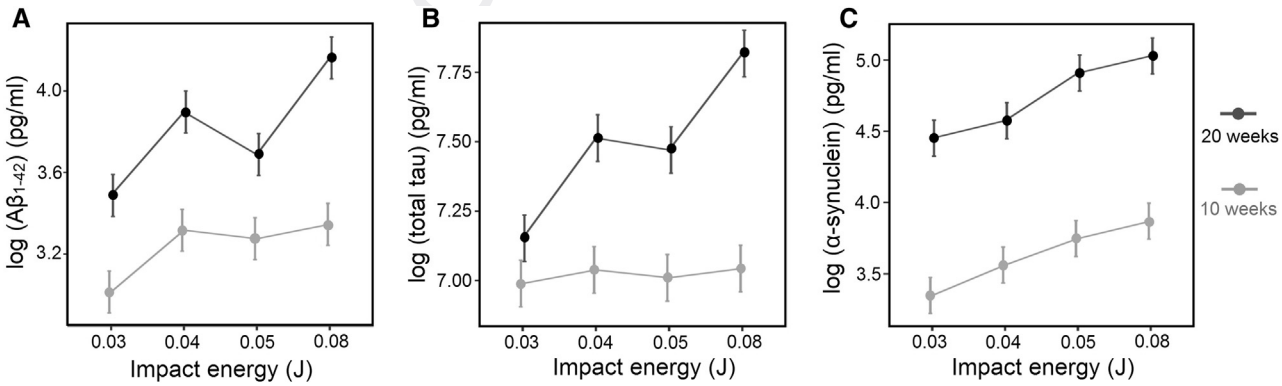

Fig. 4. Main and interaction effects of impact energy and duration of exposure on log-transformed values of (A) $A\beta_{1–42}$, (B) total tau, and (C) α-synuclein levels. There was a highly significant main effect of energy impact ($A\beta_{1–42}$, $F_{3,62} = 41.4$; tau, $F_{3,62} = 29.8$; and α-synuclein, $F_{3,62} = 37.2$; $P < .001$) and duration of exposure ($A\beta_{1–42}$, $F_{1,62} = 296$; tau, $F_{1,62} = 300$; and α-synuclein; $F_{1,62} = 740$; $P < .001$). Energy × duration of exposure interaction was significant for $A\beta_{1–42}$ ($F_{3,62} = 7.2$, $P < .001$) and tau ($F_{3,62} = 21.3$, $P < .001$) but not for α-synuclein ($P = 1.0$). Abbreviation: $A\beta_{1–42}$, amyloid-$\beta_{1–42}$.

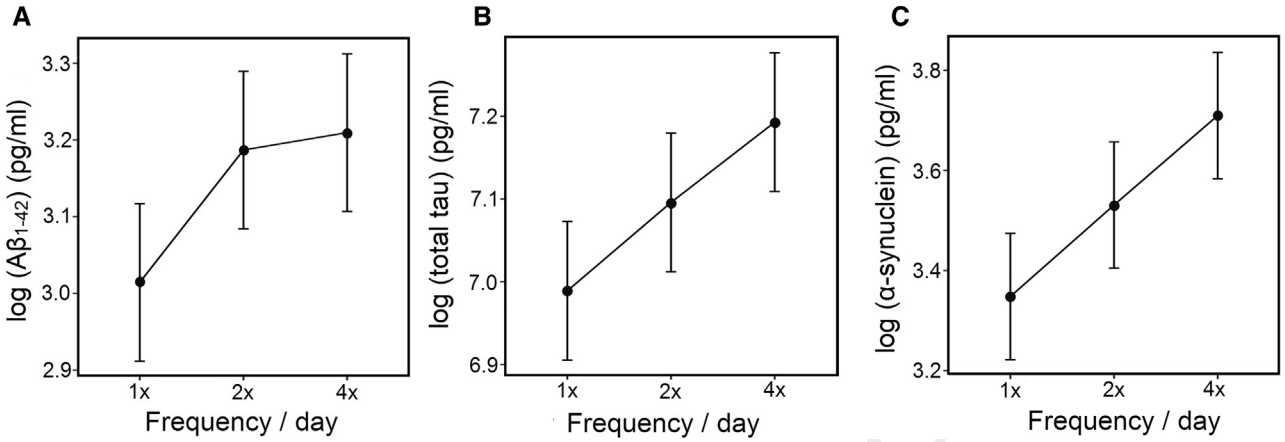

Fig. 5. Main effects of daily frequency of impacts on log-transformed values of (A) $A\beta_{1–42}$, (B) total tau, and (C) α-synuclein levels. There was a highly significant main effect of impact frequency on $A\beta_{1–42}$, ($F_{2,62} = 13.8$, $P < .001$), total tau ($F_{2,62} = 18.9$, $P < .001$), and α-synuclein ($F_{2,62} = 26.3$; $P < .001$) levels. No significant interaction effects were found for frequency versus impact energy ($A\beta_{1–42}$, $P = .11$; tau, $P = .28$; and α-synuclein, $P = .8$) nor frequency versus duration of exposure ($A\beta_{1–42}$, $P = .11$; tau, $P = .28$; and α-synuclein, $P = .8$). Abbreviation: $A\beta_{1–42}$, amyloid-$\beta_{1–42}$.

0.03 to 0.08 J. Table 1 summarizes the results regarding brain concentrations of $A\beta_{1–42}$, total tau, and α-synuclein assessed quantitatively by ELISA in all groups 10 days after final injury as a function of rmTBI parameters. The Kruskal–Wallis test revealed significant intergroup differences among sham, 0.03, 0.04, 0.05, and 0.08 J impact groups ($A\beta_{1–42}$, $P < .001$; tau, $P = .004$; α-synuclein, $P < .001$); sham, 1×, 2×, and 4×/day groups ($A\beta_{1–42}$, $P = .001$; tau, $P = .004$; α-synuclein, $P < .001$); and sham, 10- and 20-week groups ($A\beta_{1–42}$, tau, and α-synuclein, $P < .001$). Supplementary Table S1 shows median, minimum, and maximum values for protein levels and fold changes relative to sham, for the purpose of comparison, as a function of the 24 rmTBI paradigms. Among all experimental groups, injured mice receiving 20 g – 40 cm impacts 4×/day for 20 weeks (560 impacts) had the highest levels of $A\beta_{1–42}$ (92.2 pg/mL), tau (3738.3 pg/mL), and α-synuclein (258.7 pg/mL) compared with sham, representing a fold increase of ∼5.0, 3.4, and 30.2, respectively.

3.2. Prolonged rmTBI increases brain levels of $A\beta_{1–42}$, total tau, and α-synuclein

The effect of exposure to rmTBI versus sham, the effect of duration of exposure between 10- and 20-week experimental groups, and their interaction were highly significant for all protein levels ($P < .001$). The effect of rmTBI exposure on protein levels for 10- and 20-week rmTBI groups compared to controls are shown in Fig. 3A–3C. In sham mice, there was no significant time-dependent difference in brain levels of $A\beta_{1–42}$ ($P = .464$), total tau ($P = .841$), or α-synuclein ($P = .572$) between the 10-week group and the 20-week group. After 10 weeks of sustaining rmTBI, injured mice (n = 36) had increased $A\beta_{1–42}$, tau, and α-synuclein levels ($P < .001$) compared to sham mice (n = 3). The effects of rmTBI on the 20-week group (n = 36) was also significant compared to sham ($P < .001$). Moreover, the 20-week group had significantly higher levels of $A\beta_{1–42}$, tau, and α-synuclein compared to the 10-week group ($P < .001$). The minimum amount of impact energy required to achieve significant differences between rmTBI and sham groups was 0.03 J for $A\beta_{1–42}$ ($P = .001$), tau ($P = .003$), and α-synuclein ($P \leq .001$).

3.3. Cumulative and dose-dependent effect of rmTBI on brain levels of $A\beta_{1–42}$, total tau, and α-synuclein

The main and interaction effects of rmTBI parameters are represented in Fig. 4A–4C and Fig. 5A–5C. The results of the final three-way analysis of variance showed a highly significant main effect of impact energy ($A\beta_{1–42}$, $F_{3,62} = 41.4$; tau, $F_{3,62} = 29.8$; α-synuclein, $F_{3,62} = 37.2$; $P < .001$), frequency ($A\beta_{1–42}$, $F_{2,62} = 13.8$; tau, $F_{2,62} = 18.9$; α-synuclein; $F_{2,62} = 26.3$; $P < .001$ [Fig. 5A–5C]), and duration of exposure ($A\beta_{1–42}$, $F_{1,62} = 296.6$; tau, $F_{1,62} = 299.6$; α-synuclein; $F_{1,62} = 739.9$; $P < .001$). Energy × exposure interaction was significant for $A\beta_{1–42}$ ($F_{3,62} = 7.2$, $P < .001$ [Fig. 4A]) and tau ($F_{3,62} = 21.3$, $P < .001$ [Fig. 4B]), but not for α-synuclein ($F_{3,62} = 0.7$, $P = 1.0$ [Fig. 4C]). No significant interaction effects were found regarding energy × frequency ($A\beta_{1–42}$, $P = .105$; tau, $P = .275$; α-synuclein, $P = .801$) or for frequency × exposure ($A\beta_{1–42}$, $P = 1.0$; tau, $P = .178$; α-synuclein, $P = 1.0$). For all proteins, the duration of exposure had the major effect size ($A\beta_{1–42}$, $\omega^2 = 0.55$; tau, $\omega^2 = 0.54$; α-synuclein, $\omega^2 = 0.76$). These results suggest the possibility of a dose-dependent and cumulative influence of prolonged rmTBI on brain levels of $A\beta_{1–42}$, total tau, and α-synuclein.

4. Discussion

4.1. Implications of the results

In the present study, we investigated the effect of mechanical stress induced by rmTBI on the key molecular

biomarkers of neurodegenerative disorders. We hypothesized that mechanical stress triggers and/or accelerates neurodegeneration as a direct consequence of biochemical cascades initiated at the time of impact, reflected by initial changes in tau and α-synuclein concentrations in brain tissues. To test this hypothesis, we chose a mechanical stress model [18] that allowed us to create multiple regimens of repeated subconcussive brain injury [12–14] in nontransgenic animals. Thus, the duration of exposure to injury used in our study was considerably greater than that typically used in other rmTBI studies [19]. The increasing effect of impact energy and frequency of injuries (Figs. 4 and 5) suggests a dose-dependent influence of rmTBI on $A\beta_{1-42}$, tau, and α-synuclein levels [20–25]. The highly significant effect of duration of exposure to rmTBI (Fig. 3) in addition to the energy × exposure interaction for $A\beta_{1-42}$ and t-tau (Fig. 4) also suggest a cumulative effect of mechanical stress on biomarker levels.

4.2. Comparison with results from prior TBI studies

Mechanical stress, induced by TBI, accelerates the production of $A\beta_{1-42}$ in transgenic mice that express mutant human amyloid precursor protein (APP) [10,34–36]. In one study, APP mutation has shown to induce a fourfold increase in soluble brain $A\beta_{1-42}$ levels in aging transgenic APP mice [10]. Our mechanical stress model induced a fivefold increase in $A\beta_{1-42}$, in the absence of amyloid-type mutation. Shorter weight-drop regimens of 5 to 8 days of exposure to rmTBI in nonmutated mice have not demonstrated a significant increase in brain Aβ levels, measured by ELISA [19,37]. Controlled cortical impact injury, in turn, can induce a two to threefold increase in $A\beta_{1-42}$ in APP transgenic mice [38,39] and a 50% increase in $A\beta_{1-42}$ in wild-type mice.

The microtubule-associated protein tau has six isoforms in humans, and it is a normal constituent of axons. After TBI, tau dissociates from the microtubules and is dispersed by interneuronal transfer and via glial to glial spread [40,41]. Several studies have used transgenic mice in the assessment of tau pathology after TBI. Most of these models used the controlled cortical impact mechanism to demonstrate increased total tau, cleaved-tau, and/or phosphorylated-tau immunoreactivity within the first postinjury weeks [9]. Controlled cortical impact also induces severity-dependent increased cleaved-tau levels in the cortex and hippocampus of injured rats, 1.5- to eightfold higher compared with shams [42]. Unlike our results, one study using wild-type mice subjected to rmTBI (4×/day, 1 day/week for 4 weeks) did not reproduce postinjury tau changes [11].

Brain concentrations of α-synuclein largely reflect cell death occurring after TBI as a result of the primary injury and widespread postinjury neurodegeneration [43]. In a study using 24-month-old mice that underwent cortical impact injury, α-synuclein immunoreactivity increased in the neutrophil of the cortex, stratum, and hippocampus [44]. With an *in vitro* scratch injury model and *in vivo* mouse weight-drop model, Surgucheva *et al.* [45] showed that TBI causes alterations in the expression and localization of synucleins near the impact-damaged area. Before the injury, α-synuclein is diffused in the cytoplasm of neurons. After the injury, it forms punctate structures in the cytoplasm that keep increasing for up to 24 h. It is known that the extent of α-synuclein fibrillation, which precedes aggregation, depends on the initial protein concentration [46]. A key point highlighted in our study is the disproportionate changes in α-synuclein levels (30.2-fold increase) relative to the changes in brain concentrations of $A\beta_{1-42}$ and tau (5- and 3.4-fold increase respectively) compared to sham mice. It suggests that the effect of mechanical stress on α-synuclein levels is considerably more significant than on $A\beta_{1-42}$ and tau levels.

4.3. Possible biological interpretations

Amyloidosis, tauopathy, and synucleinopathy may be influenced by independent and/or common risk factors. Biochemical processes that initiate these proteinopathies may occur in parallel, and their onset and rate could be under the influence of environmental risk factors [47], such as mechanical stressors [48]. Neuronal structures are highly vulnerable to mechanical insults, even to physiological cellular energy loads [49]. Concerning amyloidosis, not only does Aβ accumulate after TBI but so do the necessary APP enzymes responsible for Aβ production: BACE1 protein (β-secretase) and the γ-secretase complex protein presenilin-1 [50–52]. We hypothesize that transmembrane enzymes BACE and presenilin-1 are mechanosensitive enzymes, as TBI causes changes in cell membrane integrity [53], and membrane deformation strongly relates to Aβ concentrations [54]. Structurally, an injured axon can undergo progressive ultrastructural changes after mechanical stress, including microtubule fragmentation, leading to degradation of the cytoskeleton. Thus, it is possible that tau fragmentation [55] in acute brain injury will somehow increase protein concentration in brain tissues. It raises the possibility that mechanical stress might trigger molecular pathways that result in the overproduction of proteins prone to pathological accumulation in neurodegenerative disease including tau, $A\beta_{1-42}$, and α-synuclein.

4.4. Confounding factors

Some limitations to our study should be considered. First, while $A\beta_{1-42}$, tau, and α-synuclein exist in a number of different forms, including oligomeric (Aβ and α-synuclein) and phosphorylated forms (tau and α-synuclein), the assay used in this study was primarily designed for the detection of the total concentration of the protein. A high brain level of $A\beta_{1-42}$ is a necessary condition for aggregation and accumulation, but it is not sufficient [56]. So, we could not

evaluate the threshold level of $A\beta_{1-42}$, as well as the tau and α-synuclein concentrations necessary to cause lesions, as biochemical changes may not persist at chronic time points after injury [31]. It was outside the scope of this study to specifically identify Aβ, tau, or α-synuclein pathology by immunohistochemistry methods that guarantee eliability and consistency inr terms of results in protein accumulation.

Human postmortem studies on rmTBI can show combinations of proteinopathies, indicating that the neuropathology of TBI is best described as a polypathology [8]. Modulation of Aβ secretion by α-synuclein exemplifies the cooperation between amyloid and Lewy pathology [57]. The synergy between the Aβ and tau pathophysiology has been largely documented in the "amyloid cascade hypothesis," which states that the accumulation of Aβ peptide is the cause of a cascade of reactions that lead to tau pathology [58]. Thus, the ability of α-synuclein, Aβ, or tau to directly or indirectly affect each other through interaction processes might contribute to the overlap of increasing levels of these biomarkers in our study. Finally, the ELISA methods employed in different studies, in terms of the antibodies and the detection method used, and the different protocols for sample collection and processing could account for discrepancies in concentration measurements in brain homogenates [59].

4.5. Conclusion

The main finding to emerge from this rmTBI study in nontransgenic mice is a dose-dependent and cumulative influence of prolonged rmTBI exposure on brain levels of $A\beta_{1-42}$, total tau, and α-synuclein. These observations raise the possibility that rmTBI-induced mechanical stress could trigger and/or accelerate neurodegeneration as a direct or indirect consequence of biochemical cascades initiated at the time of impact, reflected by initial changes in $A\beta_{1-42}$, tau, and α-synuclein concentrations in brain tissues. Human postmortem studies of chronic traumatic encephalopathy confirmed coaccumulation of these three proteins in brain tissues [7]. Otherwise, epidemiological, neuropathological, and microstructural studies largely support the notion of mechanical stress–induced neurodegeneration, and further investigations should provide more mechanistic insights into this hypothesis.

Acknowledgments

We sincerely thank Prof. Charles Duyckaerts for his significant contribution to this project.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2017.11.003.

RESEARCH IN CONTEXT

1. Systematic review: Epidemiological and case-control human studies, neuropathological evidence, and preclinical data suggest that repetitive mild traumatic brain injury can trigger neurodegenerative conditions, such as Alzheimer's disease and Parkinson's disease. The extent to which mechanical dynamics of repetitive mild traumatic brain injury influence neurodegenerative cascades and even normal aging remains a mystery.
2. Interpretation: Our findings suggest that continuous, repetitive, and long-term exposure to mechanical stress induced by repetitive mild traumatic brain injury promote increased concentrations of hallmark proteins associated with the development of neurodegenerative diseases.
3. Future directions: Further histopathological and mechanobiological investigations using this experimental model should provide more mechanistic insights into the effect of the mechanical energy on protein misfolding, spreading, aggregation, and deposition.

References

[1] Plassman BL, Havlik RJ, Steffens DC, Helms MJ, Newman TN, Drosdick D, et al. Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. Neurology 2000; 55:1158–66.
[2] Goldman SM, Tanner CM, Oakes D, Bhudhikanok GS, Gupta A, Langston JW. Head injury and Parkinson's disease risk in twins. Ann Neurol 2006;60:65–72.
[3] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002; 297:353–6.
[4] Schmidt ML, Zhukareva V, Newell KL, Lee VM, Trojanowski JQ. Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease. Acta Neuropathol 2001;101:518–24.
[5] Uryu K, Chen X-H, Martinez D, Browne KD, Johnson VE, Graham DI, et al. Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in humans. Exp Neurol 2007;208:185–92.
[6] Johnson VE, Stewart W, Smith DH. Widespread τ and amyloid-β pathology many years after a single traumatic brain injury in humans. Brain Pathol 2012;22:142–9.
[7] McKee AC, Stern RA, Nowinski CJ, Stein TD, Alvarez VE, Daneshvar DH, et al. The spectrum of disease in chronic traumatic encephalopathy. Brain J Neurol 2013;136:43–64.
[8] Washington PM, Villapol S, Burns MP. Polypathology and dementia after brain trauma: does brain injury trigger distinct neurodegenerative diseases, or should they be classified together as traumatic encephalopathy? Exp. Neurol 2016;275:381–8.
[9] Tsitsopoulos PP, Marklund N. Amyloid-β peptides and tau protein as biomarkers in cerebrospinal and interstitial fluid following traumatic

brain injury: a review of experimental and clinical studies. Front Neurol 2013;4:79.

[10] Uryu K, Laurer H, McIntosh T, Praticò D, Martinez D, Leight S, et al. Repetitive mild brain trauma accelerates Aβ deposition, lipid peroxidation, and cognitive impairment in a transgenic mouse model of Alzheimer amyloidosis. J Neurosci 2002;22:446–54.

[11] Yoshiyama Y, Uryu K, Higuchi M, Longhi L, Hoover R, Fujimoto S, et al. Enhanced neurofibrillary tangle formation, cerebral atrophy, and cognitive deficits induced by repetitive mild brain injury in a transgenic tauopathy mouse model. J Neurotrauma 2005;22:1134–41.

[12] Xu L, Nguyen JV, Lehar M, Menon A, Rha E, Arena J, et al. Repetitive mild traumatic brain injury with impact acceleration in the mouse: multifocal axonopathy, neuroinflammation, and neurodegeneration in the visual system. Exp Neurol 2016;275 Pt 3:436–49.

[13] Tang YP, Noda Y, Hasegawa T, Nabeshima T. A concussive-like brain injury model in mice (I): impairment in learning and memory. J Neurotrauma 1997;14:851–62.

[14] Kane MJ, Angoa-Pérez M, Briggs DI, Viano DC, Kreipke CW, Kuhn DM. A mouse model of human repetitive mild traumatic brain injury. J Neurosci Methods 2012;203:41–9.

[15] Houpt TA, Kwon B, Houpt CE, Neth B, Smith JC. Orientation within a high magnetic field determines swimming direction and laterality of c-Fos induction in mice. Am J Physiol Regul Integr Comp Physiol 2013; 305:R793–803.

[16] Petraglia AL, Plog BA, Dayawansa S, Dashnaw ML, Czerniecka K, Walker CT, et al. The pathophysiology underlying repetitive mild traumatic brain injury in a novel mouse model of chronic traumatic encephalopathy. Surg Neurol Int 2014;5:184.

[17] Briggs DI, Angoa-Pérez M, Kuhn DM. Prolonged repetitive head trauma induces a singular chronic traumatic encephalopathy-like pathology in white matter despite transient behavioral abnormalities. Am J Pathol 2016;186:2869–86.

[18] Levy Nogueira M, Epelbaum S, Steyaert J-M, Dubois B, Schwartz L. Mechanical stress models of Alzheimer's disease pathology. Alzheimers Dement 2016;12:324–33.

[19] Mannix R, Meehan WP, Mandeville J, Grant PE, Gray T, Berglass J, et al. Clinical correlates in an experimental model of repetitive mild brain injury. Ann Neurol 2013;74:65–75.

[20] Luo J, Nguyen A, Villeda S, Zhang H, Ding Z, Lindsey D, et al. Long-term cognitive impairments and pathological alterations in a mouse model of repetitive mild traumatic brain injury. Front Neurol 2014; 5:12.

[21] DeFord SM, Wilson MS, Rice AC, Clausen T, Rice LK, Barabnova A, et al. Repeated mild brain injuries result in cognitive impairment in B6C3F1 mice. J Neurotrauma 2002;19:427–38.

[22] Manley GT, Rosenthal G, Lam M, Morabito D, Yan D, Derugin N, et al. Controlled cortical impact in swine: pathophysiology and biomechanics. J Neurotrauma 2006;23:128–39.

[23] Long JB, Bentley TL, Wessner KA, Cerone C, Sweeney S, Bauman RA. Blast overpressure in rats: recreating a battlefield injury in the laboratory. J Neurotrauma 2009;26:827–40.

[24] Budde MD, Shah A, McCrea M, Cullinan WE, Pintar FA, Stemper BD. Primary blast traumatic brain injury in the rat: relating diffusion tensor imaging and behavior. Front Neurol 2013;4:154.

[25] Turner RC, Naser ZJ, Logsdon AF, DiPasquale KH, Jackson GJ, Robson MJ, et al. Modeling clinically relevant blast parameters based on scaling principles produces functional & histological deficits in rats. Exp Neurol 2013;248:520–9.

[26] Bolton AN, Saatman KE. Regional neurodegeneration and gliosis are amplified by mild traumatic brain injury repeated at 24-hour intervals. J Neuropathol Exp Neurol 2014;73:933–47.

[27] Laurer HL, Bareyre FM, Lee VM, Trojanowski JQ, Longhi L, Hoover R, et al. Mild head injury increasing the brain's vulnerability to a second concussive impact. J Neurosurg 2001;95:859–70.

[28] Mouzon B, Chaytow H, Crynen G, Bachmeier C, Stewart J, Mullan M, et al. Repetitive mild traumatic brain injury in a mouse model produces learning and memory deficits accompanied by histological changes. J Neurotrauma 2012;29:2761–73.

[29] Fujita M, Wei EP, Povlishock JT. Intensity- and interval-specific repetitive traumatic brain injury can evoke both axonal and microvascular damage. J Neurotrauma 2012;29:2172–80.

[30] Bailes JE, Petraglia AL, Omalu BI, Nauman E, Talavage T. Role of subconcussion in repetitive mild traumatic brain injury. J Neurosurg 2013;119:1235–45.

[31] Turner RC, Lucke-Wold BP, Logsdon AF, Robson MJ, Lee JM, Bailes JE, et al. Modeling chronic traumatic encephalopathy: the way forward for future discovery. Front Neurol 2015;6:223.

[32] Weber JT. Experimental models of repetitive brain injuries. Prog Brain Res 2007;161:253–61.

[33] Cernak I. Blast injuries and blast-induced neurotrauma: overview of pathophysiology and experimental knowledge models and findings. In: Kobeissy FH, ed. Brain neurotrauma: molecular neuropsychological and rehabilitation aspects. Boca Raton (FL): CRC Press/Taylor & Francis, 2015. Available at: http://www.ncbi.nlm.nih.gov/books/NBK299193/. Accessed February 15, 2016.

[34] Iwata A, Chen X-H, McIntosh TK, Browne KD, Smith DH. Long-term accumulation of amyloid-beta in axons following brain trauma without persistent upregulation of amyloid precursor protein genes. J Neuropathol Exp Neurol 2002;61:1056–68.

[35] Loane DJ, Pocivavsek A, Moussa CE-H, Thompson R, Matsuoka Y, Faden AI, et al. Amyloid precursor protein secretases as therapeutic targets for traumatic brain injury. Nat Med 2009;15:377–9.

[36] Stone JR, Okonkwo DO, Singleton RH, Mutlu LK, Helm GA, Povlishock JT. Caspase-3-mediated cleavage of amyloid precursor protein and formation of amyloid Beta peptide in traumatic axonal injury. J Neurotrauma 2002;19:601–14.

[37] Mouzon BC, Bachmeier C, Ferro A, Ojo J-O, Crynen G, Acker CM, et al. Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model. Ann Neurol 2014; 75:241–54.

[38] Abrahamson EE, Ikonomovic MD, Dixon CE, DeKosky ST. Simvastatin therapy prevents brain trauma-induced increases in beta-amyloid peptide levels. Ann Neurol 2009;66:407–14.

[39] Smith DH, Nakamura M, McIntosh TK, Wang J, Rodríguez A, Chen XH, et al. Brain trauma induces massive hippocampal neuron death linked to a surge in beta-amyloid levels in mice overexpressing mutant amyloid precursor protein. Am J Pathol 1998;153:1005–10.

[40] Tran HT, LaFerla FM, Holtzman DM, Brody DL. Controlled cortical impact traumatic brain injury in 3xTg-AD mice causes acute intra-axonal amyloid-β accumulation and independently accelerates the development of tau abnormalities. J Neurosci Off J Soc Neurosci 2011;31:9513–25.

[41] Tran HT, Sanchez L, Esparza TJ, Brody DL. Distinct temporal and anatomical distributions of amyloid-β and tau abnormalities following controlled cortical impact in transgenic mice. PLoS ONE 2011; 6:e25475.

[42] Gabbita SP, Scheff SW, Menard RM, Roberts K, Fugaccia I, Zemlan FP. Cleaved-tau: a biomarker of neuronal damage after traumatic brain injury. J Neurotrauma 2005;22:83–94.

[43] Mondello S, Buki A, Italiano D, Jeromin A. α-Synuclein in CSF of patients with severe traumatic brain injury. Neurology 2013; 80:1662–8.

[44] Uryu K, Giasson BI, Longhi L, Martinez D, Murray I, Conte V, et al. Age-dependent synuclein pathology following traumatic brain injury in mice. Exp Neurol 2003;184:214–24.

[45] Surgucheva I, He S, Rich MC, Sharma R, Ninkina NN, Stahel PF, et al. Role of synucleins in traumatic brain injury — An experimental in vitro and in vivo study in mice. Mol Cell Neurosci 2014;63:114–23.

[46] van Raaij ME, van Gestel J, Segers-Nolten IMJ, de Leeuw SW, Subramaniam V. Concentration dependence of alpha-synuclein fibril length assessed by quantitative atomic force microscopy and statistical-mechanical theory. Biophys J 2008;95:4871–8.

[47] Chetelat G. Alzheimer disease: Aβ-independent processes-rethinking preclinical AD. Nat Rev Neurol 2013;9:123–4.
[48] Levy Nogueira M, da Veiga Moreira J, Baronzio GF, Dubois B, Steyaert J-M, Schwartz L. Mechanical stress as the common denominator between chronic inflammation, cancer, and Alzheimer's disease. Front Oncol 2015;5:197.
[49] Tyler WJ. The mechanobiology of brain function. Nat Rev Neurosci 2012;13:867–78.
[50] Nadler Y, Alexandrovich A, Grigoriadis N, Hartmann T, Rao KSJ, Shohami E, et al. Increased expression of the gamma-secretase components presenilin-1 and nicastrin in activated astrocytes and microglia following traumatic brain injury. Glia 2008;56:552–67.
[51] Blasko I, Beer R, Bigl M, Apelt J, Franz G, Rudzki D, et al. Experimental traumatic brain injury in rats stimulates the expression, production and activity of Alzheimer's disease beta-secretase (BACE-1). J Neural Transm (Vienna) 2004;111:523–36.
[52] Chen X-H, Siman R, Iwata A, Meaney DF, Trojanowski JQ, Smith DH. Long-term accumulation of amyloid-beta, beta-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. Am J Pathol 2004;165:357–71.
[53] Arun P, Abu-Taleb R, Oguntayo S, Tanaka M, Wang Y, Valiyaveettil M, et al. Distinct patterns of expression of traumatic brain injury biomarkers after blast exposure: role of compromised cell membrane integrity. Neurosci Lett 2013;552:87–91.
[54] Pannuzzo M, Milardi D, Raudino A, Karttunen M, La Rosa C. Analytical model and multiscale simulations of Aβ peptide aggregation in lipid membranes: towards a unifying description of conformational transitions, oligomerization and membrane damage. Phys Chem Chem Phys 2013;15:8940–51.
[55] Higuchi M, Lee VMY, Trojanowski JQ. Tau and axonopathy in neurodegenerative disorders. Neuromolecular Med 2002;2:131–50.
[56] Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, et al. High-level neuronal expression of a 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J Neurosci 2000;20:4050–8.
[57] Duyckaerts C, Potier M-C, Delatour B. Alzheimer disease models and human neuropathology: similarities and differences. Acta Neuropathol 2008;115:5–38.
[58] Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci 1991; 12:383–8.
[59] Emmanouilidou E, Elenis D, Papasilekas T, Stranjalis G, Gerozissis K, Ioannou PC, et al. Assessment of α-synuclein secretion in mouse and human brain parenchyma. PLoS One 2011;6:e22225.