

Featured Article

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

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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.

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Keywords:

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

1. Introduction

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 $A\beta$, tau, and α -synuclein [5–7]. The frequent association found between chronic traumatic encephalopathy and other neurodegenerative disorders suggests that repetitive mild

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TBI (rmTBI), the most common form of head injury in humans, can promote the accumulation of multiple proteins 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 A β , tau, and α -synuclein concentrations in brain tissues [9]. Pre-clinical rmTBI studies using transgenic mice models of amyloidosis or tauopathy produce elevated brain A β 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 β 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 A β , 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

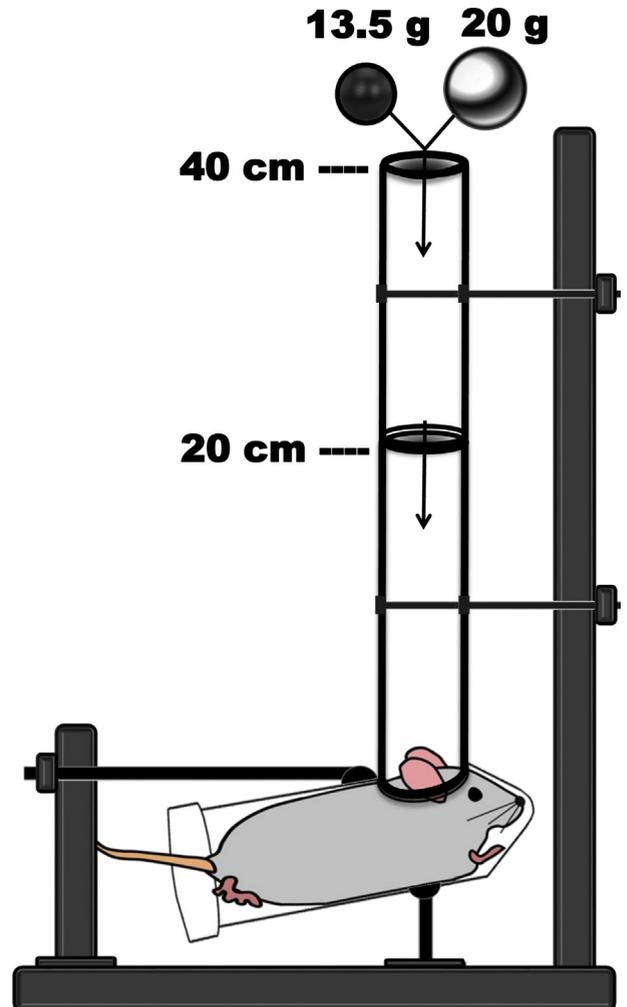


Fig. 1. Schematic illustration of the weight-drop device showing the essential components of the apparatus that comprised a vertical guide tube for the dropped weight situated above the mouse stage. Mice were restrained in a 50-mL conical polypropylene tube of 30 mm in diameter and 115 mm in length equipped with an $\sim 1 \times 1$ 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.

tubes, 30 mm in diameter and 115 mm in length, and featuring an opening of $\sim 1 \times 1$ 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.

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 pm 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

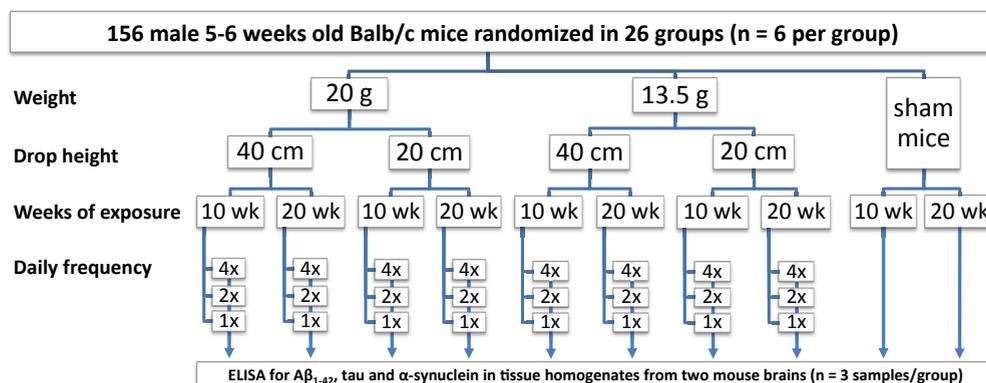


Fig. 2. Study flow diagram. Abbreviations: $A\beta_{1-42}$, amyloid β_{1-42} ; ELISA, enzyme-linked immunosorbent assay.

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

	Sham	Impact energy				<i>P</i> value*	Frequency/day			<i>P</i> value*	Duration of exposition		
		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		1×	2×	4×		10 weeks	20 weeks	<i>P</i> value*
N	6	18	18	18	18		24	24	24		36	36	
A β_{1-42}													
Median	18.8	28.6 [‡]	39.5	39.3	46.1	<.001	32.2	37.4	38.9	.001	29.9	46.0	<.001
(Q1, Q3)	(18.2, 20.1)	(23.6, 36.1)	(31.0, 57.2)	(30.4, 44.5)	(33.6, 69.7)		(25.4, 44.5)	(32.7, 50.5)	(31.8, 50.5)		(23.8, 33.2)	(39.4, 59.2)	
Fold-change [†]	-	1.52	2.10	2.09	2.09		1.71	1.99	2.07		1.47	2.50	
Total Tau													
Median	1082	1309 [‡]	1633	1467	1692.0	.004	1313	1403	1500	.004	1230	1930	<.001
(Q1, Q3)	(1061, 1113)	(1208, 1393)	(1262, 2072)	(1224, 1857)	(1274, 2841)		(1205, 1754)	(1261, 1969)	(1304, 2245)		(1139, 1314)	(1526, 2215)	
Fold-change [†]	-	1.21	1.51	1.37	1.6		1.21	1.30	1.39		1.14	1.76	
α -synuclein													
Median	8.0	64.2 [‡]	64.9	84.0	117.8	<.001	58.6	80.1	87.3	<.001	44.8	150.4	<.001
(Q1, Q3)	(7.1, 8.7)	(35.0, 99.7)	(41.3, 109)	(51.4, 157)	(59.2, 165)		(38.6, 103)	(44.7, 135)	(53.9, 162)		(35.4, 53.6)	(100, 164)	
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 β_{1-42} , *P* = .001, tau; *P* = .003; and α -synuclein, *P* ≤ .001).

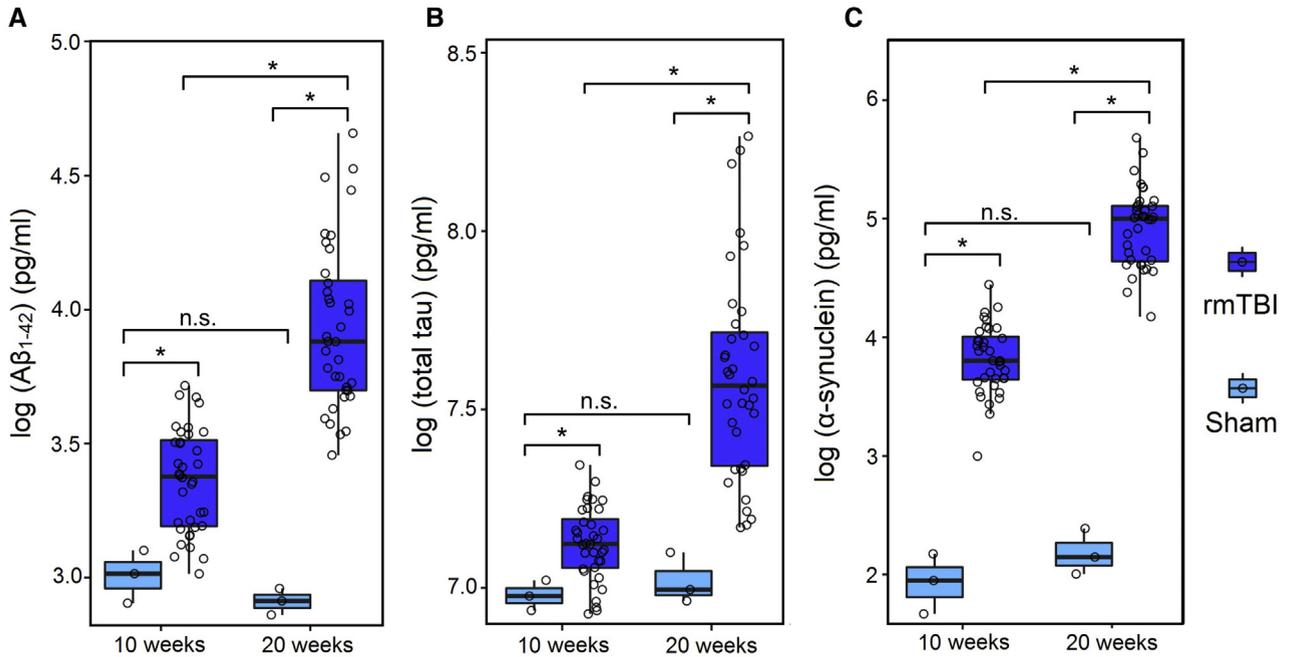


Fig. 3. Enzyme-linked immunosorbent assay testing for (A) $A\beta_{1-42}$, (B) total tau, and (C) α -synuclein in brain homogenates 10 days after final injury. In sham mice, there was no difference in brain levels of $A\beta_{1-42}$ ($P = .464$), total tau ($P = .841$), or α -synuclein ($P = .572$) in 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- β_{1-42} ; rmTBI, repetitive mild traumatic brain injury. * $P < .001$.

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, 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 en-

ergy, 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

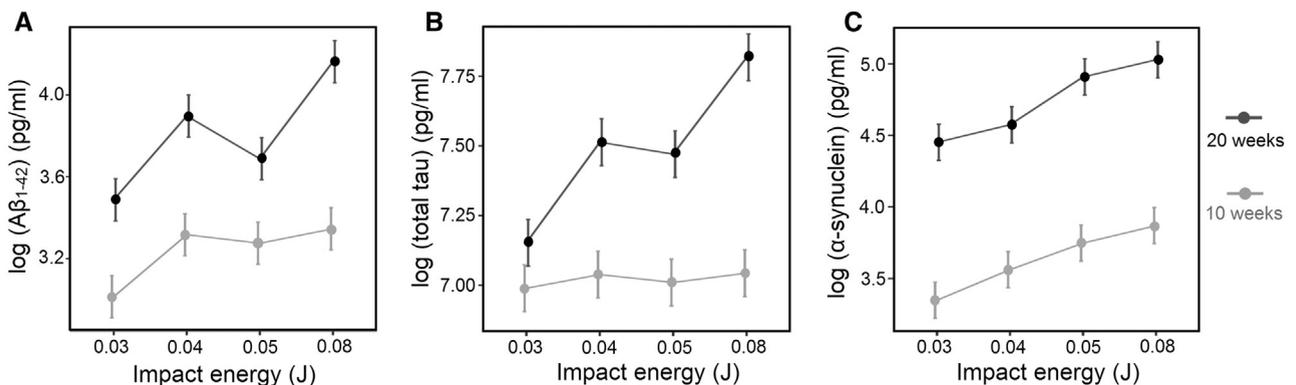


Fig. 4. Main and interaction effects of impact energy and duration of exposure on log-transformed values of (A) $A\beta_{1-42}$, (B) total tau, and (C) α -synuclein levels. There was a highly significant main effect of energy impact ($A\beta_{1-42}$, $F_{3,62} = 41.4$; tau, $F_{3,62} = 29.8$; and α -synuclein, $F_{3,62} = 37.2$; $P < .001$) 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 \times 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- β_{1-42} .

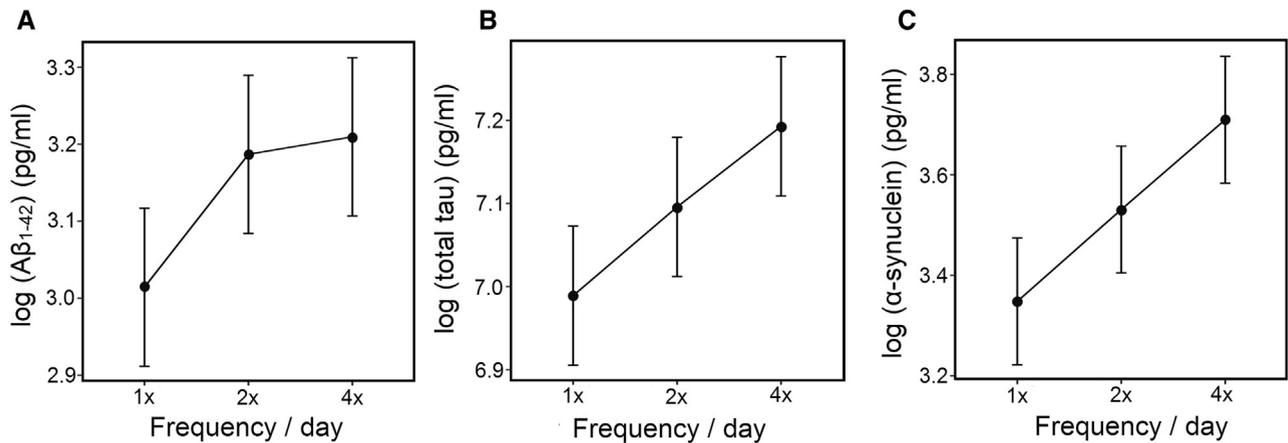


Fig. 5. Main effects of daily frequency of impacts on log-transformed values of (A) Aβ₁₋₄₂, (B) total tau, and (C) α-synuclein levels. There was a highly significant main effect of impact frequency on Aβ₁₋₄₂ ($F_{2,62} = 13.8$, $P < .001$), total tau ($F_{2,62} = 18.9$, $P < .001$), and α-synuclein ($F_{2,62} = 26.3$; $P < .001$) levels. No significant interaction effects were found for frequency versus impact energy (Aβ₁₋₄₂, $P = .11$; tau, $P = .28$; and α-synuclein, $P = .8$) nor frequency versus duration of exposure (Aβ₁₋₄₂, $P = .11$; tau, $P = .28$; and α-synuclein, $P = .8$). Abbreviation: Aβ₁₋₄₂, amyloid-β₁₋₄₂.

40 cm to impart different forces of impact ranging from 0.03 to 0.08 J. Table 1 summarizes the results regarding brain concentrations of Aβ₁₋₄₂, 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 (Aβ₁₋₄₂, $P < .001$; tau, $P = .004$; α-synuclein, $P < .001$); sham, 1×, 2×, and 4×/day groups (Aβ₁₋₄₂, $P = .001$; tau, $P = .004$; α-synuclein, $P < .001$); and sham, 10- and 20-week groups (Aβ₁₋₄₂, 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 Aβ₁₋₄₂ (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 Aβ₁₋₄₂, 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 Aβ₁₋₄₂ ($P = .464$), 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 Aβ₁₋₄₂, 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 Aβ₁₋₄₂, 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 Aβ₁₋₄₂ ($P = .001$), tau ($P = .003$), and α-synuclein ($P \leq .001$).

3.3. Cumulative and dose-dependent effect of rmTBI on brain levels of Aβ₁₋₄₂, 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 (Aβ₁₋₄₂, $F_{3,62} = 41.4$; tau, $F_{3,62} = 29.8$; α-synuclein, $F_{3,62} = 37.2$; $P < .001$), frequency (Aβ₁₋₄₂, $F_{2,62} = 13.8$; tau, $F_{2,62} = 18.9$; α-synuclein; $F_{2,62} = 26.3$; $P < .001$ [Fig. 5A–5C]), and duration of exposure (Aβ₁₋₄₂, $F_{1,62} = 296.6$; tau, $F_{1,62} = 299.6$; α-synuclein; $F_{1,62} = 739.9$; $P < .001$). Energy × exposure interaction was significant for Aβ₁₋₄₂ ($F_{3,62} = 7.2$, $P < .001$ [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 (Aβ₁₋₄₂, $P = .105$; tau, $P = .275$; α-synuclein, $P = .801$) or for frequency × exposure (Aβ₁₋₄₂, $P = 1.0$; tau, $P = .178$; α-synuclein, $P = 1.0$). For all proteins, the duration of exposure had the major effect size (Aβ₁₋₄₂, $\omega^2 = 0.55$; 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 Aβ₁₋₄₂, 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 [17] 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 [18]. 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 [19–24]. The highly significant effect of duration of exposure to rmTBI (Fig. 3) in addition to the energy \times 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) [10,25–27]. 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 $A\beta$ levels, measured by ELISA [18,28]. Controlled cortical impact injury, in turn, can induce a two to threefold increase in $A\beta_{1-42}$ in APP transgenic mice [29,30] 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 [31,32]. 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 post-injury 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 [33]. Unlike our results, one study using wild-type mice subjected to rmTBI (4 \times /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 [34]. 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 [35]. With an *in vitro* scratch injury model and *in vivo* mouse weight-drop model, Surgucheva *et al.* [36] 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 [37]. 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 [38], such as mechanical stressors [39]. Neuronal structures are highly vulnerable to mechanical insults, even to physiological cellular energy loads [40]. Concerning amyloidosis, not only does $A\beta$ accumulate after TBI but so do the necessary APP enzymes responsible for $A\beta$ production: BACE1 protein (β -secretase) and the γ -secretase complex protein presenilin-1 [41–43]. We hypothesize that transmembrane enzymes BACE and presenilin-1 are mechanosensitive enzymes, as TBI causes changes in cell membrane integrity [44], and membrane deformation strongly relates to $A\beta$ concentrations [45]. 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 [46] 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 ($A\beta$ 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 [47]. 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 [48]. It was outside the scope of this study to specifically identify $A\beta$, tau, or α -synuclein pathology by immunohistochemistry methods that guarantee reliability and consistency in 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 poly pathology [8]. Modulation of $A\beta$ secretion by α -synuclein exemplifies the cooperation between amyloid and Lewy pathology [49]. The synergy between the $A\beta$ and tau pathophysiology has been largely documented in the “amyloid cascade hypothesis,” which states that the accumulation of $A\beta$ peptide is the cause of a cascade of reactions that lead to tau pathology [50]. Thus, the ability of α -synuclein, $A\beta$, 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 [51].

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.

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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.

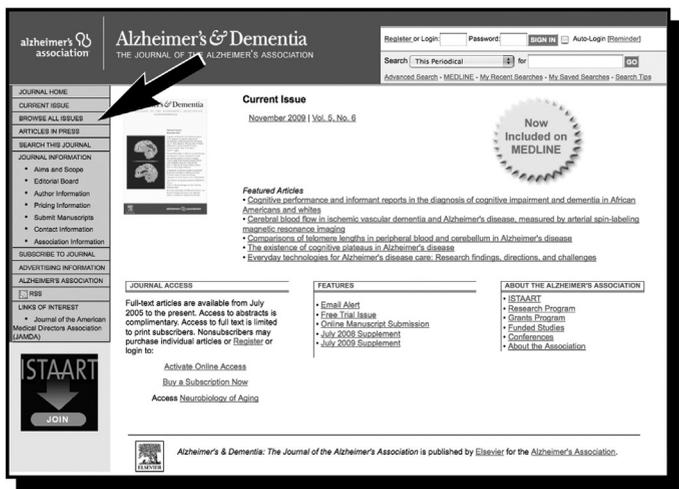
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