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Histo-neuro-behavioral effects of a single, very mild trauma on senescent rodent brains: a systematic review

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Abstract

Post-concussion syndrome, recently recognized as a complication of mild traumatic brain injury, is considered a consequence of the summative effect of multiple concussions received over lifetime. In elderlies, the main mild brain trauma mechanism is fall (low impact force). Many falls are often not reported or noticed but may generate serious medical and medico-legal consequences.

Our research question was to find if a single, very mild brain trauma can induce neuro-behavioral consequences in elderlies. One database was queried (PubMed – MeSH terminology) looking for histopathological, neuro-cognitive and behavioral changes that can be generated by sub-concussional trauma in senescent rodents, in comparison with young animals.

41 published research articles were selected. 17 of them used very mild brain trauma in young and senescent animals, in the same experiment (6 rats and 11 mice). 24 articles evaluated the effect of sub-threshold brain trauma in adult animals (no control group). Five trauma models were used (blast models were excluded). Neuro-inflammatory changes were detected immediate after very mild primary impact. In young animals, observed pathology disappeared fast (after 3 to 7 days). Increased apoptosis, mild axonal injury in white matter tracts plus maladaptive astrogliosis and microglial activation was stronger in aged animals, persisted over time (8 months) and significantly altered animals' cognition and behavior. Associated preexisting pathology (hypertension, tau protein deposits, microbleeds, reactive inflammation) was often responsible for amplification of the primary impact results.

As translation of observation is the weak spot of pathology and behavior animal research, further investigation is needed before to conclude that even a single, very mild brain trauma may have medical consequences on human senescent brain.

Keywords:

Very mild Traumatic Brain Injury, Animal models (rodents), Post-concussion syndrome, neuro-behavioral changes, "inflammaging", brain apoptosis

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Introduction

Traumatic brain injury (TBI) is a common medical condition that may lead to long-term disability or death. Often seen in clinical practice, the complex, heterogenous consequences of TBI are still a subject of research and debate [1]. TBI is labeled in many ways. The most popular classification system is the Glasgow Coma Scale (GCS). Under it, the broad spectrum of brain trauma pathology is classified as mild, moderate and severe. Mild TBI (mTBI), the least severe form of head trauma, represents more than 80% of the cases. Most of the patients with mTBI have a favourable evolution with full, rapid recovery. A minority of patients (10-20%) will develop prolonged neurocognitive and behavioral changes [2]. For these patients, neurological, cognitive, emotional and behavioral changes may organize in time, under the label of various chronic neuropsychiatric conditions [3]. Many factors may dictate the heterogenous clinical response after mTBI: age at the moment of impact [4], type and intensity of trauma, existence of associated pathology [5] or the number of hits over lifetime [6].

mTBI has a recognized bimodal distribution. Young (bellow 21 years) and old people (65 years and above) are more frequently affected [7]. When effects of mTBI are compared in the two age groups, elderlies (65 and over) have a worse immediate clinical outcome, a higher degree of medical resource utilization (and medical related costs), an increased higher risk for progressive cognitive decline in time and a reduced response to medical rehabilitation [8,9]. In geriatric patients, fall is the recognized cause of the mTBI. 35% of old people (65 and over) living alone, in long term care facilities or in assisted living housing fall at least once per year. This percentage increase to 50%- 60% for the group 80 years and over [10]. These numbers can be

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even higher as not all fall victims are medically evaluated if the trauma is very mild (sub-concussion), if the fall is unwitnessed and not mentioned to the caregivers or family [11].

Beside immediate and medium-term complications after mTBI, in elderlies, a progression to chronic traumatic encephalopathy (CTE) [12], Alzheimer Disease (AD) [13] or Parkinson disease (PD) [14] was described, frequently in association with pre-existing pathology [15].

CTE (sometimes named post-concussion syndrome) was a subject of medical controversy for more than 130 years [16]. Only recently CTE was recognized as one of major chronic neuro-psychologic complication of mTBI [17]. CTE is considered now a spectrum disease [18] where progressive brain atrophy is associated with progressive cognitive decline. Initially was linked with professional exposure (military personnel or professional athletes) to multiple mild head injuries over a lifetime [19]. It is widely accepted now that CTE may appear in geriatric population without history of repeated head trauma [20,21].

We hypothesized that a single sub-concussive trauma (without any loss of conscience, GCS =15), in specific conditions (old, frail geriatric patients with associated pathology) may contribute to CTE development that often remains undetected but may have serious clinical and medico-legal consequences.

In order to evaluate the importance of sub-concussive impact on aged brain, we reviewed published research data reporting induced mTBI on senescent animal (rodents). There are limitations in translating results from animals to humans but it is widely accepted that adequate rodent models may mimic human mTBI and will allow alignment of the mechanical impact with specific, measurable neuronal and behavioral consequences [22]

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Method

Our research was performed under PRISMA recommendations [23]. We evaluated one publication database (PubMed) using a Boolean research strategy based on the following MeSH terminology: (((animals) AND (mice) OR (rats) OR (rodents)) AND (aging) AND ((mild traumatic brain injury) OR (concussion)) AND (head injury, closed))). 329 publications were initially found. 125 articles were eliminated as referring to humans, sport, blast injuries, military, treatment, paediatrics, protective medication or review articles. 204 articles (75 of them evaluating mice, 98 rats, 2 mice and rats and 30 rodents) were reviewed based on abstract. Finally, 41 articles were selected as reflecting the neurobehavioral consequences of a single, sub-concussive or mild brain trauma in adult or aged rodents. Articles were finally divided, based on published content, in main studies (responding to our research question) and associated studies (partially responding to our research question).

Results

Based on our specific selection criteria (single, mild brain trauma in senescent animals compared with a group of young animals) 17 research papers were selected by both investigators in the main review arm. 6 of them used a rat experimental model: 5 articles reported results after the use of lateral or medial fluid percussion (FP) (single trauma, 1-2 atm, craniotomy positive). 1 article reported closed head injury using weight drop (*table 1*). 5 evaluated histologic brain changes. In 5 articles, neuro-behavioral changes were analyzed as early as after 7 days after the impact.

11 articles evaluated mTBI in senescent mice vs young animals. 7 of these used controlled cortical impact (CCI) (craniotomy positive, 3-6 m/s, under 1 mm brain tissue deformation for a

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time between 50-400ms) and 3 the closed head injury model (CHI) (craniotomy negative, CHIMERA model in 2 cases and weight drop in one case). One article evaluated the consequences of laser produced cerebral trauma in aged animals. 9 articles evaluated neurobehavioral consequences usually 1 month after the primary impact. Longest survival time was 8 months after he primary injury and reported long lasting cognitive-behavioral consequences (*table 2*).

24 articles were considered associated studies as research was performed on adult rodents and no young control group was described (*table 3*). 7 reports evaluated mTBI in rats and 17 and in mice. Force varied from extremely mild (under 1 atm, less than 5 m/s or weight under 15 grms in mice, under 450 grms in rats) to moderate (under 2 atm, more than 5 m/s or weight of more than 15 grms). Longest survival time was 24 months after the primary impact (reflecting aging after single mTBI and showing cognitive and behavioral deficits). One article was selected as it evaluated the structure of the normal aging in young and adult brain (cortical structure and ventricular volumes) comparing cortex and ventricular dimensions using MRI and histology measurements.

Discussion

Mild brain trauma is not a trivial disease. The notion of mild is referring only to the intensity of traumatic event and the immediate clinical picture. mTBI has an uncomplicated evolution in more than 80% of cases. In a minority of patients, evolution can be complicated by long-lasting neurobehavioral symptomatology [24]. In rare cases, chronic invalidating complications may develop. Between these complications, CTE was recently included. CTE was initially described

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as an occupational injury (military or sport related) of young people that suffered repeated mild head trauma over their lifetime. It was demonstrated that mTBI complications can affect seniors as well, usually in relation with same-level (low intensity) falls. In elderlies, the central dogma of CTE (summation of multiple mild hits) is under scrutiny as it was observed that similar histopathological picture (neuronal tissue chronic alteration) may develop even after one single mild brain trauma [25]. It is a recognized connection between mTBI and dementia [26] but early phases of dementia (mild cognitive impairment) are difficult to diagnose and to tie causally with mTBI and falls [27].

Our research hypothesis is that a single, sub-concussive head trauma may generate specific CTE histopathological and neuro-behavioral changes in senescent subjects, when pre-existing pathology exists.

We evaluated published research data in order to find histopathological and neuro-behavioral effects of a single, very mild (sub-concussive) head trauma in aged rodents in comparison with the neuro-behavioral picture generated by similar forces in young animals. The difficulty of this review study resides in accommodating and analyzing data coming from a large number of impact models (five), a well-recognized challenge that reflects the complexity of the head trauma pathology research. We deliberately eliminated the blast mechanism as not frequently seen in geriatric populations.

There are several ways to produce brain trauma in rodents. The open-head injury model (craniotomy+) includes FP model (median or lateral) and CCI model. The external force (fluid or mechanical) is applied against intact dura. The impact is a combination of speed, depth and time. The closed-head injury (CHI) models use either gravity (classical weight drop – “Marmarou”

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model) or an external mechanical force impacting the intact skull. In most models, head is blocked in a stereotaxic frame. One external closed head mechanical model (Closed-Head Impact Model of Engineered Rotational Acceleration - CHIMERA) allows a free movement of the head in the moment of the impact.

In closed head models, the intensity of head injury is judged based on general reported outcomes like well-being of the animal (blood pressure, spontaneous respirations or recovery of free movements) or recognized measurables like the righting reflex [28]. All animals that had an open-skull surgery were allowed to recover after trauma. Animals age was judged based on published average lifespan (36 months for rats and 24 months for mice) [29].

Three rat studies evaluated inflammatory and apoptotic gene expressions, early after primary impact (PMID: 24385964, 23238576, 31039431). All senescent mice studies performed extensive neuronal tissue inflammatory biomarkers evaluation (chemokines, cytokines) and gene expression in support of histopathological observation (immunohistology). In young animals, after mild trauma proinflammatory cytokines had an early increase followed by rapid decrease. In aged animals, same proteins increased slower and did not decreased even after one month after the primary impact. Specific markers ($p16^{inka}$) doubled in aged animals after trauma; $p21^{cip1a}$ increased in old animals but not in relation with mTBI (30904769). Other apoptotic related proteins (hippocalcin, LANP, Heat Shock Protein 27) were detected significantly increased in aged brains in precise areas (dentate gyrus) after very mild trauma (32290848).

In young/adult rodents, very mild trauma generated an acute reactive astrogliosis and activated microglia in cortex and evidence of axonal injury in the corpus callosum. Changes were

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discovered in specific brain regions and were proportional with trauma intensity (22245525, 21704658, 17174280). Transitory neuro-behavioral changes were reported in adult rodents, mainly in relation with very mild trauma, in response to white matter (axonal) alterations (29993324, 24550885 and 29376093). Long-term cognitive and behavioral changes were observed after 4 weeks after mild to moderate injuries (26774527, 28910378).

In senescent animals, 15 studies reported similar histopathological changes. Increased apoptosis and mild axonal injury were associated with maladaptive astrogliosis and microglial activation. Edema and neuronal tissular destruction were significantly stronger in aged animal groups and persisted over time (8 months). Several studies (22952778, 32290848, 30904769) reported similar microglia senescent changes and increased, exacerbated secondary neuro-inflammatory response (“inflammaging”). Trauma intensity was responsible for the location of lesions: very mild trauma affected only hippocampus and corpus callosum of aged animals (2328576, 1335138). 30486287 reported an increased complement activation with specific local lesion evolution (lesion peaked and associated cavitation increased), permanent hippocampus involvement and persistent neuronal cognitive and behavioral changes in aged mice.

12 studies evaluated neuronal (open field, rotarod and balance beam), cognitive (passive avoidance, mazes, novel objects avoidance) and behavioral changes (social, memory). Specific hippocampal inflammation and white matter destruction were associated with alteration of cognitive and executive functions (30904769, 29848996 and 22952778). 30998995 evaluated executive function in adult rats after mTBI and observed that age and not traumatic intensity was responsible for long term changes (12 months).

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Several studies evaluated the consequences of mild trauma in specific circumstances. 29269117 observed “lifelong” neuro-behavioral consequences in mutated mice (APP/PS KI) after very mild brain trauma. Using similar mutated APP/PS KI mice, 25904805 reported a delayed but persistent inflammatory response that involved both astrocytes and microglia and generated very long-lasting cognitive deficits (3 months) after trauma. 31262044 reported destruction of blood brain barrier and persistent neuro-inflammatory response after very mild trauma in spontaneous hypertensive rats, also associated with chronic cognitive impairment. 23953759, using a different open-skull injury technique (laser injuries in young, adult and aged mice) observed significant maladaptive response of microglia (increased soma and reduced processes) after injuries in senescent animals. 29808778 showed metabolic long-time changes in aged injured brain that worsen in time. Study 32641073 reported an increased number of microbleeds, cortical thinning and increased ventricular volume in aged, non-injured animals. 31039431 reported that, in aged animals, an associated external trauma (limb fractures) may exacerbate neuroinflammatory response.

The complexity of brain trauma mechanism is well recognized. Closed head injuries alter brain tissue in a two steps mechanism. The primary impact will produce direct (primary) brain tissue destruction that will be followed by a cascade of pathophysiologic and neurochemical (secondary) events that can be highly variable among patients [29]. The association between neuronal death (produced eighter by apoptosis or necrosis), neuro-inflammation and white matter, axonal alterations in different brain areas is probably related with neuro-behavioral changes observed in humans and animals after mild brain trauma.

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Most of the brain trauma studies performed on animal models used young or adult subjects and evaluated the consequences of mild or moderate impacts. It was not clear if a single, very mild trauma (sub-concussive) can persistently alter cognition and behavior in aged animals. Our literature research revealed that a single, sub-concussional trauma will induce only minimal, transitory changes in young or adult animals. These lesions can also "prime" the brain for further traumatic events allowing CTE development as a summation of trauma over time. In senescent animals, maladaptive neuro-inflammatory response with increased sensibility to apoptosis, a weakened blood brain barrier, an increased propensity to brain edema formation, the presence of microbleeds and altered local metabolism with increased reactive oxygen species generation are responsible for the development of a serious histopathologic picture associated with long term neuro-cognitive consequences.

Our study has several limitations. Our literature review was performed on only one, English speaking, database. The low number of true long-lasting senescent rodent studies was perceived as a serious limitation. There are several models that are currently used for modelling consequences of mTBI in rodents, reflecting both the complexity of the brain trauma and specific research interests. As there are not accepted guidelines for mTBI research design, there are not many similitudes between research centers. In our review, we did not separate mTBI consequences based on subjects' sex but in aged humans there were reported serious differences. Translation of the results from rodents to humans is a well-recognized basic science challenge, mainly when cognition and behavior are evaluated [30].

In conclusion, apoptosis and inflammation were contributing factors for neuro-cognitive alterations discovered after very mild brain trauma in rodent models. Chronic inflammation

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status (“inflammaging”), coexistence of peripheric traumatic pathology, the summative effects of repeated head trauma, abnormal brain protein deposits and associated cardio-vascular pathology were aggravating factors. In senescent animals, neuro-cognitive changes were observed long time after the primary mild impact. As translation of research results from animal models to humans is challenging, further studies are needed before to conclude that very mild brain trauma may be involved in the development of mild cognitive impairment in senescent patients.

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Table 1

PMID	1st Author, year, Reference	Age_1	Age_2	Method	No. Hits	Survival	Cranio tomy	Histo (*)	Neuro	Behave	Obs.
24385964	Sun_D, 2013 [31]	1 mo	24 mo	LFP 2 atm	1	2 d	Yes	Yes, proteomics + Dentate gyrus	NA	NA	Increased apoptosis in aged group,
23238576	Titus 2013 [32]	3 mo	19 mo	LFP mild 1.5 and moderate 1.9 atm	1	1-3 d	Yes	Yes, proteomics + hippocamp	NA	Yes	mTBI lowered cAMP in aged animals hippocamp, proportional cu impact (low impact affects just aged, High impact, both groups)
31039431	Sun_M 2018 [33]	3mo	12mo	LFP 2 atm	1	7 d	Yes	Yes hippocamp MRI	Yes	Yes, Worse in aged	Bone fracture may exacerbate brain inflammation worse behavioral
27449121	Rowe, 2016 [34]	Rats from <1 mo to 6 mo	6 mo	MidFP 1.5-2 atm (age dependent)	1	6 mo max	Yes	NA	Yes	No depression, anxiety if mTBI in adulthood	5 groups, aim was to evaluate age at injury and aging with mTBI using acute and chronic behavior batteries
22374222	Itoh, 2013 [35]	2 1/2 mo	24 mo	CHI	1	1,3,7 d	No	Yes	NA	Yes, cognitive Impaired in old	free radicals increased in aged group
1335138	Hamm, 1993 [36]	3mo	20 mo	LFP, medium 1.7-1.8 atm	1	5 d motor, 12 d cognitive	Yes	Yes	Yes	Yes, Significant cognitive impairment in aged	Selective cognitive deficits hippocampus related. No motor or cognitive in young group

Table 1. Single mild brain trauma in senescent rats. (*): axonal destruction, apoptosis, activation of astrogliosis and microgliosis, inflammation, LFP: lateral fluid percussion, MidFP: midline fluid percussion, CHI: closed head injury, d: days, mo: months. Histo: histology; Neuro: neurologic; Behave: Behavioral

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Table 2.

PMID	1st Author, Year, Reference	Age_1	Age_2	Method	No.	Survival	Cranio	Histo (*)	Neuro	Behave	Obs.
32290848	Early, 2020 [37]	Adult 4mo	Old 18 mo	CCI, 4m/s, 0.9, 30ms	1	1, 3, 7 d	Y	Y Transcriptomics+	NA	NA	aged increased altered edema, inflammation and astrocytes function (aquaporin 4)
30904769	Ritzel 2019 [38]	Adult 3mo	Old 18 mo	CCI, 6m/s, 1mm, 50 ms	1	3 d	Y (sham no cranio)	Y Cytokines+ Transcriptomics+	Y	Anxiety	Microglial sensitivity and peripheral immune dysfunction
23273602	Kumar 2013 [39]	3 mo	24 mo	CCI 6m/s, 2 mm	1	1, 7 d	Y	Y Proteomics+	NA	NA	Highly reactive macrophage and microglia, hippocamp
27090212	Morganti 2016 [40]	3 mo	23 mo	CCI 4, 0.95 300ms	1	1 d	Y	Y Proteomics+	NA	NA	Peripheral CCR2 macro recruit increased in aged brains
29848996	Chou 2018 [41]	3-6 mo	20-25 mo	CCI 4m/s, 0.95, 300ms	1	4, 7, 28d	Y	Y Proteomics+	NA	Y (spatial learning and memory)	CCR2/5 response increased in aged reducing behavioral recovery
30486287	Krukowski 2018 [42]	NA	19mo	CCI 4m/s, 0.95, 300ms	1	1 mo	Y	Y Complement C1q, C3, CR3	Y	Y (spatial learn and memory)	Complement activation, loss of synapses, increased macrophages MRI-T2: lesion size peaked, cavitation size increase, hippocamp CD11 increase
22952778	Timaru-Kast 2012 [43]	2mo	21mo	CCI, 8m/s, 1mm, 150 m/s	1	1h, 24h, 3 d	Y	Y, IL1, IL6 TNF Proteomics+	Y	Y	Age effect on Brain Edema Formation, Secondary Brain Damage and Inflammatory Response inflammaging = increase of IL1, IL6, TNF x2 in aged animals

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29269117	Cheng 2018 [44]	3mo	13mo	CHIMERA, 50 gr, 0.5m/s (0.6J)	2	14 d - 8 mo	No	Y Proteomics+	Y	Y	APP vs WT. In WT, microgliosis and axonal injury may persist 8 mo after injury
30636629	Cheng 2018 [45]	3 mo	13 mo	CHIMERA, 0.1-0.7J	1	14 d	No	Y	Y impact = >0.5 J	Y	threshold increase of Iba and GFAP in corpus callosum
25904805	Webster 2015 [46]	APP1 (8mo)	WT	CHI 5m/s, 1mm, 100 ms	1	9h, 1d, 7d, 1 mo 3 mo	No	Y Proteomics+	Y	Y	APP/PS1 KI AD model. One single hit delays the inflammatory response and produce more inflammation vs WT
23953759	Hefendehl 2014 [47]	3mo, 12 mo, 27 mo	NA	Laser (910 nm, 15 s)		21 d	Yes	Y	NA	NA	Microglia soma increased, process length decreased, low responsiveness, no homogenous distrib. (photon microscopy)

Table 2. Single mild brain trauma in senescent mice (*): axonal destruction, apoptosis, activation of astrogliosis and microgliosis, inflammation; APP/PS1: Alzheimer disease mouse model, WT: wild type, CCI: controlled cortical impact, CHI: closed head injury, CHIMERA: Closed-Head Impact Model of Engineered Rotational Acceleration, Y=Yes, NA=not available, AD: Alzheimer disease, nm: nanometers, s: seconds, d:days, mo: months. Histo: histology; Neuro: neurologic; Behave: Behavioral

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Table 3

PMI	1st Author	Age_1	Age_2	Method	Number Hits	Survival	Craniotomy	Histo (*)	Neuro	Behave	Obs
22245525	Shultz 2012 [48]	Adult rat (300 grm)	NA	LFP, very mild (0.5 atm)	1	4days/ 1month	Yes	Yes	No	No	Increased microglial/ macros and reactive astrogliosis
21704658	Shultz 2011 [49]	Adult rat	NA	LFP 1 atm	1	24h/1 mo	Yes	Yes	NA	Cognitive, anxiolytic	Astrogliosis and microgliosis in corpus callosum
21933013	Shultz 2012 [50]	Adult rat (repeated)	NA	LFP 1 atm	1-3-5	5d/2 mo	Yes	Yes	Yes	Yes	Apoptosis, cortex and hippocamp
8155285	Hamm 1993 [51]	Adult rats	NA	LFP 2 atm	1	7d/12d	Yes	Yes	Yes	Yes	Selective hippocampal modification with neurobehavioral changes of the environment and a flexible response to it
30998995	Arulsamy 2019 [52]	Rats 12mo	NA	WD (450 gr, 0.75 m)	1	12 mo	No	Yes Proteomics +	Yes	Yes	Age and not mTBI intensity affect executive function
28855139	Collins-Praino [53]	Rats 3 mo	NA	WD	1	3 mo	No	Yes IL1, IL6, TNF (9 cytokines)	No	Yes	mTBI induces changes when microglial priming exists
20528171	Spain 2010 [54]	Adult mice	NA	LFP, 0.9 atm	1	4h – 1½ mo	Yes	Yes	Yes	Yes, Learning	PT changes unmyelinated axons in both ipsi and contra
26774527	Muccigrosso 2016 [55]	Adult mice	NA	MidPF 1.2 atm	1	1 mo	Yes	(IL-1β, CCL2, TNFα	Yes	Yes, at 1 mo	Hippocampal Learning after 1-week, anterograde learning after 1 mo
28910378	Taib 2016 [56]	Adult mice	NA	CCI, 3.5 m/s, 1mm, 50ms	1	1w-3 mo	Yes	IL1b and CD11b increased max at 3 days + demyelination at 3 mo in CC Proteomics+	Signific. at 3 mo	Yes, + at 3 mo	Neuro-inflammation (microglia) and WMI. Myelin sheath defects in corpus callosum at 3 months (em)
24289885	Fenn 2014 [57]	3 mo, mice	NA	LPF 2 atm	1	7d - 1 mo	Yes	Yes	Yes Motor	Yes, depressive like	primed microglia reduced motility and increased soma
24223856	Tajiri 2013 [58]	Adult mice	NA	CCI4.0 m/s, 1 mm, 150 ms	1	1 ½ mo	Yes	Increased Abeta deposits		Significant errors	Tg2576 APP mice, more Abeta in both cortex and hippocam
29993324	Mouzon 2019 [59]	3 mo, mice	NA	CHI 5m/s 1mm, 200 ms	1 or 5	12 mo	No	Yes CA1/CA3 hippocamp	Yes	Yes, Cognitive impair at single impact	hTau minimal after 1 impact, WMI present

29376093	Mouzon 2017 [60]	3 mo, mice	NA	CHI 5m/s 1mm, 200 ms	1	24 mo	No	Yes Astrogliosis and reduced cc thick	Yes	Yes, increased risk behavior at 24 mo	Iba-1, GFAP+, la 24 months after single impact
29808778	Lyons 2018 [61]	4 mo mice	NA	CHI 5m/s, 1, 100ms	1	3d, 28d	No	Yes	NA	Learning & spatial memory	mitochondria metabolism deficit after single impact, worsen in time. MRI
12732240	Zohar 2003 [62]	2 mo mice	NA	WD 20,25,30 grms	1	7d, 1 mo, 2 mo, 3 mo	No	Yes	No difference	Learning and spatial memory	MRI ex-vivo
21499325	Zohar 2011 [63]	2 mo mice	NA	WD 20,25,30 grms	1	7d, 1 mo, 2 mo, 3 mo	No	No	No	depressive like	
24312187	Rachmany 2013 [64]	2 mo mice	NA	WD 30 grms	1	3 d, 7 d, 1 mo	No	Yes, diffuse	Yes	Yes	cell culture, p53
22892942	Rachmany 2012 [65]	2 mo mice	NA	WD 30 grms	1	24h, 3 d, 7 d	No	Yes, diffuse	No	Yes	
17174280	Tashlykov 2006 [66]	2 mo mice	NA	WD 5-30 grms	1	3d	No	Yes, cingulate	No	Yes	Apoptosis hippocamp and cortex prop. with trauma intensity.
18651249	Tashlykov 2008 [67]	2 mo mice	NA	WD 5-30 grms	1	3d	No	Yes p53 and Bcl-2	NA	NA	minimal trauma (>10 grms) induces apoptosis in dentate and hippocamp
25879458	Baratz 2015 [68]	2 mo mice	NA	WD 50 grms	1	1-18h, 72 h, 7 days	No	Yes, GFAP	NA	Cognitive impairment	Apoptosis, TNF, astrogliosis
24550885	Luo 2014 [69]	3 mo mice	NA	CHI, 3-5 m/s, 200ms, 0.9mm	1-3	1 d 6 mo	No	Yes, CREB	No changes after single	Cognitive impair after repeated	Short term inflammation exists after single
32641073	Taylor 2020 [70]	3 mo, mice 17 mo, 25 mo	NA	NA	No	NA	NA	Reduce cortical thickness and increase ventricular volume	NA	NA	Cerebral microbleeds (MRI in-vivo and ex-vivo + histo) without previous trauma.
24756076	Aungst 2020 [71]	3 mo rats	NA	LFP 1.1 atm	1-3	28 days	Yes	neuronal cell loss increased numbers of activated microglia	Yes	Yes	Focussed on hippocamp. Spatial learning, memory, observed only after repetitive trauma in young animals

Table 3. Associated studies related to mTBI in adult and senescent rats (*) axonal destruction, apoptosis, activation of astrogliosis and microgliosis, inflammation; LFP: lateral fluid percussion, MFP: midline fluid percussion, WD: weight drop, CHI: closed head injury, NA - not available, PT: posttraumatic, MRI: magnetic resonance imaging, em: electronic microscopy, CC: corpus callosum Histo: histology; Neuro: neurologic; Behave: Behavioral

References

1. Snell, D.L., Sandy Macleod, A.D. and Anderson, T. (2016) Post-Concussion Syndrome after a Mild Traumatic Brain Injury: A Minefield for Clinical Practice. Journal of Behavioral and Brain Science, 6, 227-232. http://dx.doi.org/10.4236/jbbs.2016.66023

2. Daneshvar DH, Riley DO, Nowinski CJ, et al. Long-term consequences: effects on normal development profile after concussion. Phys Med Rehabil Clin N Am. 2011b; 22:683–700. ix.

3. 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 Jan;275 Pt 3(0 3):381-388. doi: 10.1016/j.expneurol.2015.06.015.

4. Hukkelhoven, C. W., E. W. Steyerberg, A. J. Rampen, E. Farace, J. D. Habbema, L. F. Marshall, G. D. Murray and A. I. Maas (2003). "Patient age and outcome following severe traumatic brain injury: an analysis of 5600 patients." J Neurosurg 99(4): 666-673

5. Mckee AC, Daneshvar DH. The neuropathology of traumatic brain injury. Handb Clin Neurol. 2015;127:45-66. doi: 10.1016/B978-0-444-52892-6.00004-0

6. Guskiewicz KM, Marshall SW, Bailes J, McCrea M, Cantu RC, Randolph C, et al: Association between recurrent concussion and late-life cognitive impairment in retired professional football players. Neurosurgery 57:719–726, 2005

7. Langlois JA, Rutland-Brown W, Wald MW. The epidemiology and impact of traumatic brain injury. J Head Trauma Rehabil. 2006;21(5):375–78

8. Gardner RC, Dams-O'Connor K, Morrissey MR, Manley GT. Geriatric Traumatic Brain Injury: Epidemiology, Outcomes, Knowledge Gaps, and Future Directions. J Neurotrauma. 2018 Apr 1;35(7):889-906. doi: 10.1089/neu.2017.5371.

9. Peters ME, Gardner RC. Traumatic brain injury in older adults: do we need a different approach? Concussion. 2018 Sep 20;3(3): CNC56. doi: 10.2217/cnc-2018-0001.

10. Rojas-Fernandez C, Dadfar F, Wong A, Brown SG. Use of fall risk increasing drugs in residents of retirement villages: a pilot study of long-term care

and retirement home residents in Ontario, Canada. BMC Res Notes. 2015 Oct 14;8:568. doi: 10.1186/s13104-015-1557-2.

11. Gordon KE. The Silent Minority: Insights into Who Fails to Present for Medical Care Following a Brain Injury. Neuroepidemiology. 2020;54(3):235-242. doi:10.1159/000503579

12. Baugh CM, Stamm JM, Riley DO, et al. Chronic traumatic encephalopathy: Neuro-degeneration following repetitive concussive and subconcussive brain trauma. Brain Imaging Behav 2012;6:244–54

13. Sivanandam T. Thakur MK. Traumatic brain injury: a risk factor for Alzheimer's disease Neurosci Biobehav Rev. 2012;36:1376– 810

14. (Donnemiller E, Brenneis C, Wissel J, et al. Impaired dopaminergic neurotransmission in patients with traumatic brain injury: a SPECT study using 1231-beta-CIT and 1231-IBZM. Eur J Nucl Med. 2000;27(9):141–4

15. Noy S., Krawitz S., DelBigioMR. Chronic traumatic encephalopathy-like abnormalities in a routine neuropathology service. J Neuropathol Exp Neurol 2016;75:1145–54

16. Evans RW. The post-concussion syndrome: 130 years of controversy. Semin Neurol. 1994;14:32-39

17. Gardner RC, Yaffe K. Epidemiology of mild traumatic brain injury and neurodegenerative disease. Mol Cell Neurosci. 2015 May;66(Pt B):75-80. doi: 10.1016/j.mcn.2015.03.001

18. McKee AC, Stern RA, et al, The spectrum of disease in chronic traumatic encephalopathy. Brain. 2013 Jan;136(Pt 1):43-64. doi: 10.1093/brain/aws307.

19. Guskiewicz KM, Marshall SW, Bailes J, et al. Association between recurrent concussion and late-life cognitive impairment in retired professional football players. Neurosurgery. 2005; 57:719–726. discussion 726

20. Baugh CM, Robbins CA, Stern RA, et al. Current understanding of chronic traumatic encephalopathy. Curr Treat Opt Neurol 2014;16:306

21. Iverson GL, Luoto TM, Karhunen PJ, Castellani RJ. Mild Chronic Traumatic Encephalopathy Neuropathology in People With No Known Participation in Contact Sports or History of Repetitive Neurotrauma. J Neuropathol Exp Neurol. 2019 Jul 1;78(7):615-625. doi: 10.1093/jnen/nlz045.

22. Xiong Y, Mahmood A, Chopp M. Animal models of traumatic brain injury. Nat Rev Neurosci. 2013 Feb;14(2):128-42. doi: 10.1038/nrn3407

23. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

24. McMahon, P.; Hricik, A.; Yue, J.K et al. Symptomatology and Functional Outcome in Mild Traumatic Brain Injury: Results from the Prospective TRACK-TBI study. J. Neurotrauma 2014, 31, 26–33.

25. Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. Brain J Neurol. 2013; 136(Pt 1):28–42

26. Gardner RC, Burke JF, Nettiksimmons J, Kaup Aet al., Dementia risk after traumatic brain injury versus non brain trauma: the role of age and severity. JAMA Neurology 2014;71(12):1490-1497. doi:10.1001/jamaneurol.2014.2668.

27. Heim LR, Bader M, Edut S, Rachmany L, et al., The Invisibility of Mild Traumatic Brain Injury: Impaired Cognitive Performance as a Silent Symptom. J Neurotrauma. 2017 Sep;34(17):2518-2528. doi: 10.1089/neu.2016.4909. Epub 2017 Jul 19. PMID: 28514188.

28. Rowe RK, et al: Recovery of neurological function despite immediate sleep disruption following diffuse brain injury in the mouse: clinical relevance to medically untreated concussion. Sleep 2014; 37: 743–752.

29. Sengupta P. The Laboratory Rat: Relating Its Age With Human's. Int J Prev Med. 2013 Jun;4(6):624-30. PMID: 23930179; PMCID: PMC3733029.

30. Marklund N, Hillered L. Animal modelling of traumatic brain injury in preclinical drug development: where do we go from here? Br J Pharmacol. 2011 Oct;164(4):1207-29. doi: 10.1111/j.1476-5381.2010.01163.x.

31. Sun D, McGinn M, et al Aging- and injury-related differential apoptotic response in the dentate gyrus of the hippocampus in rats following brain trauma. Front Aging Neurosci. 2013 Dec 18;5:95. doi: 10.3389/fnagi.2013.00095

32. Titus DJ, Furones C, et al Age-dependent alterations in cAMP signaling contribute to synaptic plasticity deficits following traumatic brain injury. Neuroscience. 2013;231:182-194. doi:10.1016/j.neuroscience.2012.12.002

33. Sun, M., Brady, R. D, et al Aged rats have an altered immune response and worse outcomes after traumatic brain injury. Brain, behavior, and immunity, 2019, 80, 536–550. https://doi.org/10.1016/j.bbi.2019.04.038

34. Rowe, R. K., Ziebell, J. M., Aging with Traumatic Brain Injury: Effects of Age at Injury on Behavioral Outcome following Diffuse Brain Injury in Rats. 2016, Developmental neuroscience, 38(3), 195–205. https://doi.org/10.1159/000446773

35. Itoh T, Imano M, Nishida S, et al. Increased apoptotic neuronal cell death and cognitive impairment at early phase after traumatic brain injury in aged rats. Brain Struct Funct. 2013;218(1):209-220. doi:10.1007/s00429-012-0394-5

36. Hamm RJ, White-Gbadebo DM, Lyeth BG, et al. The effect of age on motor and cognitive deficits after traumatic brain injury in rats. Neurosurgery. 1992;31(6):1072-1078. doi:10.1227/00006123-199212000-00013

37. Early AN, Gorman AA, Van Eldik LJ, et al Effects of advanced age upon astrocyte-specific responses to acute traumatic brain injury in mice. J Neuroinflammation. 2020;17(1):115. doi:10.1186/s12974-020-01800-w

38. Ritzel RM, Doran SJ, Glaser EP, et al. Old age increases microglial senescence, exacerbates secondary neuroinflammation, and worsens neurological outcomes after acute traumatic brain injury in mice. Neurobiol Aging. 2019;77:194-206. doi:10.1016/j.neurobiolaging.2019.02.010

39. Kumar A, Stoica BA, et al Traumatic brain injury in aged animals increases lesion size and chronically alters microglial/macrophage classical and alternative activation states. Neurobiol Aging. 2013;34(5):1397-1411. doi:10.1016/j.neurobiolaging.2012.11.013

40. Morganti JM, Riparip LK, et al. Age exacerbates the CCR2/5-mediated neuroinflammatory response to traumatic brain injury. J Neuroinflammation. 2016;13(1):80. Published 2016 Apr 18. doi:10.1186/s12974-016-0547-1

41. Chou A, Krukowski K, Morganti JM et al. Persistent Infiltration and Impaired Response of Peripherally-Derived Monocytes after Traumatic Brain

Injury in the Aged Brain. Int J Mol Sci. 2018;19(6):1616. Published 2018 May 30. doi:10.3390/ijms19061616

42. Krukowski K, Chou A, Feng X, et al. Traumatic Brain Injury in Aged Mice Induces Chronic Microglia Activation, Synapse Loss, and Complement-Dependent Memory Deficits. Int J Mol Sci. 2018;19(12):3753. Published 2018 Nov 26. doi:10.3390/ijms19123753

43. Timaru-Kast R, Luh C, Gotthardt P, et al. Influence of age on brain edema formation, secondary brain damage and inflammatory response after brain trauma in mice. PLoS One. 2012;7(8):e43829. doi:10.1371/journal.pone.0043829

44. Cheng WH, Stukas S, Martens KM, et al. Age at injury and genotype modify acute inflammatory and neurofilament-light responses to mild CHIMERA traumatic brain injury in wild-type and APP/PS1 mice. Exp Neurol. 2018;301(Pt A):26-38. doi:10.1016/j.expneurol.2017.12.007

45. Cheng WH, Martens KM, Bashir A, et al. CHIMERA repetitive mild traumatic brain injury induces chronic behavioural and neuropathological phenotypes in wild-type and APP/PS1 mice. Alzheimers Res Ther. 2019;11(1):6. Published 2019 Jan 12. doi:10.1186/s13195-018-0461-0

46. Webster SJ, Van Eldik LJ, Watterson DM, Bachstetter AD. Closed head injury in an age-related Alzheimer mouse model leads to an altered neuroinflammatory response and persistent cognitive impairment. J Neurosci. 2015;35(16):6554-6569. doi:10.1523/JNEUROSCI.0291-15.2015

47. Hefendehl JK, Neher JJ, et al. Homeostatic and injury-induced microglia behavior in the aging brain. Aging Cell. 2014;13(1):60-69. doi:10.1111/acel.12149

48. Shultz SR, MacFabe DF, et al Sub-concussive brain injury in the Long-Evans rat induces acute neuroinflammation in the absence of behavioral impairments. Behav Brain Res. 2012;229(1):145-152. doi:10.1016/j.bbr.2011.12.015

49. Shultz SR, MacFabe DF, et al. A single mild fluid percussion injury induces short-term behavioral and neuropathological changes in the Long-Evans rat: support for an animal model of concussion. Behav Brain Res. 2011;224(2):326-335. doi:10.1016/j.bbr.2011.06.012

50. Shultz SR, Bao F, et al, Repeated mild lateral fluid percussion brain injury in the rat causes cumulative long-term behavioral impairments, neuroinflammation,

and cortical loss in an animal model of repeated concussion. J Neurotrauma. 2012;29(2):281-294. doi:10.1089/neu.2011.2123

51. Hamm RJ, Lyeth BG, Jenkins LW, O'Dell DM, Pike BR. Selective cognitive impairment following traumatic brain injury in rats. Behav Brain Res. 1993;59(1-2):169-173. doi:10.1016/0166-4328(93)90164-l

52. Arulsamy A, Corrigan F, Collins-Praino LE. Age, but not severity of injury, mediates decline in executive function: Validation of the rodent touchscreen paradigm for preclinical models of traumatic brain injury. Behav Brain Res. 2019;368:111912. doi:10.1016/j.bbr.2019.111912

53. Collins-Praino LE, Arulsamy A, Katharesan V, Corrigan F. The effect of an acute systemic inflammatory insult on the chronic effects of a single mild traumatic brain injury. Behav Brain Res. 2018;336:22-31. doi:10.1016/j.bbr.2017.08.035

54. Spain A, Daumas S, Lifshitz J, et al. Mild fluid percussion injury in mice produces evolving selective axonal pathology and cognitive deficits relevant to human brain injury. J Neurotrauma. 2010;27(8):1429-1438. doi:10.1089/neu.2010.1288

55. Muccigrosso MM, Ford J, Benner B, et al. Cognitive deficits develop 1month after diffuse brain injury and are exaggerated by microglia-associated reactivity to peripheral immune challenge. Brain Behav Immun. 2016;54:95-109. doi:10.1016/j.bbi.2016.01.009

56. Taib T, Leconte C, Van Steenwinckel J, et al. Neuroinflammation, myelin and behavior: Temporal patterns following mild traumatic brain injury in mice. PLoS One. 2017;12(9):e0184811. Published 2017 Sep 14. doi:10.1371/journal.pone.0184811

57. Fenn AM, Gensel JC, Huang Y, Popovich PG, Lifshitz J, Godbout JP. Immune activation promotes depression 1 month after diffuse brain injury: a role for primed microglia. Biol Psychiatry. 2014;76(7):575-584. doi:10.1016/j.biopsych.2013.10.014

58. Tajiri N, Kellogg SL, et al. Traumatic brain injury precipitates cognitive impairment and extracellular Aβ aggregation in Alzheimer's disease transgenic mice. PLoS One. 2013;8(11):e78851. Published 2013 Nov 4. doi:10.1371/journal.pone.0078851

59. Mouzon B, Bachmeier C, Ojo J, et al. Chronic White Matter Degeneration, but No Tau Pathology at One-Year Post-Repetitive Mild Traumatic Brain Injury in a Tau Transgenic Model. J Neurotrauma. 2019;36(4):576-588. doi:10.1089/neu.2018.5720

60. Mouzon BC, Bachmeier C, Ojo JO, et al. Lifelong behavioral and neuropathological consequences of repetitive mild traumatic brain injury. Ann Clin Transl Neurol. 2017;5(1):64-80. Published 2017 Dec 14. doi:10.1002/acn3.510

61. Lyons DN, Vekaria H, Macheda T, et al. A Mild Traumatic Brain Injury in Mice Produces Lasting Deficits in Brain Metabolism. J Neurotrauma. 2018;35(20):2435-2447. doi:10.1089/neu.2018.5663

62. Zohar O, Schreiber S, et al, Closed-head minimal traumatic brain injury produces long-term cognitive deficits in mice. Neuroscience. 2003;118(4):949-955. doi:10.1016/s0306-4522(03)00048-4

63. Zohar O, Rubovitch V, et al., Behavioral consequences of minimal traumatic brain injury in mice. Acta Neurobiol Exp (Wars). 2011;71(1):36-45.

64. Rachmany L, Tweedie D, Rubovitch V, et al. Cognitive impairments accompanying rodent mild traumatic brain injury involve p53-dependent neuronal cell death and are ameliorated by the tetrahydrobenzothiazole PFT-α. PLoS One. 2013;8(11):e79837. Published 2013 Nov 28. doi:10.1371/journal.pone.0079837

65. Rachmany L, Tweedie D, Li Y, et al. Exendin-4 induced glucagon-like peptide-1 receptor activation reverses behavioral impairments of mild traumatic brain injury in mice. Age (Dordr). 2013;35(5):1621-1636. doi:10.1007/s11357-012-9464-0

66. Tashlykov V, Katz Y, Gazit V, Zohar O, Schreiber S, Pick CG. Apoptotic changes in the cortex and hippocampus following minimal brain trauma in mice. Brain Res. 2007;1130(1):197-205. doi:10.1016/j.brainres.2006.10.032

67. Tashlykov V, Katz Y, Volkov A, et al. Minimal traumatic brain injury induce apoptotic cell death in mice. J Mol Neurosci. 2009;37(1):16-24. doi:10.1007/s12031-008-9094-2

68. Baratz R, Tweedie D, Wang JY, et al. Transiently lowering tumor necrosis factor-α synthesis ameliorates neuronal cell loss and cognitive impairments induced by minimal traumatic brain injury in mice. J Neuroinflammation. 2015;12:45. Published 2015 Mar 7. doi:10.1186/s12974-015-0237-4

69. Luo J, Nguyen A, Villeda S, et al. Long-term cognitive impairments and pathological alterations in a mouse model of repetitive mild traumatic brain injury. Front Neurol. 2014;5:12. Published 2014 Feb 4. doi:10.3389/fneur.2014.00012

70. Taylor EN, Huang N, Wisco J, Wang Y, Morgan KG, Hamilton JA. The brains of aged mice are characterized by altered tissue diffusion properties and cerebral microbleeds. J Transl Med. 2020;18(1):277. Published 2020 Jul 8. doi:10.1186/s12967-020-02441-6

71. Aungst SL, Kabadi SV, Thompson SM. Repeated mild traumatic brain injury causes chronic neuroinflammation, changes in hippocampal synaptic plasticity, and associated cognitive deficits. J Cereb Blood Flow Metab. 2014 Jul;34(7):1223-32. doi: 10.1038/jcbfm.2014.75.