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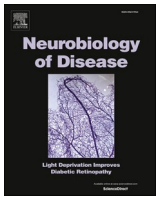

Thoracic trauma promotes alpha-Synuclein oligomerization in murine Parkinson's disease

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ABSTRACT

Background: Systemic and neuroinflammatory processes play key roles in neurodegenerative diseases such as Parkinson's disease (PD). Physical trauma which induces considerable systemic inflammatory responses, represents an evident environmental factor in aging. However, little is known about the impact of physical trauma, on the immuno-pathophysiology of PD. Especially blunt chest trauma which is associated with a high morbidity and mortality rate in the elderly population, can induce a strong pulmonary and systemic inflammatory reaction. Hence, we sought out to combine a well-established thoracic trauma mouse model with a well-established PD mouse model to characterize the influence of physical trauma to neurodegenerative processes in PD.
Methods: To study the influence of peripheral trauma in a PD mouse model we performed a highly standardized blunt thorax trauma in a well-established PD mouse model and determined the subsequent local and systemic response.
Results: We could show that blunt chest trauma leads to a systemic inflammatory response which is quantifiable with increased inflammatory markers in bronchoalveolar fluids (BALF) and plasma regardless of the presence of a PD phenotype. A difference of the local inflammatory response in the brain between the PD group and non-PD group could be detected, as well as an increase in the formation of oligomeric pathological alpha-Synuclein (asyn) suggesting an interplay between peripheral thoracic trauma and asyn pathology in PD.
Conclusion: Taken together this study provides evidence that physical trauma is associated with increased asyn oligomerization in a PD mouse model underlining the relevance of PD pathogenesis under traumatic settings.

1. Background

Worldwide, trauma in geriatric patients is frequent, and the health-related quality of life in old people is still rather poor after major trauma (Freigang et al., 2020). In this context, elderly with comorbidities and high frailty are notably prone to posttraumatic complications (Zhao et al., 2022). Concerning the trauma patterns, especially chest trauma is associated with a high morbidity and mortality irrespective of whether it occurs as mono-, combined- or multiple-injury (Eghbalzadeh et al., 2018). The lungs represent a major target and actor of a direct, parenchymal, or indirect, remote trauma because they are centrally involved in the early immuno-pathophysiological response (Huber-Lang et al., 2018). Exposure to damage-associated molecular patterns (DAMPS) activates the fluid phase and cellular immune response within

Abbreviations: PD, Parkinson's disease; asyn, alpha-synuclein; BALF, bronchoalveolar fluids; DAMPS, damage-associated molecular patterns; ARDS, adult respiratory distress syndrome; TXT, thorax trauma; SEC, size-exclusion chromatography; MPO, myeloperoxidase; KC, keratinocyte chemoattractant; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; IL-1ß, interleukin-1ß; GFAP, glial fibrillary acidic protein.

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the broncho-alveolar space resulting in recruitment of inflammatory cells, disruption of the air-blood barrier, development of endotheliopathy and further damage to the lung tissue, escalating in respiratory dysfunction and adult respiratory distress syndrome (ARDS) (Huber-Lang et al., 2018). In addition, there exists a tight neuro-pulmonary crosstalk which can significantly contribute to the development of acute lung injury. For example, damage to the brain can result in broncho-alveolar recruitment of leukocytes (Hu et al., 2017) breakdown of the air-blood barrier, and clinically signs of pneumonia in terms of "neurogenic acute lung injury" (Yasui et al., 2016) (Dai et al., 2013). Consequently, any preexisting neurodegenerative disease, such as Parkinson's or Alzheimer's, may in principle suppress or exacerbate the posttraumatic response of the lungs and other organs. Especially Parkinson's disease (PD) with dysphagia and associated high risk of aspiration pneumonia as well as sensorimotor cough dysfunction (Borders et al., 2021) are known to directly affect the lung immune- and respiratory function and thus result in an enhanced morbidity and mortality rate (Dilmaghani et al., 2021). Furthermore, PD often is associated with pulmonary dysfunction based on both obstructive and restrictive lung alterations (Docu Axelerad et al., 2021). In addition, PD also features some age-related pathophysiological vicious circles (Belikov, 2019) supposedly feeding in trauma-caused immune-pathophysiological alterations within the lungs. Clinically, a higher risk for development of pneumonia, urinary tract infection, and bedsores, were also reported after hip fractures in PD patients (Critchley et al., 2015).

However, the consequences of PD as pre-existing condition on the local and systemic posttraumatic response after thorax trauma are still in the dark and neither clinically nor experimentally addressed so far. Therefore, we combined a well-established blunt chest trauma model (Weckbach et al., 2013) with a well-established PD model in mice and investigated the posttraumatic response with focus on the directly and indirectly injured organs. We report for the first time in PD mice so far unknown synchronic early effects on the lungs and brain after blunt thorax trauma (TXT).

2. Methods

2.1. Animals

The study protocol was approved by the University Care Committee and the federal authorities for animal research, Tuebingen, Germany (Permit No. 1576). The experiments were performed in accordance with the National Institutes of Health Guidelines on the use of laboratory animals and included male and female mice. The mouse cohorts at the age of 18–22 months were used in the study (sham non-PD $n = 5$; sham-PD $n = 8$–9; TXT non-PD n = 8–9; TXT PD $n = 10$). Since we used male and female mice in our study, we tested whether sex plays a role in our experimental set up. In all assays used in this study we did not detect any confounding effects based on sex.

2.2. Alpha-Synuclein protein complementation assay

To quantify the oligomerization of asyn a well-established bioluminescent protein complementation assay was used. C-terminal and N-terminal halves of Gaussia luciferase were merged with wild-type human asyn. Oligomerization of asyn leads to the reconstitution of Gaussia luciferase, resulting in a bioluminescence signal in the presence of the substrate coelenterazine (Danzer et al., 2011; Kiechle et al., 2019).

2.3. PD mouse model

A transgenic mouse model with asyn fused to N-terminal half of human Gaussia luciferase (S1) and asyn fused to C-terminal part of human Gaussia luciferase (S2) with its expression regulated by tetracycline (Tet-off) was generated for in vivo use (Gossen and Bujard, 1992). S1 and S2 were placed under the control of a tetracycline response element (TRE) with a bidirectional CMV promoter, which regulates the expression of both S1 and S2. These single transgenic S1/S2 mice were then crossbred with the CaMKIIa-tTA driver line to induce S1 and S2 expression in neurons of different forebrain areas (Mayford et al., 1996). After a symptom free prodromal phase of about 7 months these mice showed reduced locomotor activity, exploratory behavior and decreased motor coordination. Additionally, olfactory dysfunction could be shown which is an early symptom of PD. Furthermore, this mouse model showed age dependent asyn oligomer formation with a reduction of striatal dopaminergic fibers and a significant reduction of striatal dopamine and elevated levels of striatal dopamine metabolites. This PD mouse model has been described in detail previously (Kiechle et al., 2019).

2.4. Induction of the thorax trauma

Thorax trauma was induced by blunt bilateral chest trauma according to a standardized protocol. 30 min prior to the trauma or sham treatment, as analgesia 0.05 mg/kg body weight buprenorphine (Temgesic, RB Pharmaceuticals Limited, Slough, GB) was injected subcutaneously (s.c.) to the mice. Mice were anesthetized with a 3% sevoflurane (Baxter Healthcare, Glenview, USA) - 97% oxygen mixture. Thorax chest trauma was induced by exposure to a single blast-wave onto the chest. The anesthetized mice, placed in a supine position under the cylinder, was hit by a blast wave of 1–2 ms duration (as defined by the bursting of a 50A Mylar Polyester membrane, Fa. Du Pont Luxemburg) generated by compressed air, as described in detail elsewhere (Knöferl et al., 2003), (Knöferl et al., 2004), (Perl et al., 2006). Sham animals underwent the same procedure, except for the induction of the blast injury.

2.5. Euthanasia and cardiac puncture

Three hours after thorax trauma or sham treatment, mice in deep anesthesia were sacrificed by cardiac puncture and blood drawing. EDTA blood was centrifuged for 5 min at 800 $\times g$ at 4 °C. Subsequently, the supernatant was centrifuged (2 min at 13,000 $\times g$ at 4 °C) and stored at −80 °C until further analysis.

2.6. Bronchoalveolar lavage

After exsanguination, the trachea of the animals was carefully exposed and cannulated. 500 µl ice cold phosphate-buffered saline (PBS) (Gibco, Eggenstein, Germany) was carefully injected, retrieved, and collected in a tube with 10 µl 1:1000 broad-spectrum protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA). The rinsing with PBS was repeated two times for gathering the alveolar cells. The bronchoalveolar fluid (BALF) was centrifuged at 380 x*g* for 10 min at 4 °C, and the supernatant was aliquoted and stored at −80 °C until analysis. For cell counting, the pellets of the BAL were resuspended in PBS, stained with trypan blue, and counted in the Neubauer chamber.

2.7. Preparation of lung homogenates

The lobes of the lungs were homogenized in a proteinase inhibitor cocktail (Roche complete, Roche diagnostics, Mannheim, Germany) with an Ultra-Turrax disperser (IKA, Staufen, Germany) followed by ultrasonic treatment with a sonifier (Branson, Danbury, Conn). After centrifugation (16,000 x*g*; 15 min; 4 °C), supernatants were stored at −80 °C.

2.8. Preparation of brain homogenates

Brain tissue was dissected and homogenized in PBS (Gibco, Eggenstein, Germany) using a TissueLyser II (Qiagen, Venlo, Netherlands) for 2 × 2 min at 25 Hz with stainless steel beads (Qiagen, Hilden, Germany). Samples were centrifuged at 20,800 x*g* for 15 min at 4 °C. After

centrifugation supernatants were stored at −80 °C for further use.

2.9. Protein concentration measurement

Protein concentrations in BALF, lung homogenates and brain homogenates were analyzed using the Pierce BCA Protein Assay Kit (Pierce Protein Research Products, Rockford, USA). The analysis was performed according to the manufacturer's recommendations.

2.10. Size-Exclusion Chromatography (SEC)

3 mg protein of brain homogenates in 600 µl PBS (Gibco, Eggenstein, Germany) was loaded onto a PBS equilibrated 24 ml Superdex 200 (10/300 GL) column (GE Healthcare, Chicago, IL, USA) connected to an ÄKTApurifier (GE Healthcare, Chicago, USA). The protein eluate was monitored by UV absorbance at 280 nm and 215 nm wavelength with a flow rate of 0.75 ml/min and fractions of 500 µl were collected for luciferase activity measurement (100 µl, duplicates). Molecular mass of protein eluate was estimated using the Gel Filtration Calibration Kit (GE Healthcare, Chicago,IL, USA) with the following standard proteins: Ferritin (440 kDa), Aldolase (158 kDa), Conalbumin (75 kDa), Ovalbumin (44 kDa),Carbonic anhydrase (29 kDa), Ribonuclease A (13.7 kDa) and protinin A (6.5 kDa).

2.11. Dot Blot

100 µl of SEC fractions were analyzed using a Dot blot apparatus (Whatman Schleicher & Schuell Minifold I Spot-Blot system, GE Healthcare, Chicago, IL, USA). Briefly, samples were applied on a nitrocellulose membrane (Amersham Protran 0.2, GE Healthcare, Chicago, IL, USA) and the liquid was filtered through the membrane by gentle vacuum. Specific antigens were detected with mouse anti-asyn clone 42 (#610787; BD, Franklin Lakes, NJ, USA; 1:1000). HRP-conjugated goat anti mouse secondary antibodies were supplied from Promega. Images were acquired on a FUSION SOLO S (Vilber, Eberhardzell, Germany) with Luminata Forte HRP substrate (Merck, Darmstadt, Germany). Densitometry of immunoblots was performed using ImageJ (NIH).

2.12. Western Blot

30 µg protein samples were separated on NuPAGE™ 4–12% Bis-Tris gels by using a XCell SureLock™ Mini-Cell Electrophoresis system and transferred to a nitrocellulose membrane, using a XCell SureLock™ XCell II™ Blot Module (Thermo Fisher Scientific, Waltham, MA, USA). Specific antigens were detected with the following primary antibodies: mouse anti-asyn clone 42 (#610787; BD, Franklin Lakes, NJ, USA; 1:1000), mouse anti-asyn O2 (#847602, Biolegend, Amsterdam, Netherlands, 1:1000) and rabbit anti-GAPDH 10494–1-AP (Proteintech, Rosemont, IL, USA). HRP-conjugated goat secondary antibodies were supplied from Promega. Images were acquired on a FUSION SOLO S (Vilber, Eberhardzell, Germany) with Luminata Forte HRP substrate (Merck, Darmstadt, Germany). Densitometric analysis of western blot analysis was performed with FusionCaptAdvance software from Vilber Smart Imaging. Extracted values were analyzed with a paired *t*-test.

2.13. Human Gaussia luciferase assay

0.5 mg protein of brain homogenates in 100 µl PBS (Gibco, Eggenstein, Germany) (duplicates) and 100 µl eluate of SEC fractions (duplicates) were used for luciferase activity measurement in an automated plate reader (Victor X3 microplate reader, Perkin-Elmer, Waltham, USA) with automated injection of the cell permeable substrate coelenterazine (40 µM, P.J.K., Kleinblittersdorf, Germany) and a signal integration time of 1 s.

2.14. Measurement of cytokine concentrations in plasma, BALF, lung and brain homogenates

Concentrations of keratinocyte chemoattractant (KC) (Mouse KC R&D DuoSet, R&D Systems, Minneapolis, USA), interleukin-6 and MCP-1 (Mouse IL-6 and MCP-1 BD OptEIA, BD Pharmingen, San Diego, USA), tumor necrosis factor α (TNF-α) (ELISA MAX Deluxe, Biolegend, San Diego, CA), interleukin-1β (Mouse IL-1β ELISA MAX Deluxe, Biolegend, San Diego, USA) and glial fibrillary acidic protein (Mouse GFAP, Lifespan Biosciences Inc., Seattle, WA, USA) were determined by sandwich-enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's recommendations.

2.15. Analysis of myeloperoxidase activity (MPO) in lung and brain homogenates

For determination of lung MPO activity, lungs were homogenized (ULTRA-TURRAX, IKA, Staufen, Germany) in KH_2PO_4 buffer (Merck, Darmstadt, Germany) and incubated for 2 h at 60 °C and centrifuged at 3950 x*g* at room temperature for 15 min. 25 µl supernatant were mixed with 25 µl tetramethylbenzidine (Sigma-Aldrich, St. Louis, MO, USA) and 200 µl 0.002% H_2O_2 (Fluka, Deisenhofen, Germany). After an incubation time of 5 min at 37 °C, the extinction was read at 450 nm (Tecan Sunrise Reader, Tecan, Crailsheim, Germany).

2.16. Statistical analyses

Statistical analyses were performed with GraphPad Prism (Edition 9.3; GraphPad Software, USA). All results are presented as mean ± SEM. To eliminate outliers, the *Z*-score test was used. To compare multiple cohorts, data sets were analyzed using two-way analysis of variance (ANOVA), followed by Holm-Sidak test, provided that a normal distribution could be assumed. For comparison of differences between two normally distributed groups, Student's *t*-test was used. Mann-Whitney *U* test was applied if two comparing groups had no parametric distribution. A global significance level was defined as $p \leq 0.05$. All statistical tests were two-tailed.

3. Results

3.1. No differences in the acute trauma response of PD and non-PD mice

To determine if pre-existing PD aggravates the early consequences of a blunt chest trauma to lungs and the brain in a PD mouse model, we applied the well-established thoracic trauma (TXT) (Fig. 1A) (Maitz et al., 2021) to asyn overexpressing mice described earlier (Kiechle et al., 2019) at the symptomatic age (18–22 month of age). Aggregation of asyn is the main hallmark of PD pathology and oligomeric asyn is regarded as the pathological species in PD. These PD mice were recently generated based on human asyn protein complementation expressing asyn split *Gaussia* luciferase that allows a highly sensitive assessment of asyn oligomers. The expression of non-bioluminescent asyn constructs is neuron specific and inducible, they can reconstitute when brought together by asyn-asyn interactions (Tet Off System)(Kiechle et al., 2019). At the age of 18–22 months when PD mice show a full-blown motoric phenotype as reported previously(Kiechle et al., 2019). PD mice and non-PD controls were exposed to either thorax trauma (TXT) or control treatment (sham) (Fig. 1A). Immediately after blunt chest trauma mice respond with an acute onset apnea phase. We measured this time span in the PD and non-PD group after the thoracic trauma and could not observe any significant differences (Fig. 1B). Macroscopical analysis of the injury degree and pattern (hematoma, free blood, atelectasis) revealed no differences within the traumatized animals and we did not observe any immediate fatalities in both groups (Fig. 1C).

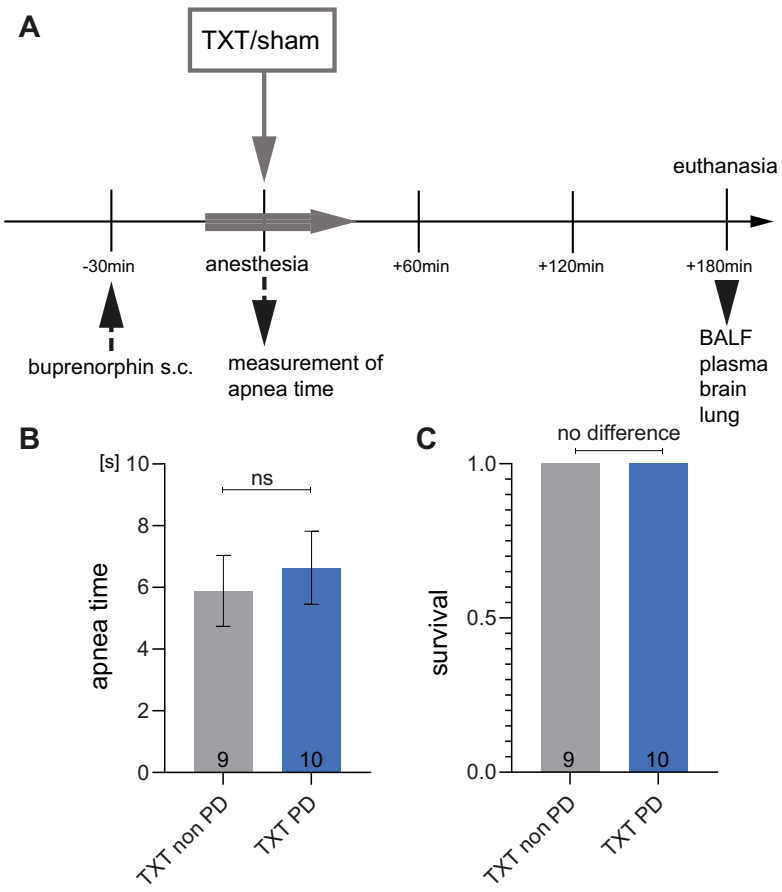

Fig. 1. (A) Schematic overview of the experimental workflow. 30 min prior to treatment mice received a subcutaneous injection of the opioid buprenorphine. Before treatment anesthesia with isoflurane was applied. (B) During treatment apnea time was measured, which did not show any significant differences between the Parkinson's disease (PD) thorax trauma (TXT) and the non-PD thorax trauma group. (C) 180 min after treatment mice were sacrificed and sampling of bronchoalveolar fluid (BALF), serum and brain was performed. No difference in the survival rate between the groups (thoracic trauma: PD vs non-PD) was observed. Values are presented as ± SEM, significances were tested using Student's *t*-test, (TXT group $n = 10$ mice, Sham group = 9 mice).

3.2. Peripheral inflammatory profiling confirms increased pulmonary and systemic interleukins following thoracic trauma

In contrast to an increased immune cell count in the bronchoalveolar fluid (BALF) and a posttraumatic elevation of chemokine KC and cytokine IL-6 characteristic for TXT, we did not observe any immunological peripheral differences in the presence or absence of PD (Fig. 2A-C). As expected, we could also confirm peripheral elevation of KC in the plasma of the trauma group vs sham-treated groups, both in the PD mice expressing asyn and in the non-transgene expressing group (Fig. 2G). In the next step we immuno-profiled lung tissue homogenates. Consistent with our previous studies, thorax trauma mildly increased MPO levels, whereas chemokine KC and IL-6 in lung tissue homogenate in trauma treated animals did not reach statistical significance (Fig. 2 D—F).

3.3. Central nervous interleukin profiling reveals altered (PD) immunological precondition but no acute trauma-induced interleukin alteration

As trauma-induced alterations of interleukin concentrations in BALF and the peripheral blood were found, we also measured interleukins in the brain tissue. While there was no significant trauma-associated change of the IL-1β, lL-6, TNF-alpha, and MCP-1 levels we could detect increased cytokine levels of IL-1β, IL-6 and TNF-alpha as well as MCP-1 in brain homogenates of PD mice compared to non-PD mice, suggesting an altered inflammatory state in the presence of asyn oligomers (Fig. 3 A-D). Brain GFAP and MPO-1 were not significantly altered in PD mice with or without TXT (Fig. 3 E, F) suggesting that activation of astrocytes, microglia and neutrophils does not play a major role in these conditions early after trauma.

3.4. Increased high- and medium molecular asyn oligomers after thoracic trauma

To determine if prior TXT has an impact on asyn oligomer formation we performed size-exclusion chromatography (SEC) combined with luciferase activity measurement indicative for asyn oligomers with brain homogenates of PD mice exposed to either TXT or sham treatment. Interestingly, we found an increase in high molecular weight and medium molecular weight asyn oligomers (7–9 ml and 10–13 ml, respectively) corresponding to >30mers and 4–16-mer oligomeric species. The presence of asyn in the respective fractions was also verified by asyn specific Dot blots (Fig. 4 A). In addition, this enrichment in asyn oligomers in the PD/TXT group could also be confirmed using immunoblotting with an asyn oligomer specific antibody Syn—O2, whereas no differences were seen in the non-PD groups (Fig. 4 B, D, E). The total amount of asyn was not significantly altered using immunoblot analysis with an antibody detecting total asyn suggesting that specifically asyn oligomers are increased in TXT treated PD mice (Fig. 4 C).

4. Discussion

In this study we have combined a standardized thoracic trauma model with a well-established PD mouse model and investigated the effects of a severe tissue trauma on peripheral inflammation, neuroinflammation and protein aggregation. We demonstrate that a blunt chest trauma results in acute traumatic peripheral inflammation in both PD and non-PD mice. During the onset of trauma, no immediate differences in the response to the trauma could be observed between the PD and non-PD group early after the trauma impact. The apnea time in both groups was similar, there were no trauma-associated fatalities in both groups. We could show that there is an early inflammatory response

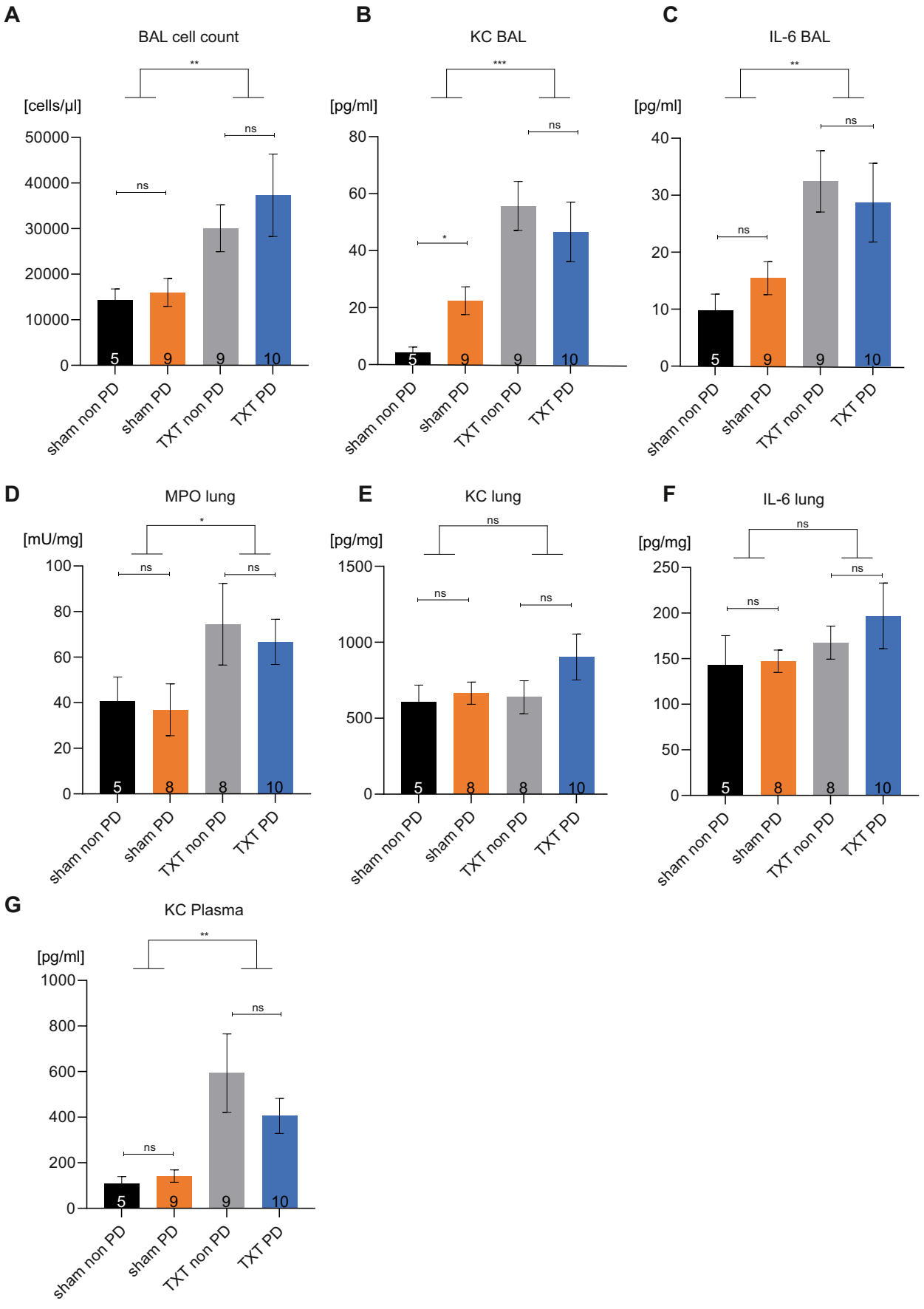

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Fig. 2. Systemic inflammation induced by blunt thorax trauma. (A) bronchoalveolar (BAL) cell count is increased in the trauma groups compared to sham treated animals (B, C) Thoracic trauma leads to a strong inflammatory response with increased keratinocyte chemoattractant (KC) and interleukin-6 (IL-6) levels in BAL and KC in plasma (G) in the trauma group compared to the sham treated group. No significant difference between the PD and non-PD group with thorax trauma could be observed. (D—F) Thorax trauma increased mildly MPO levels, whereas chemokine KC and IL-6 in lung tissue homogenate in trauma treated animals did not reach statistical significance. There was no difference between the PD and non-PD groups. All values are presented as mean ± SEM, significances were tested using Student's t-test between PD and non-PD. Two-Way-ANOVA was used to test for effects of sham treatment vs TXT treatment (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; sham non PD group $n = 5$ mice; sham PD group $n = 8$–9 mice; TXT non PD group n = 8–9 mice, TXT PD group n = 10 mice).

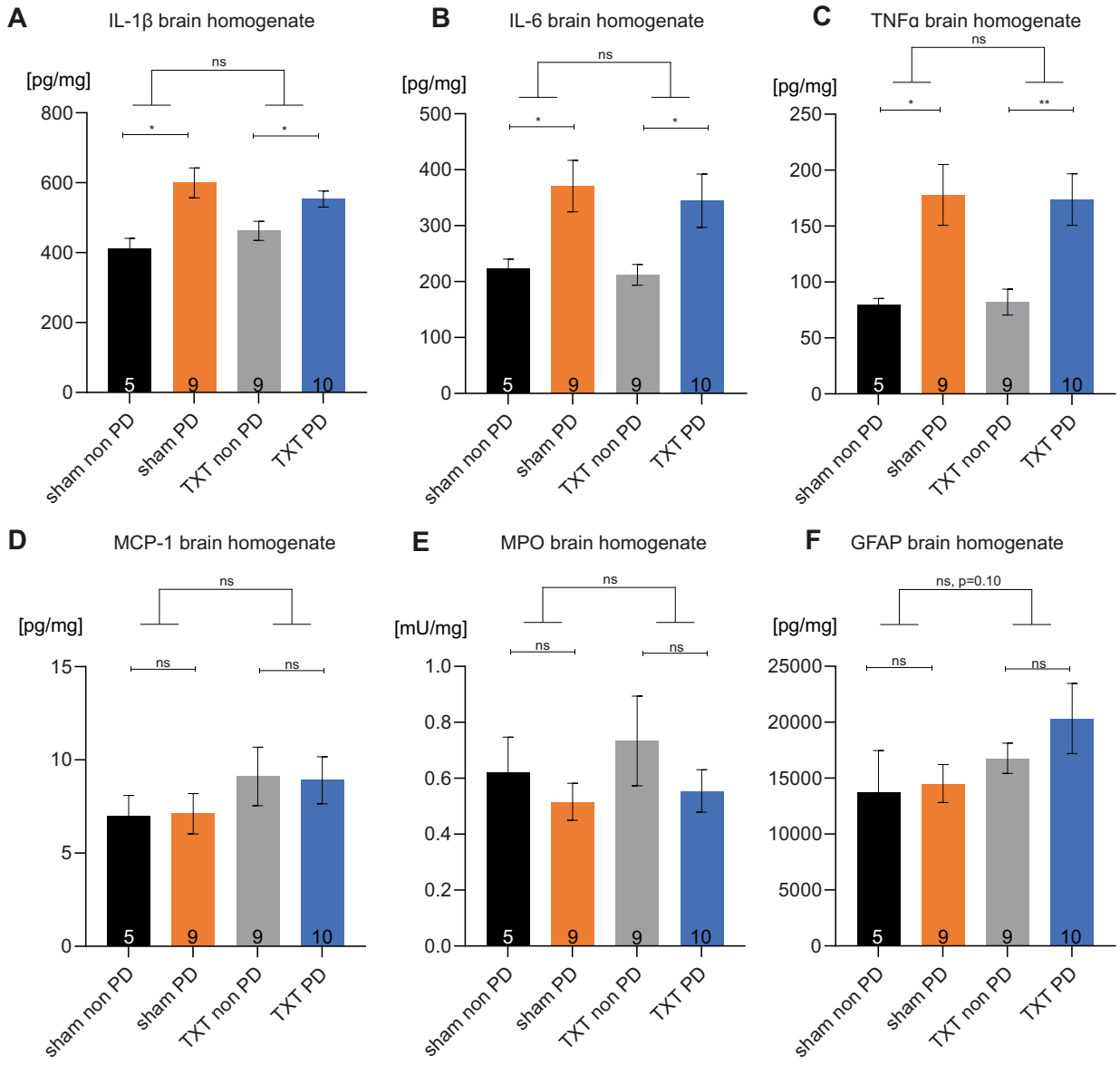
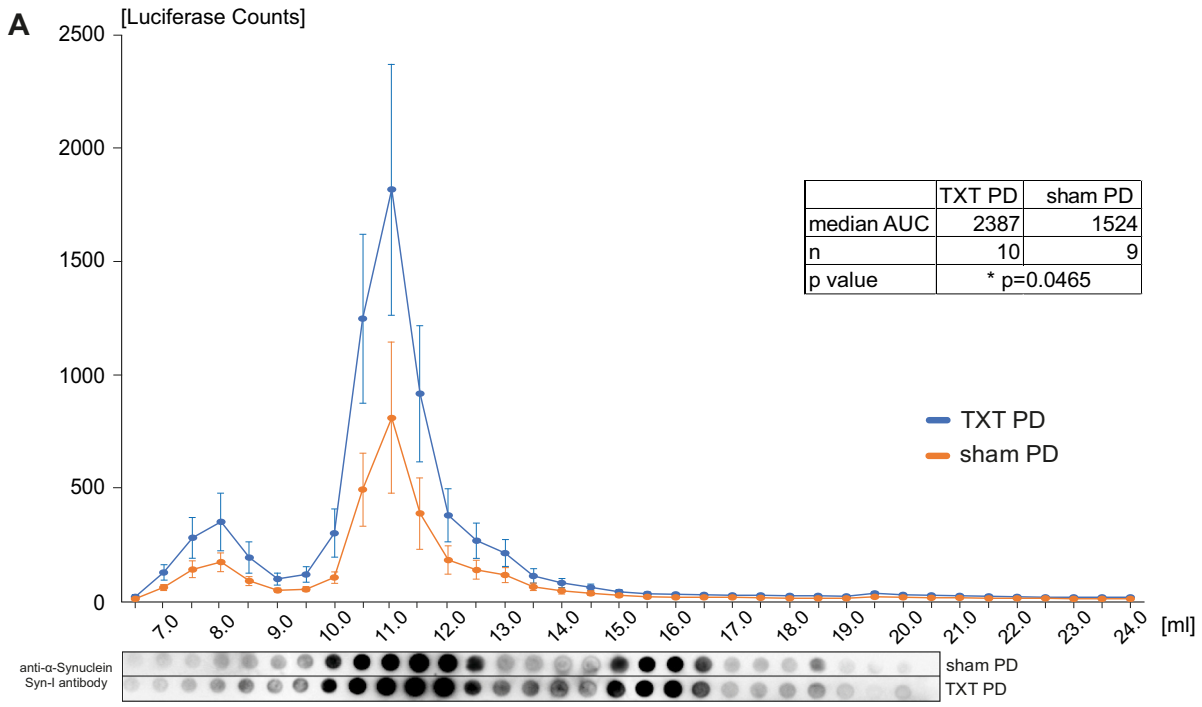


Fig. 3. Neuroinflammation in PD mice. Increased cytokine levels of interleukin-1ß (IL-1β), interleukin-6 (IL-6), tumor necrosis factor α (TNF α - as well as MCP-1 in brain homogenates of PD mice could be detected while no significant trauma-associated change of the IL-1β, lL-6, TNF-alpha, and MCP-1 levels could be observed (A-D). Brain myeloperoxidase (MPO-1) (E) and GFAP (F) were not significantly altered in PD mice with or without TXT. All values are presented as mean ± SEM and significances were tested using Student's *t*-test between PD and non-PD. Two-Way-ANOVA was used to test for effects of sham treatment vs TXT treatment (sham non PD group n = 5 mice; sham PD group $n = 9$ mice; TXT non PD group n = 9 mice, TXT PD group = 10 mice).

following a thoracic injury with increased interleukin levels in the BALF and plasma in the PD and non-PD group.

While central inflammation is elevated in PD mice, TXT did not further increase this neuroinflammatory condition in PD TXT treated mice. However, TXT trauma exposed PD mice showed an elevation of asyn oligomers in comparison to sham treated mice, suggesting that peripheral injury influences protein aggregation in the brain of PD mice.

A direct link between physical trauma and neurodegeneration has long been discussed and traumatic injuries have been shown to increase the risk of developing neurodegenerative diseases (Fleminger et al., 2003) (Rugbjerg et al., 2008) (Schmidt et al., 2010) (Wang et al., 2015). This link has been reported mainly in the context of traumatic brain injuries for which it has been shown to significantly increase the risk of developing Parkinson's disease (PD) (Jafari et al., 2013). However, peripheral injury has been associated with CNS diseases, e.g., neuropsychiatric syndromes (Karnatovskaia et al., 2015a) (Karnatovskaia et al.,



	TXT PD	sham PD
median AUC	2387	1524
n	10	9
p value	* p=0.0465	

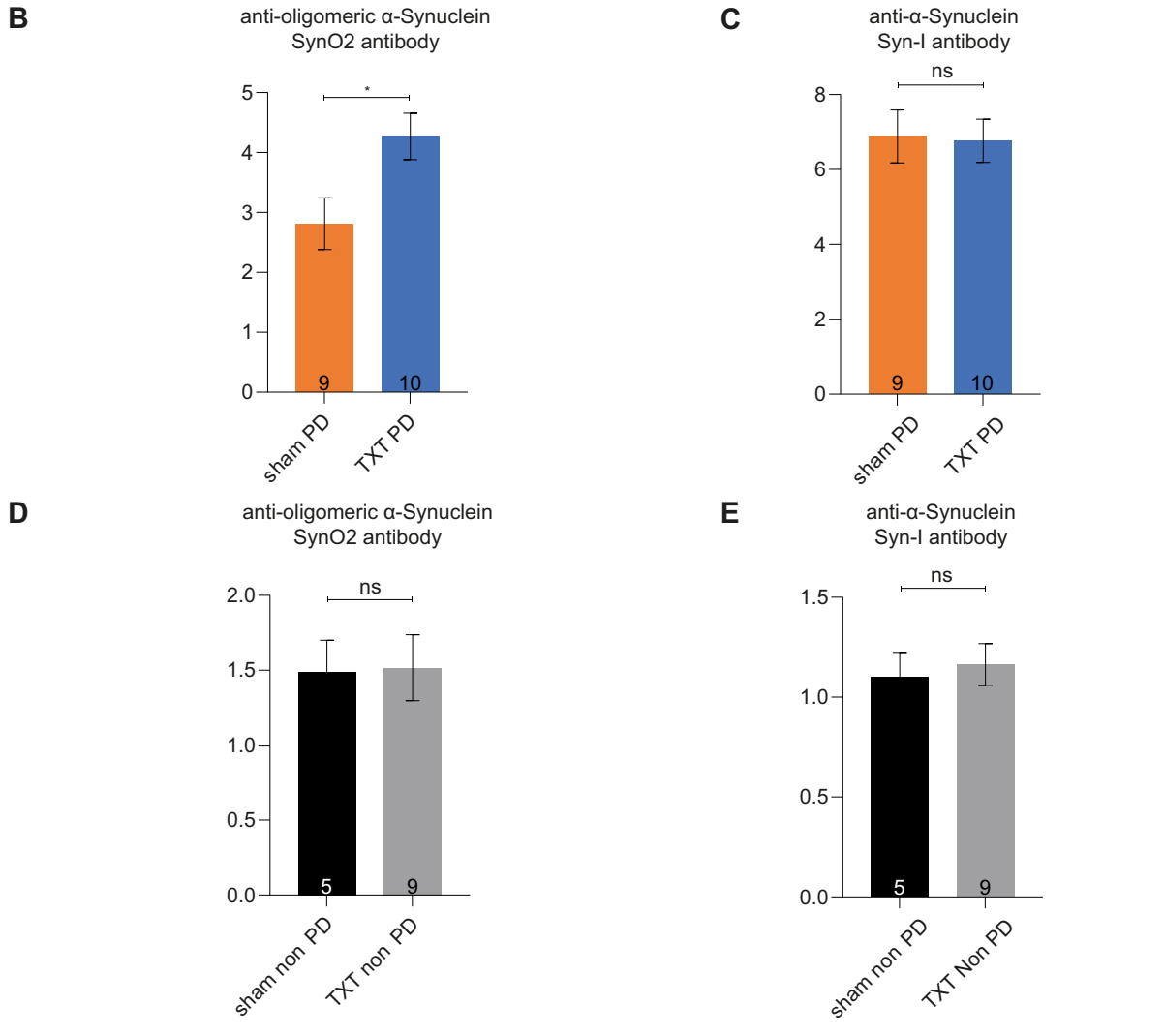


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Fig. 4. Increased luciferase signal in the alpha-synuclein (asyn) complementation assay in brain samples of mice with thoracic trauma with unchanged total asyn levels. (A) Luciferase counts of different size exclusion chromatography fractions of 3 mg brain lysates indicating increased asyn oligomerization in the brain of mice after thoracic trauma. Mann-Whitney test shows significant increased calculated area under the curve (AUC) (1.57-fold) in the thorax trauma group compared to the sham treated group (TXT group $n = 11$ mice, Sham group = 9 mice). The presence of asyn in the respective fractions was also verified by asyn specific Dot blots. (B) Densitometric analysis of immunoblots with oligomer-specific anti asyn O2- antibody normalized to GAPDH confirms increased asyn oligomerization. (C) Densitometric analysis of immunoblots of total asyn detecting antibody Syn-1 (Immunogen AA: 15–123) normalized to GAPDH shows comparable total asyn levels. (D, E) no differences in the densitometric analysis of oligomer-specific O2-anti α-Synuclein antibody and total α-Synuclein detecting antibody Syn-1 normalized to GAPDH could be observed in non-PD mice. All values are presented as mean ± SEM and significances were tested using Student's t-test. (* $p < 0.05$), (sham non PD group n = 5 mice; sham PD group n = 9 mice; TXT non PD group n = 9 mice, TXT PD group = 10 mice).

2015b) (Wiseman et al., 2013). Interestingly, on a cellular level, a peripheral trauma has been linked to synaptic alterations and even synaptic loss (Cursano et al., 2021). However, our knowledge on the pathophysiology linking peripheral inflammation to brain malfunction are limited. Some studies suggested that the brain dysfunction might be mediated through altered stress hormones (Chen et al., 2008) (Maras and Baram, 2012) (Andres et al., 2013). Peripheral immune dysfunction in PD patients has repeatedly been shown by others and our group. We have previously demonstrated that monocytes are strongly dysregulated in PD and resemble many characteristic changes of microglia in the CNS (Bliederhaeuser et al., 2016) (Grozdanov et al., 2014). In addition, specific pathological asyn oligomers are capable to induce immune cell activation, not only in the brain (Kim et al., 2013) (Zhang et al., 2005) but also to human peripheral immune cells which react to a specific pathological conformation of asyn (Grozdanov et al., 2019).

Encouraged by this strong link and possible direct interplay between central neurodegenerative processes and peripheral immune dysfunction we hypothesize that there could be an immediate ascending inflammatory reaction from the periphery that eventually reaches the CNS. However, while we could show that inflammatory markers are already elevated in the PD mice representing a preexisting inflammatory state in the CNS, we could not detect a further increase in inflammatory markers of the CNS early after a chest trauma. One possible explanation for this finding could be that this preexisting increase in interleukins in the CNS (abundance of interleukins increased by a power of ten compared to interleukins in the BALF) might mask a further significant increase of inflammatory markers in the CNS due to a systemic increase of interleukins. Whether the increased oligomerization tendency of asyn comes from these speculated peripheral augmented inflammatory processes in the CNS cannot be conclusively determined at this stage. Another hypothesis could be that DAMPs from cellular debris and protein aggregates from dead cells could enter the CNS and promote aggregation of asyn. One limitation of the study is certainly the relative short observation period (180 min post trauma) which may be too early for the detection of sustained alterations of the inflammatory response in the CNS or in peripheral organs. A further limitation of the study, the brain was only assessed globally but not the inflammatory signature in differential brain areas. Therefore, further studies are necessary which address the dynamic of these potential cross-talking processes including different brain areas.

Taken together, our study provides a novel model of acute physical stress responses and protein aggregation in the PD brain, highlighting the clinical importance of physical trauma in the elderly and patients with neurodegenerative diseases.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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