C. elegans detects toxicity of traumatic brain injury generated tau

Elisa R. Zanier [a,**], Maria Monica Barzago [b], Gloria Vegliante [a], Margherita Romeo [b], Elena Restelli [a], Ilaria Bertani [a], Carmina Natale [a,b], Luca Colnaghi [a,b], Laura Colombo [b], Luca Russo [b], Edoardo Micotti [a], Luana Fioriti [a], Roberto Chiesa [a,**], Luisa Diomede [b,*]

[a] *Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy*
[b] *Department of Molecular Biochemistry and Pharmacology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy*

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ABSTRACT

Traumatic brain injury (TBI) is associated with widespread tau pathology in about 30% of patients surviving late after injury. We previously found that TBI in mice induces the formation of an abnormal form of tau (tau[TBI]) which progressively spreads from the site of injury to remote brain regions. Intracerebral inoculation of TBI brain homogenates into naïve mice induced progressive tau pathology, synaptic loss and late cognitive decline, suggesting a pivotal role of tau[TBI] in post-TBI neurodegeneration. However, the possibility that tau[TBI] was a marker of TBI-associated neurodegeneration rather than a toxic driver of functional decline could not be excluded.

Here we employed the nematode *C. elegans* as a biosensor to test the pathogenic role of TBI generated tau. The motility of this nematode depends on efficient neuromuscular transmission and is exceptionally sensitive to the toxicity of amyloidogenic proteins, providing a tractable model for our tests. We found that worms exposed to brain homogenates from chronic but not acute TBI mice, or from mice in which tau[TBI] had been transmitted by intracerebral inoculation, had impaired motility and neuromuscular synaptic transmission. Results were similar when worms were given brain homogenates from transgenic mice overexpressing tau P301L, a tauopathy mouse model, suggesting that TBI-induced and mutant tau have similar toxic properties. P301L brain homogenate toxicity was similar in wild-type and *ptl-1* knock-out worms, indicating that the nematode tau homolog protein PTL-1 was not required to mediate the toxic effect. Harsh protease digestion to eliminate the protein component of the homogenates, pre-incubation with anti-tau antibodies or tau depletion by immunoprecipitation, abolished the toxicity. Homogenates of chronic TBI brains from tau knock-out mice were not toxic to *C. elegans*, whereas oligomeric recombinant tau was sufficient to impair their motility.

This study indicates that tau[TBI] impairs motor activity and synaptic transmission in *C. elegans* and supports a pathogenic role of tau[TBI] in the long-term consequences of TBI. It also sets the groundwork for the development of a *C. elegans*-based platform for screening anti-tau compounds.

1. Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability in young adults worldwide (Maas et al., 2017; McMillan et al., 2011). There is a growing acceptance that TBI is an acutely initiated event that results in long-lasting physical, and mental disability. In a sizeable minority TBI may also be a progressive disease, with worsening neuro-imaging findings and neurology over months and years, and/or an increased risk of late dementia (Fann et al., 2018; Nordström and Nordström, 2018). In the last decade, studies have led to the characterization of the neuropathology in individuals exposed to TBI of varying severity, and across injury survivors, from hours to many decades. These

studies have given a complex picture of pathologies, with abnormalities in tau, amyloid beta (Aβ), neuronal loss, axonal degeneration, neuro-inflammation and blood-brain barrier disruption, all changes common to wider neurodegenerative diseases (Hay et al., 2016; McKee et al., 2014, 2013; Smith et al., 2013). The development of hyper-phosphorylated tau (p-tau) is a hallmark of Alzheimer's disease, correlating to outcome and late symptomatology (Braak and Braak, 1995), and may be a crucial aspect linking TBI to late dementia. The recent addition of the positron emission tomography ligand flor-taucipir/18F-AV-1451 to map tau pathology has provided information about the temporal and spatial characteristics of tau accumulation in the living brain, showing variable increases compared to healthy volunteers years after single or repetitive TBI (Gorgoraptis et al., 2019; Stern et al., 2019; Takahata et al., 2019). However, in the clinical setting it is difficult to disentangle the precise significance of p-tau accumulation from other possible contributors to post-TBI neurodegeneration and/or dementia.

An important recent observation is that experimental TBI in wild-type (WT) mice induces the emergence and accumulation of a p-tau reminiscent of post-TBI tau pathology in humans (Kondo et al., 2015; Zanier et al., 2018). Studies from our group indicate that this TBI-induced abnormal form of tau (tau^{TBI}) spreads from the site of injury to remote regions of the brain and can be transmitted to naïve recipient mice by intracerebral inoculation, inducing memory deficits and synaptic alterations (Zanier et al., 2018). With the observation that tau^{TBI} is aggregated and partially resistant to pronase digestion, these results suggest that tau prions are generated in TBI, providing a possible explanation for how a biomechanical insult might trigger self-sustained neurodegeneration. However, it still needs to be established whether tau^{TBI} has a causal role in driving toxicity, as a primary player in the transition from an acute event to progressive neurodegenerative pathology.

To this aim we turned to the invertebrate *Caenorhabditis elegans*. Neuronal functions and genes encoding enzymes for production of neurotransmitter such as acetylcholine, glutamate, serotonin, dopamine and α-aminobutyric acid, and other elements involved in synaptic transmission, are conserved between in *C. elegans* and vertebrates. In particular, the nematode's locomotion and pharyngeal activity are controlled by acetylcholine receptors. Thus *C. elegans* motor activity and pharyngeal pumping have been used as readouts to assay the toxic activity of misfolded proteins involved in neurodegenerative diseases, including tauopathies, and peripheral amyloidosis (Brandt et al., 2007; Culetto and Sattelle, 2000; Fatouros et al., 2012; Kraemer and Schellenberg, 2007; Kraemer et al., 2003; Natale et al., 2020; Nussbaum-Krammer and Morimoto, 2014).

We established an integrated approach using brain tissue homogenates from TBI mice and *C. elegans* which allowed us to test tau^{TBI} toxicity and test pharmacological agents that interfere with TBI-associated tau toxicity. We found that *C. elegans* motility and neuromuscular synaptic transmission were impaired when the nematodes were administered brain homogenates from chronic but not acute TBI mice, or from mice in which tau^{TBI} had been transmitted by intracerebral inoculation. Notably pre-incubation with anti-tau antibodies or tau depletion by immunoprecipitation of the homogenates, abolished the toxicity, pointing to a direct pathogenic role of TBI generated tau. There is a therapeutic opportunity to intervene after TBI before significant neurodegeneration takes place, so defining the pathogenic potential of TBI-generated tau proteoforms may have important clinical implications.

2. Materials and methods

2.1. Mice

Male C57BL/6 J mice, referred to as WT mice, were purchased from Envigo (Holland). Male B6.129S4(Cg)-Mapt$^{tm1(EGFP)Klt}$/J, referred to as tau knock-out (KO) mice, were from Jackson Laboratory (#029219,

USA) (van Hummel et al., 2016). Male JNPL3 homozygous mice expressing 0N4R human tau with the P301L mutation (P301L) (Lewis et al., 2000) were obtained from Taconic Biosciences (New York, USA) (Tau- Model 2508). Controls were non-transgenic (Non-Tg) male mice with the same mixed C57BL/6, DBA/2, SW genetic background as P301L mice. Mice were housed in a specific pathogen-free animal room at a constant temperature of 21 ± 1 °C, humidity 60 ± 5%, with a 12 h light/dark cycle, and *ad libitum* access to food and water.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines at the Istituto di Ricerche Farmacologiche Mario Negri IRCCS in compliance with national (D.lgs 26/2014; Authorization n. 19/2008-A issued March 6, 2008 by Ministry of Health) and international laws and policies (EEC Council Directive 2010/63/UE; the NIH Guide for the Care and Use of Laboratory Animals, 2011 edition). They were reviewed and approved by the Mario Negri Institute Animal Care and Use Committee that includes *ad hoc* members for ethical issues, and by the Italian Ministry of Health (Decreto no. D/07/2013-B and 301/2017-PR). Animal facilities meet international standards and are regularly checked by a certified veterinarian who is responsible for health monitoring, animal welfare supervision, experimental protocols and review of procedures.

2.2. Mouse model of TBI

The controlled cortical impact brain injury mouse model used in this study replicates both the mechanical forces and the main secondary injury processes observed in severe TBI patients with brain contusion and gives clinically-relevant behavioral and histopathological outcomes (Pischiutta et al., 2018; Zanier et al., 2018). Briefly, the controlled cortical impact was induced over the left parietotemporal cortex. Homogenates from the hemisphere ipsilateral to the site of the biomechanical impact were referred as TBI$_{ipsi}$ and those from the contralateral one as TBI$_{contra}$ (see cartoon in Fig. 1A). Sham age-matched mice received identical anesthesia and surgery without brain injury; naïve mice (at 12 months of age) were not subjected to any experimental procedure. WT and tau KO mice were anesthetized by isoflurane inhalation (induction 3%; maintenance 1.5%) in an N$_2$O/O$_2$ (70%/30%) mixture and placed in a stereotaxic frame. Rectal temperature was maintained at 37 °C. Mice were then subjected to craniectomy followed by induction of controlled cortical impact brain injury as previously described (Zanier et al., 2016). Briefly, the injury was induced using a 3-mm rigid impactor driven by a pneumatic piston rigidly mounted at an angle of 20° from the vertical plane and applied to the exposed dura mater, between bregma and lambda, over the left parietotemporal cortex (antero-posteriority: −2.5 mm, laterality: −2.5 mm), at an impactor velocity of 5 m/s and deformation depth 1 mm, resulting in a severe level of injury (Smith et al., 1995). The craniotomy was then covered with a cranioplasty and the scalp sutured. Sham mice received identical anesthesia and surgery without brain injury. Mice were allocated to surgery by a list randomizer (http://www.random.org/list). All behavioral evaluations and magnetic resonance imaging (MRI) analysis were done blinded to injury status and genetic background.

2.3. Sensorimotor function

TBI-induced sensorimotor deficits were assessed on WT and tau KO mice by the Neuroscore and Simple Neuroassessment of Asymmetric Impairment (SNAP) tests 4 months post-TBI.

2.3.1. Neuroscore

Mice are scored from 4 (normal) to 0 (severely impaired) for each of the following indices: 1) forelimb function while walking on the grid and flexion function response when suspended by the tail; 2) hindlimb function while walking on the grid and extension function when suspended by the tail; and 3) resistance to lateral right and left pulsion. The maximum score per mouse is 12 (Zanier et al., 2016, 2014).
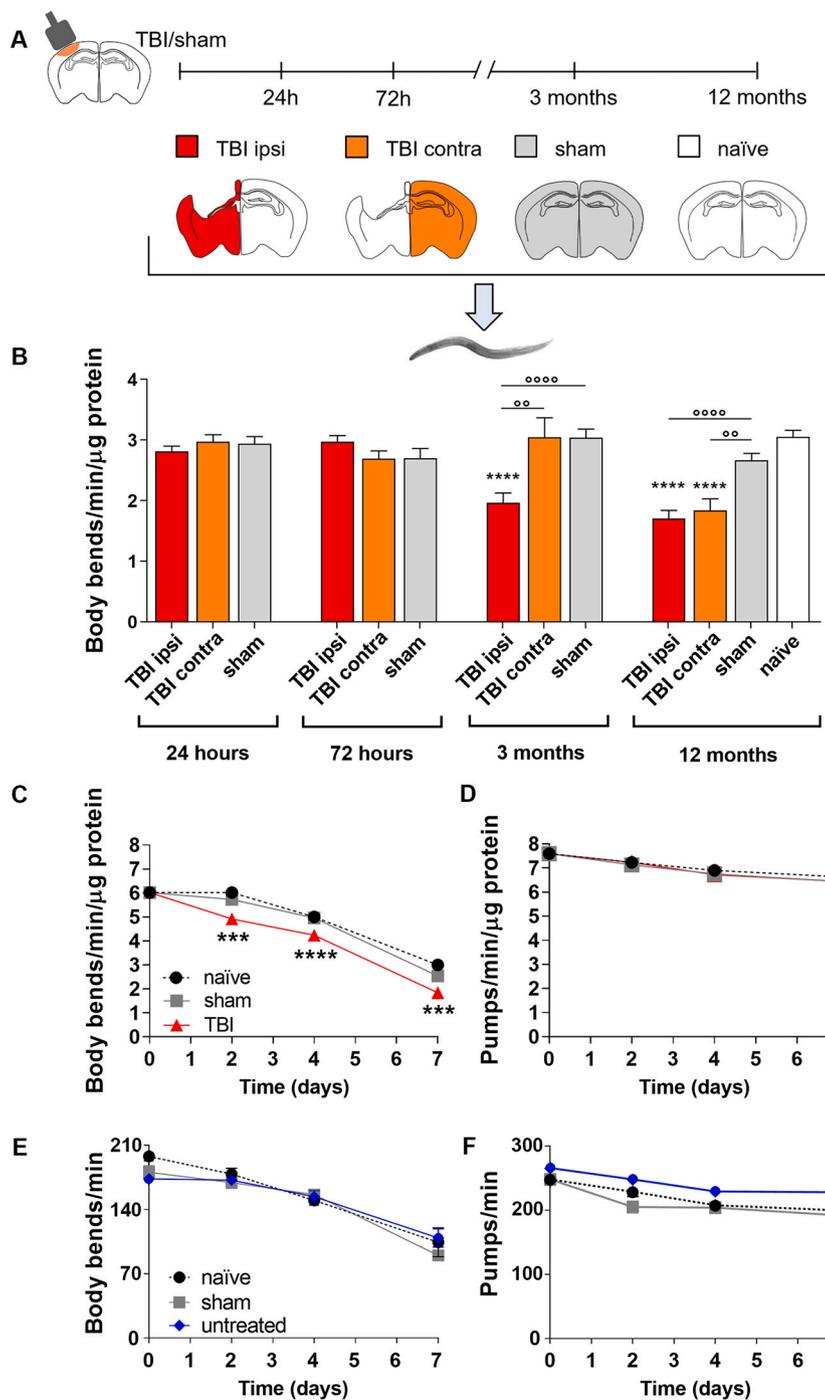
Fig. 1. Brain homogenates from chronic but not acute TBI mice are toxic to *C. elegans*. (A) Pericontusional tissue from the ipsi (TBI_{ipsi}) or contralateral (TBI_{contra}) brain areas from TBI mice 24 h, 72 h, 3 months and 12 months post-injury, were homogenized in 10 mM PBS, pH 7.4, and given to worms (30 µg protein/100 worms/100 µL). Brain homogenates from sham mice sacrificed at the same time-points of TBI animals and from naïve mice sacrificed 12 months post-injury, were used as controls. (B) Effects of the different brain homogenates on nematode locomotor activity 7 days after treatment. Data are mean ± SEM of three independent experiments using brain homogenates from three different mice/group (50 worms/group). °°$p < 0.01$ and °°°°$p < 0.0001$, **** $p < 0.0001$ *vs* naïve, one-way ANOVA and Bonferroni's *post hoc* test. (C—F) Tissue homogenates from the ipsilateral brain area from TBI mice 12 months post-injury (TBI), sham or naïve mice were given to worms (30 µg protein/100 worms/100 µL). As additional control, worms were treated with 10 mM PBS, pH 7.4 (100 worms/100 µL) (untreated). The effect on (C, E) locomotor and (D, F) pharyngeal activity was determined before (day 0) and different days after the treatment. Data are the mean ± SEM of three independent experiments with homogenates from two different mice/group (60 worms/group). ***$p < 0.001$ and ****$p < 0.0001$ *vs* sham at the corresponding time-point, one-way ANOVA and Bonferroni's *post hoc* test.

2.3.2. SNAP

The test evaluates eight neurological parameters including vision, proprioception, motor strength and posture. Score ranges from 0 (normal) to 5 (severely impaired) for each test. The scores from each of the eight tests are summed to give the total SNAP score. A neurologically intact animal would be expected to have a SNAP score of 0. The higher the score the more the asymmetric deficits (Shelton et al., 2008).

2.4. Magnetic resonance imaging

Imaging studies were done 4 months after TBI. Images were acquired on a 7 T Bruker Biospec (Ettlingen, Germany) running ParaVision 6.01, equipped with a quadrature cryogenic surface coil as transmitter and receiver. Mice were anesthetized (induction 3–4%, maintenance 1.5–2% in an air/O_2–70%/30% mixture) and body temperature was maintained at 37 °C. Acquisitions: coronal, 2D, T2-weighted RARE sequences with FOV 1.5 × 1.5 cm, matrix 150 × 150, 37 slices 300 µm thick, repetition time TR 5500 ms, echo time E = 66 ms, RARE factor of 8 and number of averages $N = 12$. Contusion volume was computed using the ITK-SNAP software.

2.5. Brain homogenates

Mice were beheaded and the brains removed from the skull, rinsed in cold PBS 1× and rapidly sectioned into different areas including the cortex (all the cortical tissue above the rhinal fissure), hippocampus,

thalamus and cerebellum, for both the left and right hemispheres (ipsi- and contralateral to the injury, respectively). Brain areas were immediately frozen and stored at −80 °C. Areas were homogenized in 10% w/v 10 mM PBS, pH 7.4, and the protein concentration was quantified with Pierce Bicinchoninic Acid Protein Assay kit (Life Technologies, Italy).

2.6. Quantitative measurement of Tau

Tau concentration in brain homogenates was determined using tau *in vitro* ab210972 Tau SimpleStep ELISA® kit (Abcam). Three brains from 9 months old Non-Tg and P301L mice and 3 pericontusional tissue homogenates from the ipsilateral brain area of 12 months post-TBI mice and sham-injured mice were homogenized in 10 mM PBS, pH 7.4. Samples were diluted at 1 μg/μL of protein in 10 mM PBS, pH 7.4, and then processed according to the kit's manufactured instructions.

2.7. Recombinant tau

Recombinant tau WT (Tau$_{WT}$) and P301L (Tau$_{P301L}$) were expressed in *E. coli* and purified by exchange chromatography followed by size-exclusion chromatography. The purification protocol was adapted from Rossi et al. (Rossi et al., 2014). Aggregation was induced by incubating the proteins with heparin (Mr 6000–20,000, Selleckchem), in a tau:heparin ratio of 4:1 (w/w) in 10 mM PBS, pH 7.4. Atomic force microscopy (AFM) was then used to visualize the morphology of Tau$_{WT}$ and Tau$_{P301L}$ immediately before (T0) and 24 h after (T24) incubation at 37 °C under shaking. Tau solutions were diluted at 1.5 μM with 50 mM phosphate buffer (PB), pH 7.4, and 30 μl of each sample was spotted onto freshly cleaved Muscovite mica disks (Assing) and incubated for 5 min. The mica disks were then washed with 10 ml Milli-Q water and dried under a gentle nitrogen flow for 10 min. Measurements were performed using 0.01–0.025 Ohm/cm antimony-doped silicon probes (T: 3.5–4.5 μm, L: 115–135 μm, W: 30–40 μm, k: 20–80 N/m, f0: 323–380 kHz, Bruker AFM probes), on a Multimode AFM, with a Nanoscope V system, operating in tapping mode, with a scan rate in the 0.5–1.2 Hz range, proportional to the area scanned. For each sample five distinct regions were scanned and AFM images were analyzed with the Scanning Probe Image Processor (SPIP, version 5.1.6, release 13 April 2011, www.imagemet.com) data analysis. To minimize possible artefacts, freshly cleaved mica and freshly cleaved mica soaked with 50 mM PB were used as controls.

The samples at T0 and T24 were examined in a detergent insolubility assay adapted from Drisaldi et al. (Drisaldi et al., 2015) and the proteins in the supernatant and pellet were analyzed by western blot using the anti-tau rabbit polyclonal antibody DAKO (Agilent Technologies Italy SpA, Milan, Italy) (1:10000 dilution). Anti-rabbit IgG peroxidase conjugate (1:20000, Sigma) was used as secondary antibody.

2.8. C. elegans studies

Bristol N2 nematodes and RB809 *ptl-1* knock-out worms (*ptl-1* KO) were obtained from the *Caenorhabditis elegans* Genetic Center (CGC, University of Minnesota, Minneapolis, MN, USA) and propagated at 20 °C on solid Nematode Growth Medium (NGM) seeded with *E. coli* OP50 (CGC) for food. We used the bleaching technique to prepare age-synchronized animals (Porta-de-la-Riva et al., 2012). *C. elegans* at the first larval stage were then transferred to fresh NGM plates and grown at 20 °C. At L3-L4 larval stage nematodes were collected with M9 buffer, centrifuged, and washed twice with 10 mM PBS, pH 7.4, to eliminate bacteria. Worms were incubated for 2 h at room temperature with orbital shaking, in the absence of *E. coli*, with homogenates from ipsi or contra areas from WT or tau KO TBI mice, the equivalent area of either WT or tau KO sham mice, homogenates of hippocampus or thalamus from mice inoculated with 12 months post-TBI or sham-injured mice sacrified 12 months post-inoculation, or brain homogenates from Non-Tg and P301L mice (30–60 μg protein/100 worms/100 μL in 10 mM

PBS, pH 7.4). Tau$_{WT}$ or Tau$_{P301L}$ (30 μg protein/100 worms/100 μL) were administered to N2 worms immediately before (T0) and after 24 h of incubation with heparin (tau:heparin 4:1, w/w) at 37 °C under shaking (T24), in 10 mM PBS, pH 7.4. Worms incubated with brain homogenates of naïve mice (30 μg protein/100 worms/100 μL) or 10 mM PBS, pH 7.4 (100 worms/100 μL) were used as negative controls. Worms were then plated onto NGM plates seeded with OP50 *E. coli*, grown at 20 °C and transferred every day to new NGM plates seeded with *E. coli* to avoid overlapping generations.

The locomotor activity and the pharyngeal function of nematodes were scored before (time 0) and 2, 4, and 7 days after the treatment (Diomede et al., 2014; Morelli et al., 2018). To measure the locomotor activity, worms were picked up, transferred into a well of a 96-well ELISA plate containing 100 μL of ddH$_2$O and their motility was evaluated by counting the number of left–right movements in 1 min (body bends/min). The pharyngeal pumping rate was measured by counting the number of times the terminal bulb of the pharynx contracted in 1 min (pumps/min).

The brain homogenate from Non-Tg and P301L mice (30 μg protein/100 worms/100 μL in 10 mM PBS, pH 7.4), were administered to N2 worms (wild-type) or *ptl-1* KO nematodes (30 μg proteins/100 worms/100 μL) and the locomotor activity was evaluated after 7 days scoring the number of body bends/min.

In some experiments, N2 nematodes were fed for 2 h with the different brain homogenates (30 μg proteins/100 worms/100 μL) previously incubated or not for 30 min at room temperature with 5–50 ng/μL anti-mouse tau monoclonal antibody T46 or anti-human tau monoclonal antibody SP70 (Rockland, Limerick, PA, USA). Antibodies alone were employed as controls. Worms were also treated in the same conditions with brain homogenates incubated for 30 min at room temperature with 10 ng/μL of rabbit IgG (Sigma Aldrich) or 10 ng/μL of T46 antibody inactivated by incubation at 100 °C for 10 min (inactivated T46) or the corresponding volume of 10 mM PBS, pH 7.4. Worms were then plated onto NGM plates seeded with OP50 *E. coli,* grown at 20 °C and transferred every day for 6 days to new NGM plates seeded with *E. coli*. Locomotor activity was rated on the seventh day. All behavioral evaluations were done blinded.

Synaptic dysfunction was evaluated by testing the sensitivity of N2 worms to aldicarb and levamisole 4 days after treatment with brain homogenates. Nematodes (50 worms/plate) on NGM agar plates with OP50 *E. coli*, were treated with 0.5 mM aldicarb (Sigma Aldrich) or 1 mM levamisole (Sigma Aldrich). The worms were prodded on the nose after 15 min and 30 min intervals for the next 4 h to determine when paralysis was complete (Mahoney et al., 2006).

For lifespan experiments, L4 N2 worms were fed for 2 h with the different brain homogenates, described above. Nematodes were then plated onto NGM plates seeded with OP50 *E. coli* and kept at 20 °C. To avoid overlapping generations, live worms were transferred daily to fresh NGM plates seeded with OP50 *E. coli* without fluorodeoxyuridine until they were all dead. Nematodes were scored as dead when there was no touch-provoked movement. The first day of adulthood is day 1 in survival curves.

2.9. Proteinase-K digestion

Brain homogenates (30 μg proteins) from the ipsi pericontusional area of 12 months post-TBI and sham-injured mice, Non-Tg and P301L were incubated for 1 h at 37 °C with 10 μg/mL proteinase-K (PK) or the same volume of water. After incubation, 2 μg of digested proteins were suspended in 4× (SDS) loading buffer, boiled for 5 min and analyzed in 10% SDS-PAGE, followed by Comassie Blue R250 staining for 30 min. Brain homogenates incubated or not with PK were then given to N2 *C. elegans* (30 μg proteins/100 worms/100 μL) as described above. Control worms were treated with 100 μL of water ±10 μg/mL PK. Worms were then plated onto NGM plates seeded with OP50 *E. coli*, grown at 20 °C and transferred every day for 6 days to new NGM plates

seeded with *E. coli*. Locomotor activity was rated on the seventh day after treatment.

2.10. Tau immunodepletion

Brain homogenates (30 μg proteins) were diluted in 50 μL of 10 mM PBS, pH 7.4, containing 0.1% Triton X-100 and were incubated overnight at 4 °C in orbital shaking with 10 μg of anti-rabbit tau DAKO polyclonal antibody or polyclonal rabbit anti-human lambda Light Chains (Dako, A 0193). At the end of incubation, samples were added with 20 μL of Protein A and Protein G resins (1:1 vol/vol) (Genespin) and incubated for 2 h at 4 °C under orbital shaking. Samples were centrifuged at 0.2 x*g* for 5 min at 4 °C, the supernatants collected and given to *C. elegans* (30 μg proteins/100 worms/100 μL) as described above. Control worms were treated with 100 μL of 10 mM PBS, pH 7.4 ± 10 μg of anti-rabbit tau DAKO polyclonal antibody or polyclonal rabbit anti-human lambda Light Chains. Worms were then plated onto NGM plates seeded with OP50 *E. coli*, grown at 20 °C and transferred every day for 6 days to new NGM plates seeded with *E. coli*. Locomotor activity was rated on the seventh day after treatment. Western blot analysis was performed on brain homogenates before and after immunoprecipitation. Samples were heated at 95 °C for 10 min in a sample buffer containing 10% sodium dodecyl sulfate (SDS) and loading buffer, and analyzed by using the anti-mouse tau monoclonal antibody T46 (Thermo Scientific) (1:1000 dilution) or anti-actin mouse monoclonal antibody (Millipore) (1:2000 dilution). Anti-mouse IgG peroxidase conjugate (1:10000, Sigma) was used as secondary antibody.

2.11. Statistical analysis

The data were analyzed using GraphPad Prism 8.0 software (CA, USA) by Gaussian distribution and Student's *t*-test, one-way or two-way ANOVA and Bonferroni's or Tukey's *post hoc* test. The median survivals were determined using the same software. A *p* value <0.05 was considered significant.

3. Results

3.1. Brain-injured tissue from chronic TBI mice impairs C. elegans motility

We previously found that a single severe TBI induced persistent and evolving tau pathology in WT mice, which progressively spread from the site of injury to ipsi and contra brain regions. P-tau deposits were seen adjacent to the site of injury 3 months post-TBI, and were widespread in the ipsi and contra hemispheres at 12 months. Tau^{TBI} could be transmitted to naïve mice by intracerebral inoculation in a prion-like manner, causing synaptic toxicity and memory deficits (Zanier et al., 2018). To test the hypothesis that tau^{TBI} is toxic and able to induce neuronal impairment, providing support to the pathogenic role of this isoform in the evolution of TBI at chronic stages, we used *C. elegans*. This nematode is susceptible to the toxicity of various amyloidogenic proteins and, due to its small size, short reproductive life cycle and lifespan, is a more tractable experimental model than mice (Diomede et al., 2014; Giorgino et al., 2019; Stravalaci et al., 2012; Zeinolabediny et al., 2017). Motility and pharyngeal contraction are controlled in worms by numerous neurons innervating body wall muscle cells and the pharyngeal nervous system, respectively, and misfolded proteins can cause their dysfunction (Dosanjh et al., 2010; Sengupta and Samuel, 2009; Wang et al., 2009). We set out to test whether the TBI mouse brains contained a toxic activity able to impair motility or pharyngeal function, and if this was ascribable to tau^{TBI}. Synchronized nematodes were incubated at L4 larval stage with brain homogenates (0.3 μg total proteins per worm) from TBI or sham mice euthanized at different times; naïve mice were used as an additional control (Fig. 1A). Locomotor activity and pharyngeal function were scored after 7 days. There was a significant

reduction in the frequency of body bends in worms exposed to homogenates from chronic (3 and 12 months) but not acute (24 h or 72 h) TBI mice (Fig. 1B). Locomotor impairment was induced by TBI$_{ipsi}$ but not TBI$_{contra}$ brain tissues collected 3 months post-TBI, and by both TBI$_{ipsi}$ and TBI$_{contra}$ tissues at 12 months (Fig. 1B), correlating with the presence of p-tau pathology (Zanier et al., 2018). The decrease in body bends was first detectable 2 days after treatment and persisted at 4 and 7 days (Fig. 1C), exacerbating the physiological age-dependent decline in nematode motility (Fig. 1E). No effect on pharyngeal pumping was observed at any time (Fig. 1D), indicating a selective neuromuscular defect. There were no changes in body bend frequency or pharyngeal pumping in worms incubated with brain homogenates from naïve or sham mice, compared to untreated controls (Fig. 1E-F). The defect in motility caused by TBI brain homogenates was not accompanied by a nematodes' lifespan shortening (median survival: 12 and 13 days for worms treated with sham and TBI brain homogenates, respectively) (Supplementary Fig. 1A).

3.2. Tau^{TBI} mediates toxicity in worms

We used different approaches to investigate whether the abnormal tau that accumulates in TBI mice at chronic stages was responsible for impairing the nematode locomotor function. First, we asked whether a deficit in motility like that induced by TBI homogenates was also seen in worms exposed to brain homogenates from P301L mice, which show robust tau pathology (Lewis et al., 2000). Worms were incubated with brain homogenates from nine-month-old P301L mice or age-matched Non-Tg controls, and body bend frequency and pharyngeal activity were scored after 7 days. Like in worms treated with TBI tissue, the P301L homogenate significantly impaired body bend frequency but not pharyngeal activity (Fig. 2 A-C). The locomotor deficit worsened significantly on doubling the amount of P301L brain homogenate, consistent with a dose-dependent effect (Fig. 2D). P301L brain homogenates, similarly to TBI ones, did not affect the lifespan of the nematodes (median survival: 13 and 14 days for worms treated with Non-Tg and P301L brain homogenates, respectively) (Supplementary Fig. 1B).

The tau levels in P301L and TBI brain homogenates were determined by ELISA and related to the locomotor dysfunction induced in worms. P301L and TBI brain homogenates had tau concentrations significantly higher than their respective Non-Tg and sham controls (Supplementary Fig. 2A). These data suggested a direct relationship between the levels of tau in brain homogenates and their toxicity in worms (Supplementary Fig. 2B).

Next, we asked whether the toxicity of TBI and P301L brains was abolished by treating them with high concentrations of PK, a broad-spectrum serine protease (Fig. 3A-B). Homogenates from TBI$_{ipsi}$ (hereafter TBI) were used in all the subsequent experiments. Protein analysis by SDS-PAGE followed by Coomassie staining indicated complete protein digestion (Fig. 3C-D), and western blot analysis with an anti-tau antibody was consistent with tau being completely degraded (not shown). PK completely abolished the ability of brain homogenates from P301L and TBI mice to induce the locomotor defect (Fig. 3E-F and Supplementary videos).

We used several complementary approaches to directly assess the role of tau. First, to see whether anti-tau antibodies could block P301L and TBI homogenate toxicities, we treated the nematodes with P301L or TBI brain homogenates preincubated with or without the T46 antibody, which recognizes the C-terminal region of both human and mouse tau, or the SP70 antibody recognizing the N-terminal domain of human but not mouse tau (Fig. 4A-B). Incubation with native but not heat-inactivated T46 antibody prevented the motility defect caused by the P301L and TBI brain homogenates (Fig. 4C, E, G). The toxicity induced by the P301L, but not TBI brain homogenates, was abrogated by the human tau-specific SP70 antibody (Fig. 4D, F). The antibodies alone had no effect (Fig. 4H, I). No protective effect was observed when P301L and TBI brain homogenates were incubated with rabbit IgG used as
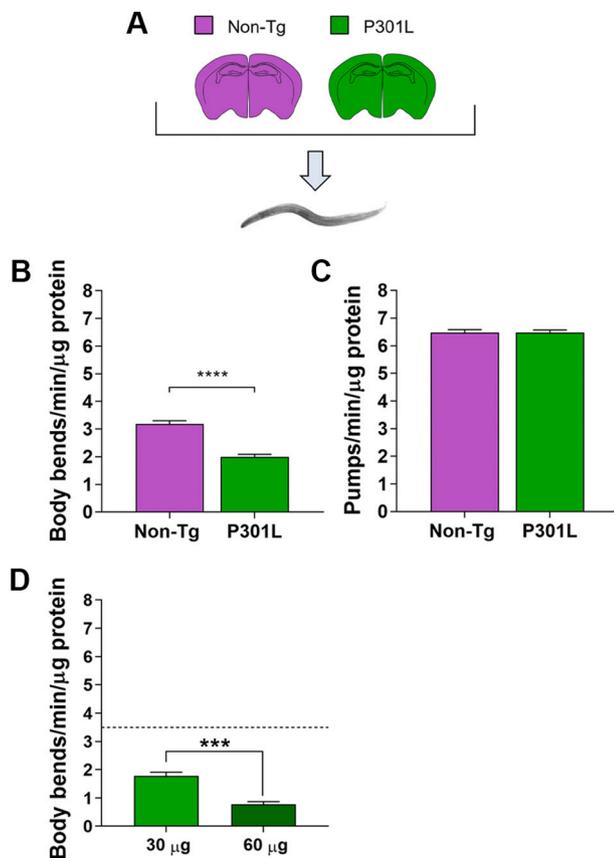
Fig. 2. Brain homogenates from transgenic P301L mice are toxic to *C. elegans*. (A) Brains from Non-Tg or P301L mice of 9 months of age were homogenized in 10 mM PBS, pH 7.4, and given to worms (30 μg protein/100 worms/100 μL). (B) Locomotor activity and (C) pharyngeal function of nematodes were rated 7 days after treatment. Data are the mean ± SEM of three independent experiments with homogenates from three different mice (70 worms/group). **** p < 0.0001 *vs* Non-Tg, Student's *t*-test. (D) Dose-dependent effect of P301L brain homogenate on *C. elegans* locomotion. Worms (100 worms/100 μL) were treated with 30 or 60 μg protein of brain homogenate from Non-Tg (dotted line) or P301L transgenic mice (green columns) and their locomotor activity was rated seven days later. Data are mean ± SEM (30 worms/group). ***p < 0.001 according to one-way ANOVA and Bonferroni's *post hoc* test.

additional negative control (Supplementary Fig. 3).

Nematodes were also treated with sham or TBI brain homogenates previously subjected to immunoprecipitation with an antibody recognizing murine tau or an antibody against human light chains used as a negative control (Fig. 5A-B). Tau immunodepletion abolished the motility defect caused by the TBI brain homogenates (Fig. 5C, D), whereas immunoprecipitation with the anti-light chain antibody had no effect (Fig. 5C, E). The antibodies alone had no effect (Fig. 5F). These results indicate a direct involvement of tau in toxicity.

C. elegans has a tau homolog protein with tau-like repeats (PTL-1) and a high level of sequence homology with the repeat region of mammalian microtubule-associated protein (MAP)2, MAP4, and tau (Goedert et al., 1996; Hashi et al., 2016). To test whether tau toxicity was mediated by PTL-1 we employed *ptl-1* KO worms. P301L brain homogenates caused a similar locomotor defect in wild-type and *ptl-1* KO nematodes (Supplementary Fig. 4) indicating that endogenous PTL-1 does not play any role in the toxic process induced by murine homogenates.

Next, we tested the toxicity of chronic TBI brain homogenates from tau KO mice. WT and tau KO mice were subjected to TBI or sham injury and euthanized after 4 months. There were no differences between WT and tau KO mice in TBI-induced sensorimotor deficits rated with the neuroscore and SNAP tests at 1 and 4 months (Fig. 6A, B) or in contusion volume assessed by T2w-MRI at 4 months (Fig. 6C, D). *C. elegans* treated with TBI homogenates from WT mice had significantly fewer body bends than those treated with WT sham homogenates, while homogenates from TBI tau KO mice had no effect (Fig. 6E, F).

Finally, we investigated whether exposing the nematodes to purified recombinant tau was sufficient to induce the deficit in body bends. To correlate tau toxicity with a specific conformational state, recombinant human 2N4R tau, either Tau_{WT} or Tau_{P301L}, was incubated with heparin and the molecular species present in the freshly dissolved solution (T0) or after 24 h (T24) of incubation at 37 °C were characterized by AFM and detergent insolubility assay. In both the freshly diluted Tau_{WT} and Tau_{P301L} solutions (T0), only monomers were observed whereas oligomers with a range of highly defined dimensions were observed at T24 (Fig. 7A, B and Supplementary Fig. 5), consistent with the time-dependent aggregation of monomeric recombinant tau into oligomers (Rossi et al., 2014). T0 and T24 tau solutions were administered to worms at the concentration of 2 μg/100 worms/100 μL to mimic the concentration of tau in P301L and TBI brain homogenates (1.46 μg and 0.84 μg, respectively), as determined by ELISA (Supplementary Fig. 2A). No change in motility was observed in worms receiving T0, Tau_{WT} or Tau_{P301L}, whereas the T24 solutions significantly reduced the frequency of body bends (Fig. 7C), without affecting pharyngeal activity (data not shown). The toxicity caused by oligomeric tau was similar to that induced by P301L or TBI brain homogenates. These findings indicate that the toxic effect of tau on the *C. elegans* neuromuscular apparatus is related to its conformational state, independently from its phosphorylation, since the recombinant protein is not phosphorylated and further support a role for misfolded tau in the locomotor deficit caused by TBI brain homogenates.

3.3. The locomotor defect is associated with impaired cholinergic neurotransmission

In *C. elegans* motility is coordinated by the neuromuscular functions driving the contraction of muscle cells due to the release of acetylcholine at neuromuscular junctions and the simultaneous relaxation caused by the release of γ-aminobutyric acid. Defects in synaptic transmission led to locomotion impairment resulting from disrupted signaling at the neuromuscular junctions. We hypothesized that the motility defect caused by P301L and TBI brain homogenates could be ascribed to neurotransmission failure at pre- and/or post-synapse. To test this, we evaluated the sensitivity to aldicarb- and levamisole-induced paralysis, which are the best and most commonly used assays to assess synaptic function in *C. elegans* (Mahoney et al., 2006).

First, we analyzed the time-course of aldicarb-induced paralysis, which is dependent on the efficacy of synaptic transmission between the motor neuron and the muscle cells and is an indicator of both the pre- and post-synaptic function. Being aldicarb an acetylcholinesterase inhibitor, the speed at which an animal becomes paralyzed depends on the nerve cell ability to secrete acetylcholine. Worms with impaired acetylcholine release accumulate less neurotransmitter in the presence of aldicarb, taking longer to become paralyzed than controls. Conversely, worms that have enhanced neurotransmitter release accumulate more acetylcholine in the presence of aldicarb and become paralyzed more rapidly (Mahoney et al., 2006). The latter was the case when worms were treated with P301L and TBI brain homogenates (Supplementary Fig. 6A, C). As shown in Fig. 8A and C, the percentage of paralyzed nematodes in the presence of aldicarb, scored 240 min after treatment, was significantly greater in P301L and TBI brain homogenate-treated worms than in those treated with Non-Tg and sham homogenates. To obtain information on the post-synapse activity, we evaluated the sensitivity of worms treated with brain homogenates to levamisole, a selective agonist of ligand-gated acetylcholine receptors located at the post-synaptic side of the neuromuscular junction. It is known that worms with pre-synaptic defect exhibit a similar rate of
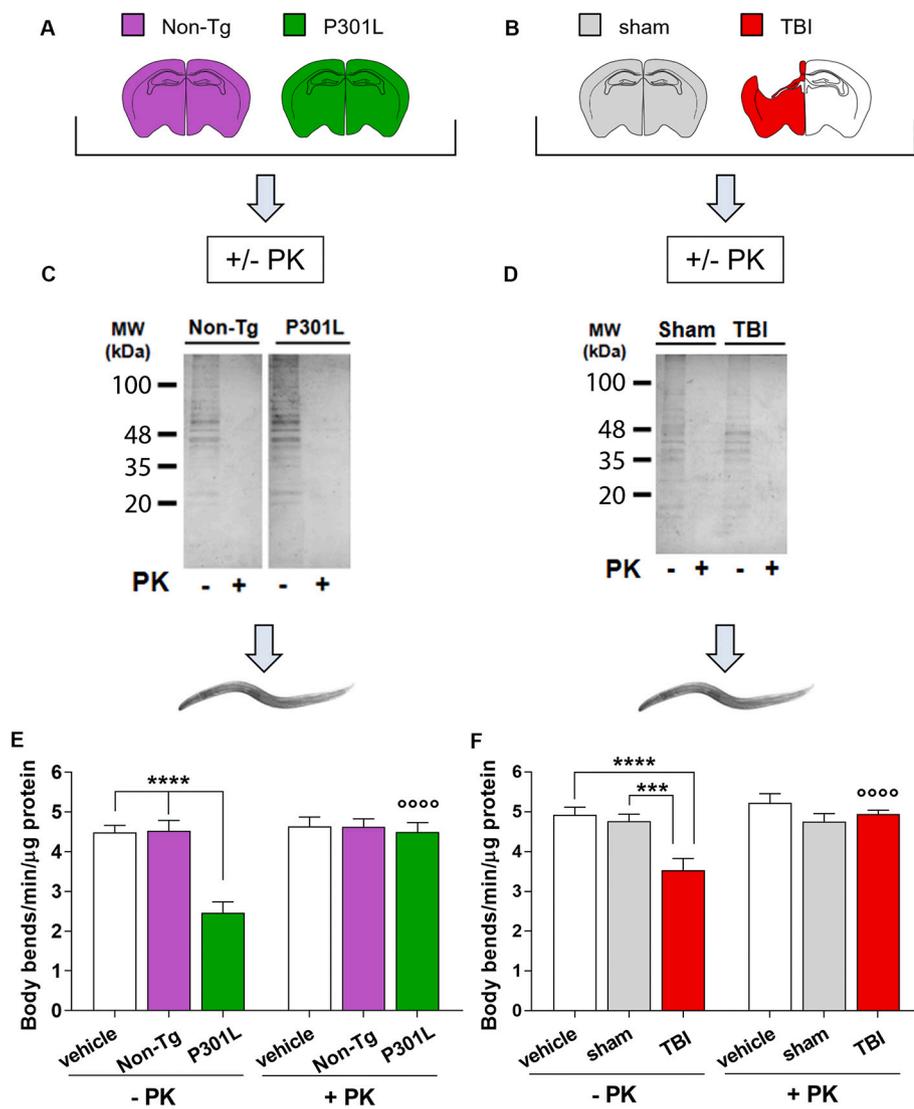
Fig. 3. P301L and TBI brain homogenate toxicities are abolished by pretreatment with proteinase-K. Tissue homogenates (30 μg proteins) from (A) Non-Tg, P301L, (B) 12 months TBI and sham mice, were incubated for 1 h at 37 °C with 10 μg/mL proteinase-K (+PK) or the same volume of water (-PK). (C, D) Protein digestion was evaluated by SDS-PAGE analysis and staining with Comassie Blue (2 μg proteins/lane). (E, F) The same samples were given to *C. elegans* (30 μg proteins/100 worms/100 μL) and the locomotor activity was rated 7 days later. Control worms were treated with 100 μL of water ±10 μg/mL PK (vehicle). Data are the mean ± SEM (50 worms/group). (E) ****$p < 0.0001$ and °°°°$p < 0.0001$ *vs* P301L-PK, by one-way ANOVA and Bonferroni's *post hoc* test. Interaction P301L/PK = 0.0017, two-way ANOVA and Bonferroni's *post hoc* test. (F) ***$p < 0.001$, ****$p < 0.0001$ and °°°° $p < 0.0001$ *vs* TBI-PK, one-way ANOVA and Bonferroni's *post hoc* test. Interaction TBI/PK ≤0.0001, by two-way ANOVA and Bonferroni's *post hoc* test.

paralysis in the presence of levamisole whereas in the presence of defects in post-synaptic function, nematodes will be either resistant or hypersensitive to levamisole (Mahoney et al., 2006). Nematodes treated with P301L and TBI brain homogenates were less sensitive than controls to the time-dependent paralysis induced by levamisole (Supplementary Fig. 6B, D). Thirty min after exposure to levamisole, the percentage of paralyzed worms was in fact significantly lower in P301L and TBI brain homogenate-treated nematodes than in their respective Non-Tg or sham-treated controls (Fig. 8B, D). These findings indicate that the motility impairment caused by brain homogenates administration is due to both pre- and post-synaptic defects at the neuromuscular junction.

### 3.4. Brain homogenates of tau^TBI^-inoculated mice are toxic to C. elegans

We previously found that the hippocampus and thalamus are two brain regions with prominent tau pathology in mice in which tau^TBI^ was transmitted by intracerebral inoculation (Zanier et al., 2018). We asked whether brain homogenates from these two brain regions were toxic to nematodes. Homogenates were administered to worms and their locomotor activity was rated 7 days later. There was a significant reduction in body bend frequency in worms treated with brain homogenates from mice inoculated with TBI but not sham tissue (Fig. 9). The motility defect caused by TBI-inoculated brain homogenates was comparable to that of worms treated with TBI homogenates (Fig. 1) indicating that the

toxic properties of tau^TBI^ remained unaltered upon transmission and self-propagation in naïve mice.

4. Discussion

This study shows that brain-injured tissue from chronic TBI mice impairs motility and synaptic transmission in *C. elegans*, and this is directly attributable to the abnormal form of tau that accumulates over time after TBI. These data indicate a pivotal role of abnormal tau conformers in the chronic consequences of TBI.

C. elegans is widely used to investigate the *cell-autonomous* and *non-cell-autonomous* mechanisms underlying the pathogenesis of human diseases (Nussbaum-Krammer and Morimoto, 2014). Its genes and pathways have a high degree of homology with those of vertebrates (Culetto and Sattelle, 2000; Siepel et al., 2005) and its small size, short reproductive life cycle and lifespan allow to give large volumes of data in a short time (Culetto and Sattelle, 2000). In the context of protein misfolding neurodegenerative diseases and systemic amyloidosis, *C. elegans* has been used as a biosensor of toxic amyloidogenic proteins, as shown by our previous studies employing synthetic Aβ, HIV-1 matrix protein p17 peptides, amyloidogenic immunoglobulin light chains purified from patients' biological fluids and recombinant gelsolin domains (Diomede et al., 2014; Giorgino et al., 2019; Stravalaci et al., 2012, 2016; Zeinolabediny et al., 2017). This simple system has helped
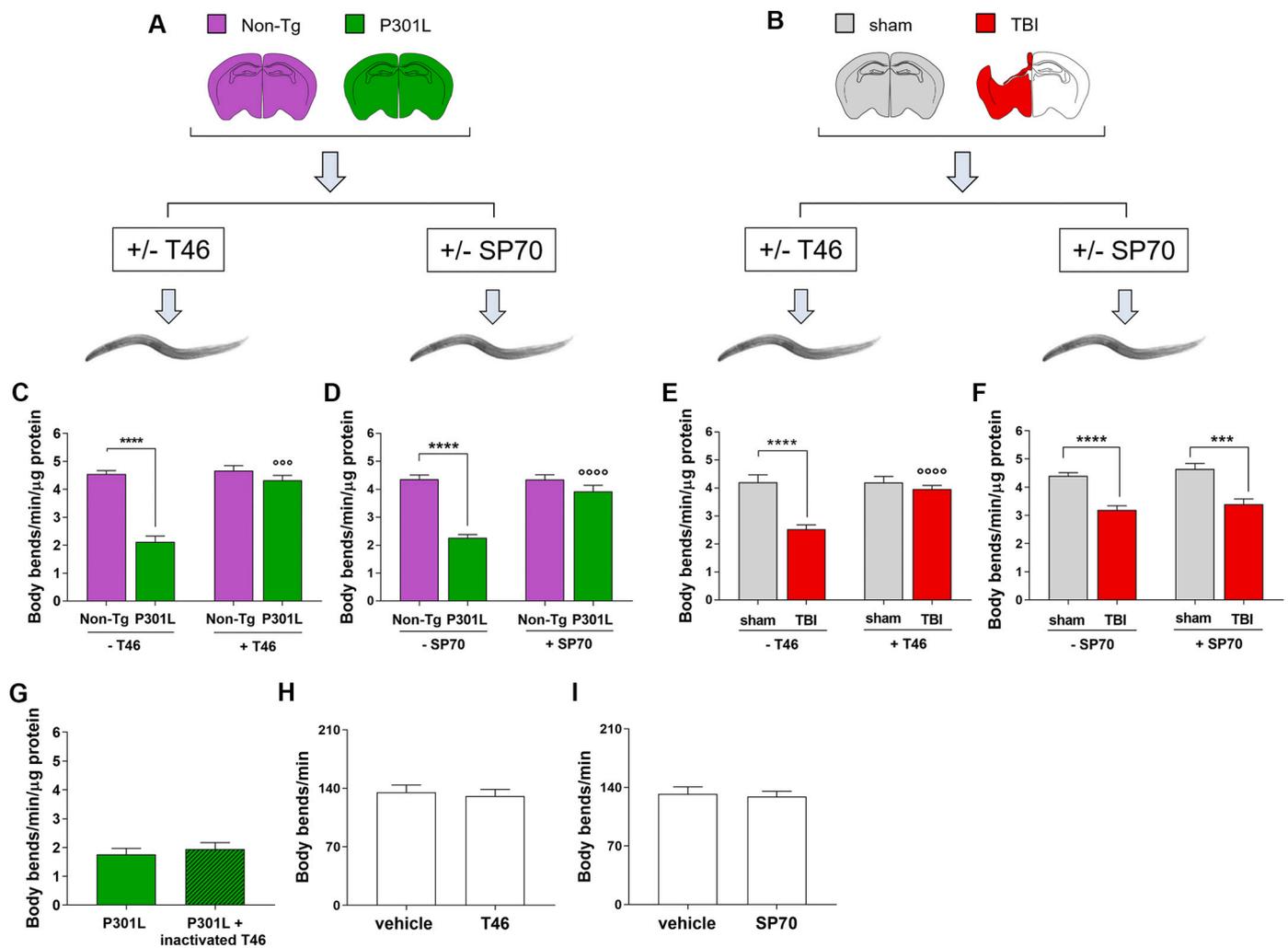
Fig. 4. Anti-tau antibodies abolish P301L and TBI brain homogenate toxicities. Tissue homogenates (30 μg proteins) from (A) Non-Tg, P301L, (B) 12 months TBI and sham mice, were incubated for 30 min at room temperature with 10 ng/μL monoclonal anti-tau T46 antibody (+T46) or the same volume of water (−T46) or 5 ng/μL monoclonal anti-tau SP70 antibody (+SP70) or the same volume of water (-SP70). (C—F) The homogenates were then given to worms (30 μg proteins/100 worms/100 μL) and locomotor activity was rated 7 days later. Data are the mean ± SEM (50 worms/group). (C and D) ****p < 0.0001, °°°p < 0.001 and °°°° p < 0.0001 *vs* P301L-T46 and P301L-SP70, respectively. Interaction P301L/T46 ≤0.0001 and interaction P301L/SP70 ≤0.0001 according to two-way ANOVA and Bonferroni's *post hoc* test. (E and F) **** p < 0.0001, ***p < 0.001 and °°°° p < 0.0001 *vs* TBI-T46. Interaction TBI/T46 = 0.0008, according to two-way ANOVA and Bonferroni's *post hoc* test. (G) The brain homogenate from P301L mice was administered to worms (30 μg proteins/100 worms/100 μL) before (P301L) or after 30 min pre-incubation at room temperature with the monoclonal anti-tau T46 antibody (10 ng/μL) previously inactivated by incubation at 100 °C for 10 min (P301L + inactivated T46). (H) The effect of antibody alone was also examined by treating worms (100 worms/100 μL) in the same experimental conditions with 10 ng/μL monoclonal anti-tau T46 antibody (T46). Control worms were treated with the same volume of water (vehicle). The locomotor activity of nematodes was rated 7 days after the treatment. Data are mean ± SEM. (I) Locomotor activity of nematodes, rated 7 days after the treatment for 30 min at room temperature with 5 ng/μL monoclonal anti-tau SP70 antibody (SP70) (100 worms/100 μL) or the same volume of water (vehicle). Data are mean ± SEM.

elucidate proteotoxic mechanisms and pave the way for novel therapeutic approaches (Diomede et al., 2014; Wechalekar and Whelan, 2017).

In the present study we exploited the biosensor properties of *C. elegans* to test the hypothesis that abnormal tau conformers play a causal role in driving toxicity in TBI. Chronic (3 and 12 months) but not acute (24 and 72 h) TBI tissue was toxic to nematodes, inducing a selective defect in locomotor activity. This indicates that toxicity is due to pathological changes occurring in the injured brain at chronic stages suggesting a negligible contribution of acute events, such as excitotoxicity, oxidative stress or acute inflammation. While at 3 months post-TBI only the ipsilateral brain homogenate was toxic, at 12 months both the ipsi and contra tissues induced the locomotor defect. This correlated with the progressive spread of tau pathology from the site of contusion to the ipsi- and contra-lateral brain hemispheres (Kondo et al., 2015; Zanier et al., 2018). This contention was further supported by the

observation that brain homogenates from P301L mice, which develop genetically determined p-tau pathology with age, had similar deleterious effects on nematode neuromuscular function. The absence of toxicity in worms fed brain homogenates from sham or Non-Tg mice indicated the specificity of the effect.
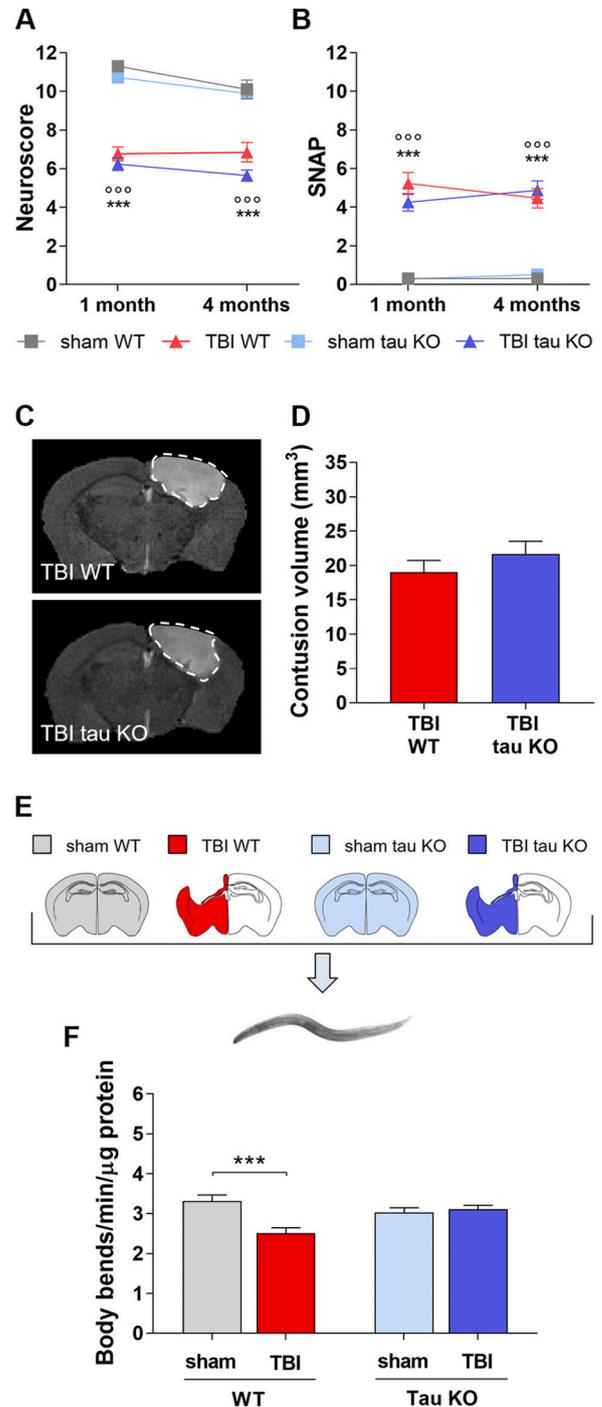
The observation that the TBI and P301L brain homogenate toxicities were abolished by anti-tau antibodies or tau immunodepletion, that brain homogenates from TBI tau KO mice were not toxic, and that recombinant tau was toxic to the worms, provided strong evidence of a direct role of tau. Whether other factors, misfolded proteins next to p-tau may also be involved in the observed toxicity, cannot be excluded. While in P301L mice proteinopathies other than tau have not been reported (Lewis et al., 2000), TBI favors misfolding of other proteins, accelerating Aβ and TDP-43 deposition (Smith et al., 2013; Tan et al., 2018). Aβ administered to *C. elegans* is associated with transient pharyngeal impairment, but no motility deficits (Stravalaci et al., 2016, 2012), and
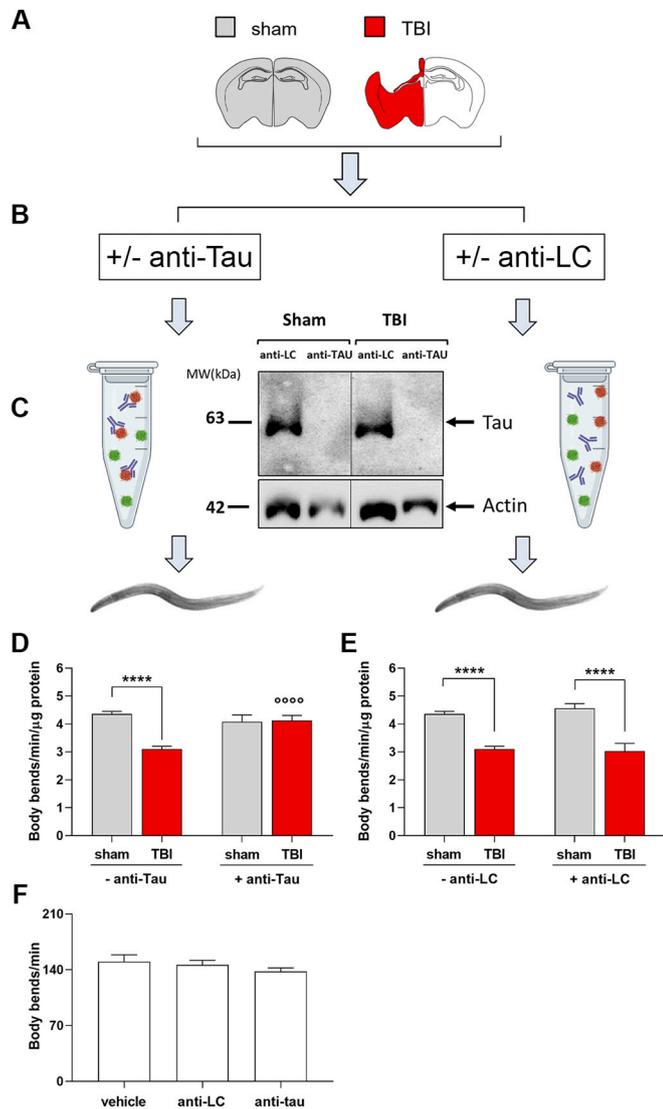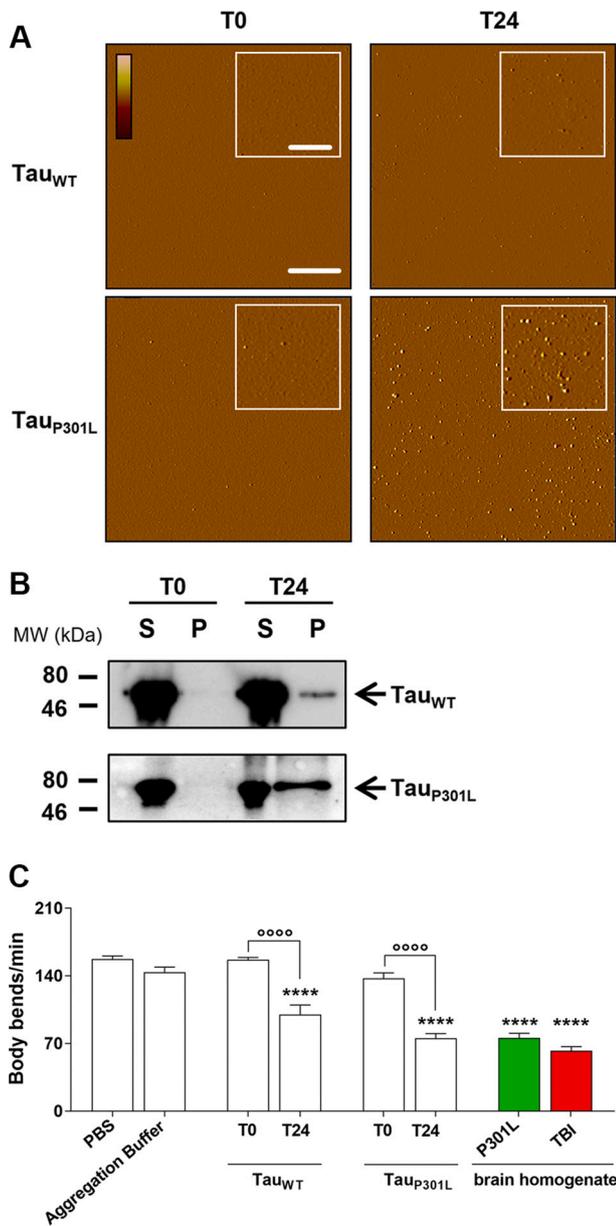
Fig. 5. Tau immunodepletion abolishes the toxicity of TBI brain homogenates. Tissue homogenates (30 μg proteins) from (A) 12 months TBI and sham mice, were (B) immunoprecipitated with 10 μg of anti-tau DAKO antibody (± anti-TAU) or anti-human lambda Light Chains antibody (±anti-LC), as described in the Methods section. (C) Tau in homogenates post-immunoprecipitation was evaluated by western blot analysis. Equal amount of proteins (3 μg) were loaded in each gel lane and immunoblotted with anti-tau antibody (T46) or anti-actin antibody. (D-E) The immunoprecipitated (+anti-Tau or + anti-LC) or untreated (−anti-TAU or -anti-LC) homogenates were given to worms (30 μg proteins/100 worms/100 μL) and locomotor activity was rated 7 days later. Data are the mean + SEM (50 worms/group). (D) ****p < 0.0001, °°°°p < 0.0001 *vs* TBI- anti-TAU and (E) ****p < 0.0001, one-way ANOVA and Bonferroni's *post hoc* test. (D) Interaction TBI/anti-TAU ≤0.0001 according to two-way ANOVA and Bonferroni's *post hoc* test. (F) Worms were treated with 100 μL of 10 mM PBS, pH 7.4 alone (vehicle) or 10 μg of anti-LC or anti-Tau antibody (100 worms/100 μL). Locomotor activity was rated 7 days later. Data are the mean± SEM (50 worms/group).

Fig. 6. Brain homogenates from TBI tau knock-out mice are not toxic to *C. elegans*. Sensorimotor function of WT and tau knock-out (tau KO) mice was assessed by (A) neuroscore and (B) SNAP tests at 1 and 4 months after severe TBI (TBI WT and TBI tau KO) or sham (sham WT and sham tau KO) injury. Data are mean ± SEM, two way-ANOVA followed by Tukey's *post hoc* test. ***p > 0.001 sham WT *vs* TBI WT, °°°p > 0.001 sham tau KO *vs* TBI tau KO. (C, D) T2w-MRI images were used to evaluate contusion volume in TBI mice 4 months post-TBI. Data are mean ± SEM, upaired t-test. (E) TBI homogenates of TBI WT or TBI tau KO, sham WT or sham tau KO mice sacrificed 4 months post injury, were given to worms (30 μg protein/100 worms/100 μL). (F) The locomotor activity of nematodes was scored 7 days later. Data are mean ±SEM of data from three independent experiments with homogenates from three different mice (30 worms/group). *** p < 0.001 *vs* sham WT, one-way ANOVA and Bonferroni's *post hoc* test.

in worms fed TBI tissue no pharyngeal impairment was observed. This confirms the primary role of tau in TBI-related toxicity and suggests that Aβ has a negligible effect if any.

Both tau^TBI and P301L tau have the ability to self-propagate as shown by transmission studies in mice (de Calignon et al., 2012; Liu et al., 2012; Zanier et al., 2018). Interestingly, tau toxicity in *C. elegans* did not depend on expression of the nematode tau homolog protein PTL-1, indicating that prion-like propagation of tau misfolding is not required in worms. This suggests a dissociation between the toxic and self-

Fig. 7. Oligomeric recombinant tau is toxic to *C. elegans.* (A) Atomic force microscopy (AFM) images of recombinant Tau$_{WT}$ and Tau$_{P301L}$ recorded immediately after addition of the co-aggregant heparin (T0) or after 24 h of incubation at 37 °C under shaking conditions (T24). The samples were diluted at 1.5 μM in 50 mM phosphate buffer. AFM images were obtained in taping mode and are shown as amplitude data (color scale: 0–150 mV). Scale bar = 1 μm, inset = 500 nm. (B) Detergent insolubility test done on Tau$_{WT}$ and Tau$_{P301L}$ at T0 or T24. The supernatant (S) and pellet (P) were analyzed on 10% SDS-PAGE followed by Western blotting using the anti-total tau DAKO antibody. (C) Aliquots of Tau$_{WT}$ and Tau$_{P301L}$ at T0 and T24 were diluted in 10 mM PBS, pH 7.4, and given to worms at the final concentration of 2 μg tau/100 worms/ 100 μL. *C. elegans* were treated in the same experimental conditions with 10 mM PBS, pH 7.4 (PBS) or co-aggregant heparin diluted in 10 mM PBS, pH 7.4 (Aggregation buffer) as negative controls. Locomotor activity was rated 7 days after treatment. The effects of brain homogenates from P301L and 12 month post-TBI mice (30 μg of total proteins/100 worms/100 μL) (Figs. 1B and 2B), are shown for comparison. Data are mean ± SEM (50 worms/group). ****p < 0.0001 *vs* PBS and °°°°p < 0.0001, one-way ANOVA and Bonferroni's *post hoc* test.
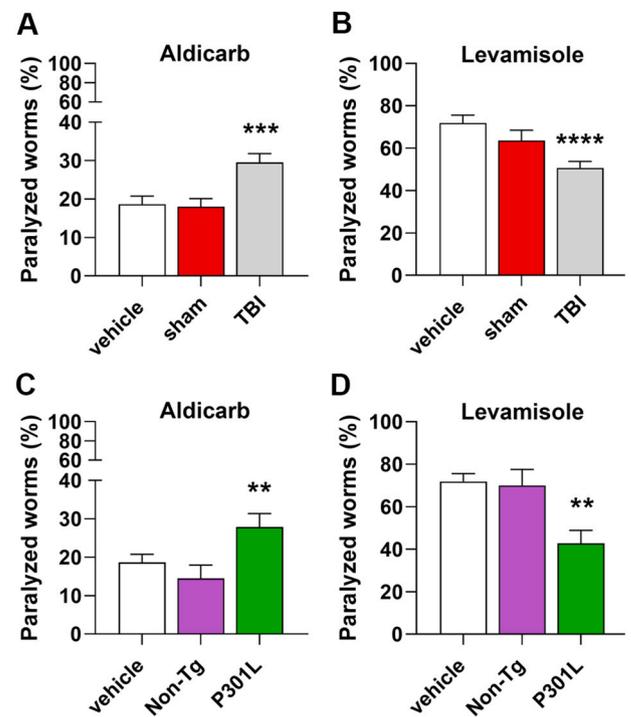
Fig. 8. TBI and P301L brain homogenates induce pre- and post-synaptic defects. Brain homogenates from (A, B) 12 months post-TBI (TBI) and sham-injured (sham) mice or (C, D) Non-Tg and P301L mice were given to worms (30 μg protein/100 worms/100 μL). Four days after treatment the sensitivity of worms to (A, C) 0.5 mM aldicarb and (B, D) 1 mM levamisole was evaluated. Control worms were treated with the same volume of 10 mM PBS, pH 7.4 (vehicle). The percentage of paralyzed worms was determined (A, C) 240 min after treatment with aldicarb and (B, D) 30 min after levamisole. Data are mean ± SEM (100 worms/group). **p < 0.001 *vs* Non-Tg, ***p < 0.001 and ****p < 0.0001 *vs* sham, according to one-way ANOVA and Bonferroni's *post hoc* test.

propagating species of tau, much like the dissociation between the toxic and infectious PrP species in the prototypical prion diseases (Benilova et al., 2020; Bouybayoune et al., 2015; Chiesa et al., 2003; Sandberg et al., 2011).

C. elegans neuromuscular integrity and synaptic function deteriorate with age, contributing to the motility decline (Mulcahy et al., 2013). The defect in motility caused by TBI and P301L brain homogenates, however, was not due to hastened aging, since the lifespan of the nematodes considered the ultimate parameter of the toxicity was not affected. The fact that the defect in motility was not accompanied by shortening of the nematodes' lifespan supports a sublethal toxicity caused by TBI and P301L brain homogenates.

The locomotor impairment was associated with defective acetylcholine-mediated synaptic transmission. Studies are now needed on the effects of tau on γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter involved in the worms' motility, to further support a synaptotoxic effect of tau.

Transgenic *C. elegans* expressing human WT tau 2N4R in neurons accumulate insoluble forms of p-tau and, like worms fed TBI and P301L brain homogenates, have no impairment in pharyngeal function but develop locomotor and pre- and post-synaptic defects (Morelli et al., 2018). This suggests that the proteotoxic mechanisms of the neuro-muscolar dysfunction induced by transgenically expressed and exogenously administered tau involve common molecular targets.

5. Conclusions

There is growing interest in the role of tau in post-TBI neuro-degeneration, and the idea that targeting pathological tau may point to a
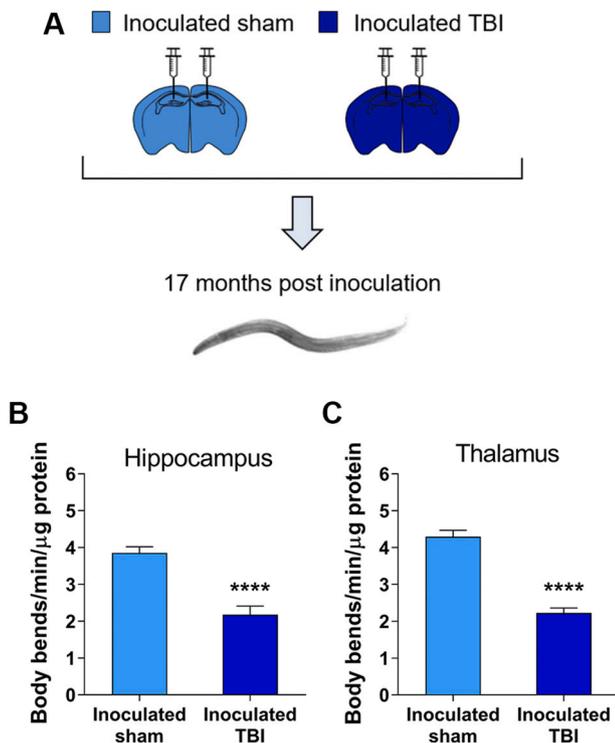
Fig. 9. Brain homogenates from mice inoculated with contused brain tissue are toxic to *C. elegans*. (A) Homogenates of hippocampus or thalamus from mice inoculated with 12 months post-TBI (Inoculated TBI) or sham-injured (Inoculated sham) mice and sacrified 12 months post-inoculation, were given to *C. elegans* (30 μg protein/100 worms/100 μL). (B, C) Locomotor activity was rated 7 days after treatment. Data are mean ± SEM from two independent experiments conducted with homogenates from three different mice (50 worms/group). **** p < 0.0001 *vs* inoculated sham, according to one-way ANOVA and Bonferroni's *post hoc* test.

therapeutic opportunity in TBI is growing (Katsumoto et al., 2019; Kondo et al., 2015; Lu et al., 2016; Rubenstein et al., 2019). The findings from this study indicate that brain-injured tissue from chronic TBI mice impairs motility in *C. elegans*, and this is directly attributable to the abnormal form of tau that accumulates over time after TBI. These data indicate a pivotal role of abnormal tau conformers in the toxic events occurring in the brain after TBI.

The *C. elegans* model of tau^{TBI} toxicity described here could be employed to rapidly screen TBI conditions involving the generation of potentially toxic tau species in both experimental and human TBI, to investigate the mechanisms underpinning chronic neurodegeneration in TBI, and to develop a high-throughput experimental platform to screen for pharmacological agents that interfere with TBI-associated tau toxicity.

Author contributions

Conceptualization; E.R.Z., R.C., and L.D; Data curation and Formal analysis: E.R.Z., R.C., and L.D; Funding acquisition; E.R.Z., R.C., L.C., L.F. and L.D; Investigation and Methodology: M.M.B., G.V., M.R., L. C., I.B., E.R., C.N., L.COLO., L.R., and E.M.; Project administratio: E. R.Z., R.C., and L.D; Resources: E.R.Z., R.C., L.D., L.C., L.F.; Supervision: E.R.Z., R.C., and L.D; Writing - original draft; E.R.Z., R.C., and L. D; Writing - review & editing: E.R.Z., R.C., and L.D.

Declaration of Competing Interest

Nothing to report.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.nbd.2021.105330.

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