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DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

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AUTHOR CONTRIBUTIONS

MCC: literature search, study design, data interpretation, writing, critical revision.

MC: experimental procedures, data collection, data analysis, data interpretation, writing, critical revision.

PB: literature search, study design, data interpretation, critical revision.

AT: experimental procedures, data collection, data interpretation, critical revision.

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ABSTRACT

Background. Early but not late tranexamic acid (TXA) after TBI preserves blood-brain-barrier integrity, but it is unclear if and how dose timing affects cognitive recovery beyond hours post-injury. We hypothesized that early (1h post-TBI) but not late (24h post-TBI) TXA administration improves cognitive recovery for 14 days.

Methods. CD1 male mice (n=25) were randomized to severe TBI (Injury, [I] by controlled cortical impact) or sham craniotomy (S) followed by IV saline at 1h (placebo, P1) or 30mg/kg TXA at 1h or 24h (TXA1, TXA24). Daily body weights, Garcia Neurological Test (GNT) scores, brain/lung water content and Morris water maze exercises quantifying swimming traffic in the platform quadrant (Z1) & platform area (Z5) were recorded for up to 14 days.

Results. Among injured groups, I+TXA1 demonstrated fastest weight gain for 14 days and only I+TXA1 showed rapid (day 1) normalization of GNT ($p=0.01$ vs. I+P1, I+TXA24). In cumulative spatial trials, compared to I+TXA1, I+TXA24 hindered learning (distance to Z5 and % time in Z1: $p<0.05$). Compared to I+TXA1, I+TXA24 showed poorer memory with less Z5 time (0.51 vs 0.16s, $p<0.01$) and Z5 crossing frequency. Unexpectedly, TXA in uninjured animals (S+TXA1) displayed faster weight gain, but inferior learning and memory.

Conclusion. Early TXA appears beneficial for cognitive and behavioral outcomes following TBI, though administration 24h post-injury consistently impairs cognitive recovery. TXA in sham animals may lead to adverse effects on cognition.

KEY WORDS

Tranexamic Acid, Traumatic Brain Injury, Water Maze, Learning, Memory

INTRODUCTION

Traumatic brain injury (TBI) is a major cause of death and disability worldwide, impacting more than 60 million people each year, with incidence rising in the last decade.(1-3) When TBI occurs, pathophysiologic effects are typically divided into a brief, initial and a secondary, elongated phase – the first, occurring within minutes of the head impact and the latter manifesting days to weeks after impact.(4, 5) TBI survivors will often experience debilitating cognitive dysfunction including deficits in memory, attention, processing speed, and executive functions.(6-8) It is in the first secondary phase hours after TBI that interventions can be applied to mitigate progression of cerebral inflammation and swelling and potentially improve outcomes.(9, 10) Presently, few proposed therapies have been successful in improving outcomes and thus none has been broadly implemented in the care of TBI patients.(7) A promising post-TBI therapy that could offer such potential is tranexamic acid (TXA).

TXA is an antifibrinolytic agent commonly used to reduce blood loss in patients experiencing critical hemorrhage post-injury.(11, 12) A synthetic lysine analog, TXA inhibits fibrinolysis by blocking lysine binding sites on plasminogen and arresting the conversion of plasminogen into plasmin.(12) Independently, TXA may also modulate plasmin's activation of complement, leukocytes and other host immune cells, thereby inhibiting the post-injury immune response and decreasing neurovascular inflammation and BBB permeability.(13, 14) Post-TBI TXA administration decreases mortality and may enhance neurological recovery, especially when administered early after injury.(13, 15, 16) Beneficial TXA effects may thus be time dependent, leading us to investigate whether the timing of post-TBI TXA administration differentially influences cognitive recovery. We hypothesized that early, but not late, TXA

administration after TBI would result in improved murine learning and memory as well as neuroclinical recovery for two weeks after injury.

MATERIALS AND METHODS

Experimental Design and Study Groups

Experimental procedures were approved by the <Blank> Institutional Animal Care and Use Committee (IACUC). SDC Content: The ARRIVE Checklist was followed, completed and submitted as SDC Content, http://links.lww.com/TA/D292. CD1 adult male mice (27-32g) (Charles River Laboratories, Wilmington, MA) were acclimatized in standard housing facilities using dark and light cycles with water and chow *ad libitum*. On day 0, all mice underwent either sham craniotomy (S) or TBI by controlled cortical impact (CCI, Injury [I]) (Figure 1).

At 1 or 24 hours after CCI, injured animals received a single intravenous (lateral tail vein) injection of either normal saline (NS, Placebo [P]) (Baxter Healthcare Corporation, Deerfield, IL) or TXA (30 mg/kg) (AuroMedics Pharma LLC, E. Windsor, NJ). Uninjured, sham animals received either NS or TXA at only 1h post-sham craniotomy. TXA dosing was derived from the standard bodyweight dosage for a 70kg human receiving a 2g (28 mg/kg) bolus.(17)

Twenty-five mice were randomly assigned to one of five groups (n=5 for each): (1) sham craniotomy and saline 1h post-craniotomy (**S+P1**); (2) sham craniotomy and TXA 1h post-

craniotomy (**S+TXA1**); (3) CCI and placebo 1h post-CCI (**I+P1**); (4) CCI and TXA 1h post-CCI (**I+TXA1**); (5) CCI and TXA 24h post-CCI (**I+TXA24**).

Convenience sampling was used to establish animal group numbers, supported by previous reports from our laboratory using identical Morris water maze investigations and data analyses. *Animal Research: Reporting of In Vivo Experiments guidelines were fulfilled in their entirety* (Supplemental Digital Content, http://links.lww.com/TA/D292).

Severe TBI Murine Model

Controlled cortical Impact (CCI) is a validated murine injury model that replicates severe TBI.(18) On Day 0, mice were anesthetized intraperitoneally with ketamine (Mylan Institutional, Rockford, IL), xylazine (Akorn Inc., Lake Forest, IL), and acepromazine (Boehringer Ingelheim, Duluth, GA) (KXA: 100, 10, 1 mg/kg, respectively). This was followed by subcutaneous injection of bupivacaine (Fresenius Kabi, Lake Zurich, IL; 0.5 mg/mL) and buprenorphine-SR (ZooPharm, Laramie, WY; 1 mg/kg) for extended analgesia. Mice were then placed prone in a stereotactic device, and a left-sided, 4mm circular craniotomy outline was marked between the bregma and lambda sutures with a 4mm trephine. A craniotomy was then created on this periosteal outline using a dental drill (Henry Schein, Melville, NY). All animals underwent craniotomy without durotomy. In TBI animals, after skull flap removal, a controlled cortical impactor (AMS201, AmScien Instruments, Richmond, VA) was used to injure the exposed left parietotemporal cortex, using standardized parameters known to replicate severe TBI (3mm-diameter impactor tip, 6 m/s impact velocity, 1mm depth cortical deformation). The scalp was sutured closed in all animals once hemostasis was achieved.

Body Weight Loss and Neurological Recovery

Animal body weights were recorded before craniotomy (W0) and daily for the subsequent 14 days, with the weight loss versus W0h expressed as a percentage: ([W0 − Wx]/W0 × 100%, where x = day after CCI/sham).

Animal neurological function was scored using the validated Garcia Neurological Test (GNT), which assesses rodent motor, sensory, reflex and balancing ability (maximum score: 18).(19) All animals underwent daily GNT scoring for 14 days after CCI or sham craniotomy. Optimal recovery was indicated by a near-maximal or maximal score (17-18). All animals had a pre-craniotomy GNT score of 18.

Brain and Lung Water Content

After the day 14 water maze trials were completed, animals were sacrificed and their brain and lungs removed. The brain was separated into injured (ipsilateral) and uninjured (contralateral) hemispheres and wet weight (WW) was determined immediately after organ procurement. Dry weight (DW) was obtained 72 hours after dehydration at 70 °C. Percent tissue water content was calculated using a wet-to-dry ratio (% water content = 100 × [(WW-DW)/WW]).

Morris Water Maze

On day 6 post-CCI/sham craniotomy, mice were introduced to daily exercise trials in a Morris Water Maze (MWM) (Figure 1) by one of the authors (Mi Co) who had no knowledge of treatment or group allocation). The MWM is a circular tub with a diameter of 100 cm, filled with

22°C water (Supplemental Digital Content, Figure A, http://links.lww.com/TA/D291) . The maze is sectioned into four equal quadrants, with a 10 cm diameter platform centrally located in the north quadrant (Zone 1, Z1). Zone 5 represents the area of the pool where the platform is placed, regardless of whether the platform is actually present. MWM exercises involved cued, spatial, and probe trials, which were conducted and scored by an operator blinded to the animals' experimental groups.

Cued Learning Trials

On days 6 and 7, mice underwent four cued trials per day (totaling 8 trials) to familiarize them with the maze and establish platform arrival as the goal. In these trials, the platform was randomly placed in a quadrant above the water level and indicated by a red flag atop the platform center (Supplemental Digital Content, Figure A, http://links.lww.com/TA/D291). The platform was set 1cm above water level, thereby making it visible to the swimming mouse; no other visual cues were utilized. Mice were randomly placed facing the walls of the maze, in different starting locations (north (N), east (E), south (S), west (W)) and given 60 seconds to reach the platform. Mice that did not reach the platform were guided to it by the operator and allowed to stay on the platform for 15 seconds before being removed from the maze and dried. Mice were then placed under a heat lamp, and given ten minutes to rest and warm between trials.

Spatial Learning Trials

On days 8-13 (totaling 20 trials), mice underwent spatial learning trials where the platform was placed solely in the north quadrant, 1cm below the waterline and without a platform flag. Colorful and distinctive spatial cues were placed on the edge of the pool wall at

each cardinal point (N, S, E, W), assisting the animals in localizing the hidden platform (Supplemental Digital Content, Figure A, http://links.lww.com/TA/D291). The purpose of spatial learning trials was to gauge the animals' ability to use visual cues to navigate to the goal, the platform. Mice underwent four trials per day, with each trial starting with the mouse facing outward at various points relative to the platform to prevent them from readily identifying the platform before entering the maze. Mice were given 60 seconds to reach the platform in each trial. If they failed to reach the platform in this timeframe, they were guided onto it and allowed 15 seconds rest before being removed from the maze. After each trial, the mice were dried, and then placed under a heat lamp for 10 minutes to warm between trials.

Probe Memory Trials

The final exercises were probe memory trials conducted on days 9-14, during which the platform was removed but the pool wall cues remained as they were for spatial trials. The aim was to assess animals' long-term memory of platform location using visual cues. Animals were released from various positions along the perimeter of the pool but only given 30 seconds of swim time, after which they were removed from the water. A daily probe trial was conducted after spatial trials on days 9-13 to assess memory from the previous day's learning trials. On day 14, four probe trials were performed without any preceding spatial trial, totaling 9 probe trials. After each trial, mice were dried, and then placed under a heat lamp for 10 minutes to warm and rest between trials.

All MWM exercises were recorded by a camera mounted above the maze. Video recording and analyses were conducted with commercial video tracking software (Ethovision,

Noldus, Leesburg, VA). Computerized analyses yielded exercise parameters aimed to quantify animal learning and memory of the platform's location, presence and absence. Analyzed parameters in each set of trials included: delay (latency) to arrive to platform/Z1/Z5 (in seconds, s), distanced swum to reach platform/Z1/Z5 (in cm), duration in zone 5 (in s, probe trials only), percent duration spent in Z1/Z5 (spatial and cued trials only) and frequency of mouse crossings into Z5 (probe trials only). Swimming velocity was measured in cm/s. For simplicity, all results for a given parameter were reported and compared as a mean summation across all days tested unless otherwise stated.

Statistical Analysis

All data analyses are presented as mean ± SEM and graphs were created in Prism (GraphPad Software, San Diego, CA 2022). A sample size of five animals per group was used as multiple previous studies (29) using the same MWM exercises demonstrated that 4-6 animals per group were sufficient in this model to elicit significant differences in both swimming distance to zone 5 and probe trial zone 1 latency. For all outcomes measured, 5 post hoc pairwise comparisons were conducted using analysis of variance with Bonferroni's correction to determine significance between group means (corrected $\alpha = 0.008$). p values less than 0.05 were considered statistically significant.

RESULTS

Body Weight Loss and Neurological Recovery

In animals subjected to TBI, extent of animal weight loss (initially), extent of subsequent body weight recovery and rapidity of this recovery over days is a surrogate of neurological (and

systemic) recovery from injury. The particularly high rate of body weight loss recovery after day 5-7 in I+TXA1 animals was distinct to that of all other injured animal groups (Figure 2). Weight loss in the initial 24h following CCI/sham craniotomy was ubiquitous with the greatest loss in I+TXA24 (-10.3 ± 1.1%) and I+P1 (-7.2 ± 2.2%, p>0.05). Initially, all groups demonstrated similar rates of weight gain but by Day 7, S+TXA1 and I+TXA1 demonstrated accelerated weight gain (Figure 2A, B). On days 12 and 13, S+TXA1 (18.5 ± 3.8%, 18.6 ± 3.1%) had increased weight gain compared to both S+P1 (5.4 ± 2.3%, p=0.04; 5.4 ± 1.7%, p=0.02) and I+TXA24 (5.7 ± 2.6%, p=0.049; 6.6 ± 2.8%, p=0.04) but not I+TXA1 (14.8 ± 2.7%, p=0.99; 14.5 ± 1.2%, p=0.99). Two weeks post-sham craniotomy/CCI, the only difference was between S+TXA1 (19.1 ± 3.5%) and S+P1 (6.1 ± 2.4%, p=0.049).

On Day 1 of GNT testing, TXA administered at 1 hour (I+TXA1: 17.6 ± 0.3) significantly improved GNT scores over those of the I+P1 animals (16.4 ± 0.4, p=0.01) , but when TXA was administered at 24 hours (I+TXA24: 15.8 ± 0.2, p=0.8 vs I+P1) GNT remained at I+P1 levels (Figure 3). I+TXA1 GNT was also significantly better than that of I+TXA24 (p<0.001). Mean GNT differences were no longer significant after Day 1 post-CCI/sham craniotomy, and all animals consistently reached maximal scores on days 7-14.

Brain and Lung Water Content

When comparing water content in all organs no differences were noted across injured groups regardless of treatment timing (Figure 4A-C). However, uninjured animals treated with TXA (S+TXA1) demonstrated significant less tissue water in sham craniotomy cerebral hemispheres (ipsilateral, 73.5 ± 0.6%, p<0.02 vs all other groups), uninjured cerebral

hemispheres (contralateral, 73.2 ± 0.6%, p<0.03 vs all other groups) and in lungs (69.3 ± 1.8%, p<0.01 vs S+P1, I+P1, I+TXA1, but p=0.06 vs I+TXA24).

Morris Water Maze

In cued learning trials, there were no observable differences in the aforementioned parameters across any group. Unexpectedly, I+TXA1 animals displayed the slowest cued trial swimming velocity (18.9 ± 0.8cm/s), significantly slower than I+P1 (27.8 ± 2.6cm/s, p<0.01), S+TXA1 (29.4 ± 1.8cm/s, p<0.01), and S+P1 (27.6 ± 1.7cm/s, p<0.01).

During spatial learning trials, no differences were found amongst groups in platform latency or platform duration, but the I+TXA24 group consistently underperformed the I+TXA1 group in mean total distance traveled to platform (32.4 ± 0.5 vs 27.0 ± 0.9cm, p=<0.001), zone 1% duration (0.21 ± 0.01% vs 0.31 ± 0.02%, p<0.0001), and zone 1 latency (8.9 ± 0.8 vs 6.1 ± 0.7s, p=0.01) (Figure 5 A-C). I+TXA1 animals also travelled a lower mean distance to reach the platform than I+P (27.0 ± 0.9 vs. 30.5 ± 0.7 cm, p<0.01) (Figure 5A). I+TXA24 mice tended to perform worse than I+P1, although not significantly (p=0.6). The S+TXA1 group performed consistently worse than S+P1 in all spatial parameters above and tended to perform worse than injured groups (Figure 5). The only significant disparity in swimming velocity across all spatial trials was noted in in the S+TXA1 group where mice swam significantly faster than in all other groups on day 13 (p<0.01 vs S+P1, p<0.01 vs I+P1, p<0.0001 vs I+TXA1, p<0.001 vs I+TXA24).

In the memory probe trials (platform removed) conducted on days 9-14 post injury, I+TXA24 (0.16 ± 0.03s) spent the least time in the platform zone (Z5) compared to I+TXA1 (0.51 ± 0.08s, p<0.01) which performed similarly to I+P1 (0.37 ± 0.05s, p=1.0) (Figure 6A). I+TXA24 mice (0.58 ± 0.12 crossings) crossed into the Z5 with the lowest frequency among all groups (p=0.02 vs I+TXA1, p=0.09 vs I+P1, p<0.0001 vs S+P1, p=0.03 vs S+TXA1) (Figure 6B). No differences were noted across any group in probe latency or distance to Z1 or Z5.

DISCUSSION

In the current study, we explored cognitive and functional neurological recovery using a validated *in vivo,* murine, severe TBI model. Our findings suggest that mice receiving TXA 24 hours post-injury performed worse in terms of learning (spatial trials) and memory (probe trials) compared to those treated with TXA 1 hour post-injury, which had similar outcomes to sham animals. Early TXA treatment also facilitated quicker neurological recovery, with improvements in functional neurological scores and body weight recovery. Finally, unexpectedly, in uninjured animals, early TXA resulted in decreased tissue edema, greater weight gain and earlier improvement of GNT scores, but consistently demonstrated hindered learning and memory MWM parameters.

Following TBI, external mechanical forces deform cerebral tissue and initiate a cascade of pathophysiologic events resulting in BBB dysfunction and a rapidly escalating local and systemic proinflammatory host immune response.(20-22) In particular, activated leukocyte (LEU) and endothelial cells (EC) interact closely in the penumbral neurovasculature, promoting release of cytokines and chemokines that further recruit other inflammatory cells advancing the

release of reactive oxygen species and cytotoxic proteases. This, in turn, results in further damage to cerebral tissue, fostering neuronal cell death, axon degeneration, oxidative stress and cerebral edema, which may ultimately lead to brain herniation if left untreated.(20, 23-25)

This self-promoting cascade of tissue level injury in TBI patients invariably manifests in downstream cognitive sequelae that often lead to acute, subacute and chronic impairment and disability. Indeed, TBI is the most important injury responsible for cognitive impairment.(26) Executive cognitive function and memory are the most vulnerable of cognitive abilities affected by TBI, with memory impairment in particular being the main source of long-term disability.(8, 26) Such impairments have a profound and pervasive negative effect on activities of daily living as memory and cognitive executive functions control learning, attention, planning, decision-making, and social behavior, all of which are required to complete routine tasks.(8) Both focal and diffuse severe TBI can influence these cognitive functions, through alterations of cerebral circuitry in the prefrontal cortex and temporal lobe. TBI also directly influences hippocampal and thalamic function causing histopathological changes, observed first during the secondary response to injury.(26, 27) In animal CCI models, the MWM has long been used to test and observe such cognitive deficits, providing understanding of functional recovery after TBI, but also aiding in the exploration of therapeutic options for brain injury.(28, 29) Similarly, but using a weight-drop TBI model, Schwarzbold *et al.* showed that mild TBI caused anxiety and depressive behaviors, while severe TBI resulted in significant memory deficits.(30) The same group additionally confirmed histologically that severe TBI resulted in extensive tissue damage to the frontal and parietal cortex, with architectural and neuronal losses in both the cortex and hippocampus.(30) Given the pervasive and significant debilitating burden of severe TBI,

identifying effective therapeutics to mitigate cognitive deficits and help improve the long term quality of life of TBI patients is imperative.

TXA is one such potential therapeutic that has recently emerged as possessing attributes able to modulate the host immune post-TBI response through inhibition of the plasminogen activation pathway. Plasminogen is transformed into plasmin by two main activators: tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), which are both inhibited by antifibrinolytics such as TXA.(31, 32) TXA is a synthetic lysine analog that competitively binds to high and medium affinity Kringle domains on plasminogen lysine binding sites, preventing plasminogen from binding to fibrin clots and being cleaved into plasmin.(32, 33) The accumulation of plasmin and other byproducts of plasminogen breakdown have been implicated in sustained tissue inflammation which is reversed by TXA. The time-dependence of these effects have been demonstrated in a host of animal and human studies and may be related to the fact that tPA and uPA achieve their peak concentrations at different times following injury.(33) tPA peaks in the immediate period after initial injury while uPA peaks several hours later. In a murine study, Hijazi *et al.* utilized an intracerebral hemorrhage TBI model and found that tPA in cerebrospinal fluid (CSF) peaked immediately after injury then fell to 50% of maximal concentration four hours later, whereas uPA CSF concentrations did not peak until eight hours post-injury.(34) This has led some investigators to posit that TXA behaves in two opposing, time-dependent manners: (1) as an antifibrinolytic when tPA-driven plasminogen activation peaks and (2) as a profibrinolytic when uPA-driven activation peaks. Similar to opposite time-dependent effects on fibrinolysis, TXA appears to have parallel effects on inflammation as well – acting as an early anti-inflammatory while later on as a proinflammatory

driver. This was corroborated in a study where TXA administration reduced C5a generation during early tPA-driven fibrinolysis (anti-inflammatory response), but increased C5a generation during later uPA-driven fibrinolysis (pro-inflammatory response).(33) Such a dichotomous pro-inflammatory and anti-inflammatory TXA effect by timing has also been observed in injury models which have noted that early TXA administration reduces IL-6 and TNF-α plasma concentrations, as well as intestinal neutrophil extracellular traps after injury compared to late administration; early administration more effectively reduces tissue edema and microvascular permeability.(13, 35, 36)

This apparent pathophysiologic time-dependent contradiction may explain the clinical observations several investigators have made in studying survival outcomes in multi-trauma and TBI patients receiving TXA. Notably, the CRASH-2 study demonstrated a significant survival advantage in multi-trauma patients receiving TXA before - but not after - three hours from injury.(37) The same initiative subsequently published the CRASH-3 trial, specifically evaluating brain injured patients and again noted a reduced risk of head injury-related mortality when TXA was administered within three hours of injury in patients with mild-to-moderate TBI.(15) While survival advantages are consistently found with early post-TBI TXA, both animal and human studies have also investigated differential functional neurological recovery with TXA after injury. Using a CCI model, Daglas and colleagues found that in male mice, TXA reduced BBB permeability while simultaneously exhibiting better motor function with improved mouse step angle and gait symmetry up to baseline levels.(38) In a similar setting, our group previously reported how early TXA administration (1 hour post-TBI) resulted in improved GNT scores at 24 and 48 hours concurrent to greater 48-hour body weight loss recovery - results that

were reproduced in the current study. Additionally, in that study also reported how *in vivo* penumbral BBB permeability significantly increased at 48 hours after CCI, and that early, but not late, TXA administration restored BBB integrity to normal.(13) In the current study, we expanded to a more robust neuroclinical evaluation over an extended period of time and found early TXA-related body weight loss recovery to persist beyond a week after injury - significantly greater in I+TXA1 compared to I+TXA24, while I+TXA24 behaved similarly to untreated injured animals (I+P1).

In human studies, effects of early TXA on neurological recovery are mixed. In a cohort of combat TBI patients, Morte and colleagues again found that early TXA patients suffered lower mortality but also reported that they manifested improved neurological outcomes at discharge measured by change in GCS.(16) Similarly, in a separate military study, patients with traumatic intracranial hemorrhage receiving TXA manifested greater neurological improvement with superior GCS recovery prior to discharge.(39) In contradistinction however, the recently published PATCH- Trauma trial did not note improved neuroclinical recovery as measured by the Glasgow Outcome Scale–Extended (GOS-E) at six months after injury in patients receiving prehospital TXA.(40) However, this population was characterized by multi-organ injury (not isolated TBI), and as the trial was not designed to enroll TBI patients, only a small percentage of enrolled patients ultimately were found to have TBI retrospectively through less reliable methodology involving AIS-head/neck or GCS scores; no information was provided regarding intracranial content, imaging, neurological exam, neurosurgical intervention(s), or ICP monitoring. In a similar North American study, the Prehospital TXA for TBI Trial, which was a randomized, double-blind, multicenter phase II trial, also found no improvement in 6-month

GOS-E in the combined TXA regimen (all bolus/infusion regimens). However, the group that received a 2-gram bolus alone demonstrated an improved Disability Rating Scale Score at 6-months.(17) This is particularly relevant as in our study the TXA regimen involved the equivalent of a 2 gram bolus in a 70-kg human and no infusions. In our study, the rate of animal body weight loss recovery showed a significantly greater positive inflection after day 4-5 but only in TBI animals receiving TXA at 1 hour, further supporting the more elaborate neurological recovery markers elicited in the MWM exercises (spatial learning and memory).

The finding that animals receiving sham craniotomy (without CCI) manifest differences when exposed to 1-hour TXA was surprising. At first, we attributed this to surgical technique or lack of randomization but noted that none of this was the case with all animals randomized to CCI or sham craniotomy and then again randomized to TXA or placebo, indicating no difference in how groups were treated, managed or analyzed. Yet, S+TXA1 animals demonstrated improved weight loss recovery, reduced cerebral and lung water; but worsened learning and memory across multiple MWM parameters. These contradictory results are perplexing and further investigation in TXA administration without TBI is needed to determine if this is a true signal or if a beneficial TXA mechanism of action exists in this less or non-injured populations.

Our study investigated a time dependent effect of TXA administration on cognitive and neurological recovery following severe TBI in mice. However, the presented murine study must be evaluated within the context of certain limitations. First, our model solely used male CD1 mice, as this has been the only mouse sex and strain that our laboratory has used for the past decade. However, other groups have found sex-dependent differences in physiological responses

to TBI and even to TXA exposure after TBI, indicating that current results may not be extrapolatable to females.(38, 41, 42) No doubt significant bias exists in animal research excluding female subjects in studies fearing excessive variability, however in the current study the decision to be single sex specific was purposeful because of known sex-related differences in TBI. Second, this was a small animal study, so the results cannot be directly extrapolated to human TBI physiological responses, and may not necessarily translate to how TXA functions in other animal species. Third, although TXA dosage utilized in our study was based on human bodyweight dosing, the most common form of human administration is an initial bolus followed by a multi-hour infusion. Due to animal husbandry constraints, only a single TXA bolus regimen was used, meaning that the response to standard bolus-plus-infusion TXA in humans could be different. Fourth, with respect to TXA effects in sham craniotomy, we only investigated TXA administration at 1 hour after craniotomy. An additional sham craniotomy group receiving TXA at 24 hours would have helped elucidate a possible delayed effect of TXA (harmful or beneficial) when brain injury was minimal or non-existent. Also, adding a TBI + placebo group at 24 hours would have better confirmed that administering placebo at 24 hours had no effects. Fifth, the time delay for the late TXA in TBI group was intentionally chosen to be very long at 24 hours. Human TBI literature (37) only shows three hours or more as being a worse time to administer TXA but it remains unknown if the harm/benefit relationship with dose timing is linear, multipeaked or follows any other relation. Additional trials will have to study this and establish when, exactly is the worse timing for TXA administration in trauma and what, if any, the time/benefit relationship is. In this proof-of-concept, preliminary study, we wanted to exaggeratedly delay timing to ensure that any signal of worse outcome with greater delay was captured. Finally, certain spatial and probe memory trial parameters compared demonstrated

trends that approached significance (i.e. p=0.09 for probe latency to Z1) greater sample sizes despite our power calculation may have elicited these to become significant differences.

CONCLUSION

Early but not late TXA administration after severe blunt TBI improves markers of learning and memory in a murine model for up to 14 days after injury. Surrogates of neuroclinical recovery after TBI show that early TXA administration also accelerates neuroclinical and overall recovery when compared to late TXA or no treatment. It remains to be determined if and why early TXA administration in sham animals alters some but not all markers of neurological recovery. This work adds to an increasing body of evidence indicating that early TXA administration appears to improve post-injury outcomes.

SUPPLEMENTAL DIGITAL CONTENT:

1) SDC Figure A: Morris Water Maze viewed from different angles as organized for cued, spatial, and probe trials.
2) JTACS disclosure forms
3) Completed ARRIVE Checklist

REFERENCES

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LEGEND OF FIGURES

Figure 1. Experimental timeline.

Figure 2. ***(A)*** Daily mean % animal body weight change for 14 days following sham craniotomy or CCI. ***(B)*** Specific depiction of panel A on Day 7 after sham craniotomy/CCI when group differences begin to amplify where animal bodyweight change is compared to pre-injury weight. **S**: Sham craniotomy animals, **I**: Injured animal, **S+P1**: Sham craniotomy and NS 1h post-craniotomy, **S+TXA1**: Sham craniotomy and TXA 1h post-craniotomy, **I+P1**: CCI (injury) and NS 1h post-injury, **I+TXA1**: CCI and TXA 1h post-injury, **I+TXA24**: CCI and TXA 24h post-injury. (*p<0.05 vs. I+P1, #p<0.05 vs. I+TXA24, ##p<0.01 vs. I+TXA24)

Figure 3. Animal functional neurological recovery 1 day after sham/injury, as calculated by the Garcia Neurological Test (GNT, max score = 18). Non statistically significant trends continued in subsequent days until day 7; after 7 days and beyond, all groups exhibited maximum GNT scores **S**: Sham craniotomy animals, **I**: Injured animal, **S+P1**: Sham craniotomy and NS 1h post-craniotomy, **S+TXA1**: Sham craniotomy and TXA 1h post-craniotomy, **I+P1**: CCI (injury) and NS 1h post-injury, **I+TXA1**: CCI and TXA 1h post-injury, **I+TXA24**: CCI and TXA 24h post-injury. (*p<0.05 vs. I+P1, **p<0.01 vs. I+P1, ##p<0.01 vs. I+TXA24, ++p<0.01 vs. S+P1)

Figure 4. Organ tissue water content: ***(A)*** Ipsilateral (Injured) cerebral hemispheric, ***(B)*** Contralateral (Uninjured) cerebral hemispheric, and ***(C)*** Pulmonary tissue water measured by wet-to-dry ratios (↑% tissue water = ↑ edema). **S**: Sham craniotomy animals, **I**: Injured animal,

S+P1: Sham craniotomy and NS 1h post-craniotomy, **S+TXA1**: Sham craniotomy and TXA 1h post-craniotomy, **I+P1**: CCI (injury) and NS 1h post-injury, **I+TXA1**: CCI and TXA 1h post-injury, **I+TXA24**: CCI and TXA 24h post-injury. (++p<0.05 vs. S+P1, I+P1, I+TXA1 and I+TXA24, +p<0.01 vs. S+P1, I+P1, I +TXA1, but p=0.06 vs I+TXA24).

Figure 5. Spatial Trials: ***(A)*** The mean distance travelled to reach the platform. ***(B)*** Summative time spent in Zone 1, the quadrant containing the platform, taken as a percentage of total trial time. ***(C)*** Summative time to reach Zone 1. **S**: Sham craniotomy animals, **I**: Injured animal, **S+P1**: Sham craniotomy and NS 1h post-craniotomy, **S+TXA1**: Sham craniotomy and TXA 1h post-craniotomy, **I+P1**: CCI (injury) and NS 1h post-injury, **I+TXA1**: CCI and TXA 1h post-injury, **I+TXA24**: CCI and TXA 24h post-injury. (*p<0.05 vs. I+P1, **p<0.01 vs. I+P1, #p<0.05 vs. I+TXA24, ##p<0.01 vs. I+TXA24, +p<0.05 vs. S+P1, ++p<0.01 vs. S+P1)

Figure 6. Probe Trials: ***(A)*** Mean amount of time spent swimming in the platform area (Zone 5). ***(B)*** Mean number of crossings into the platform area (platform removed). **S**: Sham craniotomy animals, **I**: Injured animal, **S+P1**: Sham craniotomy and NS 1h post-craniotomy, **S+TXA1**: Sham craniotomy and TXA 1h post-craniotomy, **I+P1**: CCI (injury) and NS 1h post-injury, **I+TXA1**: CCI and TXA 1h post-injury, **I+TXA24**: CCI and TXA 24h post-injury. (*p<0.05 vs. I+P1, **p<0.01 vs. I+P1, #p<0.05 vs. I+TXA24, ##p<0.01 vs. I+TXA24, +p<0.05 vs. S+P1, ++p<0.01 vs. S+P1)

Figure 1

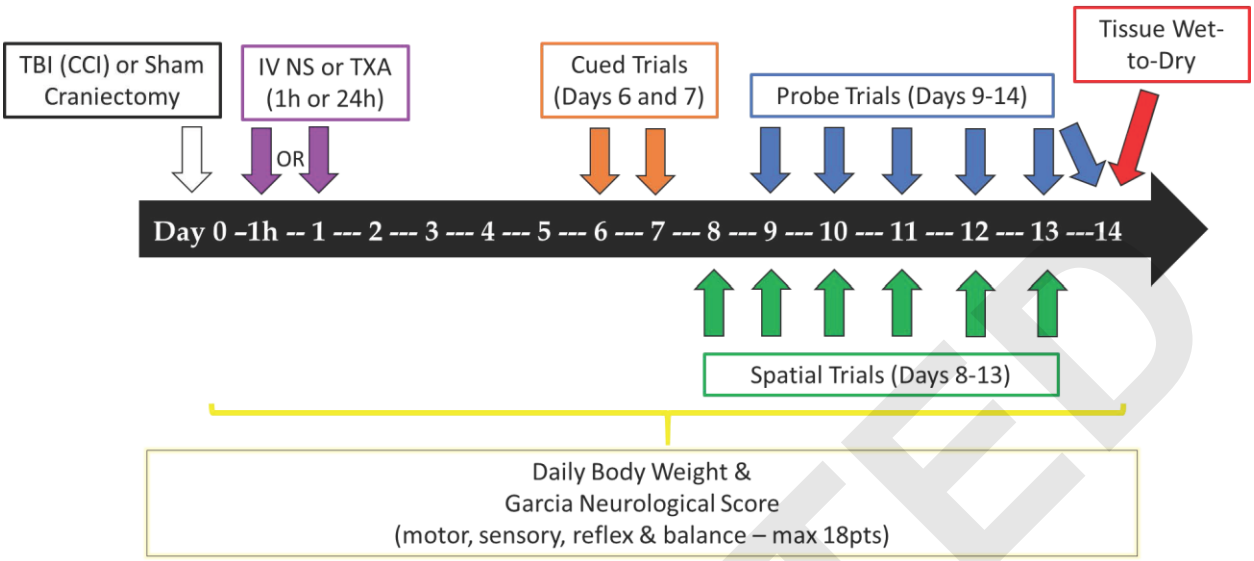


Figure 2

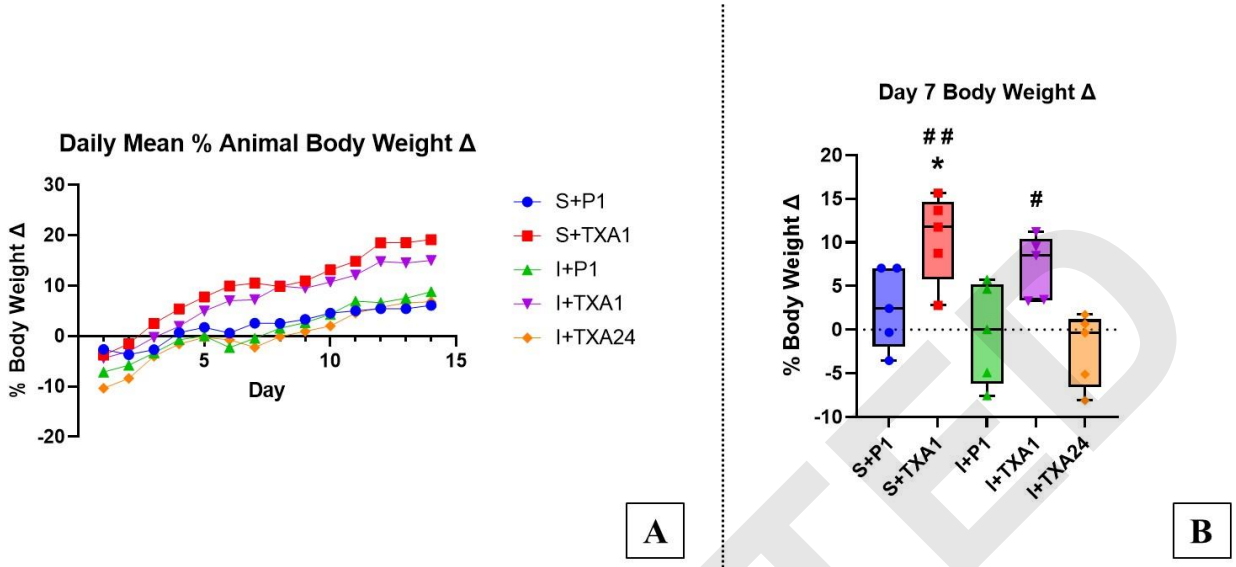
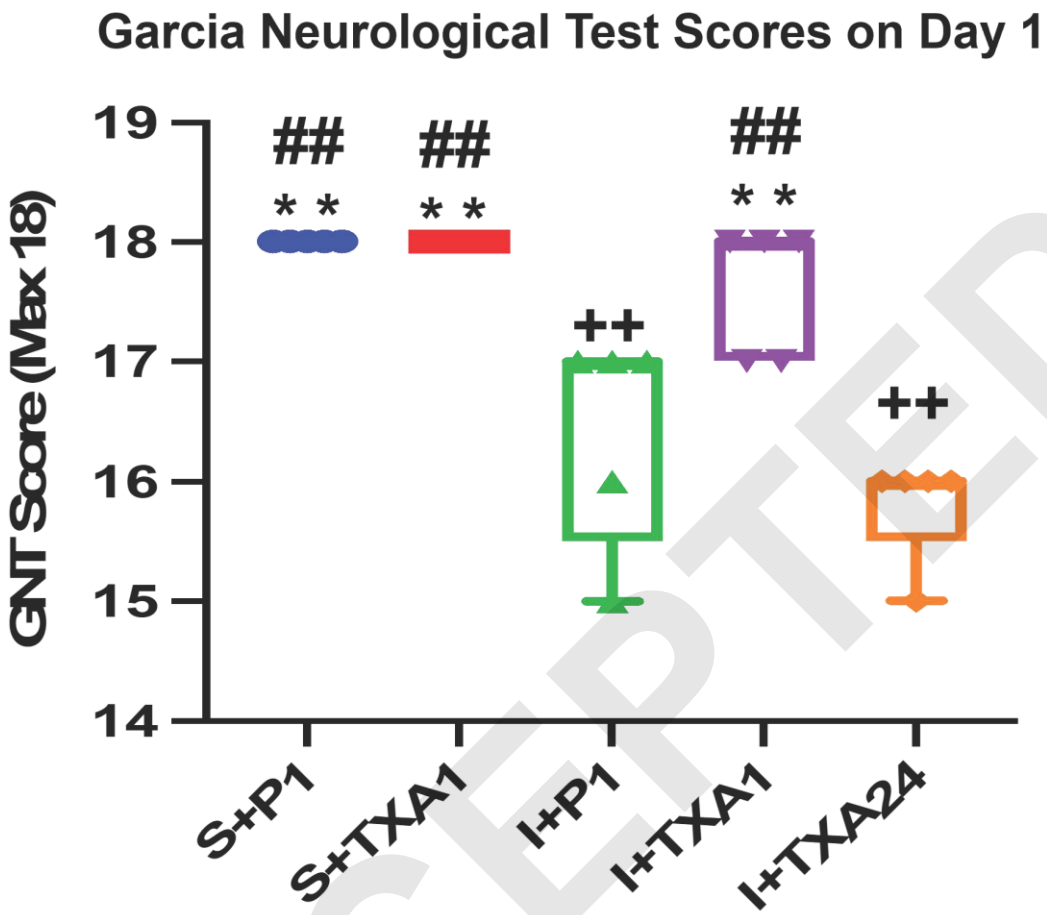


Figure 3



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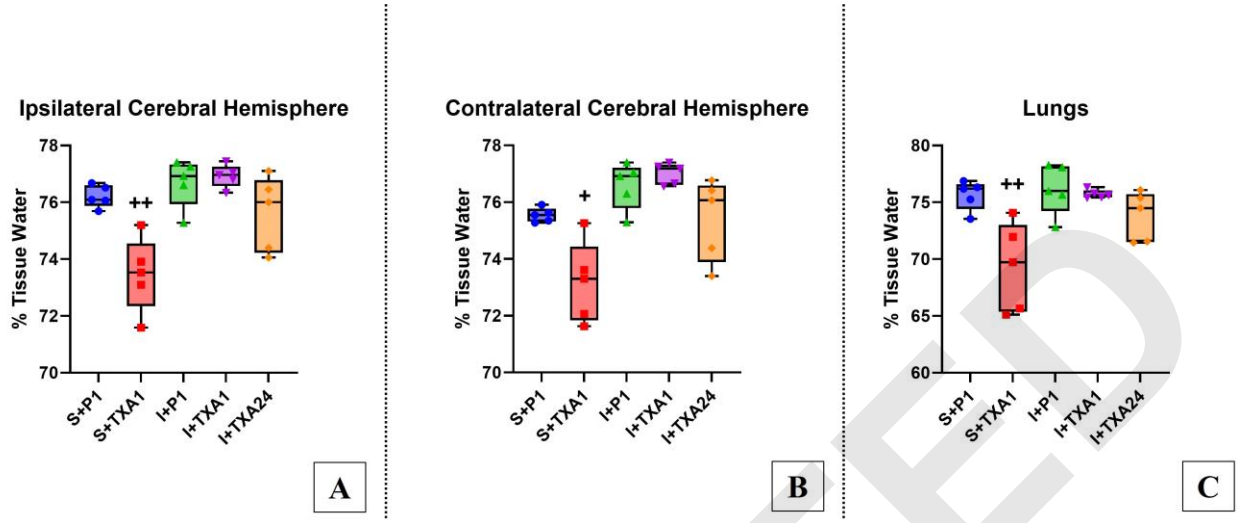


Figure 5

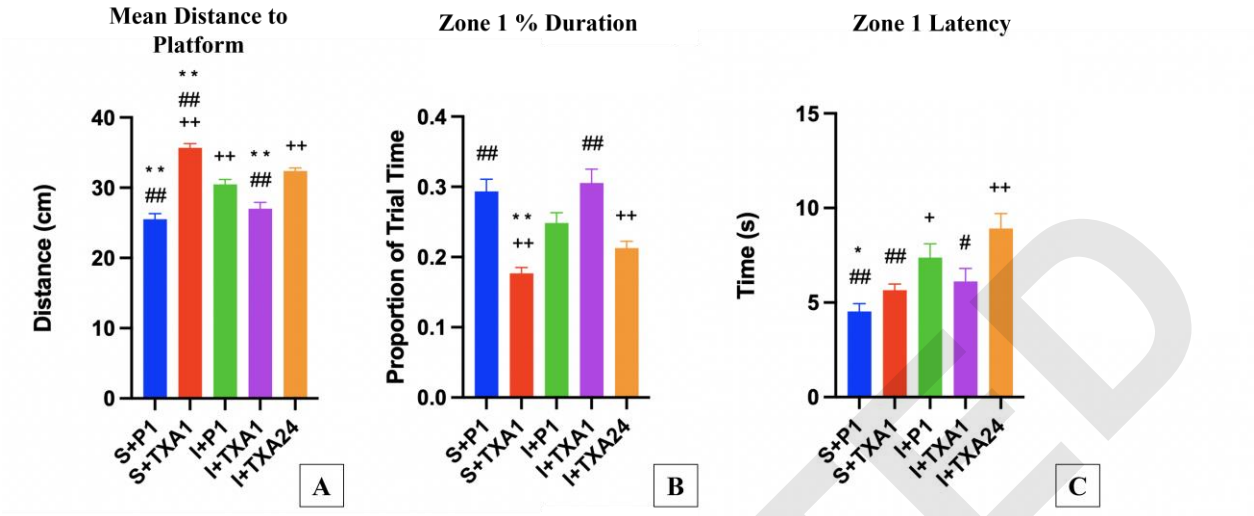
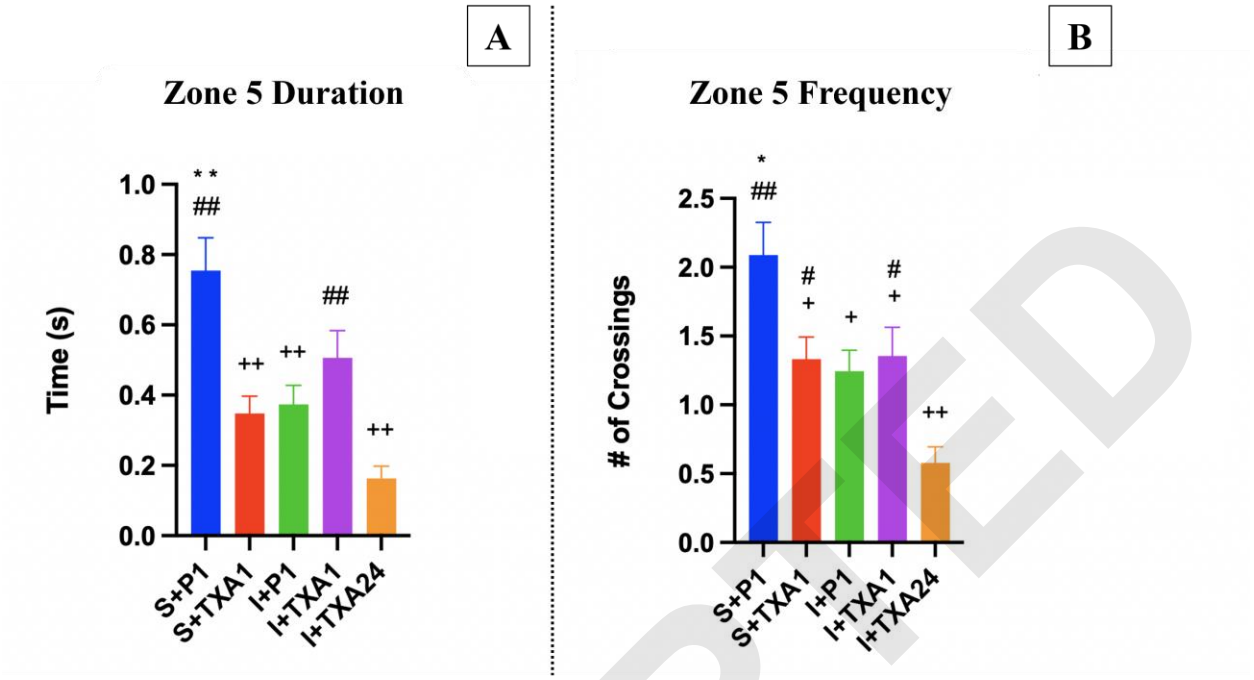


Figure 6



Reporting checklist for study using laboratory animals.

Based on the ARRIVE guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the ARRIVEreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Essential 10			
Study design	#1a	Give details of the groups being compared, including control groups. If no control group has been used, the rationale should be stated.	2
Study design	#1b	Give details of the experimental unit (e.g., a single animal, litter, or cage of animals).	2
Sample size	#2a	Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.	2-3

Sample size	#2b	Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done.	3
Inclusion and exclusion criteria	#3a	Describe any criteria used for including or excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly.	n/a
Inclusion and exclusion criteria	#3b	For each experimental group, report any animals, experimental units, or data points not included in the analysis and explain why. If there were no exclusions, state so.	n/a
Inclusion and exclusion criteria	#3c	For each analysis, report the exact value of n in each experimental group.	n/a
Randomisation	#4a	State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.	2
Randomisation	#4b	Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	2
Blinding	#5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	4
Outcome measures	#6a	Clearly define all outcome measures assessed (e.g., cell death, molecular markers, or behavioural changes).	3-6
Outcome measures	#6b	For hypothesis-testing studies, specify the primary outcome measure, i.e., the outcome measure that was used to determine the sample size.	4

Statistical methods	#7a	Provide details of the statistical methods used for each analysis, including software used.	7
Statistical methods	#7b	Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	7
Experimental animals	#8a	Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.	2
Experimental animals	#8b	Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	2
Experimental procedures	#9a	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate what was done, how it was done, and what was used.	3-4
Experimental procedures	#9b	Timing and frequency of procedures	3
Experimental procedures	#9c	Where procedures were carried out (including detail of any acclimatisation periods).	3-6
Experimental procedures	#9d	Rationale for procedures	3-6
Results	#10a	For each experiment conducted, including independent replications, report summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g., mean and SD, or median and range).	7-9 + figures
Results	#10b	If applicable, for each experiment conducted, including independent replications, report the effect size with a confidence interval.	na

Recommended set

Abstract	#11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	abs
Background	#12a	Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.	1-2
Background	#12b	Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	1-2
Objectives	#13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	2
Ethical statement	#14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	na
Housing and husbandry	#15	Provide details of housing and husbandry conditions, including any environmental enrichment.	2
Animal care and monitoring	#16a	Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering, and distress.	2-3
Animal care and monitoring	#16b	Report any expected or unexpected adverse events.	3
Animal care and monitoring	#16c	Describe the humane endpoints established for the study, the signs that were monitored, and the frequency of monitoring. If the study did not set humane endpoints, state this.	3-4
Interpretation/scientific implications	#17a	Interpret the results, taking into account the study objectives and hypotheses, current theory, and other relevant studies in the literature.	9-15
Interpretation/scientific implications	#17b	Comment on the study limitations, including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	9-15

Generalisability/translation	#18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	9-15
Protocol registration	#19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	na
Data access	#20	Provide a statement describing if and where study data are available.	na
Declaration of interests	#21a	Declare any potential conflicts of interest, including financial and nonfinancial. If none exist, this should be stated.	na
Declaration of interests	#21b	List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis, and reporting of the study.	na

Notes:

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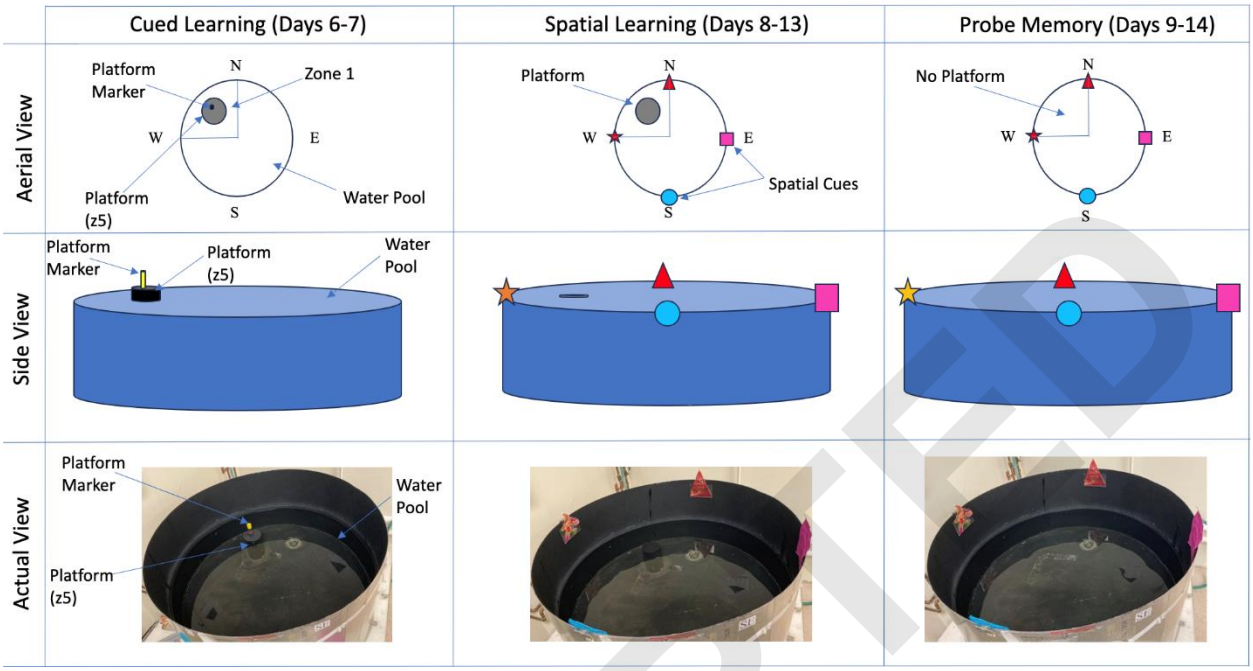
SDC Figure A



The Journal of Trauma and Acute Care Surgery

CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: Matthew Culkin

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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8	Patents planned, issued or pending	☒ None	
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ None	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☒ None	
11	Stock or stock options	☒ None	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ None	
13	Other financial or non-financial interests	☒ None	
14	Family Disclosure. Disclose any financial associations involving a spouse, partner, or children	☒ None	

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CONFLICT OF INTEREST DISCLOSURE FORM

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Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

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6	Payment for expert testimony	☒ **None**	
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11	Stock or stock options	☒ None	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ None	
13	Other financial or non-financial interests	☒ None	
14	Family Disclosure. Disclose any financial associations involving a spouse, partner, or children	☒ None	

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The Journal of Trauma and Acute Care Surgery

CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: Priyanka Bele

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: Advait Thaploo

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: Anastasia Georges

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: Erin D. Anderson

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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CONFLICT OF INTEREST DISCLOSURE FORM

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Date: 7/31/2023

Your Name: Kevin D. Browne

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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Based on ICMJE Form

Date: 7/31/2023

Your Name: Christina L. Jacovides

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: Patricia Santos Carlin

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☒ **None**	
6	Payment for expert testimony	☒ **None**	
7	Support for attending meetings and/or travel	☒ **None**	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
8	Patents planned, issued or pending	☒ None	
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ None	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☒ None	
11	Stock or stock options	☒ None	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ None	
13	Other financial or non-financial interests	☒ None	
14	Family Disclosure. Disclose any financial associations involving a spouse, partner, or children	☒ None	

Please place an "X" next to the following statement to indicate your agreement:

☒ I certify that I have answered every question and have not altered the wording of any of the questions on this form.

The Journal of Trauma and Acute Care Surgery

CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: Lewis J Kaplan, MD

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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Participants of an accredited activity must disclose all personal **financial and non-financial relationships**, over the previous 36 months with an **ineligible company** (formerly defined as a commercial interest). **Financial relationships** are those relationships in which the individual benefits by receiving a salary, royalty, consulting fee, honoraria, ownership interest (e.g., stocks, stock options or other ownership interest), or other financial benefits, and may affect activity content relevant to products or services of an **ineligible company**, defined as an entity whose primary business is producing, marketing, selling, re-selling, or distributing healthcare products used by or on patients.

Research grants from **ineligible companies** are financial relationships that should be disclosed, even if the funds go to the researcher's institution and not to the individual researcher.

The author's relationships/activities/interests should be defined broadly and not only related to the manuscript in question. For example, if your manuscript pertains to the epidemiology of shock, you should declare all relationships with manufacturers of treatments used in shock, even if that form of treatment is not mentioned in the manuscript.

According to federal regulations approved by the US Senate, any amount equal to above $10 USD must be disclosed. Although disclosure of the total amount is not required on this form. Authors are encouraged to search the CMS Open Payments Database found at https://openpaymentsdata.cms.gov and report on the JTACS Conflict of Interest Disclosure form ALL COI, and any other conflicts related or unrelated to the manuscript being submitted to the Journal for the last 36 months/3 years.

In item #1 below, report all support for the work reported in this manuscript without time limit. For all other items, the time frame for disclosure is the past 36 months.

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		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
Time frame: Since the initial planning of the work			
1	All support for the present manuscript (e.g., funding, provision of study materials,	☒ None	
			Click the tab key to add additional rows.

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
	medical writing, article processing charges, etc.) No time limit for this item.		
	Time frame: past 36 months		
2	Grants or contracts from any entity (if not indicated in item #1 above).	☒ **None**	
3	Royalties or licenses	☐ **None**	UpToDate: Royalties paid to me
4	Consulting fees	☒ **None**	
5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☒ **None**	
6	Payment for expert testimony	☐ **None**	Medical Legal consulting: Monies paid to me
7	Support for attending meetings and/or travel	☒ **None**	Society of Transplant Surgeons meeting: Travel expenses only

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
8	Patents planned, issued or pending	☒ None	
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ None	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☒ None	
11	Stock or stock options	☒ None	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ None	
13	Other financial or non-financial interests	☒ None	
14	Family Disclosure. Disclose any financial associations involving a spouse, partner, or children	☒ None	

Please place an "X" next to the following statement to indicate your agreement:

☒ **I certify that I have answered every question and have not altered the wording of any of the questions on this form.**

The Journal of Trauma and Acute Care Surgery

CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: David F Meaney

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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Time frame: Since the initial planning of the work			
1	All support for the present manuscript (e.g., funding, provision of study materials,	☒ None	
			Click the tab key to add additional rows.

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	medical writing, article processing charges, etc.) No time limit for this item.		
	Time frame: past 36 months		
2	Grants or contracts from any entity (if not indicated in item #1 above).	☒ None	
3	Royalties or licenses	☒ None	
4	Consulting fees	☒ None	
5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☒ None	
6	Payment for expert testimony	☒ None	
7	Support for attending meetings and/or travel	☒ None	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
8	Patents planned, issued or pending	☒ None	
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ None	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☒ None	
11	Stock or stock options	☒ None	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ None	
13	Other financial or non-financial interests	☒ None	
14	Family Disclosure. Disclose any financial associations involving a spouse, partner, or children	☒ None	

Please place an "X" next to the following statement to indicate your agreement:

☒ I certify that I have answered every question and have not altered the wording of any of the questions on this form.

The Journal of Trauma and Acute Care Surgery

CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: Douglas H. Smith

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related or unrelated to the content of your manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

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		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
Time frame: Since the initial planning of the work			
1	All support for the present manuscript (e.g., funding, provision of study materials,	☐ None	
		National Institutes of Health grant funding	University of Pennsylvania
		Department of Defense grant funding	University of Pennsylvania

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
	medical writing, article processing charges, etc.) No time limit for this item.	Uniformed Services University of Health Services Grant funding	University of Pennsylvania
		Department of Army USAMRAA	University of Pennsylvania
		Allen Foundation grant funding	University of Pennsylvania
		Time frame: past 36 months	
2	Grants or contracts from any entity (if not indicated in item #1 above).	☐ **None**	
		Listed above	
3	Royalties or licenses	☒ **None**	
4	Consulting fees	☒ **None**	
5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☐ **None**	
		University of Pittsburgh speaker	**Paid to Douglas Smith**
		University of Michigan speaker	**Paid to Douglas Smith**
		LEIDOS review committee	**Paid to Douglas Smith**
6	Payment for expert testimony	☒ **None**	
7	Support for attending meetings and/or travel	☒ **None**	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
8	Patents planned, issued or pending	☐ None	
		US 6264944 Mechanically Elongated Neuronal Cells 2001	No revenue generated or royalties to date.
		US 6365153 Mechanically elongated neuronal cells and methods for producing and using these cells 2002	
		US 7,429,267 Device and method using integrated neuronal cells and an electronic device 2008	
		US 7,972,367 Device and method using integrated neuronal cells and an electronic device 2011	
		US 8,400,636 Blast injury dosimeter 2013	
		US 8790666 Nerve construct containing living stretch-grown nervous tissue 2014	
		US 9,895,399 Repair of peripheral nerve injury 2015	
		CA 2,380,943 Mechanically Elongated Neuronal Cells and Methods for Producing These Cells 2010	
		Japan 4679780 Mechanically Elongated Neural Cells and Methods for Producing and Using these Cells 2011	
		EU 1225906 Mechanically Elongated Neural Cells and Methods for Producing and Using these Cells 2008	
		Australia – 766096 Mechanically Elongated Neuronal Cells and Methods for Producing These Cells 2004	
		Denmark - 1225906 Mechanically Elongated Neural Cells and Methods for Producing and Using these Cells 2008	
		US 10525085 Repair of peripheral nerve injury 2020	
		US – 17358851 Implantable Living Electrodes and Methods for use Thereof 2021	
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ None	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☐ None	
		Mind Your Brain Foundation	

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
11	Stock or stock options	☒ None	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ None	
13	Other financial or non-financial interests	☐ None	
		Axonova Medical LLC	Co-Founder; no profits or income to date.
14	Family Disclosure. Disclose any financial associations involving a spouse, partner, or children	☒ None	

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☒ I certify that I have answered every question and have not altered the wording of any of the questions on this form.

The Journal of Trauma and Acute Care Surgery

CONFLICT OF INTEREST DISCLOSURE FORM

Based on ICMJE Form

Date: 7/31/2023

Your Name: Jose L. Pascual L.

Manuscript Title: DELAYED TXA AFTER TBI IMPEDES LEARNING, MEMORY; EARLY TXA IS FAVORABLE BUT NOT IN SHAM ANIMALS

Manuscript Number (if known): TBD

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Time frame: Since the initial planning of the work			
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			Click the tab key to add additional rows.

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
	medical writing, article processing charges, etc.) No time limit for this item.		
		Time frame: past 36 months	
2	Grants or contracts from any entity (if not indicated in item #1 above).	☒ **None**	
3	Royalties or licenses	☒ **None**	
4	Consulting fees	☒ **None**	
5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	☐ **None**	
		Multiple presentations for SCCM Executive	No payments
6	Payment for expert testimony	☐ **None**	
		Multiple firms for plaintiff and defendant Council	As per standard fee schedule U Penn
7	Support for attending meetings and/or travel	☐ **None**	
		SCCM, AAST, ACS	To cover travel, lodging and registration at conference

		Name all entities with whom you have this relationship or indicate none (add rows as needed)	Specifications/Comments (e.g., if payments were made to you or to your institution)
8	Patents planned, issued or pending	☒ None	
9	Participation on a Data Safety Monitoring Board or Advisory Board	☒ None	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	☐ None	SCCM Secretary
11	Stock or stock options	☒ None	
12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	☒ None	
13	Other financial or non-financial interests	☒ None	
14	Family Disclosure. Disclose any financial associations involving a spouse, partner, or children	☒ None	

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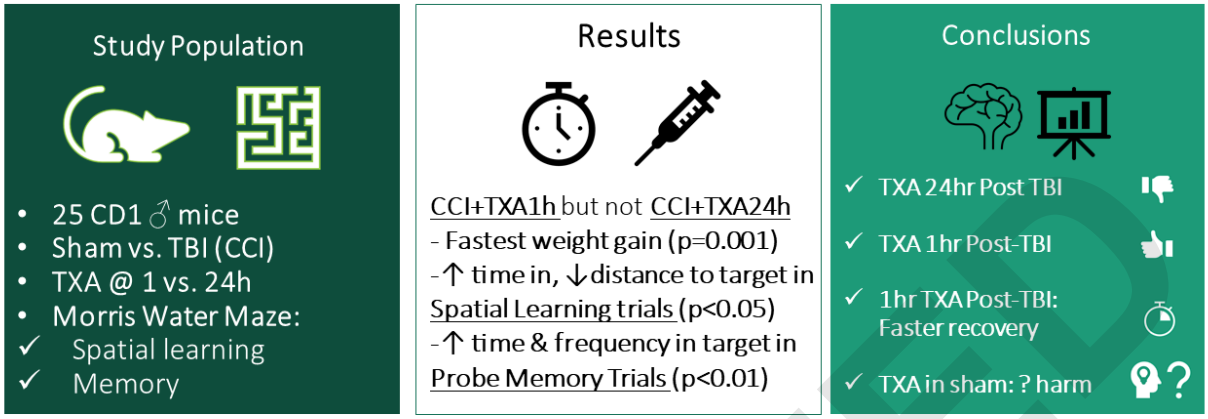

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