ARG-007 PROTECTS BRAIN CELLS IN MODERATE TRAUMATIC BRAIN INJURY MODEL

Highlights:

- *ARG-007 shown to significantly reduce damage to brain cells caused by moderate traumatic brain injury (TBI) as assessed in a preclinical rat model, compared to placebo treated controls.*

- *Damage to brain cells was assessed by measuring the accumulation of key proteins which contribute to brain cell injury and death following moderate TBI (modTBI). Importantly, the protein levels following ARG-007 treatment were* ***equivalent to non-injured animals.***

- *The level of an inflammation marker (Iba1) in the brain was* ***also significantly reduced back to non-injured levels****. Inflammation in the brain following TBI is an important cause of secondary brain injury which lasts far beyond the initial injury.*

- *ARG-007 treated animals also showed significant improvement in functional outcomes, including increased weight gain and reduced anxiety, as well as providing a more sustained improvement in balance.*

- *Taken together, this study provides evidence that ARG-007 protects against modTBI-induced brain cell damage and death, brain inflammation, weight loss, and anxiety-related movement dysfunction, providing encouraging results to progress further studies.*

Perth, Australia; 22 JUNE 2023 - Argenica Therapeutics Limited (ASX: AGN) ("Argenica" or the "Company"), a biotechnology company developing novel therapeutics to reduce brain tissue death after brain injury, is pleased to announce encouraging results from a preclinical study assessing the efficacy of ARG-007 in a rat model of moderate traumatic brain injury (modTBI).

This study, undertaken in the laboratory of Prof Lindy Fitzgerald at Curtin University and the Perron Institute for Neurological and Translational Sciences, assessed the therapeutic potential of ARG-007 in protecting brain cell (neuron) integrity and resulting behavioural outcomes following modTBI. The study was conducted in a well-characterised, impact-acceleration rat model of modTBI that results in diffuse injury to brain cells, mimicking an injury sustained during a fall, such as following a "king-hit", or in a motor vehicle accident.

ARG-007 was found to protect brain cells in the injured brain by significantly reducing the accumulation of proteins that contribute to brain cell injury and death following TBI, specifically neurofilament heavy protein (NFH) and amyloid precursor protein (APP). The level of both NFH and APP protein in ARG-007 treated animals was equivalent to animals that had not received a TBI (i.e. same as the sham controls) shown in Figure 1.

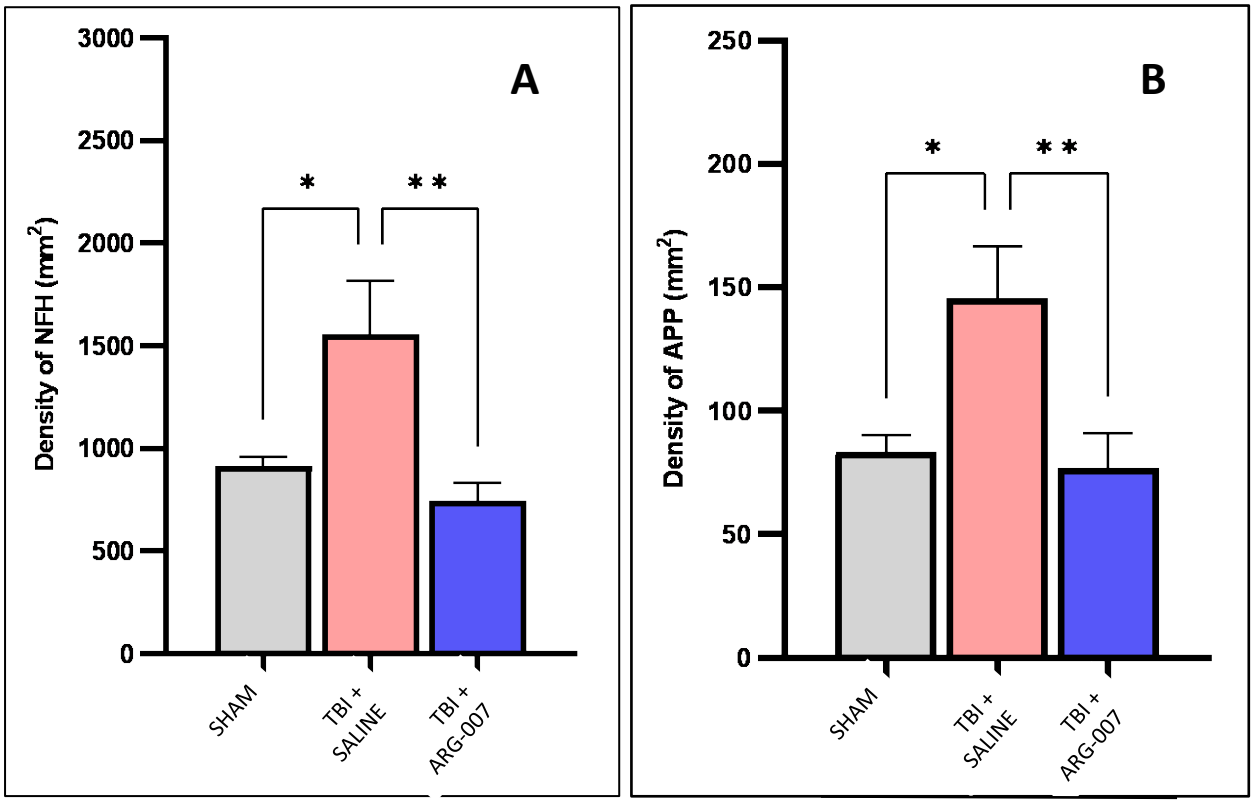

Figure 1. (A) ARG-007 protected brain cell axons in the pyramidal tract from injury as shown by a significant reduction in NFH density, and (B) ARG-007 significantly protected brain cells from neurodegeneration following modTBI. Both protein levels were reduced to Sham or non-injury controls.

Further, ARG-007 significantly reduced inflammation in the brain equivalent to non-injured animals (see Figure 3 in Appendix). Inflammation in the brain following TBI can be a significant cause of secondary brain injury which can last far beyond the acute phase of the initial injury. Importantly, the reduction in protein accumulation and inflammation in ARG-007 treated animals was also associated with significant improvements in key functional behaviours, including reduced anxiety, reduced weight loss and improved balance. These behaviours can often be adversely affected by people who suffer a TBI event.

Dr Liz Dallimore, Managing Director, said: "We are extremely pleased with the results from our first preclinical study in a moderate TBI animal model. The study indicates that injury caused by moderate traumatic brain injury may be limited by the introduction of ARG-007 shortly thereafter, so we are extremely encouraged by this data. We now look forward to

undertaking further studies, supported by funds provided by our CRC-P grant, to provide greater evidence of ARG-007's efficacy in TBI before establishing a clinical program of work."

An overview of the study is provided in Appendix 1.

NEXT STEPS

Argenica will now work with its CRC-P partners Curtin University, the University of Adelaide, AusPep and Connectivity TBI Australia to undertake further preclinical studies assessing the efficacy of ARG-007 in modTBI in ferrets, and repeated dosing following multiple mild-TBIs in rats. This data will form a comprehensive preclinical package to progress into clinicals trials in TBI.

This announcement has been approved for release by the Board of Argenica

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ABOUT ARGENICA

Argenica (ASX: AGN) is developing novel therapeutics to reduce brain tissue death after stroke and improve patient outcomes. Our lead neuroprotective peptide candidate, ARG-007 has been successfully demonstrated to improve outcomes in pre-clinical stroke models and is in the process of being verified for its safety and toxicity before commencing Phase 1 clinical trials in humans. The aim is for our therapeutic to be administered by first responders to protect brain tissue against damage during a stroke with further potential to enhance recovery once a stroke has taken place.

STUDY OVERVIEW

Background

Every year, over 69 million people in the world sustain a traumatic brain injury (TBI). Most of these people suffer ongoing neurological dysfunction, including motor problems. In addition to primary tissue damage, TBI causes secondary degeneration of initially spared tissue around the primary damage. Secondary degeneration can lead to ongoing damage to brain cells, ultimately manifesting as neurological deficits. Despite the clinical demand, neuroprotective pharmacological agents targeting axonal injury and secondary degeneration are lacking for TBI.

Diffuse axonal injury following TBI results from the rapid acceleration and deceleration forces caused by the impact, causing deformation of brain tissue through shearing forces and stretching. When brain cells stretch over each other it gives rise to widespread damage throughout the cerebral hemispheres, corpus callosum, and brain stem. This stretching of neurons causes intra-axonal changes through specific protein accumulation and inflammation leading to neurodegeneration[1].

ARG-007, a novel neuroprotective peptide being developed by Argenica, has shown preclinical efficacy in stroke and hypoxic ischaemic encephalopathy (HIE) treatment. Stroke, HIE and TBI share a number of neurodegenerative features, raising the possibility that ARG-007 could also be effective in moderate TBI (modTBI). The overarching hypothesis of this and planned studies is that ARG-007 mitigates neural death, white matter injury, related cellular pathologies, and functional deficits following TBI.

Aims

This study assessed the therapeutic potential of ARG-007 in protecting brain cells from injury and neurodegeneration, resulting in improved behavioural function, following TBI. An impact-acceleration rat model of moderate TBI (modTBI) that induces diffuse axonal injury was used to model white matter damage, as seen after falls and motor vehicle accidents.

STUDY RESULTS HIGHLIGHTS

ARG-007 Significantly Reduces Accumulation of Neurodegenerative Proteins NFH and APP

A single administered dose of ARG-007 at 30 minutes post-injury prevented the accumulation of both neurofilament heavy protein (NFH) and amyloid precursor protein (APP) in neurons, as assessed 11 days after injury, suggesting neuroprotection of axons from ARG-007 following modTBI. The level of NFH density correlates with the extent of diffuse axonal injury of neurons

[1] Plummer S, Van den Heuvel C, Thornton E, Corrigan F, Cappai R. The Neuroprotective Properties of the Amyloid Precursor Protein Following Traumatic Brain Injury. Aging Dis. 2016 Mar 15;7(2):163-79.

following TBI. The elevated density of APP following modTBI is a marker for neurodegeneration, or brain cell death.

Animals that sustained modTBI with no post-injury treatment displayed increased density of NFH in the pyramidal tract of the pons, which is an important pathway responsible for voluntary motor movement, in the brain (Fig 1b & 1c). Encouragingly, ARG-007 reduced the density of NFH to vehicle-treated sham control levels (i.e. animals that were not injured; Fig.1). In addition, modTBI with saline/placebo treatment elevated the density of APP in the pyramidal tract (Fig 2b & 2c), however ARG-007 treatment attenuated APP density to vehicle--treated sham control levels (Fig. 2b & 2c).

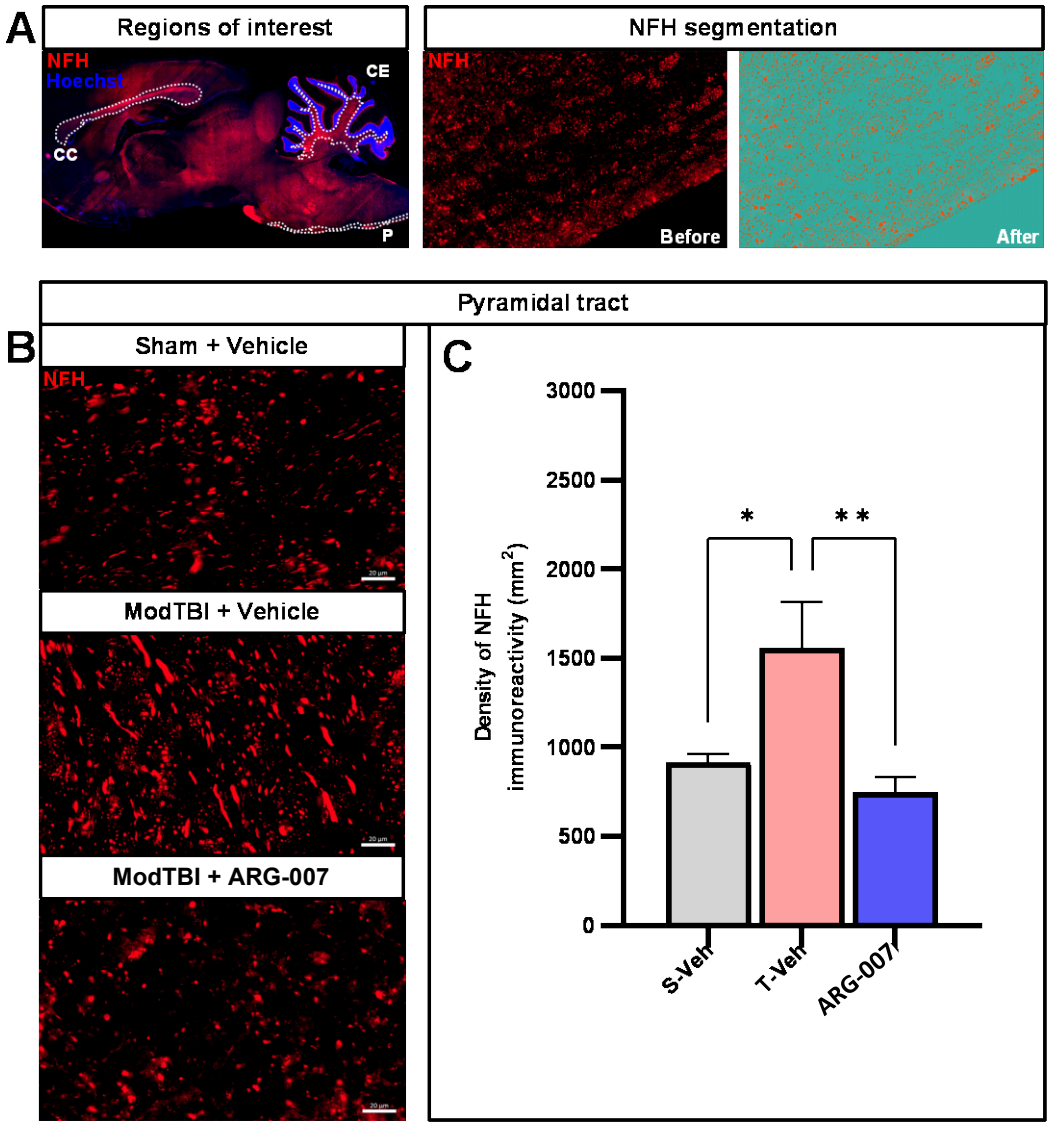

FIGURE 1: ARG-007 protected axons in the pyramidal tract from injury (**A**) Broken lines in the sagittal whole-brain image indicate white matter regions analysed in the corpus callosum (CC), cerebellum (CE) and pyramidal tract (P). NFH segmentation is demonstrated in the 'after' image with orange (positive) and aqua (background) masks. (**B**) Representative images and (**C**) bar graph showing the density of NFH immunoreactivity in the pyramidal tract of the indicated experimental groups. S = Sham. T = ModTBI. Veh = vehicle. * = $p<0.05$ and ** = $p<0.01$.

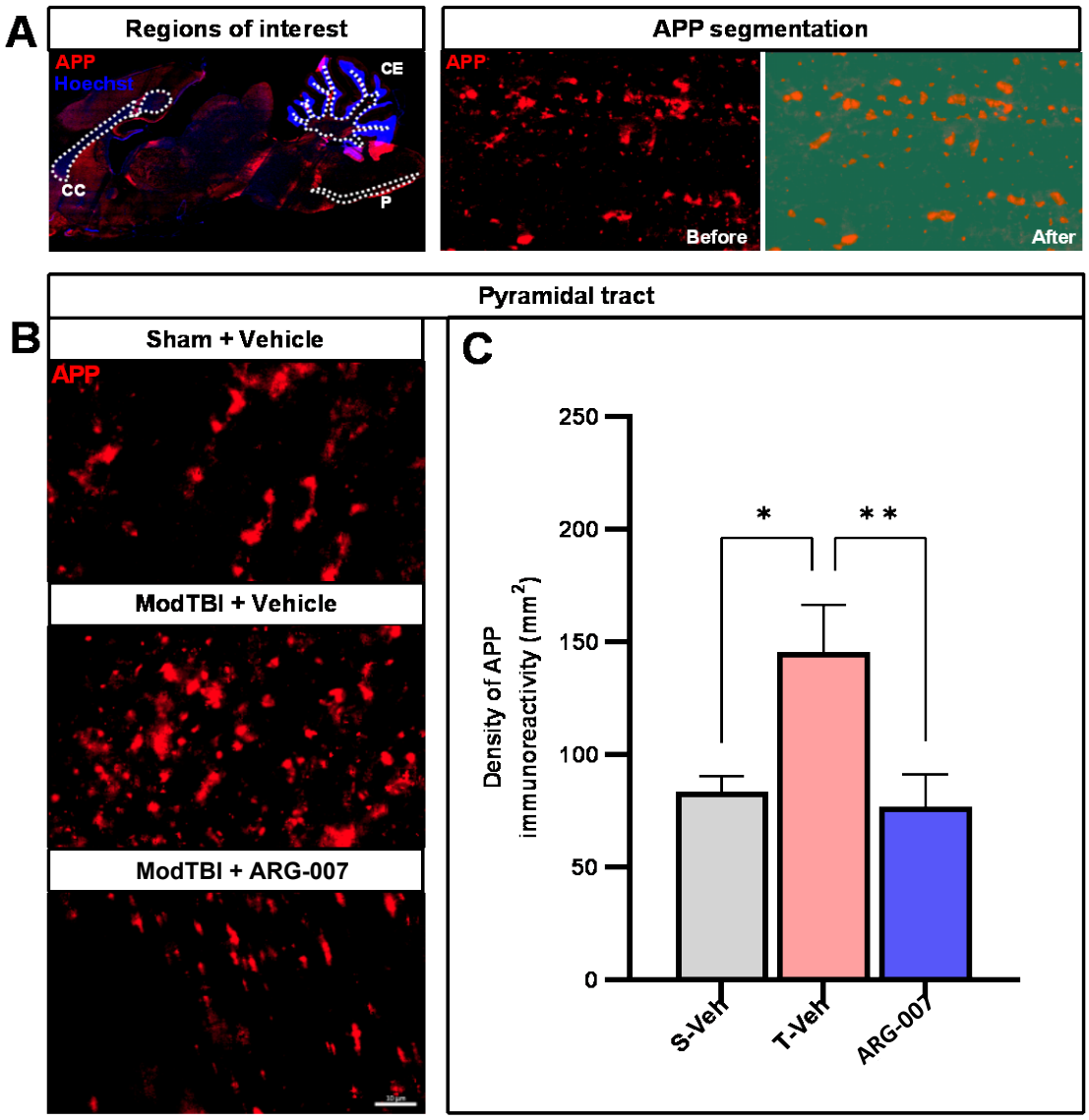

FIGURE 2: ARG-007 prevented neurodegeneration in the pyramidal tract (**A**) Broken lines in the brain image indicate regions analysed in the corpus callosum (CC), cerebellum (CE) and pyramidal tract (P). APP segmentation is demonstrated in the 'after' image with orange (positive) and green (background) masks. (**B**) Representative images and (**C**) bar graph showing the density of APP immunoreactivity in the pyramidal tract. S = Sham. T = ModTBI. Veh = vehicle * = $p<0.05$ and ** = $p<0.01$.

ARG-007 Reduces Neuroinflammation Following TBI

To investigate the effect of modTBI and ARG-007 treatment on neuroinflammation, the level of Iba1 expression, a protein marker of microglia activation, was quantified. Microglia are the immune cells in the central nervous system and are activated during the inflammatory response to enhance release of pro-inflammatory molecules. In the pyramidal tract, moderate TBI without treatment increased the density of Iba1. Importantly, ARG-001 reduced Iba1 density to a level significantly lower than that of the vehicle-treated modTBI group, and on par with the level in the sham injured controls (Fig. 3).

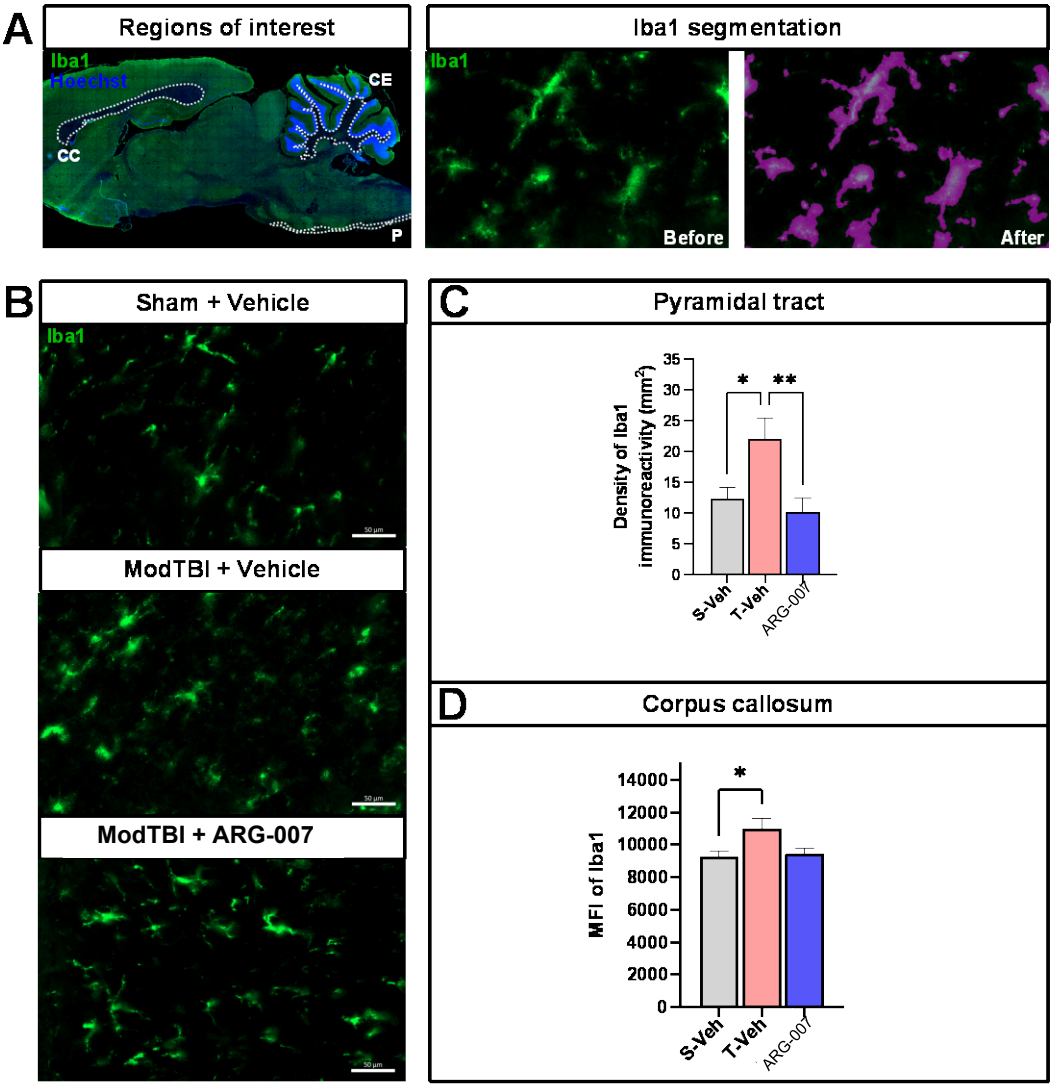

FIGURE 3: ARG-007 reduced neuroinflammation in the pyramidal tract (**A**) Broken lines in the sagittal whole-brain image indicate the white matter regions analysed in the corpus callosum (CC), cerebellum (CE) and pyramidal tract (P). The accuracy of Iba1 segmentation is demonstrated in the 'after' image with the purple (positive) mask. (**B**) Representative images and (**C**) bar graph showing Iba1 immunoreactivity in the pyramidal tract for each experimental group. (**D**) Bar graph shows the mean fluorescence intensity (MFI) of Iba1 in the corpus callosum. * = $p<0.05$ and ** = $p<0.01$.

ARG-007 Prevented Weight Loss and Reduced Anxiety Following ModTBI

Moderate/severe TBI can cause weight loss[2,3], and post-injury weight loss has been associated with greater neurological deficit and mortality[2]. ModTBI caused weight loss that occurred periodically throughout the acute phase (days 1-2), and which did not recover to pre-injury levels until the subacute phase (day 9) of injury. This is indicated by the weight loss in vehicle-treated modTBI rats relative to vehicle-treated sham controls at day 2, 4, 5, 8 and 10.

ARG-007 prevented modTBI-induced weight loss. This is demonstrated by the lack of weight loss in ARG-007-treated modTBI rats at any stage of the experiment. ARG-007 also shortened the time it took for rats to regain their pre-injury weight by 3 days.

Further, moderate and severe TBI can induce anxiety-related behaviour, such as increased velocity and distance of movement, in a novel and potentially stressful environment like an open field[4]. To test the efficacy of ARG-007 in reducing TBI related anxiety, rats that received ARG-007 moved less overall at 10 days post-injury and moved more slowly. Over time, sham rats demonstrated greater anxiety, indicated by increased movement and less centre entries. In contrast, rats that received ARG-007 appeared calm and inquisitive – whisking and grooming, not actively searching for an escape route. Visual inspection of movement traces also showed that rats treated with ARG-007 had a reduced tendency to walk close to the walls of the arena (thigmotaxis), potentially indicative of calm and non-anxious behaviour as thigmotaxis is more prominent in stressed and anxious rodents.

CONCLUSIONS

Taken together, this study provides evidence that ARG-007 protects against modTBI-induced weight gain retardation, anxiety-related movement dysfunction, axonal damage, neurodegeneration and neuroinflammation, particularly in the pyramidal tracts, which regulate voluntary movement patterns[5].

[2] Lapinlampi N, Andrade P, Paananen T, Hämäläinen E, Ekolle Ndode-Ekane X, Puhakka N, et al. Postinjury weight rather than cognitive or behavioral impairment predicts development of posttraumatic epilepsy after lateral fluid-percussion injury in rats. Epilepsia. 2020 Sep 1;61(9):2035–52.

[3] Kahriman A, Bouley J, Bosco DA, Salman Shazeeb M, Henninger N. Differential association of baseline body weight and body-weight loss with neurological deficits, histology, and death after repetitive closed head traumatic brain injury. Neurosci Lett. 2022 Feb 6;771:136430.

[4] Yan EB, Hellewell SC, Bellander BM, Agyapomaa DA, Morganti-Kossmann MC. Post-traumatic hypoxia exacerbates neurological deficit, neuroinflammation and cerebral metabolism in rats with diffuse traumatic brain injury. J Neuroinflammation. 2011 Oct 28;8(1):1–16.

[5] Lemon RN, Griffiths J. Comparing the function of the corticospinal system in different species: Organizational differences for motor specialization? Muscle Nerve. 2005 Sep 1;32(3):261–79.