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Research paper

Rotational TBI causes neuronal stress and changes in the monoamine and galanin systems

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ABSTRACT

Traumatic brain injury (TBI) is frequently followed by persistent affective symptoms. Dysregulation of monoaminergic and galanin signalling has been implicated but it is unclear whether such changes generalize across distinct biomechanical injury modes. Here we examined a rotational head-acceleration model of mild–moderate injury and directly compared radioactive in situ hybridization (rISH) with a non-radioactive method, alongside immunohistochemistry (IHC), in adult male rats. We quantified transcripts and proteins/peptides of tyrosine hydroxylase (TH), tryptophan hydroxylase-2 (TPH2), and galanin in locus coeruleus (LC) and dorsal raphe nucleus (DRN) and assessed the neuronal stress marker activating transcription factor-3 (ATF3).

rISH revealed a rapid, bilateral rise of TH and galanin mRNA in LC at one day post-injury (dpi) and transient increases of TPH2 and galanin mRNA at 1 dpi in mid-DRN. Non-radioactive ISH confirmed these patterns, although modest temporal differences were observed. Peptide measurements showed a similar pattern of increase as their transcripts: TH- and galanin-immunoreactivity in LC increased at 3 dpi, and galanin also rose at 7 dpi in DRN, while TPH2 remained stable. Finally, ATF3 was robustly induced in LC neuronal nuclei at 1 dpi and remained elevated thereafter, indicating activation of a conserved stress response and possible axonal injury.

These findings demonstrate that rotational head acceleration triggers selective, time-dependent modulation of monoaminergic and galanin signalling — paralleling prior blast models and confirm ATF3 as an informative marker of injury-activated neuronal states. The concordance across injury models highlights the monoamine and galanin systems as translatable targets for mitigating post-injury affective disturbances.

1. Introduction

Traumatic brain injury (TBI) is a debilitating disease that impacts life quality of millions of people worldwide. TBI exists on a spectrum, ranging from mild to severe, and the most common type is mild TBI (mTBI) (Bruns Jr. and Hauser, 2003). Given the often-lacking structural changes in the brain, hampering real-time diagnosis, mTBI presents a particular challenge to researchers and clinicians (Capizzi et al., 2020). Even when early diagnosis is possible, no effective pharmacological treatments are currently available. Many patients are diagnosed retrospectively, once a range of neuropsychological symptoms have already developed. For some individuals, these symptoms are mild and self-limiting, whereas for others they become chronic, and severely debilitating (Brenner et al., 2009; Vaishnavi et al., 2009; Hoge et al., 2004).

The most frequently reported post-mTBI symptoms are broadly categorized as cognitive (e.g., impaired attention, concentration, or memory), emotional (e.g., mood and anxiety disorders), and physical (e.g., headache, fatigue, or lethargy) (Hoge et al., 2004; Agoston et al., 2019; Galgano et al., 2017; Clark et al., 2022; Schneiderman et al., 2008; Scofield et al., 2017). Together, these are often referred to as post-concussive syndrome (PCS) (Nguyen et al., 2016). Depending on the circumstances of the initial injury, specifically extremely stressful and/or mentally traumatic events, these symptoms may be concomitant with post-traumatic stress disorder (PTSD) (Yehuda et al., 1998; Fann et al., 2004; Simmons and Matthews, 2012).

The aetiologies of anxiety and depression are not fully understood, but dysregulation of monoamine systems is been implicated in the pathophysiology of affective disorders, both related to noradrenaline (NA) (Schildkraut, 1965; Bremner et al., 1996; Goddard et al., 2010) and serotonin (5-hydroxytryptamine, 5-HT) (Maes and Meltzer, 1995; Mann,

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1999) neurons, as well as neuropeptides (Holmes et al., 2003). This is underscored by the partial symptom relief observed in many patients following pharmacological interventions targeting monoamine systems and their receptors (Millan, 2006; Mathew et al., 2008).

We previously examined changes in the NA and 5-HT systems as well as the peptide galanin (Tatemoto et al., 1983) after primary blast exposure using two mild blast TBI (mbTBI) rat models: one with military grade explosives (Kawa et al., 2015; Kawa et al., 2016; Kawa et al., 2020) and one using a compressed-gas shock tube (Kawa et al., 2018). Significant alterations in these systems, such as an immediate rise in levels of tyrosine hydroxylase (TH), the catecholamine synthesis rate-limiting enzyme (Grima et al., 1987; Nagatsu et al., 1964), in the locus coeruleus (LC), elevated NA levels in several forebrain regions, and anxiety-linked increased climbing in the forced swim test were observed (Kawa et al., 2015; Kawa et al., 2016; Kawa et al., 2020). Similarly, transcript levels for TH and tryptophan hydroxylase 2 (TPH2), the rate-limiting enzyme for 5-HT synthesis (Walther et al., 2003), rose transiently in the LC and the dorsal raphe nucleus (DRN), respectively, and this was the case also for galanin in both regions (Kawa et al., 2018).

It remains unclear whether the extensive alterations observed in these systems after blast-induced diffuse mTBI also occur following other forms of TBI. This distinction is important, as the majority of TBIs result from blunt trauma, and most blunt head injuries involve significant rotational forces (Kleiven, 2007). Moreover, most prior studies reporting changes in TH, TPH2, and galanin transcripts used radioactive in situ hybridization (rISH). While sensitive and reliable, this method is now largely replaced by modern non-radioactive approaches such as RNAscope and ViewRNA (Cassidy and Jones, 2014).This shift raises concerns about the comparability of results, as it remains unclear whether findings obtained using radioactive in situ hybridization is reliably replicated with non-radioactive methods, and to what extent data from these different approaches are directly comparable.

We previously developed an experimental TBI model, where the injury is induced by rotational acceleration and shown to cause increased levels of β-APP, COX2 and axonal degeneration following injury (Davidsson and Risling, 2011; Davidsson and Risling, 2019; Antona-Makoshi et al., 2014). The severity of this injury in this model is graded by varying the level of acceleration, and a threshold was identified, at which discrete axonal injuries appear in white matter tracts, accompanied by transient memory deficits (Davidsson and Risling, 2019; Rostami et al., 2012; Risling et al., 2019). This model thus serves as an experimental paradigm for mild-moderate TBI, allowing us to investigate any changes of monoaminergic system to a very frequent TBI. In humans, rapid head rotation results from e.g. traffic accidents, contact sports, and falls. While such events may cause mild-moderate TBI, more forceful accelerations can lead to diffuse axonal injury, vascular rupture, and severe TBI. Traumatic brain injury induces a conserved transcriptional stress response in neurons (Ng and Lee, 2019). For example, activation of transcription factor 3 (ATF3), injury-responsive bZIP transcription factor, increases sharply after CNS trauma (Katz et al., 2022) and serves as a sensitive marker for neuronal stress response and injury.

The primary objective of this study was to investigate the acute to subacute effects of rotational TBI (rTBI) on the expression of TH, TPH2, and galanin. Transcript levels were assessed using ISH and corresponding protein/peptide levels using immunohistochemistry (IHC). To determine whether these effects are conserved across injury models, we compared our findings with previously reported results from mbTBI. Moreover, we directly compared two ISH approaches, radioactive ISH (rISH) and one non-radioactive to evaluate their concordance and sensitivity in detecting changes in transcript levels. Finally, we included ATF3 as a highly conserved immediate early transcription factor that is rapidly induced by neuronal injury and stress and widely used as a bona fide marker of axonal damage and neuronal stress responses (Hunt et al., 2012).

2. Materials and methods

2.1. Animal groups and manipulations

Forty-four adult male Sprague–Dawley rats (Taconic, Ry, Denmark) with a mean body weight of 388 ± 36 g were used in this study: 20 for radioactive ISH and 24 for non-radioactive ISH and IHC. All procedures were conducted in accordance with the Swedish National Guidelines for Animal Experiments and approved by the Stockholm Animal Care and Use Ethics Committee. Animals were housed in groups of three to four per Type IV Makrolon® cage under standardized conditions (12 h light/dark cycle, lights on at 06:00; 22 ± 0.5 °C; 40–50% relative humidity) with food and water available ad libitum.

The rat's skull is exposed, and a metallic plate glued on, which is then attached to a lever. A modified air gun is used to fire a projectile, which once it strikes the lever, results in a combination of rotational and linear acceleration of the rat head in the sagittal plane. In these experiments it is set to induce a mild-moderate rotational traumatic brain injury.

2.2. Injury conditions

The rotational TBI was induced under anaesthesia, using a model (Fig. 1) and the same conditions as described in detail by Davidsson and Risling (Davidsson and Risling, 2011; Davidsson and Risling, 2019) and in previously published papers using this model (Rostami et al., 2012; Losurdo et al., 2020). Briefly, a midline incision was made through the skin and periosteum to expose the frontal, nasal, and parietal bones of the skull vault. The exposed bone was treated with a weak phosphoric acid solution, dried, and gently sanded to prepare the surface. A curved aluminium plate (35 mm long, 2–9 mm wide) was then affixed to the skull using Dental glue (Super-Bond C and B; SunMedical Co., Shiga, Japan). This aluminium plate was connected to a rotating bar, allowing free rotation of the head around its horizontal axis. During the trauma, a striker was propelled by a custom-built air-driven accelerator to impact a rubber block mounted on a striker target connected to the rotating bar. This impact generated an impulse that subjected the rotating bar—and consequently the animal's head—to a rearward rotational acceleration in the sagittal plane, with a peak value of 1,46 ± 0,34 Mrad/s^2, lasting approximately 0.4 ms. The sham animals received identical anaesthesia and surgical procedure as described, but without the rotational head trauma. Experimental time points were chosen to match those used in previously published studies: 1 day, 3 days, and 7 days post-exposure. To reduce the number of animals used, only 1 day and 3 days survival time was used for rISH.

2.3. Tissue collection and sectioning

On the day of sacrifice, animals were anesthetized with isoflurane and administered 1.5–2.0 mL pentobarbital. After decapitation, brains were rapidly removed, frozen on dry ice, and stored at −70 °C until sectioning. Serial coronal sections (14 μm) were cut on a Cryo-Star HM 560 M (MICROM International GmbH, Heidelberg, Germany) at the level of the LC (bregma −10.52 to −9.16 mm) and DRN (bregma −8.30 to −7.30 mm) (Fig. 2G-H), according to reference (Paxinos and Watson, 2007). Three sections were thaw-mounted per slide for both injured and sham animals, with slide selection performed randomly. No macroscopic, nor signs of injury on hematoxylin-eosin stained sections are seen in this injury model (Fig. 2 A-F).

2.4. Radioactive in situ hybridization

Radioactive ISH was performed as previously described in detail (Kawa et al., 2015; Kawa et al., 2016). Three slides per animal were processed. Oligonucleotides complementary to rat TH, TPH2, and galanin mRNA were 3′-end–labeled with [α-ˆ32P] deoxyadenosine 5′-triphosphate (PerkinElmer, Boston, MA) using terminal

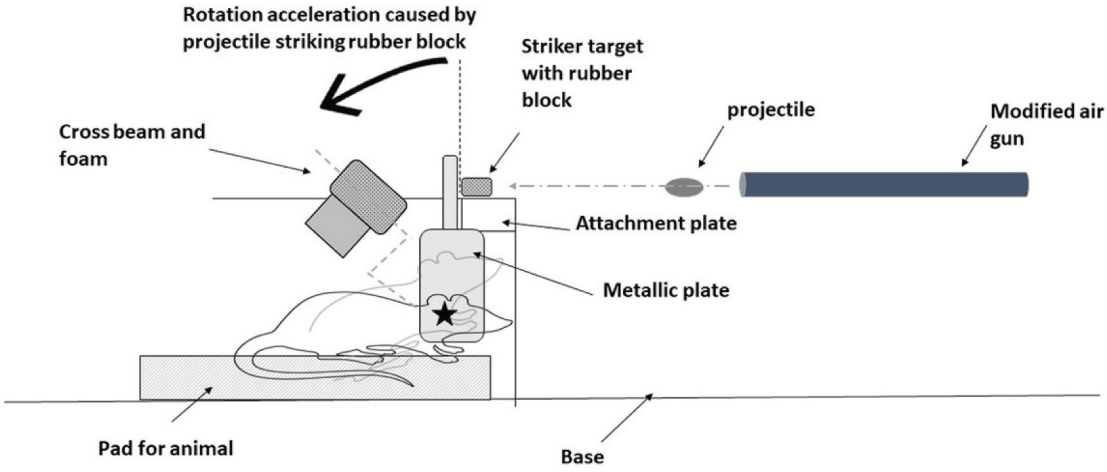

Fig. 1. Schematic drawings showing the rotational traumatic brain injury model for rodents.

deoxynucleotidyl transferase. Optimal exposure time was determined by placing slides on BAS-SR imaging plates (Fujifilm, Tokyo, Japan). Slides were developed with D19 developer and AL-4 fixative (Kodak) and mounted in glycerol-phosphate. Dark-field photomicrographs were captured using a Nikon Eclipse E-600 microscope connected to a Digital Sight U1 camera (Nikon). Primers used for oligo in situ are shown in Supplementary Fig. 1.

2.5. Non-radioactive in situ hybridization

For non-radioactive ISH, the ViewRNA™ Multiplex Assay Kit (QVT4800) was used with a modified protocol. In short, slides were transferred from −80 °C storage into ice-cold 4% formalin and fixed for 19 h at 4 °C, followed by a 1 min PBS wash and 30 min air-dry. Sections were then incubated with protease (1:100 in pre-warmed PBS) for 10 min at 40 °C, washed, post-fixed in 4% formalin (5 min), and hybridized with probes for galanin (VC1–11006-VT), TH (VC6–15462-VC), Tph2 (VC6–3228851-VC), and Ppib (VC4–13411-VC) (1:500, Ppib 1:40) for 2 h at 40 °C. After washing, slides were incubated with Pre-Amplifier, Amplifier, and Label Probe Mixes (types 1, 4, 6; 1:25, 30 min each, 40 °C), counterstained with DAPI (1:100), and mounted with ProLong. Imaging was performed on a Nikon Eclipse Ni-E fluorescent microscope with an Andor Zyla camera using NIS Elements software. For each ROI, defined by the borders of the LC or DRN, 3–4 images were acquired at 10× or 20× magnification with FITC, DAPI, and TexasRed filters using identical settings across groups.

2.6. Immunohistochemistry

IHC was performed on brain sections from the same animals used for non-radioactive ISH. Slides were air-dried for 1 h at room temperature, fixed in 4% formalin for 20 min, and washed 3 × 10 min in PBS. Sections were blocked for 45 min in primary buffer (0.01 M PBS, 0.1% NaN_3, 0.3% Triton X-100, 5% BSA) and incubated with primary antibodies at 4 °C for 24 h. Primary antibodies and dilutions were: galanin (Sigma Merck AB2233, 1:500; Novus Bio NBP1–45217, 1:500), TH (Invitrogen MA124654, 1:2000), TPH2 (Invitrogen MA124654, 1:1000), ATF3 (NovusBio NBP1–85816, 1:200), and NeuN (Sigma MAB377, 1:4000).

After 3 × 10 min PBS washes, slides were incubated for 1 h at room temperature with secondary antibodies (1:500 in secondary buffer: 0.01 M PBS, 0.1% NaN_3, 0.3% Triton): donkey anti-mouse Alexa Fluor 488 (ThermoFisher A10037), donkey anti-rabbit Alexa Fluor 555 (Life Technologies A-31572), and donkey anti-mouse Alexa Fluor 647 (Life Technologies A-31571). Specificity of galanin IHC was confirmed using two antibodies and preabsorption controls with galanin peptide (NBP1–45217) (Supplementary 2). Slides were then washed (3 × 10 min PBS), counterstained with DAPI (5 min), washed again, and mounted with ProLong Diamond Antifade Mountant (Life Technologies P36961). Imaging parameters were similar to those used for non-radioactive ISH.

2.7. Image analysis

All image analyses were performed automatically using a custom-made macro in Fiji v2.14.0 In short, mean integrated intensity of the positive signal within each ROI was used to quantify mRNA and peptide levels. Images of poor quality (e.g., with artifacts, blurring, bubbles, absent staining, or poor tissue integrity) were excluded by a blinded examiner.

2.8. Statistical analysis

Statistical analyses were performed in GraphPad Prism v10 (GraphPad Software, CA). Injured and sham groups were compared using two-way ANOVA, followed by Tukey's post hoc test for multiple comparisons when ANOVA indicated significance. As no left–right differences were found in the LC, mean values of the left LC and right LC were used. For the exposed group, Pearson's correlation was used to assess the relationship between TH mRNA levels and peak rotational acceleration. Data are presented as mean ± SD, with p-values from Tukey's tests reported for significant findings. Significance levels are indicated as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3. Results

3.1. TH exhibit early transcriptional induction followed by delayed peptide elevation in LC

Both radioactive and non-radioactive in situ hybridization revealed a robust and transient increase in TH mRNA expression in the LC at 1 days post injury (dpi) in rTBI animals compared with sham controls ($p < 0.01$; Fig. 3A-B). TH transcript levels returned to baseline by 3 dpi, with no differences detected at later time points.

In contrast to the early mRNA changes, TH peptide levels displayed a delayed temporal profile. Immunohistochemical analysis demonstrated a significant increase in TH immunoreactivity at 3 dpi in rTBI animals relative to sham ($p < 0.01$; Fig. 3C), whereas no differences were observed at 1 or 7 dpi.

3.2. Galanin transcript levels and peptide levels exhibit same elevation-pattern as TH

Radioactive ISH revealed a significant elevation of galanin mRNA in

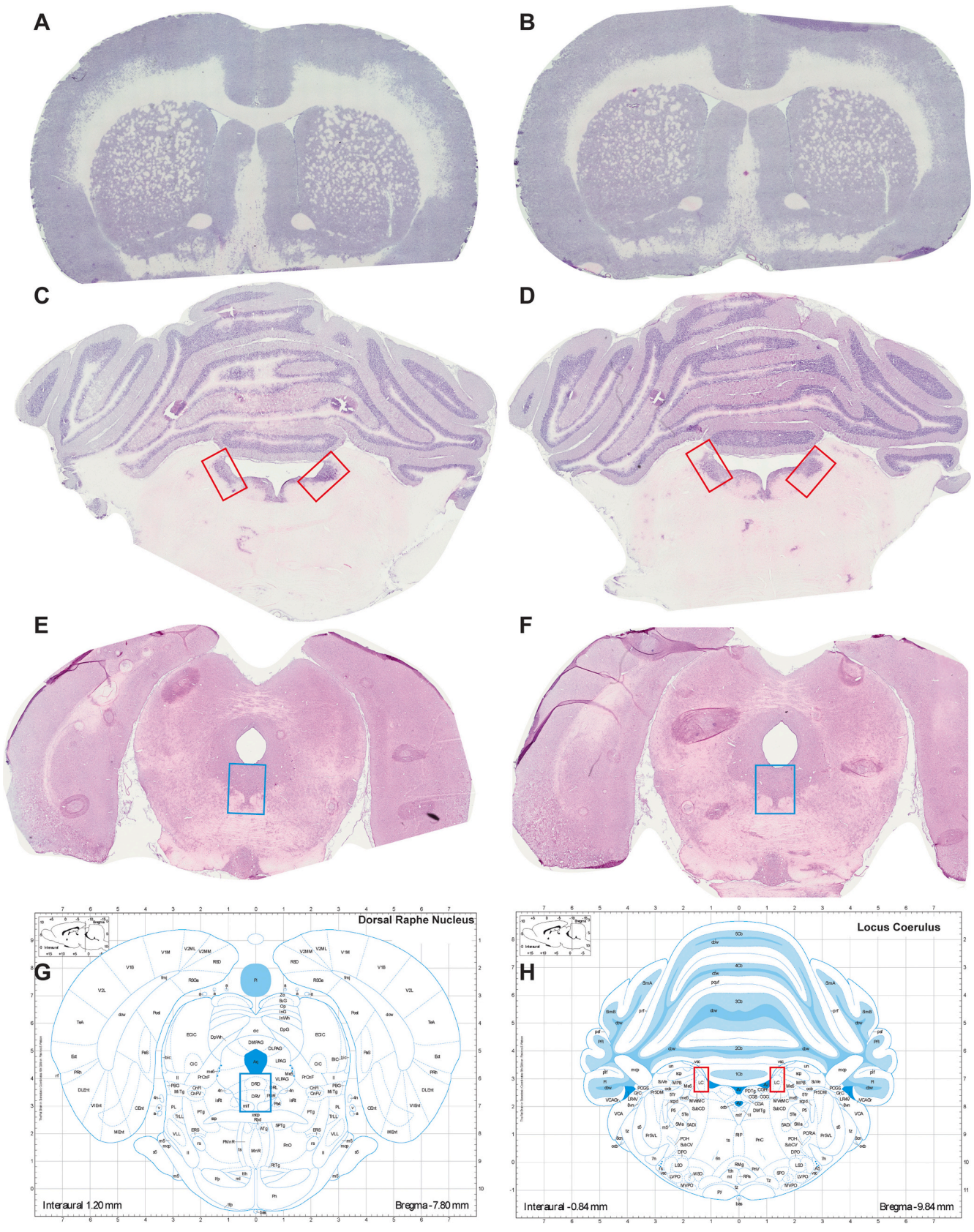

Fig. 2. Hematoxylin-Eosin overview sections.
Representative H&E-stained coronal sections from the cerebrum of sham (A) and rTBI (B) animals at 1 day post-injury (1 DPI). Adjacent sections showing the analyzed locus coeruleus (LC) region in sham (C) and rTBI (D) animals at 1 DPI. Adjacent sections showing the analyzed mid-dorsal raphe nucleus (mDRN) region in sham (E) and rTBI (F) animals at 1 DPI. Atlas reference images indicating the exact coordinates for the dorsal raphe nucleus (DRN) (G) and LC (H) are shown from the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007).

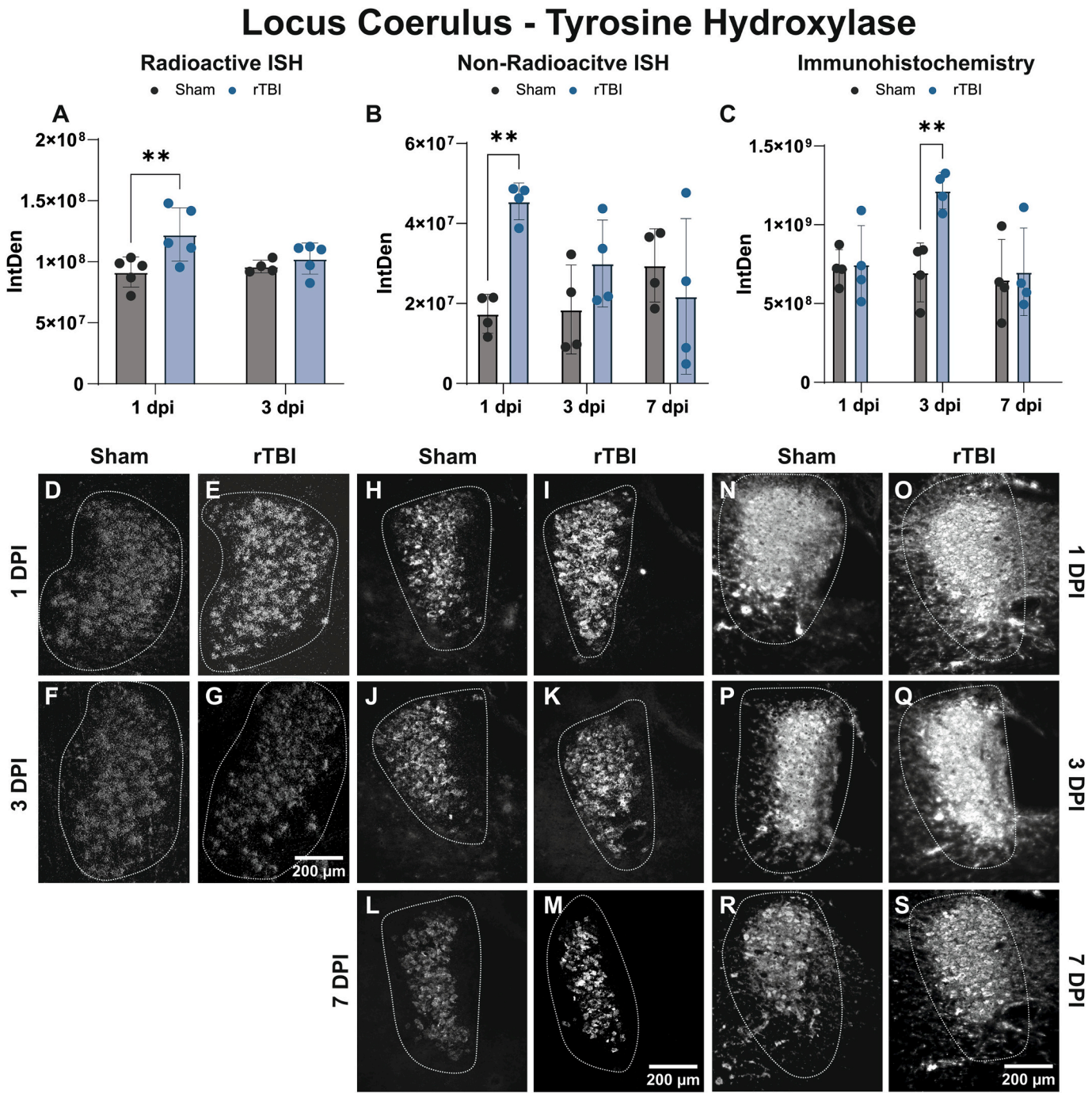

Fig. 3. Tyrosine hydroxylase in LC. (A) Radioactive ISH quantification of TH mRNA (integrated density; IntDen) in LC at 1 and 3 dpi in sham vs rTBI. (B) Non-radioactive ISH quantification of TH mRNA in LC at 1, 3, and 7 dpi. (C) Immunohistochemistry (IHC) quantification of TH immunoreactivity in LC at 1, 3, and 7 dpi. Representative LC images are shown beneath each quantification panel for the corresponding method and time point (radioactive ISH: D–G; non-radioactive ISH: H–M; IHC: N-S). Scale bars are indicated in panels.

the LC at 1 dpi following rTBI ($p < 0.01$), which normalized by 3 dpi (Fig. 4A). Using non-radioactive ISH, galanin transcriptional changes were more prolonged, with significant increases detected at both 1 and 3 dpi ($p < 0.05$), followed by normalization at 7 dpi (Fig. 4B). These results indicate an early galanin transcriptional response in LC, with assay-dependent sensitivity revealing differences in temporal resolution.

At the peptide level, galanin immunoreactivity in the LC was significantly increased at 3 dpi in rTBI animals compared with sham ($p < 0.01$; Fig. 4C). Although levels declined by 7 dpi, values remained slightly elevated relative to sham, suggesting a delayed and partially sustained peptide response. This was confirmed by using an additional galanin antibody which showed significantly higher levels of galanin at 3dpi (Supplementary Fig. 3A). Collectively, LC galanin exhibited a temporal profile closely paralleling TH, characterized by early mRNA induction followed by delayed peptide elevation.

3.3. Transient increase in TPH2 mRNA levels in mDRN

In the mDRN, TPH2 mRNA expression was transiently increased at 1 dpi in rTBI animals relative to sham, as detected by both radioactive and non-radioactive ISH ($p < 0.05$; Fig. 5A,B). By 3 dpi, TPH2 transcript levels had returned to baseline, and no differences were observed at 7

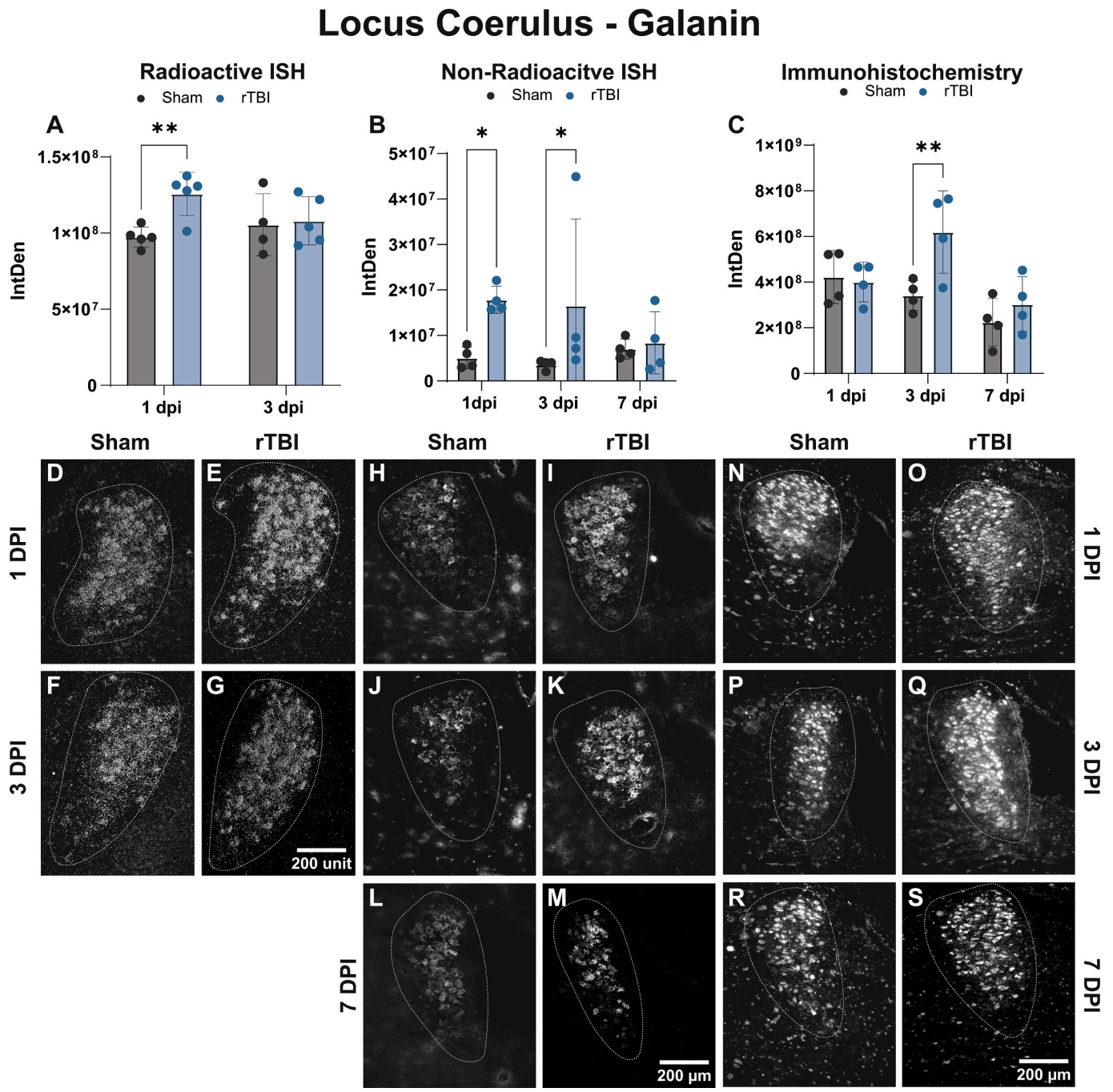

Fig. 4. Galanin in LC. (A) Radioactive ISH quantification of galanin mRNA (IntDen) in LC at 1 and 3 dpi in sham vs rTBI. (B) Non-radioactive ISH quantification of galanin mRNA in LC at 1, 3, and 7 dpi. (C) IHC quantification of galanin immunoreactivity in LC at 1, 3, and 7 dpi. Representative LC images are shown beneath each quantification panel for the corresponding method and time point (radioactive ISH: D–G; non-radioactive ISH: H–M; IHC: N–S).

dpi, indicating a brief injury-induced transcriptional response.

Despite these early mRNA changes, TPH2 immunoreactivity in the mDRN remained unchanged across all examined time points (Fig. 5C).

3.4. Galanin levels increases in mDRN

Radioactive ISH demonstrated a significant increase in galanin mRNA in the mDRN at 1 dpi following rTBI ($p < 0.05$), which was no longer present at 3 dpi (Fig. 6A). In contrast, non-radioactive ISH revealed a significant elevation of galanin mRNA at 7 dpi ($p < 0.05$), while no differences were detected at earlier time points (Fig. 6B), indicating a delayed transcriptional component detectable with this method.

At the peptide level, galanin immunoreactivity in the mDRN showed a trend toward increase at 7 dpi, but this did not reach statistical significance (Fig. 6C). Thus, while galanin transcription in the mDRN exhibits both early and delayed components depending on assay sensitivity, these changes do not translate into robust peptide-level alterations under the present experimental conditions.

3.5. TPH2 and galanin in the rDRN remain unaltered following rTBI

In the rDRN, no significant changes in TPH2 or galanin expression were detected at any time point following rTBI. Both radioactive and

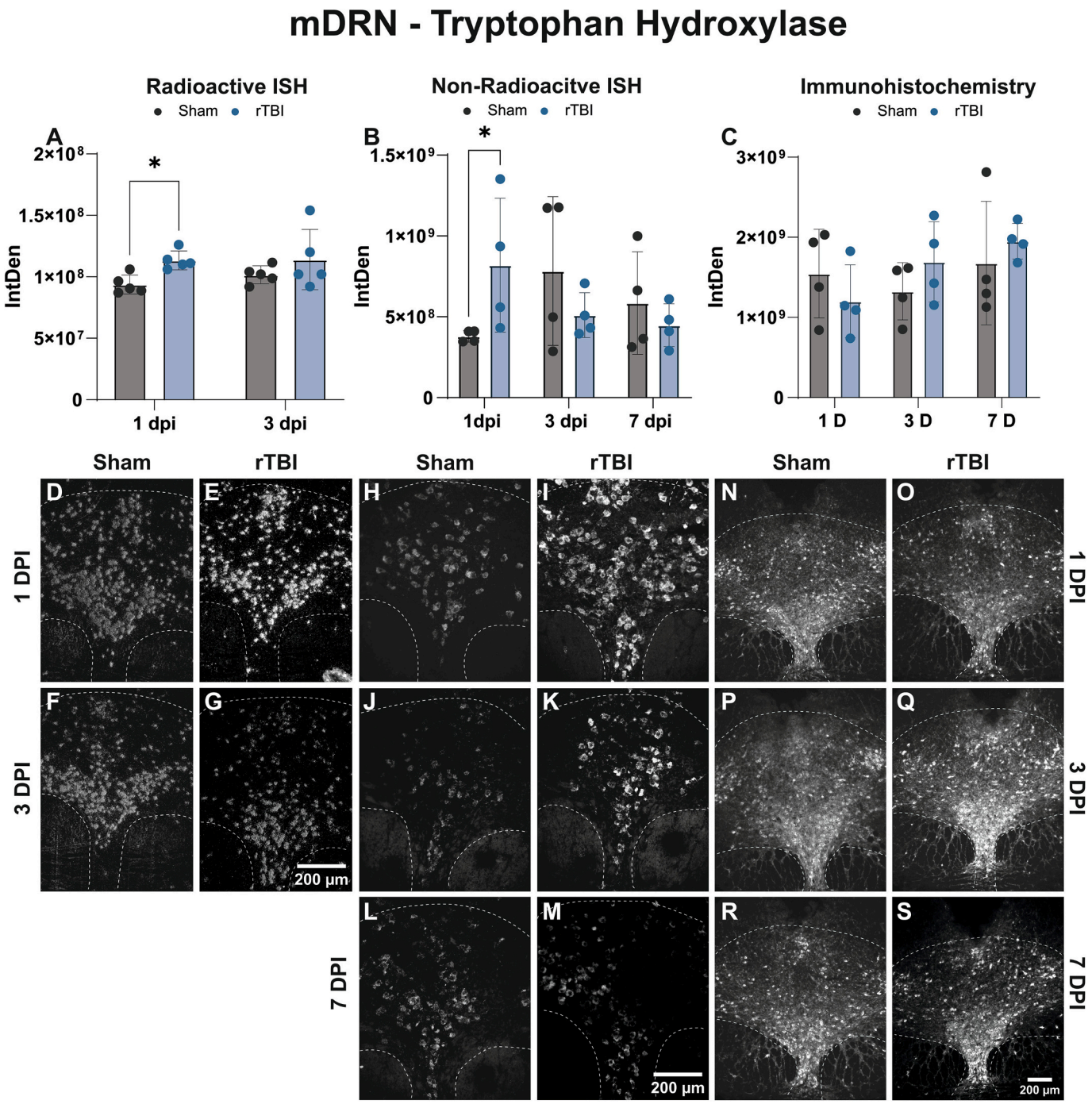

Fig. 5. TPH2 levels in mDRN. (A) Radioactive ISH quantification of TPH2 mRNA (IntDen) in mDRN at 1 and 3 dpi in sham vs rTBI. (B) Non-radioactive ISH quantification of TPH2 mRNA in mDRN at 1, 3, and 7 dpi. (C) IHC quantification of TPH2 immunoreactivity in mDRN at 1, 3, and 7 dpi. Representative mDRN images are shown beneath each quantification panel for the corresponding method and time point (radioactive ISH: D–G; non-radioactive ISH: H–M; IHC: N–S). Scale bars as indicated. Significance markers as shown.

non-radioactive ISH analyses revealed stable transcript levels across 1, 3, and 7 dpi, and immunohistochemical assessment confirmed the absence of peptide-level alterations (Supplementary Fig. 4). These findings indicate a regional specificity of monoaminergic and peptidergic responses to rotational TBI, with the rDRN remaining comparatively resistant.

3.6. *ATF3 levels in LC indicate neuronal stress response following rTBI*

Immunohistochemical analysis revealed a marked increase in ATF3 expression in the LC following rTBI (Fig. 7). Quantification showed a significant elevation at 1 dpi compared with sham controls ($p < 0.001$), indicating a rapid neuronal stress response. Although statistical significance was not maintained at later time points, ATF3 levels remained elevated at 3 and 7 dpi, suggesting a prolonged activation of stress-associated transcriptional pathways in LC neurons after injury.

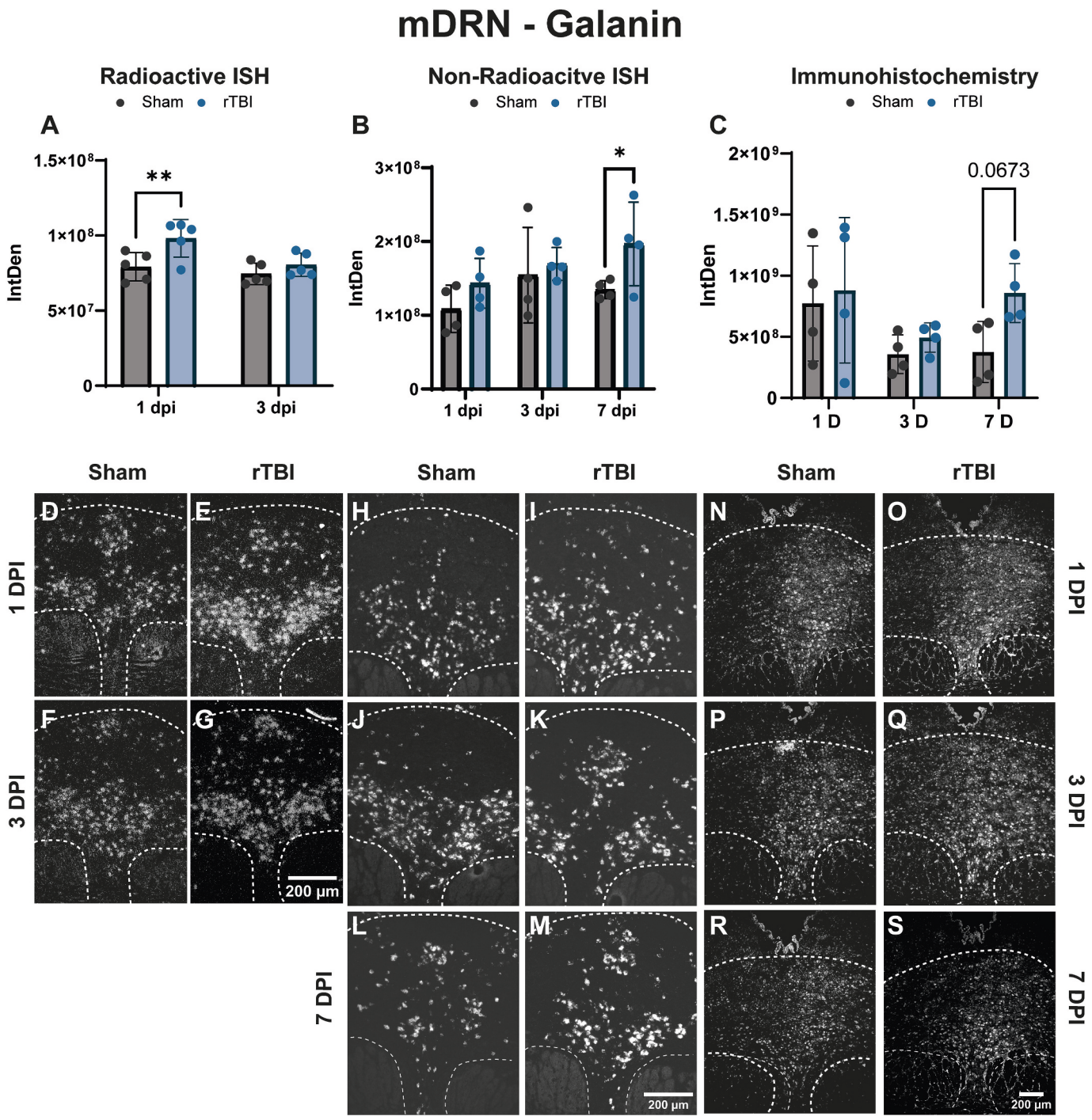

Fig. 6. Galanin levels in mDRN. (A) Radioactive ISH quantification of galanin mRNA (IntDen) in mDRN at 1 and 3 dpi in sham vs rTBI. (B) Non-radioactive ISH quantification of galanin mRNA in mDRN at 1, 3, and 7 dpi. (C) IHC quantification of galanin immunoreactivity in mDRN at 1, 3, and 7 dpi (*p* value/trend indicated in panel). Representative mDRN images are shown beneath each quantification panel for the corresponding method and time point (radioactive ISH: D–G; non-radioactive ISH: H–M; IHC: N–S). Scale bars as indicated. Significance markers as shown.

3.7. Correlations between analyzed markers and peak acceleration values within rTBI

A subanalysis using Pearson's correlation revealed a positive association between peak acceleration (m/s^2) and transcript levels of TH and galanin in the LC (Supplementary Fig. 5C). In contrast, no such relationship was observed for ATF3 in the LC (Supplementary Fig. 5I). In the DRN, TPH2 and galanin transcript levels instead showed a negative correlation with peak acceleration. Given the small group size and the pooling of multiple timepoints in the correlation analyses, these findings should be interpreted cautiously and viewed primarily as indicative of within-group variability potentially driven by differences in peak acceleration. To better visualise these patterns in relation to time-dependent effects, 3D plots for each analyzed marker are provided in Supplementary Figs. 5B, 5D, 5E, and 5H.

4. Discussion

This study provides the first demonstration that mild-to-moderate rotational TBI elicits selective and time-dependent alterations in

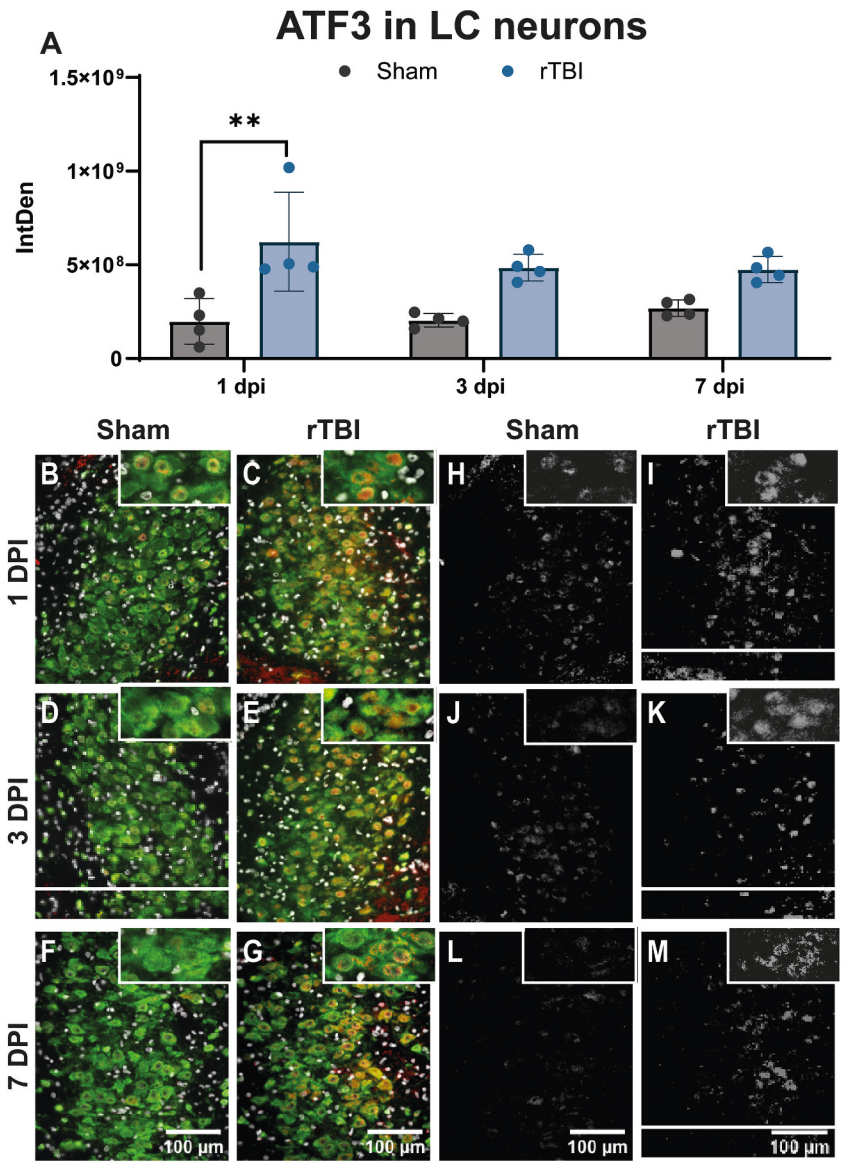

Fig. 7. ATF3 in LC: strong early induction with persistent elevation. (A) Quantification of ATF3 signal (IntDen) in LC across sham and rTBI groups at 1, 3, and 7 dpi. (B–M) Representative LC images across time points and conditions illustrating ATF3 signal; insets show higher-magnification examples as displayed. Scale bars as indicated. Significance markers as shown.

monoaminergic and galanin signalling in the brainstem. We show that TH and galanin transcripts in the LC are transiently upregulated at 1 dpi, accompanied by delayed increases in their peptides. These transcriptional responses in the LC correlated with injury acceleration, directly linking molecular changes to biomechanical severity and were paralleled by a robust induction of ATF3, marking neuronal stress, and possibly axonal damage. In the mDRN, TPH2 show only transient increase with no detectable changes at the peptide level as assessed by immunohistochemistry. Similarly, galanin transcript levels were elevated, although detected at different time points when assessed by non-radioactive ISH versus radioactive ISH, with no corresponding changes observed at the peptide level. Taken together, the two ISH approaches were largely concordant in detecting early, transient increases in TH, TPH2, and galanin transcripts after rTBI. However, non-radioactive ISH revealed modest temporal extensions of these responses, such as persistent galanin expression in the LC at 3 dpi. In the mDRN, non-radioactive ISH also resolved a delayed galanin peak at 7 dpi, which could not be assessed by rISH since this time point was not included in the radioactive series. Thus, the apparent discrepancies mainly reflect the extended temporal sampling and potentially higher sensitivity of non-radioactive ISH, while both methods consistently captured the principal early injury-induced transcriptional dynamics. No significant effects were observed in the rDRN. Together, these findings establish rotational TBI as a previously unexplored mechanism driving conserved, but distinct neurochemical adaptations, closely paralleling those observed after blast injury, and point to a common molecular pathway that may contribute to post-injury affective and behavioral disturbances.

4.1. Comparing effects of rotational TBI and mild blast TBI on galanin, TH and Tph2

Apart from temporal differences, our findings are consistent with previous reports of transcriptional changes in monoamine- and galanin-related genes in the lower brainstem following blast mTBI (Table 1). This convergence across distinct injury paradigms strengthens the evidence that the noradrenergic and galanin systems are core components of the molecular response to TBI, with at least part of this involvement

Table 1
Comparison of observed changes in gal, TH and TPH2 levels post injury in rotational TBI and blast TBI. Summary of changes for TH, GAL and TPH2 in LC and DRN (middle and rostral subdivisions) at 1-, 3-, and 7-days post-injury. Blast TBI data (left columns) are derived from previously published studies (Kawa et al., 2016; Kawa et al., 2018), whereas rotational TBI data (right columns) represent findings from the current model using rISH, non-radioactive ISH and IHC. Red arrows indicate significant increases relative to sham controls; "–" indicates no significant change. Blast exposure produced robust early (1–3 d) upregulation of TH, TPH2, and GAL in both LC and mDRN, whereas the rotational model showed a more transient and restricted increase, primarily in TH and TPH2 expression at 1–3 d post-injury. No significant changes were observed in the rostral rDR for either injury paradigm.

	Blast TBI (radioactive ISH)			Rotational TBI (radioactive ISH)		Rotational TBI ViewRNA			Rotational TBI (IHC)		
	1d	*3d*	*7d*	*1d*	*3d*	*1d*	*3d*	*7d*	*1d*	*3d*	*7d*
LC											
TH	↑	↑	-	↑	-	↑	-	-	-	↑	-
Galanin	↑	↑	↑	↑	-	↑	↑	-	-	↑	-
DRM (mid/caudal)											
TPH2	↑	-	-	↑	-	↑	-	-	-	-	-
Galanin	↑	↑	↑	↑	-	-	-	↑	-	-	-
DRR (rostral)											
TPH2	-	-	-	-	-	-	-	-	-	-	-
Galanin	-	↑	-	-	-	-	-	-	-	-	-

conserved across different models of traumatic injury.

The bTBI and rTBI models share certain biomechanical features but also differ in important respects. Different parts of the brain move differently, and one important factor is the distance to the axis of rotation (Davidsson and Risling, 2011; Antona-Makoshi et al., 2014). Another factor to consider is the greater complexity of the sham procedures in the rotational TBI model, which involve multiple steps within a surgical intervention, whereas the sham condition in the blast TBI model consists only of an anaesthesia session. This disparity may contribute to elevated baseline transcript levels in sham animals of the rotational model, thereby attenuating the relative differences observed between rTBI and sham compared to those between bTBI and sham.

In our previous blast studies, the primary shock wave had a duration of <0.5 ms and induced a comparatively slow lateral head rotation that did not produce overt signs of axonal injury. By contrast, the rotational TBI model produces a brief but rapid head movement, during which the brain lags slightly behind the skull. This relative motion between brain tissue and bone leads to axonal injury (Antona-Makoshi et al., 2014) and an increase in axon related biomarkers has previously been reported in this model of rotational TBI (Rostami et al., 2012; Losurdo et al., 2020). In line with this, the present study shows a significant increase in ATF3 expression at 1 dpi, coinciding with the timepoint when most transcripts are affected, which remains elevated at all subsequent timepoints. This is consistent with prior reports of rapid ATF3 induction after TBI, where its deficiency exacerbates inflammation and motor deficits (Forstner et al., 2018). Moreover, recent lineage-tracing work showed that ATF3 marks stressed cortical neurons (Alkaslasi et al., 2025), suggesting that ATF3 functions as a general stress sensor rather than a deterministic death signal. Our findings therefore support the notion that ATF3 is a conserved injury-responsive transcription factor in the nervous system (Katz et al., 2022; Hunt et al., 2012) which may contribute both to neuronal adaptation and to modulation of post-injury inflammation (Ma et al., 2022).

Thus, changes in transcripts for monoamines and galanin may represent a general response to TBI (O'Connell et al., 2024a) (Zhuang et al., 2025), independent of the specific mechanism of head impact. These findings, which are consistent across various TBIs models (rotational, blast, shock tube and cortical impact, closed head injury), across sexes, and following repeated exposures (Kawa et al., 2015; Kawa et al., 2018; Zhuang et al., 2025; Kawa et al., 2016; O'Connell et al., 2024b; Yan et al., 2001), may have important implications and provide further impetus for exploring pharmacological strategies aimed at alleviating PCS.

4.2. *The locus coeruleus and dorsal raphe nuclei*

These two brain nuclei have received increasing interest over the last decades because of their signalling via noradrenaline and serotonin, respectively. The LC is a compact nucleus expressing noradrenaline (Dahlstroem and Fuxe, 1964; Moore and Bloom, 1979; Ungerstedt, 1971) and has a complex structure, built up of modules with specific afferents, and efferents as well as functions (Aston-Jones and Cohen, 2005; Chandler et al., 2019; Poe et al., 2020). This modular organization, together with its widespread efferent projections, may contribute to the strong and transient transcriptional activation of TH and galanin we observed in the LC following rTBI, as even subtle perturbations may engage a large portion of the network. The DRN is a more complex structure than LC: it is part of the midbrain periaqueductal gray organized in longitudinal columns stretching over considerable distances, whereby DRN is in the ventral midline, the median raphe nucleus being its ventral extension (Keay and Bandler, 2004). The DRN can be subdivided into subnuclei according rostrocaudal localizations, as middle DRN (mDRN) and rostral DRN (rDRN). Apart from the well-established serotonin populations of the dorsal raphe complex (Dahlstroem and Fuxe, 1964; Steinbusch, 1981; Jacobs and Azmitia, 1992), there are other transmitter-specific subpopulations with specific localizations in the dorsal raphe region, also characterized by different neuropeptides (Sutin and Jacobowitz, 1988; Van den Bergh et al., 1988; Smith et al., 1994; Xu et al., 1998a; Fu et al., 2010). This cellular and anatomical heterogeneity may explain why rTBI induced only modest and regionally restricted changes in TPH2 and galanin transcripts in the DRN compared with the more uniform and consistent response seen in the LC.

4.3. *The monoamines and their synthesizing enzymes*

We have previously shown that exposure of rats to a single mild blast alters classical noradrenergic and serotonergic neurotransmitter systems in forebrain regions, as well as their rate-limiting enzymes in the lower brainstem. Also in other animal models, elevated transcript levels of TH and TPH2 have been reported following exposure to a variety of stressors (George et al., 2013; Chamas et al., 2004; Evans et al., 2009; Ong et al., 2014; Pavcovich et al., 1990).

This surge in neurotransmitter release, followed by compensatory re-synthesis, could explain the observed increases in transcript levels of the biosynthetic enzymes and the concomitant decreases in metabolite levels, as cells attempt to replenish depleted stores. Indeed, the sudden and potentially traumatic nature of TBIs, even in the absence of overt structural injury, can precipitate acute anxiety and contribute to sustained emotional impairment (Bryant et al., 2010).

Together, these data suggest that even mild rotational forces can trigger stress-related activation of monoaminergic systems, producing transient transcriptional adaptations that may set the stage for longer-term behavioral consequences. However, these effects were not uniformly expressed across brainstem nuclei.

Changes in the DRN are more limited in the rotational model. However, this is consistent with findings from other stress paradigms, indicating that the serotonergic system is generally less sensitive to stress than the noradrenergic system (Kuteeva et al., 2008; Landgraf and Neumann, 2004; Wilkinson and Jacobs, 1988). Although we previously observed an early and transient increase in TPH2 transcript levels in the blast model, and a more pronounced increase in female rats with the same model (Kawa et al., 2020), it remains to be determined whether similar responses occur in females following rotational injury.

4.4. Galanin

Galanin is a 29 (30 in humans) amino acid peptide (Tatemoto et al., 1983) acting through three receptors, GalR1–3 (Lang et al., 2015). Electrophysiological studies indicate that galanin exerts predominantly inhibitory modulatory effects on both NA and 5-HT systems (Xu et al., 1998b). Galanin has been implicated in mood disorders (Holmes et al., 2003; Kuteeva et al., 2008; Fuxe et al., 1991; Khoshbouei et al., 2002; Weiss et al., 1998; Lu et al., 2005; Weinshenker and Holmes, 2016; Hokfelt et al., 2018), in part due to its extensive co-localization with noradrenergic and serotonergic neurons in the rat LC and DRN, respectively (Melander et al., 1986).

Changes in galanin transcripts also appear across both TBI models, although they are more modest and transient in the rotational model compared with the blast model. Interestingly, this increase was more widespread in the bTBI model, extending across both the mid/caudal and rostral DRN. Experimental evidence demonstrates that stress can alter galanin expression, its transcript and peptide levels, and those of its receptors (Sweerts et al., 1999; Sweerts et al., 2000). In fact, changes in the galanin system were reported in a rat model of PTSD (Barnabas et al., 2016).

4.5. Mood and anxiety disorders

TBI is highly prevalent in society and remarkably heterogeneous, with outcomes ranging from mild to severe (Goldstein et al., 2010). The initial 'primary injury,' lasting only a few milliseconds, initiates a cascade of secondary injury processes and divergent recovery pathways that may persist for hours, days, or even a lifetime (Giza and Hovda, 2001). Development and persistence of PCS in an estimated 15–30% of affected individuals may result from metabolic and structural pathways initiated during the recovery phase that fail to return the brain to homeostasis (Gysland et al., 2012). In previous studies, we detected signs of increased anxiety like behavior in rats that had an increase in transcript levels of TH, galanin and TPH2 following bTBI (Kawa et al., 2015). Similar findings have been reported by other groups, highlighting the monoaminergic system as a mediator in development of anxiety like behavior in rats following TBI (Tsuda et al., 2025; Donner et al., 2012; Tsuda et al., 2020). While most of these studies have employed non-rotational injury models, the present data demonstrate that the mood-regulatory monoaminergic and galanin systems are also transiently affected following rTBI, supporting the notion that rotational forces alone are sufficient to engage these stress-related neurochemical pathways.

4.6. Limitations / potential next steps

The present findings add novel data to an important gap in TBI research, where molecular and behavioral outcomes frequently diverge across models, species, and time points. Several limitations should be noted. These experiments were intended as an initial step and were therefore mainly deals with transcript levels of selected biosynthetic enzymes and one neuropeptide during the acute phase, up to three days post-injury. Future studies should extend these analyses to protein expression as well as to monoamines and their metabolites, as previously examined in the blast model (Kawa et al., 2015; Kawa et al., 2016; Kawa et al., 2020; Kawa et al., 2018; Davidsson et al., 2019), and evaluate trajectories over longer post-injury intervals.

Moreover, given our prior observation of more pronounced alterations in female rats relative to males—specifically in TPH2 transcript levels within the DRN—it will be important to determine whether similar sex-specific responses occur in this model. This is particularly relevant considering the limited changes observed in males exposed to the rotational model. Extending the analysis to forebrain regions innervated by these brainstem systems, and incorporating assessments of functional outcomes, will also be essential, as behavioral phenotypes do not necessarily align with molecular alterations.

Some studies are now attempting to separate animals based on 'low' and 'high' responses to stress, for example immobility in the forced swim test (Barnabas et al., 2016) or through selective breeding for these traits (Vanderheyden et al., 2021). As in humans, not all animals respond uniformly to stress and/or injury; thus, heterogeneity in animal responses may partly explain the divergence in findings across studies. Importantly, the number of experimental rTBI models are few, and most experimental studies are not studying rTBI due to many challenges in modelling rTBI (Freeman-Jones et al., 2023; Davidsson and Risling, 2016). To our study is, to our knowledge, the first to show that mild-to-moderate rTBI elicits rapid, transient transcriptional changes in monoaminergic and galanin systems in the brainstem, and to directly compare two ISH approaches within the same model. By also linking molecular responses to biomechanical severity and incorporating ATF3 as a marker of neuronal stress, these findings provide a unique bridge between mechanistic injury parameters and neurochemical outcomes.

4.7. Concluding remarks

A wide range of experimental models has been developed to advance understanding of TBI pathophysiology, with the overarching goal of informing interventions that can mitigate the considerable social and economic burden of this condition. Replication of findings across models, species, and sexes remains essential to establish generalizable mechanisms and to contextualize the translational relevance of preclinical research.

In the present study, we demonstrate that despite marked differences in injury mechanics, including the introduction of a novel rotational paradigm, both models elicited comparable changes in monoamine- and galanin-related transcripts. The ability of the rotational model to reproduce molecular responses previously observed in more established paradigms underscores its utility and strengthens confidence in its translational value. These convergent findings reinforce prior observations across experimental systems, sexes, and post-injury intervals, and further highlight the robustness of these neurochemical alterations. Importantly, the data implicate monoaminergic and galanin-related signalling pathways as consistently responsive to TBI during the acute to subacute phase. Future studies integrating behavioral outcomes and longer post-injury intervals will be necessary to determine the functional relevance of these molecular changes and their potential involvement in persistent post-concussive symptoms.

CRediT authorship contribution statement

Zabih Aurfan: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lizan Kawa:** Methodology. **Johan Davidsson:** Writing – review & editing, Methodology. **Linda Karlsson:** Methodology. **Sebastian Thams:** Writing – review & editing. **Mattias Günther:** Writing – review & editing, Funding acquisition. **Tomas Hökfelt:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Mårten Risling:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

All experimental procedures were conducted in accordance with the Swedish National Guidelines for Animal Experiments and were approved by the Stockholm Animal Care and Use Ethics Committee (Stockholm, Norra Djurförsöksetiska Nämnd). Ethical permit numbers: NG22/10, N248/11, and N81/13.

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Declaration of competing interest

The authors declare no conflict of interest, except that T.H. has shares in Bioarctic and Lundbeck.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.expneurol.2026.115711.

Data availability

Data will be made available on request.

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