ORIGINAL ARTICLE

CONTENTS

Carbacetam attenuates biphasic neuroendocrine dysregulation and improves neurobehavioral recovery after closedhead traumatic brain injury in rats

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ABSTRACT

Aim: To elucidate neurohormonal mechanisms underlying the development of higher-order behavioral and cognitive disturbances after traumatic brain injury and to substantiate a pathogenetic approach to their correction using carbacetam.

Materials and Methods: We examined neuroendocrine dynamics after closedhead TBI in rats and assessed pathogenetically oriented correction with carbacetam – an endogenous modulator of the Type-A γ-aminobutyric acid (GABA-A)-benzodiazepine receptor complex – compared with deproteinized calf blood hemoderivative (DCBH). One hundred seventy adult male outbred rats were used.

Results: TBI caused a 5.4-5.9fold rise in neurological deficit on Day 1 with incomplete spontaneous recovery (still 3.4fold above intact on Day 7). Carbacetam accelerated recovery (57% reduction by Day 7 vs 43% in vehicle; $p < 0.05$) and improved orienting-exploratory behavior and eightarm maze performance; DCBH improved most behavioral outcomes but was less effective for exploratory activity. Endocrine profiling revealed an acute hyperreactive response (ACTH, corticosterone and vasopressin surges on Day 1) followed by delayed central depletion at Day 14 and persistent suppression of growth hormone (to 48% of intact at Day 30; $p < 0.05$).

Conclusions: Carbacetam attenuated early hyperactivation, prevented delayed depletion and preserved somatotropic signaling, whereas DCBH primarily mitigated the delayed depletion phase. Mortality was 50% in vehicle, 16% with carbacetam and 36% with DCBH ($p = 0.002$). Immunohistochemistry on Day 7 showed reduced GFAP/S100 and increased NSE under carbacetam, consistent with attenuation of degenerative changes and preservation of neuronal metabolic activity.

KEYWORDS: Brain injuries, traumatic, cognitive dysfunction, hypothalamo-hypophyseal system, pituitary-adrenal system, vasopressins, carbolines, receptors, GABA-A, carbacetam

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INTRODUCTION

Traumatic brain injury (TBI) is one of the most prevalent and consequential neurological conditions worldwide and remains a major driver of premature mortality and long-term disability. Contemporary syntheses emphasize that TBI should be viewed not only as an acute event but often as a chronic disorder with persistent neurocognitive and neuropsychiatric sequelae, substantial societal costs, and major unmet needs in follow-up care and rehabilitation [1, 2].

The burden of TBI is further amplified in settings of armed conflict, where blast exposure, high-energy mechanisms, and polytrauma increase both the incidence and the complexity of post-traumatic brain disorders. Since 2014 – and especially following the large-scale escalation of the war in Ukraine in 2022 – health systems have faced sustained demand for trauma care and rehabilitation, including for head injuries among both military personnel and civilians. In this context, blast-related TBI is of particular concern because it may be accompanied by long-term cognitive and neuropsychiatric consequences that can remain under-recognized in the acute phase amid competing injuries and resource constraints [3, 4].

Closed (non-penetrating) TBI constitutes the dominant phenotype in civilian practice and a frequent component of blast-related trauma. A key clinical challenge is the high prevalence and heterogeneity of persistent cognitive dysfunction after TBI, spanning processing speed, memory, and executive control, and only partly explained by conventional measures of injury severity. Large prospective cohorts demonstrate that, even at 6 months after injury, distinct profiles of objective cognitive impairment are common across TBI severities, underscoring the need to identify biological determinants of divergent recovery trajectories and treatment responsiveness [5].

Among the biological systems that remain insufficiently integrated into mechanistic models of cognitive recovery is neuroendocrine regulation. The hypothalamic-pituitary region is vulnerable to mechanical and vascular injury, and TBI can lead to acute and chronic disturbances of pituitary-dependent hormonal systems (including hypothalamic-pituitary-adrenal axis dysregulation) that may adversely affect metabolism, mood, and cognition – domains that critically shape functional outcomes and rehabilitation potential [6].

Despite advances in neurocritical care and rehabilitation, no disease-modifying pharmacotherapy has been approved to prevent or reverse the complex secondary injury cascades underlying long-term cognitive deficits after TBI. The persistent translational gap – including the repeated failure of Phase III neuroprotection trials – supports the need for mechanistically grounded, multi-target approaches that engage both neuronal network dysfunction and systemic stress responses [1, 7]. In this regard, inhibitory neurotransmission – particularly γ-aminobutyric acid (GABA) signaling – has been highlighted as a plausible nexus linking network-level dysfunction to neurohormonal regulation after TBI. Recent analyses argue that rationally designed GABA-A receptor modulators may represent an underexploited therapeutic direction, provided that limitations of classical benzodiazepine-site agents (e.g., sedation and cognitive adverse effects) are addressed by improved pharmacological profiles and targeted use [8].

Carbacetam is an endogenous modulator of the GABA-benzodiazepine receptor complex. Preclinical evidence suggests that carbacetam may attenuate neurodestructive processes after experimental TBI, supporting further investigation of its pathogenetically oriented potential to influence neurohormonal responses and higher-order behavioral/cognitive outcomes after closed TBI [9].

AIM

To elucidate neurohormonal mechanisms underlying the development of higher-order behavioral and cognitive disturbances after traumatic brain injury and to substantiate a pathogenetic approach to their correction using carbacetam.

MATERIALS AND METHODS

ANIMALS AND ETHICAL APPROVAL

The study was conducted in adult male outbred (non-inbred) white rats (age: 6 months; body mass: 190-210 g). Animals were housed under standard vivarium conditions (temperature 18-22 °C) with *ad libitum* access to food and water and were acclimatized for 2 weeks before experimental procedures. To reduce circadian variability, handling and all functional assessments were performed in the morning (08:00-10:00).

All animal procedures were conducted in accordance with the "General Ethical Principles of Experiments on Animals" (Ukraine, 2001), consistent with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Law of Ukraine No. 3447IV dated 21.02.2006 "On Protection of Animals from Cruelty," Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 dated 01.03.2012 "Procedure for Conducting Experiments on Animals by Scientific Institutions," and Law of Ukraine "On Protection of Animals from Cruelty" No. 440IX dated 14.01.2020. Approval for the study was obtained from the Commission on Bioethics and Academic Integrity of O.O. Bogomolets National Medical University (Kyiv, Ukraine).

STUDY DESIGN, GROUPS, AND TIMELINE

A total of 170 rats were used. Closed-head traumatic brain injury (TBI) was induced in 150 animals, which were allocated to three parallel experimental groups (n = 50 per group): Carbacetam (experimental group 1); Deproteinized calf blood hemoderivative (DCBH; experimental group 2); Vehicle (0.9% NaCl; comparison group). In addition, 15 sham-injured animals underwent identical handling and procedures without the impact, and 5 intact animals served as reference controls for baseline functional values (Table 1). Functional outcomes were assessed longitudinally at predefined time points after injury. Mortality was recorded throughout follow-up.

CLOSED-HEAD TBI MODEL

Closed-head TBI was induced using a weight-drop paradigm in which a 66.7 g weight impacted the head fixed in a holder, producing an impact energy of 0.425 J and resulting in a moderate-severity injury. In previous

Table 1. Experimental groups, interventions, and sample sizes

Group	Injury status	Treatment	Dose and regimen	Allocated n
Intact reference	No TBI	None	–	5
Sham-injured	Sham (no impact)	Vehicle	0.9% NaCl, i.p.	15
Vehicle (comparison)	Closed-head TBI	Vehicle	0.9% NaCl, 1 mL i.p., once daily for 10 days	50
Carbacetam	Closed-head TBI	Carbacetam	5 mg/kg i.p., once daily for 10 days	50
Deproteinized calf blood hemoderivative (DCBH)	Closed-head TBI	DCBH	16.5 mg/kg i.p., once daily for 10 days	50
Total				**170**

Note: Closed-head TBI was induced in 150 animals (three parallel treatment arms; n=50 per arm). Additional controls comprised sham-injured animals (n=15) and intact reference animals (n=5). Longitudinal neurological and behavioral assessments were performed at predefined post-procedure time points; due to mortality and scheduled terminal sampling for endocrine analyses, the number of animals contributing to later time points may vary (exact n is reported alongside Results where applicable).
Source: compiled by the authors of this study

calibrations of this model, this injury severity is associated with 30-50% mortality. Sham-injured animals underwent the same procedure sequence without delivery of the impact.

DRUG ADMINISTRATION

Carbacetam (an endogenous modulator of the GABA-benzodiazepine receptor complex; β-carboline derivative) was administered intraperitoneally (i.p.) at 5 mg/kg once daily for 10 consecutive days after TBI (in 0.9% NaCl; injection volume 1 mL per animal). The comparator drug was deproteinized calf blood hemoderivative (Actovegin®, Nycomed, Switzerland), administered i.p. at 16.5 mg/kg once daily for 10 consecutive days after TBI (in 0.9% NaCl; injection volume 1 mL per animal). Vehicle-treated animals received 1 mL i.p. 0.9% NaCl on the same schedule (Table 1).

NEUROLOGICAL ASSESSMENT

Neurological deficit was assessed using a 100-point neurological deficit score structured across five domains: consciousness (20 points), motor function (30), cranial nerve function (20), sensorimotor integration (20), and autonomic signs (10). Each component included predefined operational criteria. Higher total scores indicated more severe neurological deficit. Neurological scoring was performed at baseline (pre-injury) and at prespecified post-injury time points (3 h, days 1, 3, 7, 14, and 30). Assessments were performed at the same time each day by a blinded investigator. Inter-rater reliability was tested in a subset of animals (ICC >0.85). All animals were randomized using a computer-generated sequence.

BEHAVIORAL TESTING

Animals were habituated to the experimental environment and test handling prior to injury (≥10 habituation sessions). Behavioral testing was performed at the same predefined time points as neurological scoring.

Open-field test was used to quantify spontaneous locomotion and exploratory activity, recorded as number of squares crossed and number of holes explored. Light-dark transition/"burrow reflex" latency (latency to enter the dark compartment from the light compartment) was recorded: longer latency interpreted as altered avoidance/anxiety-like behavior and/or impaired adaptive responding, depending on the overall behavioral context. Eight-arm radial maze was used to assess spatial learning and memory. The primary recorded endpoint was the number of correct entries into baited arms without repetition, reflecting working memory performance and task acquisition.

BLOOD SAMPLING AND HORMONE MEASUREMENTS

Endocrine measurements were performed in pre-specified subsets of animals withdrawn at each time point. On post-injury days 1, 3, 7, 14, and 30, five animals per group per time point were euthanized for blood sampling (total n=75 across the three TBI treatment groups). Animals were anesthetized with ether and euthanized by decapitation; trunk blood was collected into pre-labelled tubes containing Na-EDTA anticoagulant.

Plasma hormone concentrations were determined by ELISA using commercial rat-specific assay kits for: adrenocorticotropic hormone (ACTH), corticosterone, vasopressin, and growth hormone.

Absorbance was measured using a microplate reader (RT2100C; Rayto Life and Analytical Sciences, China) and concentrations were calculated according to kit protocols.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

For morphological and immunohistochemical analyses, brains were collected on post-injury day 7 from a subset of animals in the carbacetam group (n=5) and the vehicle comparison group (n=5) after deep thiopental anesthesia (50 mg/kg). Brain regions of interest included the hypothalamus (supraoptic and paraventricular nuclei) and hippocampus (CA3 region). Tissues were fixed, processed routinely, paraffin embedded, and sectioned at 3-4 μm. Hematoxylin and eosin staining was used for general morphology.

For immunohistochemistry, sections were treated with proteinase K (DAKO) and incubated with primary antibodies against: S100 (code Z0311), neuron-specific enolase (NSE) (code N1557), glial fibrillary acidic protein (GFAP) (code IS524).

Signal detection used a polymer HRP/DAB system. Slides were evaluated by light microscopy with digital image acquisition.

STATISTICAL ANALYSIS

Statistical processing was performed using licensed software (SPSS 11.0, MedStat, and MedCalc). Data are presented as mean ± SEM unless stated otherwise. Prior to inferential testing, distributions were evaluated using the Shapiro–Wilk test and Q–Q plots; homogeneity of variance was examined using Levene's test. All tests were twosided, and statistical significance was set at $p < 0.05$.

Relative values were calculated as follows: % of intact reference = (individual value / mean intact value) × 100; % of sham (for behavioral outcomes) = (individual value / timematched sham mean) × 100; % of Day 1 = (value at a given time point / value on Day 1) × 100; % of previous measurement = (value at a given time point / value at the preceding time point) × 100. Intact reference values were obtained from a separate intact cohort (n = 5) and were used as a timeinvariant baseline for normalization and "vs intact" comparisons throughout the study.

Cumulative mortality across injured groups was compared using the χ^2 test of independence (or Fisher's exact test when expected cell counts were <5). Where pairwise comparisons were performed, pvalues were adjusted for multiple testing using the Holm-Šidák procedure.

Neurological deficit scores and behavioral endpoints (openfield activity, hole exploration, light-dark transition latency, and radialmaze successful entries) were measured serially over time. Group and time effects were evaluated using a mixedeffects model (fixed effects: group, time, and group × time interaction; random effect: animal as a random intercept), which permits analysis in the presence of missing observations arising from mortality and scheduled terminal sampling without imputation. When a significant group × time interaction was detected, prespecified post hoc comparisons were performed using Šidákadjusted pairwise tests. Planned contrasts included: each injured group vs sham at the same time point (behavioral outcomes), and carbacetam vs vehicle and DCBH vs vehicle at the same time point.

Hormone concentrations were measured using terminal sampling (independent animals at each time point; n = 5 per group per time point). Therefore, hormone data were analyzed crosssectionally at each time point using oneway ANOVA with treatment group as the factor. Comparisons vs intact reference were performed using Dunnett's test (vs intact), and pairwise comparisons among injured groups were performed using Tukey's HSD (or Šidákadjusted pairwise tests). If assumptions for parametric analysis were violated, the Kruskal-Wallis test with Dunn's post hoc correction was used.

Histology and immunohistochemistry were evaluated descriptively from representative sections (Day 7; n = 5 per group) and are presented qualitatively as representative micrographs.

RESULTS AND DISCUSSION

IN SILICO RATIONALE AND TARGET PROFILING

βCarboline derivatives represent a "privileged" neuroactive scaffold with broad CNS pharmacology. Their tricyclic heteroaromatic core has been exploited to design ligands that engage multiple CNS-relevant targets, including the benzodiazepine site of the GABA-A receptor, and several βcarbolines have demonstrated cognitive- and neuroprotectionrelevant effects in preclinical settings. Such properties make the scaffold particularly attractive for drug development in conditions where anxiety, stress-reactivity, and cognitive impairment coexist – limitations that remain clinically relevant for classical benzodiazepines because of tolerability and cognitionrelated adverse effects during sustained use [10-12].

Carbacetam is a βcarboline isostere positioned as an endogenous modulator of the GABA-benzodiazepine receptor complex. In prior behavioral profiling in rodent models of CNS dysfunction, carbacetam was associated with improved performance in tasks reflecting higher

Table 2. Docking-derived binding energies (kcal/mol) of carbacetam and reference ligands across CNS targets

Target	PDB ID	Ligand	Binding energy (kcal/mol)
Type-A γ-aminobutyric acid (GABA-A) receptor (α1β3γ2)	6HUO	Carbacetam	-10,513
		Alprazolam (reference)	-10,663
		Diazepam (reference)	-8,253
Type-B γ-aminobutyric acid (GABA-B) receptor (GBR1, GBR2)	4MS4	Carbacetam	-8,325
		Baclofen (reference)	-7,602
Translocator protein (TSPO)	N/A	Carbacetam	-9,053
		Diazepam (reference)	-6,804
AMPA-subtype GluA2 receptor	5L1F	Carbacetam	-8,258
		Perampanel (reference)	-9,069
The voltage-gated sodium channel Nav1.2	6J8E	Carbacetam	-9,420
		Lamotrigine (reference)	-6,841
Orexin-1 Receptor (Site 1)	6TOT	Carbacetam	-8,190
		Lemborexant (reference)	-9,615
Orexin-1 Receptor (Site 2)	6TOT	Carbacetam	-8,464
		Lemborexant (reference)	-9,646

Note: Values represent Docking-derived binding energies for the bestranked pose (more negative values indicate more favorable predicted binding within the applied scoring function). "Reference" ligands are benchmark compounds used for contextual comparison within each target system.
Source: compiled by the authors of this study

nervous activity and memory retention, supporting the concept that its activity profile may extend beyond "pure" anxiolysis toward cognitionsupporting effects [13].

COMPREHENSIVE MOLECULAR DOCKING SCREEN ACROSS CNS TARGETS

To explore a plausible polypharmacological basis for the observed neurobehavioral profile, we performed a docking-based screen of carbacetam against a panel of CNS targets implicated in inhibitory and excitatory neurotransmission, neuroinflammation/stress signaling, and arousal regulation. Docking-derived binding energies for the top-ranked poses of carbacetam and reference ligands are summarized in Table 2.

The docking screen suggested that carbacetam may engage the benzodiazepine site of the GABA-A receptor with a highly favorable predicted binding energy (-10.513 kcal/mol), comparable to alprazolam and more favorable than diazepam in this docking setup (Table 2). Importantly, the predicted interaction profile was not limited to a single target. Carbacetam also demonstrated a favorable docking score at the GABA-B receptor (-8.325 kcal/mol), exceeding the reference agonist baclofen (-7.602 kcal/mol), and at the translocator protein TSPO (-9.053 kcal/mol), with a markedly more favorable score than diazepam (-6.804 kcal/mol) (Table 2). Given the established interest in TSPO ligands as modulators of neurosteroidogenesis, stress-related neurobiology, and neuroinflammation, these findings warrant consideration as a mechanistically relevant signal rather than a definitive proof of target engagement [12].

Beyond ligand-receptor systems, carbacetam also exhibited favorable docking scores in ion channel binding sites. Notably, the predicted binding energy within the Nav1.2 pore (-9.420 kcal/mol) was more favorable than that of lamotrigine (-6.841 kcal/mol) in this model (Table 2). In addition, carbacetam showed detectable predicted affinity across both identified binding sites of the orexin receptor 1 (OX1R) (Table 2), supporting a working hypothesis of broader CNS target coverage. Collectively, the docking results support a polypharmacology-oriented interpretation; however, docking scores should be considered hypothesis-generating and require orthogonal experimental validation (e.g., binding assays and functional readouts).

IN SILICO ADMET PROFILE AND INTEGRATIVE POLYPHARMACOLOGY

The *in silico* ADMET profiling suggested that carbacetam has a generally favorable physicochemical and early safety/PK risk profile for a CNSdirected small molecule (Table 3). In particular, the compound was predicted to display high gastrointestinal absorption and blood–brain barrier permeability, while showing a low probability of mutagenicity in the Ames test, cardiotoxicity related to hERG inhibition, and drug induced

Table 3. *In silico* predicted physicochemical and ADMET properties of carbacetam

Parameter	Carbacetam	Interpretation
Physicochemical properties		
Molecular weight (g/mol)	278.36	CNSactive small molecule range
cLogP	4.18	Lipophilicity consistent with CNS exposure
Lipinski ruleoffive violations	0	Druglikeness criterion met
Synthetic accessibility score (SAscore)	0.20	Lower values indicate easier synthesis
Pharmacokinetics		
GI absorption (HIA)	High	Predicted high intestinal absorption
BBB permeability	Yes	Predicted blood-brain barrier penetration
Pglycoprotein substrate	Yes	Potential efflux liability
Toxicity		
Ames mutagenicity	No	Low predicted genotoxicity risk
hERG blockade	No	Low predicted cardiotoxicity risk (screen)
Hepatotoxicity (DILI)	No	Low predicted DILI risk (screen)
Tox-score	0.51	Lower values indicate more favorable profile

Note: ADMET – absorption, distribution, metabolism, excretion, toxicity; HIA – human intestinal absorption; BBB – blood-brain barrier; Pgp – Pglycoprotein; DILI – druginduced liver injury. *In silico* predictions are hypothesissupporting and should be interpreted qualitatively.
Source: compiled by the authors of this study

liver injury liability (Table 3). These outputs collectively support the feasibility of systemic administration and CNS exposure in vivo, while also emphasizing that experimental confirmation (PK and targetengagement studies) remains necessary before translational conclusions can be drawn.

When interpreted alongside the docking screen (Table 2), the data support a working mechanistic concept in which carbacetam may act as a multitarget CNS modulator, rather than a "singletarget" benzodiazepinelike ligand. The predicted high-affinity interaction with the benzodiazepine site of the GABA-A receptor (comparable to alprazolam and stronger than diazepam in the present docking set; Table 2) provides a molecular rationale for an anxiolyticlike phenotype, whereas the additional predicted engagement of GABA-B receptors, TSPO, and voltagegated sodium channels suggests plausible complementary pathways that could influence posttraumatic network excitability, stressaxis signaling, and neuroinflammatory tone.

A notable additional signal in the docking profile was the predicted interaction of carbacetam with the Nav1.2 pore region (Table 2). Voltagegated sodium channels (including Nav1.2) are central determinants of neuronal excitability and are increasingly recognized as modulators of downstream injury cascades when ionic homeostasis becomes destabilized. Recent syntheses of the field emphasize that sodium channel subtypes contribute in distinct ways to neuronal firing properties and can be pharmacologically leveraged to dampen pathological hyperexcitability. In experimental TBI, targeting injuryassociated dysregulation of sodium channel expression has been shown to improve structural and functional outcomes, supporting the broader principle that sodium channel modulation can be neuroprotective in the context of neurotrauma. Accordingly, the predicted sodium channel engagement by carbacetam (Table 2) may represent a mechanistically relevant component of its pleiotropic profile rather than a docking "offtarget" artifact [14, 15].

In parallel, the predicted high affinity of carbacetam for TSPO (Table 2) is mechanistically noteworthy because TSPOlinked pathways are positioned at the interface of mitochondrial function, neurosteroid biology, and neuroinflammation. Contemporary reviews highlight that neurosteroid replacement strategies and/or TSPO targeting have been proposed as means to restore altered neurosteroid signaling under neuroinflammatory conditions, while also acknowledging contextdependence and methodological complexity in translating neurosteroidome findings. Importantly for traumatic brain injury, neuroactive steroid receptor modulators (including agents enhancing GABA-A receptor signaling via neurosteroid mechanisms) are actively discussed as pleiotropic candidates capable of addressing multiple posttraumatic pathological processes (edema, inflammation, delayed cell death, neurovascular disruption), a conceptual framework aligned with the present study's rationale for testing a multimechanism compound after closedhead TBI [16, 17].

Taken together, the docking and ADMET results support a testable, integrated hypothesis: carbacetam's

Table 4. Neurological deficit score after closedhead TBI (mean ± SEM)

Group	Day 1	Day 3	Day 7
Intact reference	3.6 ± 2.3	–	–
Vehicle (comparison)	21.3 ± 1.2	21.3 ± 2.1	12.1 ± 1.2
Carbacetam	19.9 ± 1.3	16.4 ± 1.3	8.6 ± 0.9
DCBH	19.5 ± 1.6	18.8 ± 1.6	10.3 ± 1.1

Source: compiled by the authors of this study

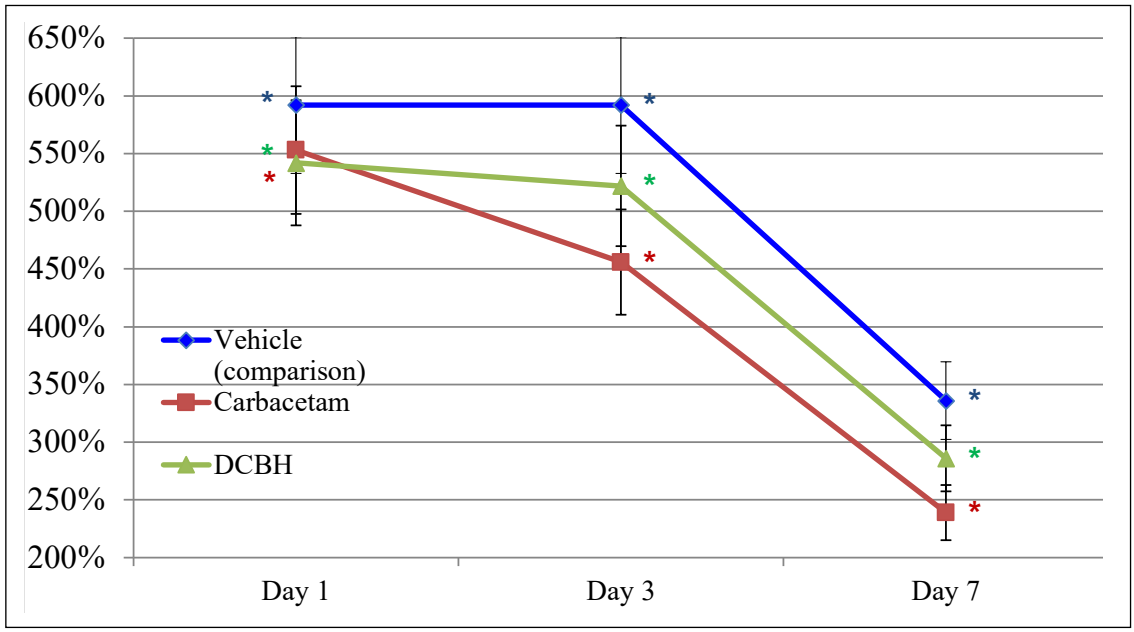

Fig. 1. Neurological deficit dynamics after closed-head TBI. Neurological deficit is presented as percentage of intact reference animals (intact = 100%). Data are shown for Vehicle (comparison), Carbacetam, and DCBH groups at the indicated post-injury time points. Values are mean ± SEM. Absolute neurological deficit scores (points) are provided in Table 4. *p < 0.05 vs intact reference, based on planned contrasts within a mixedeffects model (fixed effects: group, time, and group × time; random effect: animal), with multiplicityadjusted post hoc testing as specified in the Statistical analysis subsection (twosided).
Source: Authors' own work

therapeutic phenotype may arise from synergistic modulation across several CNS targets, in which GABA-A receptor modulation provides an anxiolytic core mechanism, while concurrent engagement of GABA-B receptors, TSPOlinked neurosteroid/neuroinflammatory pathways, and sodium channeldependent excitability may contribute to neuroprotection and cognitive support. This multinode concept is consistent with the broader need for pleiotropic interventions in TBI, where singlepathway neuroprotection has repeatedly underperformed clinically, and it provides a mechanistic scaffold for interpreting the *in vivo* endocrine and behavioral findings presented below.

MORTALITY

Cumulative mortality over the observation period differed significantly across the three injured treatment arms (n = 50 per group at allocation; Table 1). Mortality was highest in the Vehicle group (50%), compared with 16% in the Carbacetam group and 36% in the DCBH group (overall contingency analysis, p = 0.002). Pairwise comparisons indicated a robust reduction in mortality with carbacetam *versus* vehicle (3.1fold lower mortality, p = 0.001), whereas DCBH *versus* vehicle showed a smaller reduction (1.4fold) that did not reach statistical significance (p = 0.096).

NEUROLOGICAL DEFICIT

MOTOR AND REFLEX DOMAINS

On Day 1 after closedhead TBI, all injured groups exhibited a marked increase in neurological deficit relative to intact rats (3.6 ± 2.3 points). In the Vehicle group, the deficit reached 21.3 ± 1.2 points, corresponding to a 5.9fold elevation (i.e., 592% of intact, p < 0.05 vs intact). The Carbacetam group demonstrated a deficit of 19.9 ± 1.3 points (5.5fold; 553% of intact, p < 0.05 vs intact), and the DCBH group showed 19.5 ± 1.6 points (5.4fold; 542% of intact, p < 0.05 vs intact). At this early

Table 5. Behavioral outcomes across groups over time (mean ± SEM)

Outcome	Group	Day 1	Day 3	Day 7	Day 14	Day 30
Open field: squares crossed (count)	Sham	15.7 ± 1.5	15.8 ± 1.6	14.7 ± 1.4	13.7 ± 1.0	12.6 ± 1.3
	Vehicle	6.8 ± 0.5[a]	6.9 ± 0.4[a]	6.7 ± 0.7[a]	5.6 ± 0.5[a]	9.4 ± 1.0[a]
	Carbacetam	6.6 ± 0.4[a]	7.1 ± 0.6[a]	7.2 ± 0.4[a]	5.8 ± 0.4[a]	10.4 ± 0.7
	DCBH	6.5 ± 0.4[a]	6.7 ± 0.4[a]	6.7 ± 0.5[a]	6.6 ± 0.5[a]	9.4 ± 0.8
Open field: holes explored (count)	Sham	13.1 ± 1.2	13.1 ± 1.2	11.4 ± 1.3	9.4 ± 1.0[d]	10.3 ± 0.7[d]
	Vehicle	2.7 ± 0.2[a]	2.9 ± 0.2[a]	3.9 ± 0.3[a]	4.9 ± 0.5[a]	6.6 ± 0.4[a]
	Carbacetam	3.1 ± 0.2[a]	3.4 ± 0.2[a]	6.3 ± 0.4[ab]	6.4 ± 0.5[ab]	8.8 ± 0.6[b]
	DCBH	2.9 ± 0.2[a]	3.0 ± 0.2[a]	4.0 ± 0.2[ac]	4.9 ± 0.4[ac]	6.7 ± 0.5[ac]
Light–dark transition latency (s)	Sham	108 ± 10	108 ± 10	85 ± 11	67 ± 8[d]	55 ± 8[d]
	Vehicle	203 ± 11[a]	188 ± 15[a]	138 ± 12[a]	86 ± 6	70 ± 5
	Carbacetam	180 ± 10[a]	166 ± 10[a]	116 ± 7[a]	84 ± 5	63 ± 4
	DCBH	190 ± 9[a]	176 ± 10[a]	121 ± 7[a]	76 ± 4	59 ± 4
Eightarm radial maze: successful entries (count)	Sham	6.7 ± 0.7	7.1 ± 0.6	7.5 ± 0.6	7.5 ± 0.5	7.6 ± 0.7
	Vehicle	3.2 ± 0.2[a]	4.1 ± 0.3[a]	4.4 ± 0.4[a]	5.7 ± 0.4[a]	6.2 ± 0.7[a]
	Carbacetam	3.4 ± 0.1[a]	4.4 ± 0.2[a]	4.9 ± 0.3[a]	6.8 ± 0.4	7.4 ± 0.6
	DCBH	3.3 ± 0.2[a]	4.1 ± 0.3[a]	4.5 ± 0.3[a]	5.9 ± 0.5[a]	6.7 ± 0.7

Note: Sham animals underwent anesthesia and all experimental handling procedures without impact. Light-dark transition latency corresponds to the latency to enter the dark compartment (burrowreflex paradigm). Successful entries in the eightarm radial maze represent the number of correct entries into baited arms without repetition (index of working memory performance/task acquisition). Values are mean ± SEM. Superscripts indicate statistically significant differences (twosided $p < 0.05$) according to the statistical approach specified in the Statistical analysis subsection (group × time model with appropriate post hoc comparisons/multiplicity control). Superscripts (multiple may apply):
[a] $p < 0.05$ vs Sham at the same time point;
[b] $p < 0.05$ vs Vehicle at the same time point;
[c] $p < 0.05$ vs Carbacetam at the same time point;
[d] $p < 0.05$ vs Day 1 within the same group (habituation in Sham).
Source: compiled by the authors of this study

time point, neurological deficit did not differ significantly among the three injured groups.

By Day 3, neurological deficit in the Vehicle group remained essentially unchanged (21.3 ± 2.1 points), i.e., 100% of the Day1 value (still 592% of intact, $p < 0.05$ vs intact), indicating minimal spontaneous improvement during the first 72 hours. In contrast, the Carbacetam group declined to 16.4 ± 1.3 points, corresponding to 82% of the Day1 value (an 18% reduction vs Day 1) and 456% of intact ($p < 0.05$ vs intact). Compared with vehicle at the same time point, the deficit under carbacetam was 23% lower (reported as 77% of the vehicle value, $p < 0.05$). Clinically, animals in the carbacetam group appeared more stable in consciousness and adaptive behavior, maintained posture, and showed only minimal ataxia. In the DCBH group on Day 3, neurological deficit was 18.8 ± 1.6 points, representing 96% of the Day1 value (a 4% reduction vs Day 1) and 522% of intact ($p < 0.05$ vs intact). At this time point, the DCBH group did not differ significantly from vehicle or carbacetam.

On Day 7, neurological deficit decreased in all injured groups, reflecting partial recovery over the first posttraumatic week. In the Vehicle group, deficit fell to 12.1 ± 1.2 points, i.e., 57% of the Day1 value (a 43% reduction vs Day 1) and 336% of intact ($p < 0.05$ vs intact). In the Carbacetam group, the deficit declined further to 8.6 ± 0.9 points, corresponding to 52% of the Day3 value (a 48% reduction vs Day 3), 43% of the Day1 value (a 57% reduction vs Day 1), and 239% of intact ($p < 0.05$ vs intact). Relative to vehicle, the Day7 deficit under carbacetam was 29% lower (71% of the vehicle value, $p < 0.05$). In the DCBH group, deficit reached 10.3 ± 1.1 points, i.e., 55% of the Day3 value (a 45% reduction vs Day 3), 53% of the Day1 value (a 47% reduction vs Day 1), and 286% of intact ($p < 0.05$ vs intact). Despite a numerically lower score than vehicle (approximately 85% of the vehicle value), this difference was not statistically significant.

Collectively, closedhead TBI induced a 5.4-5.9fold increase in neurological deficit within 24 hours, followed by regression over the first week; however, without pharmacological correction the deficit remained 3.4fold

above intact at Day 7. Both active interventions promoted recovery, but the trajectory differed: carbacetam produced an earlier and more pronounced reduction (already evident by Day 3 and reaching a 57% reduction from Day 1 by Day 7), whereas DCBH showed a slower improvement pattern and did not yield a statistically significant advantage over vehicle in the neurological deficit score at the measured time points.

HIGHER-ORDER BEHAVIORAL AND COGNITIVE PERFORMANCE

(OPEN FIELD, LIGHT-DARK TRANSITION, EIGHTARM RADIAL MAZE)

To probe posttraumatic disturbances of higher-order behavioral and cognitive function – a clinically salient domain of TBI outcomes [5] – we used an integrated battery comprising the openfield test (locomotor activity and exploratory drive), the light-dark transition latency ("burrow reflex" paradigm; emotional reactivity/anxiety-like avoidance), and the eightarm radial maze (correct, nonrepeated entries as an index of task acquisition/working memory performance). Because repeated testing itself can modify behavior over time, we first evaluated the Sham group across the 30day observation period. In sham animals, the number of squares crossed showed a nonsignificant downward trend, reaching ~80% of Day1 values by Day 30. In contrast, hole exploration decreased significantly, to 72% of Day1 values on Day 14 and 79% on Day 30 ($p < 0.05$ for both), consistent with habituation to the testing environment. A similar habituation pattern was evident for the light-dark transition: latency to enter the dark compartment decreased to 62% of Day1 values by Day 14 and to ~51% by Day 30 ($p<0.05$ for both).

In the Vehicle group, behavioral performance was markedly impaired already on Day 1 after TBI compared with timematched sham values ($p < 0.05$ for all reported differences). Locomotor activity (squares crossed) fell to 43% of sham, exploratory activity (holes explored) to 21%, and radialmaze successful entries to 48%. In parallel, light-dark transition latency increased to ~188% of sham (an ~88% prolongation), indicating posttraumatic suppression of adaptive exploratory behavior together with increased avoidance/emotional reactivity. Across the 30day followup, partial recovery occurred but remained incomplete. By Day 30, squares crossed increased to 9.4 ± 1.0, i.e., ~138% of the Vehicle Day1 value, yet still only ~75% of the sham Day30 level ($p<0.05$). Holes explored rose to 6.6 ± 0.4 (~244% of Vehicle Day1), but remained ~64% of sham ($p<0.05$). Radialmaze performance improved to 6.2 ± 0.7 (~194% of Vehicle Day1), but remained ~82% of sham ($p<0.05$). The only parameter that approached normalization without treatment was light-dark latency, decreasing to 70 ± 5 s (~34% of Vehicle Day1; ~66% reduction) and reaching ~127% of sham Day30, which did not differ significantly ($p > 0.05$). Overall, these data indicate that spontaneous recovery after closedhead TBI was insufficient to restore exploratory behavior and workingmemory performance to control levels by one month, consistent with the recognized persistence of cognitivebehavioral sequelae after TBI [5].

In the Carbacetam group, Day1 impairment was comparable to the Vehicle group, supporting baseline comparability of injury severity: squares crossed were ~42% of sham, holes explored ~24%, successful entries ~51%, and latency increased to ~167% of sham. Under carbacetam treatment, recovery became more pronounced over time. By Day 30, locomotor activity reached 10.4 ± 0.7, corresponding to ~158% of Day1 and ~83% of the sham Day30 value (difference not significant, $p > 0.05$). Exploratory activity reached 8.8 ± 0.6, i.e., ~284% of Day1 and ~85% of sham Day30 ($p > 0.05$). Light–dark latency declined to 63 ± 4 s (~35% of Day1) and was ~115% of sham Day30 ($p > 0.05$). Radialmaze performance increased to 7.4 ± 0.6 (~218% of Day1) and approximated sham values (~97% of sham Day30; $p > 0.05$). A particularly early treatment signal was observed for exploratory activity: by Day 7, holes explored under carbacetam (6.3 ± 0.4) were ~162% of the Vehicle value at the same time point (i.e., ~62% higher), indicating earlier restoration of exploratory drive.

In the DCBH group, Day1 performance was also comparable to Vehicle and Carbacetam, again supporting standardization of injury induction. By Day 30, locomotor activity, light-dark transition latency, and radialmaze performance showed substantial improvement and approached sham levels overall (Table 5). However, holes explored remained reduced, reaching 6.7 ± 0.5, i.e., ~231% of DCBH Day1, yet only ~65% of the sham Day30 level ($p < 0.05$), indicating a persistent deficit in exploratory behavior under DCBH. Between the two active interventions, most behavioral endpoints did not differ significantly at any time point. The key exception was again exploratory activity (holes explored): starting at Day 7, DCBH values were consistently lower than under carbacetam ($p < 0.05$). Expressed as deltas, holes explored in the DCBH group were ~63% of carbacetam on Day 7, and ~77% and ~76% of carbacetam on Days 14 and 30, respectively – i.e., ~37% lower at Day 7 and ~23-24% lower at Days 14-30 (Table 5). This pattern supports a more robust restoration of exploratory activity with carbacetam, with the divergence emerging by the end of the first posttraumatic week.

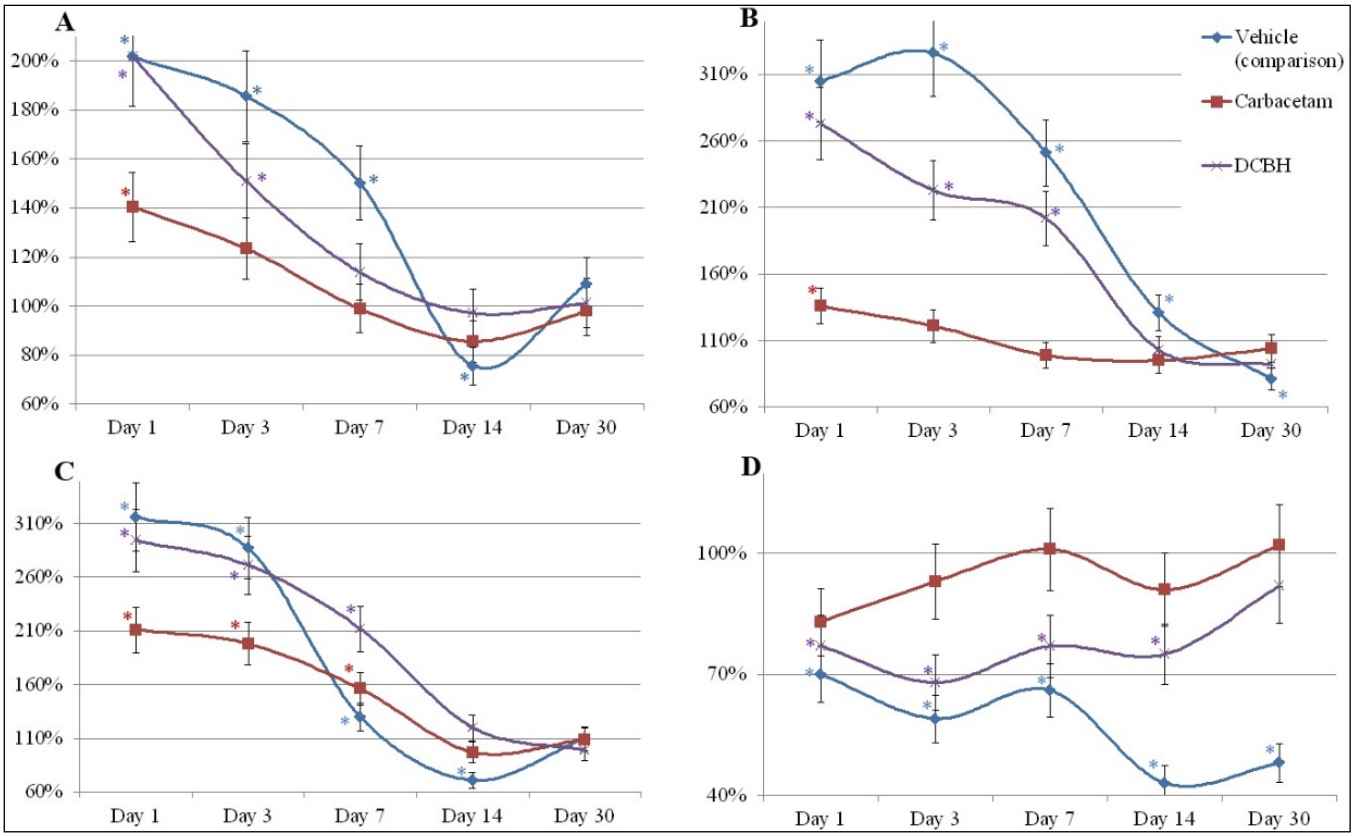

Fig. 2. Neuroendocrine response to closed-head TBI and modulation by carbacetam and DCBH. Plasma hormone levels are shown as percentage of intact reference animals (intact = 100%). Data are shown for Vehicle (comparison), Carbacetam, and DCBH groups at the indicated post-injury time points using terminal sampling (n = 5 per group per time point). Values are mean ± SEM. Absolute values are provided in the text. A – Adrenocorticotropic hormone (ACTH); B – Corticosterone; C – Vasopressin; D – Growth hormone (GH). *p < 0.05 vs intact reference (oneway ANOVA at each time point with Dunnett's post hoc test vs intact; pairwise treatment comparisons performed as described in Statistical analysis subsection).
Source: Authors' own work

NEUROENDOCRINE DYNAMICS

VEHICLE (COMPARISON) GROUP

Closedhead TBI triggered a pronounced acute stress-neuroendocrine response in vehicletreated animals, followed by a delayed suppression ("depletionlike") phase of central neurohormonal output and a later decline of the adrenal component (Fig. 2).

At 24 hours postinjury, the Vehicle group demonstrated a sharp rise in ACTH from 41.8 ± 2.9 pmol/L (intact) to 84.7 ± 8.6 pmol/L, i.e., 203% of intact (+102%, p < 0.05 vs intact). This was accompanied by a disproportionate increase in corticosterone from 379 ± 21 nmol/L to 1156 ± 42 nmol/L, i.e., 305% of intact (+205%, p < 0.05 vs intact). In axis terms, this pattern is consistent with strong feedforward activation along the hypothalamic-pituitary-adrenal (HPA) pathway, with a larger peripheral glucocorticoid output relative to the rise in ACTH. Vasopressin (AVP) also surged from 21.0 ± 1.9 pmol/L to 66.3 ± 4.7 pmol/L, i.e., 316% of intact (+216%, p < 0.05 vs intact), indicating early hypersecretion of posterior pituitary/hypothalamic neurosecretory output. In contrast to the catabolic axes, growth hormone (GH) decreased from 1.45 ± 0.11 μg/L to 1.01 ± 0.11 μg/L, i.e., 70% of intact (~30% decrease, p < 0.05 vs intact), suggesting early suppression of the somatotrophic/anabolic axis after injury.

By Day 3, ACTH began to decline but remained elevated at 77.6 ± 6.1 pmol/L, i.e., 186% of intact (+86%, p < 0.05 vs intact). In contrast, corticosterone continued to rise, reaching 1235 ± 48 nmol/L, i.e., 326% of intact (+226%, p < 0.05 vs intact), consistent with a sustained peripheral glucocorticoid drive and/or emerging decoupling of adrenal output from central ACTH signaling. AVP decreased slightly relative to Day 1 but remained markedly elevated at 60.2 ± 5.8 pmol/L, i.e., 287% of intact (+187%, p < 0.05 vs intact). GH suppression progressed: GH fell to 0.85 ± 0.04 μg/L, i.e., 59% of intact (~41% decrease, p < 0.05 vs intact). Physiologically, this constellation supports an ongoing catabolic stress response with persistent neurosecretory activation and a parallel suppression of anabolic/plasticitysupporting signaling.

At Day 7, ACTH declined to 62.9 ± 5.3 pmol/L, i.e., 151% of intact (+50%, p < 0.05 vs intact), whereas corti-

costerone remained high at 952 ± 40 nmol/L, i.e., 251% of intact (+151%, $p < 0.05$ vs intact). The persistence of hypercorticosteronemia despite a smaller ACTH elevation is compatible with impaired negative feedback within the HPA axis and/or altered glucocorticoid binding/clearance kinetics after injury (interpretation at the level of mechanism). AVP continued to normalize, falling to 27.2 ± 1.8 pmol/L, i.e., 130% of intact (+30%, $p < 0.05$ vs intact). GH showed partial recovery compared with Day 3 but remained 34% lower than intact ($p < 0.05$ vs intact), indicating that restoration of the somatotrophic axis lagged behind normalization of AVP.

At Day 14, ACTH dropped below intact values to 31.3 ± 2.9 pmol/L, i.e., 75% of intact (-25%, $p < 0.05$ vs intact). AVP similarly fell to 14.9 ± 0.8 pmol/L, i.e., 71% of intact (-29%, $p < 0.05$ vs intact). This biphasic course – early hypersecretion followed by a delayed suppression – supports the concept of a posttraumatic transition from acute hyperreactivity to central downregulation/exhaustion of neurosecretory output. Despite this central decline, corticosterone remained elevated at 497 ± 36 nmol/L, i.e., 131% of intact (+31%, $p < 0.05$ vs intact), indicating a sustained hypercorticosteronemic state across the first two postinjury weeks. GH reached its lowest observed level at 0.62 ± 0.04 µg/L, i.e., 43% of intact (-57%, $p < 0.05$ vs intact), aligning temporally with the central suppression phase and supporting prolonged impairment of anabolic and reparative signaling.

By Day 30, ACTH recovered to 45.6 ± 4.7 pmol/L and AVP to 23.1 ± 1.1 pmol/L, with no significant differences vs intact ($p > 0.05$), indicating restoration of central secretory output at the group level. In contrast, corticadrenergic output appeared reduced: corticosterone fell to 307 ± 22 nmol/L, i.e., 81% of intact (-19%, $p < 0.05$ vs intact), consistent with a later decline in the peripheral (adrenal) component after a prolonged period of hyperactivation. GH remained substantially suppressed at 0.70 ± 0.03 µg/L, i.e., 48% of intact (~52% decrease, $p < 0.05$ vs intact), indicating a prolonged somatotrophic deficit extending through the 30day posttraumatic period.

Conclusively, in vehicletreated animals, closedhead TBI produced a biphasic neuroendocrine trajectory: early hyperactivation of ACTH-corticosterone and AVP secretion with concurrent GH suppression (Days 1-3); a delayed suppression of ACTH and AVP below intact values by Day 14, while corticosterone remained elevated; by Day 30, central ACTH/AVP secretion normalized, but corticosterone declined below intact, alongside persistent GH suppression. This pattern is consistent with contemporary concepts of posttraumatic neuroendocrine dysfunction spanning both acute and subacute/chronic phases, where hypothalamic-pituitary axis perturbations (including somatotrophic and posterior pituitary components) can evolve over time and plausibly modulate recovery trajectories [18, 19].

MODULATION OF POSTTRAUMATIC NEUROENDOCRINE DYNAMICS BY CARBACETAM

Compared with the Vehicle group (described above), carbacetam produced an early and sustained "normalizing" shift of the posttraumatic neuroendocrine response, affecting both the central component of the HPA axis (ACTH), the peripheral adrenal response (corticosterone), and hypothalamic vasopressin (AVP) secretion, while largely preserving the somatotropic axis (GH).

At 24 h postTBI, ACTH in the Carbacetam group increased to 58.6 ± 6.5 pmol/L, i.e., 140% of intact (+40% vs intact, $p < 0.05$). Importantly, this early rise was 31% lower than the corresponding Vehicle value (58.6 vs 84.7 pmol/L; $p < 0.05$), indicating that the very first administration of carbacetam materially attenuated the acute central HPA response. A parallel pattern was observed for corticosterone: corticosterone increased to 515 ± 32 nmol/L, i.e., 136% of intact (+36% vs intact, $p < 0.05$), but was ~55% lower than Vehicle at the same time point (515 vs 1156 nmol/L; $p < 0.05$). Thus, both the central drive (ACTH) and peripheral output (corticosterone) were substantially less hyperreactive under carbacetam. AVP also increased, but the magnitude of hypersecretion was attenuated: AVP rose to 44.3 ± 3.9 pmol/L, i.e., 211% of intact (+111% vs intact, $p < 0.05$), yet remained 33% lower than Vehicle (44.3 vs 66.3 pmol/L; $p < 0.05$). This indicates moderated activation of hypothalamic neurosecretory processes (relative to Vehicle) already during the acute postinjury phase. For GH, the carbacetam group showed no statistically significant deviation from intact values and did not differ significantly from Vehicle on Day 1 (as per your comparisons), suggesting that early somatotropic suppression was not a prominent feature under carbacetam at this time point.

By Day 3, ACTH declined to 51.6 ± 4.6 pmol/L, corresponding to 123% of intact (+23% vs intact, not significant) and remained 34% lower than Vehicle (51.6 vs 77.6 pmol/L; $p < 0.05$). Corticosterone decreased to 458 ± 51 nmol/L, i.e., 121% of intact (not significant), and was ~63% lower than Vehicle (458 vs 1235 nmol/L; $p < 0.05$). AVP was 41.6 ± 4.4 pmol/L, i.e., 198% of intact (+98% vs intact; $p < 0.05$) and 31% lower than Vehicle (41.6 vs 60.2 pmol/L; $p<0.05$). A key divergence from Vehicle concerned the somatotropic axis: GH in the carbacetam group was 1.35 ± 0.06 µg/L, not significantly different from intact, but 59% higher than Vehicle at the

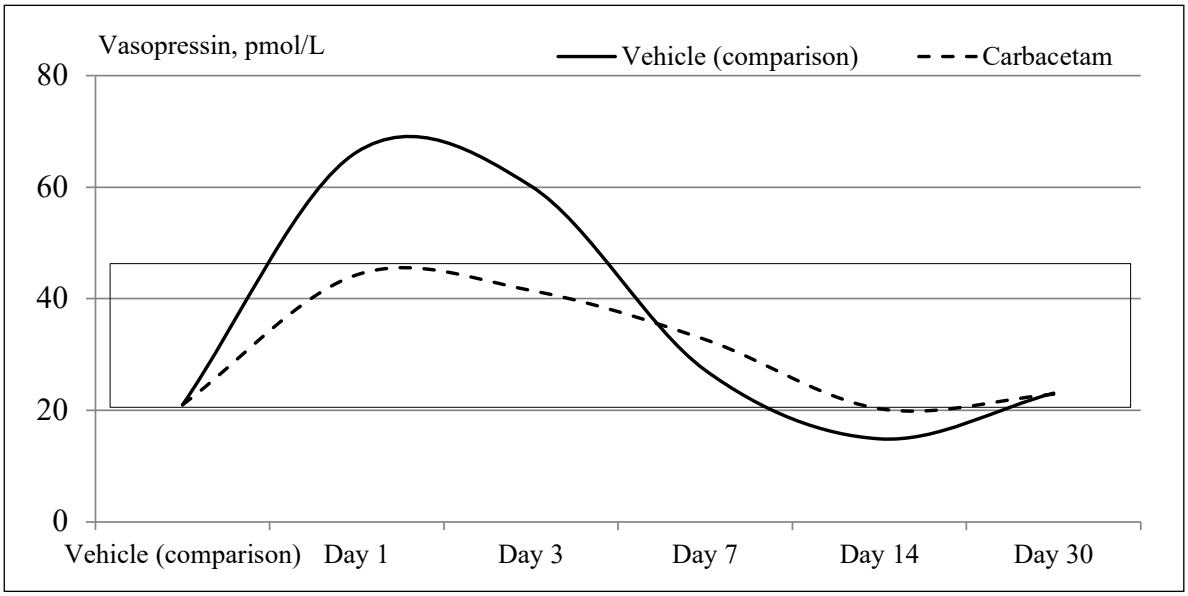

Fig. 3. Carbacetam attenuates early vasopressin hypersecretion and prevents delayed neurosecretory depletion after TBI. Vasopressin dynamics are presented as absolute concentrations (pmol/L) in the Vehicle and Carbacetam groups at post-injury Days 1, 3, 7, 14, and 30 (terminal sampling; n = 5 per group per time point). The boxed/outlined interval highlights the phase interpreted as attenuation of early posttraumatic hyperactivation and prevention of delayed depletion of vasopressin neurosecretion. Data are mean ± SEM. Group differences at each time point were assessed using oneway ANOVA (or Kruskal-Wallis where applicable) with multiplicity adjustment as described in Statistical analysis subsection.
Source: Authors' own work

same time point ($p < 0.05$), indicating preservation of GH secretion during the early subacute phase.

At Day 7, ACTH under carbacetam reached 41.3 ± 3.4 pmol/L, essentially normalized to intact (no significant difference), and remained 34% lower than Vehicle ($p<0.05$). Corticosterone was 374 ± 18 nmol/L, also not different from intact, and ~61% lower than Vehicle ($p < 0.05$). AVP was 32.7 ± 3.4 pmol/L, remaining 56% above intact ($p < 0.05$); however, it did not differ significantly from Vehicle at this time point (despite a numerically higher mean). In parallel, GH was 1.47 ± 0.07 µg/L, again not different from intact and ~53% higher than Vehicle ($p < 0.05$), reinforcing that carbacetam prevented the prolonged suppression of GH observed in Vehicle animals.

At Day 14, the contrast with Vehicle became particularly mechanistically informative. In the Vehicle group, ACTH and AVP fell below intact (a depletionlike phase), whereas under carbacetam both hormones remained within (or close to) the intact range. Specifically, ACTH under carbacetam was 35.8 ± 3.1 pmol/L (no significant difference vs intact and vs Vehicle). Corticosterone was 361 ± 18 nmol/L, not different from intact but 27% lower than Vehicle ($p < 0.05$), consistent with prevention of sustained posttraumatic hypercorticosteronemia. Critically, AVP was 20.3 ± 1.8 pmol/L, not different from intact, but 36% higher than Vehicle ($p < 0.05$), indicating prevention of the delayed AVP decline that characterized the Vehicle group at two weeks (Fig. 3).

GH at Day 14 was 1.32 ± 0.12 µg/L, again not significantly different from intact, but 113% higher than Vehicle ($p < 0.05$), showing that carbacetam prevented the pronounced GH nadir observed in untreated animals during the subacute period.

At Day 30, ACTH under carbacetam was 40.9 ± 3.8 pmol/L, with no significant differences vs intact or Vehicle. Corticosterone was 394 ± 19 nmol/L, not different from intact but 28% higher than Vehicle ($p < 0.05$), consistent with prevention of the late decline in adrenal output that emerged in the Vehicle group. AVP was 22.8 ± 1.9 pmol/L, not different from intact or Vehicle. GH remained preserved: 1.48 ± 0.09 µg/L, again not different from intact and 111% higher than Vehicle ($p<0.05$).

Taken together, across the 30day period, carbacetam acted as a modulator of posttraumatic neuroendocrine stress systems, with two clinically relevant features: immediate attenuation of primary hyperactivation (Day1 reductions vs Vehicle for ACTH, corticosterone, and AVP: -31%, -55%, and -33%, respectively), and prevention of delayed depletion of central neurosecretory output (notably AVP at Day 14), together with sustained preservation of GH across the entire followup (Day 14 and Day 30 GH exceeding Vehicle by +113% and +111%, respectively). These patterns align with contemporary concepts that neuroendocrine dysfunction after TBI is dynamic and phasedependent, spanning acute hyperreactivity and delayed pituitary/hypothalamic dys-

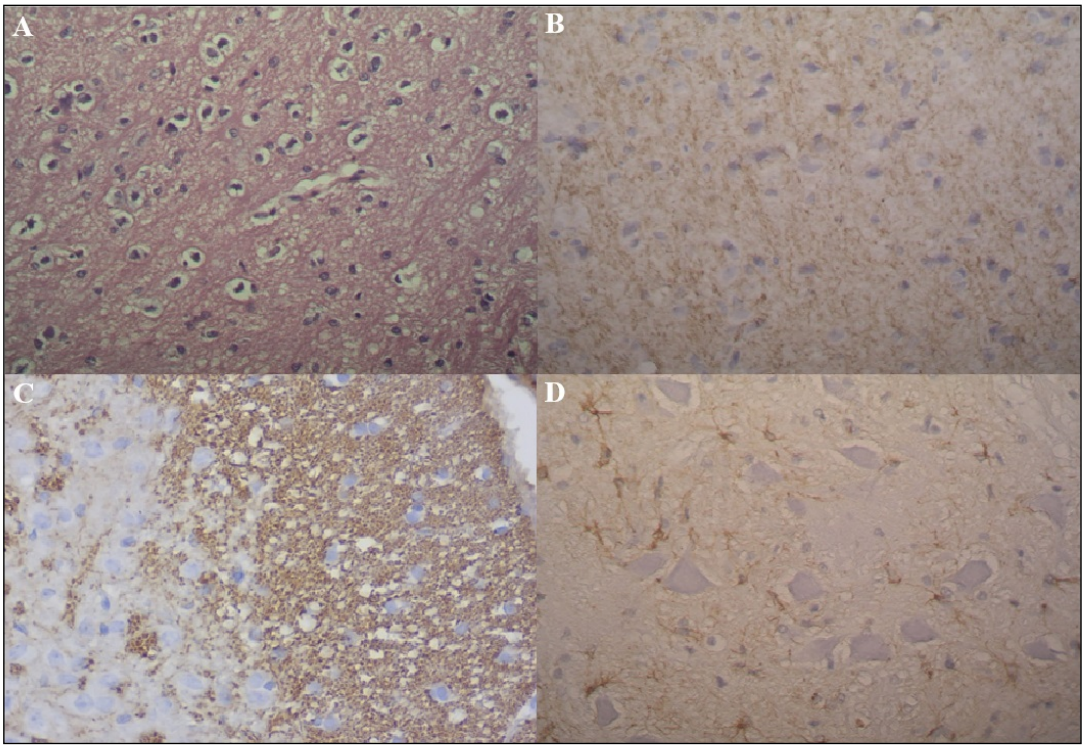

Fig. 4. Representative hypothalamic morphology and immunohistochemistry on Day 7 after closed-head TBI. Representative micrographs obtained on post-injury Day 7 (n=5 per group for tissue analyses). A – Paraventricular nucleus (PVN), H&E staining, carbacetam-treated animal; B – PVN, neuron-specific enolase (NSE) immunohistochemistry, vehicle group; C – PVN, NSE immunohistochemistry, carbacetam group; D – Supraoptic nucleus (SON), glial fibrillary acidic protein (GFAP) immunohistochemistry, vehicle group. Immunoreactivity was visualized using HRP/DAB with hematoxylin counterstain; ×400 magnification.
Source: Authors' own work

regulation. Carbacetam shifted this trajectory toward a more adaptive profile, limiting both the early surge and the later exhaustionlike phase [19].

NEUROENDOCRINE RESPONSE UNDER DCBH

(COMPARATIVE PATTERN)

The endocrine profile under DCBH differed qualitatively from carbacetam: DCBH did not suppress the primary hyperactivation of the central HPA component and AVP secretion during the acute phase, but effectively prevented the delayed depletion at approximately two weeks postinjury. In addition, relative to Vehicle, the reduction of GH secretion was less pronounced under DCBH. These groupwise trajectories are summarized in Fig. 2 (A-D).

HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

On postinjury Day 7, histological assessment of the neuroendocrine regions most directly implicated in the observed hormonal dynamics – namely the paraventricular nucleus (PVN) and supraoptic nucleus (SON) – together with the hippocampal CA3 field, showed a heterogeneous pattern of posttraumatic tissue injury in the vehicle (comparison) group (Fig. 4B, D). A subset of neurons within these regions displayed morphological signs consistent with cellular stress/injury, including distorted neuronal contours, hyperchromatic (dark) nuclei, and heterochromatin condensation. In parallel, immunohistochemistry supported the presence of a marked glial response: GFAP and S100 immunoreactivity (glial markers commonly used to capture astroglial activation and disturbance of glial-neuronal homeostasis) appeared increased in the hypothalamic neurosecretory nuclei and hippocampus, consistent with reactive astrogliosis and disrupted neuron-glia coupling after TBI. Such astrocytic responses are now understood as contextdependent (potentially both adaptive and maladaptive) components of secondary injury and repair cascades after brain trauma. NSE immunoreactivity within hypothalamic nuclei appeared comparatively weak in this posttraumatic setting, whereas hippocampal NSE staining remained more evident – suggesting regionspecific differences in metabolic compromise across structures involved in neuroendocrine regulation versus cognitive circuitry [20, 21].

Against carbacetam treatment, neurons in the PVN/SON and hippocampal CA3 more frequently exhibited a morphology closer to an "intactlike" profile – round/oval nuclei, higher euchromatin content, and fewer overt dys-

trophic features – consistent with a relative preservation of neuronal functional capacity (Fig. 4A). In the PVN, NSE immunoreactivity in carbacetamtreated animals appeared more pronounced than in the vehicle group (Fig. 4C vs B), which is compatible with better preservation of neuronal metabolic activity under treatment. Qualitatively, serial sections also suggested lower GFAP and S100 immunoreactivity within hypothalamic neurosecretory nuclei under carbacetam versus vehicle, indicating attenuation of the astroglial stress response in the very regions that coordinate HPAaxis signaling and vasopressin neurosecretion. In contrast, in the hippocampus, GFAP/S100 expression remained elevated despite treatment – an observation that can be interpreted in a mechanistically plausible way within modern frameworks: sustained astrocyte activation in hippocampal networks may represent a prorecovery glial program supporting synaptic/metabolic homeostasis and compensation rather than a simple marker of ongoing degeneration. Importantly, this regionspecific pattern provides a coherent morphological correlate to the endocrine phenotype described above – namely, a normalization/modulation of hypothalamic neurosecretion under carbacetam, while hippocampal glial remodeling may persist as part of longerterm plasticity after TBI [20].

CONCLUSIONS

1. Closedhead traumatic brain injury (TBI) induced a marked increase in neurological deficit on Day 1 (5.4-5.9fold vs intact; $p < 0.05$), followed by partial spontaneous regression; however, without treatment the deficit remained significantly elevated on Day 7 (3.4fold vs baseline/intact; $p < 0.05$). The early deficit was driven predominantly by impairment within the reflex domain (37.6% of the total deficit structure). Both carbacetam and deproteinized calf blood hemoderivative (DCBH) were associated with a reduction of neurological deficit, with a larger effect for carbacetam: by Day 7 the deficit decreased by 57% under carbacetam, 47% under DCBH, and 43% in the vehicle group ($p < 0.05$ for the reported withingroup changes).
2. On Day 1 afterTBI, behavioral indices of higherorder CNS function were significantly impaired ($p < 0.05$ vs sham): squares crossed decreased to 43%, holes explored to 21%, and successful entries in the eightarm maze to 48% of sham; light-dark transition latency (burrowreflex paradigm) increased by 88% ($p < 0.05$). During follow-up, these measures partially recovered but, without pharmacological correction, did not fully normalize to sham levels by Day 30 (with persistent deficits in exploratory/locomotor and maze indices, whereas latency approached sham values). Carbacetam restored orienting-locomotor performance to nearcontrol levels (83% for squares crossed and 85% for holes explored vs sham; $p > 0.05$) and normalized the eightarm maze and light–dark latency to controlrange values (97% and 115%, respectively; $p > 0.05$). DCBH also improved behavioral outcomes, but did not normalize holes explored; this parameter remained higher with carbacetam than with DCBH by 24-37% ($p < 0.05$), indicating a more robust recovery of exploratory activity under carbacetam.
3. Following TBI, the hypothalamic-pituitary-adrenal (HPA) axis and vasopressin secretion showed an acute hyperreactive response followed by secondary depletion at approximately two weeks postinjury. A roughly twofold rise in ACTH was accompanied by an approximately threefold rise in corticosterone, consistent with the development and maintenance of a posttraumatic hypercorticosteronemic state. Across the entire observation period, growth hormone (GH) secretion was progressively suppressed, reaching 48% of baseline by Day 30 ($p < 0.05$). Carbacetam exerted an early modulatory effect (evident after the first administration), preventing primary hyperactivation and subsequent depletion of central neuroendocrine outputs, attenuating the development of secondary posttraumatic hypercorticosteronemia, and preventing sustained suppression of GH secretion. DCBH did not attenuate the initial hyperactivation of central HPA signaling and vasopressin secretion, but effectively prevented their secondary depletion; GH remained reduced, but less markedly than in the vehicle group.
4. Immunohistochemical assessment using GFAP, S100, and NSE supported the presence of posttraumatic degenerative/glialreactive changes: GFAP and S100 immunoreactivity was increased (consistent with disturbed neuron–glia interactions), whereas NSE immunoreactivity was reduced in the injury setting. Carbacetam was associated with a decrease in GFAP and S100 and an increase in NSE, indicating attenuation of degenerative/glialstress features and better preservation of neuronal metabolic activity. In hippocampal CA3, carbacetam exerted a protective effect predominantly on the neuronal component of the tissue.
5. Cumulative mortality after TBI was 50% in the vehicle group, compared with 16% under carbacetam and 36% under DCBH (overall $p=0.002$). Carbacetam reduced mortality 3.1fold versus vehicle ($p=0.001$), whereas DCBH reduced mortality 1.4fold ($p=0.096$). These findings are consistent with the interpretation that carbacetam – and to a lesser extent DCBH – promoted a shift of neurohumoral regulatory responses toward a more adaptive pattern (restoration of central and peripheral endocrine components), which was accompanied by improved survival, neurological recovery, and behavioral outcome after closedhead TBI.

REFERENCES

1. Maas AIR, Menon DK, Manley GT, Abrams M, Åkerlund C, Andelic N et al.; InTBIR Participants and Investigators. Traumatic brain injury: progress and challenges in prevention, clinical care, and research. Lancet Neurol. 2022 Nov;21(11):1004-1060. doi: 10.1016/S1474-4422(22)00309-X. Epub 2022 Sep 29. Erratum in: Lancet Neurol. 2022 Dec;21(12):e10. doi: 10.1016/S1474-4422(22)00411-2. DOI
2. GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators. Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019 Jan;18(1):56-87. doi: 10.1016/S1474-4422(18)30415-0. Epub 2018 Nov 26. Erratum in: Lancet Neurol. 2021 Dec;20(12):e7. doi: 10.1016/S1474-4422(21)00383-5. DOI
3. Zhang JK, Botterbush KS, Bagdady K, Lei CH, Mercier P, Mattei TA. Blast-Related Traumatic Brain Injuries Secondary to Thermobaric Explosives: Implications for the War in Ukraine. World Neurosurg. 2022 Nov;167:176-183.e4. doi: 10.1016/j.wneu.2022.08.073. DOI
4. World Health Organization Regional Office for Europe. Three years of war: rising demand for mental health support, trauma care and rehabilitation. Published February 24, 2025. URL: https://www.who.int/europe/news/item/24-02-2025-three-years-of-war-rising-demand-for-mental-health-support-trauma-care-and-rehabilitation
5. Bryant AM, Rose NB, Temkin NR, Barber JK, Manley GT, McCrea MA et al. Profiles of Cognitive Functioning at 6 Months After Traumatic Brain Injury Among Patients in Level I Trauma Centers: A TRACK-TBI Study. JAMA Netw Open. 2023 Dec 1;6(12):e2349118. doi: 10.1001/jamanetworkopen.2023.49118. DOI
6. Tanriverdi F, Schneider HJ, Aimaretti G, Masel BE, Casanueva FF, Kelestimur F. Pituitary dysfunction after traumatic brain injury: a clinical and pathophysiological approach. Endocr Rev. 2015 Jun;36(3):305-42. doi: 10.1210/er.2014-1065. DOI
7. Hiskens MI. Targets of Neuroprotection and Review of Pharmacological Interventions in Traumatic Brain Injury. J Pharmacol Exp Ther. 2022 Aug;382(2):149-166. doi: 10.1124/jpet.121.001023. DOI
8. Witkin JM, Shafique H, Cerne R, Smith JL, Marini AM, Lipsky RH et al. Mechanistic and therapeutic relationships of traumatic brain injury and γ-amino-butyric acid (GABA). Pharmacol Ther. 2024 Apr;256:108609. doi: 10.1016/j.pharmthera.2024.108609. DOI
9. Ziablitsev SV, Starodubska OO, Bogza SL. Influence of carbacetam on neurologic destruction processes under the experimental traumatic brain injury. Journal of Education, Health and Sport (formerly of Health Sciences). 2017;7(2):601–611.
10. Ayipo YO, Mordi MN, Mustapha M, Damodaran T. Neuropharmacological potentials of β-carboline alkaloids for neuropsychiatric disorders. Eur J Pharmacol. 2021 Feb 15;893:173837. doi: 10.1016/j.ejphar.2020.173837. DOI
11. Beato A, Gori A, Boucherle B, Peuchmaur M, Haudecoeur R. β-Carboline as a Privileged Scaffold for Multitarget Strategies in Alzheimer's Disease Therapy. J Med Chem. 2021 Feb 11;64(3):1392-1422. doi: 10.1021/acs.jmedchem.0c01887. DOI
12. Rupprecht R, Wetzel CH, Dorostkar M, Herms J, Albert NL, Schwarzbach J et al. Translocator protein (18kDa) TSPO: a new diagnostic or therapeutic target for stress-related disorders? Mol Psychiatry. 2022 Jul;27(7):2918-2926. doi: 10.1038/s41380-022-01561-3. DOI
13. Kmet OG, Ziablitsev SV, Filipets ND, Kmet TI, Slobodian XV. Carbacetam effect on behavioral reactions in experimental Alzheimer's disease. Arch Balk Med Union. 2019;54(1):124–129. doi: 10.31688/ABMU.2019.54.1.17. DOI
14. Li N, Yan L, Peng A, Fu X, Qin H, Yao K. Voltage-gated sodium channels in the nervous system: Molecular physiology to therapeutic interventions. Neural Regen Res. 2025 Aug 13. doi: 10.4103/NRR.NRR-D-25-00260. DOI
15. Huang XJ, Li WP, Lin Y, Feng JF, Jia F, Mao Q et al. Blockage of the upregulation of voltage-gated sodium channel nav1.3 improves outcomes after experimental traumatic brain injury. J Neurotrauma. 2014 Feb 15;31(4):346-57. doi: 10.1089/neu.2013.2899. DOI
16. Angeloni E, Germelli L, Costa B, Martini C, Da Pozzo E. Neurosteroids and Translocator Protein (TSPO) in neuroinflammation. Neurochem Int. 2025 Jan;182:105916. doi: 10.1016/j.neuint.2024.105916. DOI
17. Verdoorn TA, Parry TJ, Pinna G, Lifshitz J. Neurosteroid Receptor Modulators for Treating Traumatic Brain Injury. Neurotherapeutics. 2023 Oct;20(6):1603-1615. doi: 10.1007/s13311-023-01428-7. DOI
18. Magyar-Sumegi ZD, Stankovics L, Lendvai-Emmert D, Czigler A, Hegedus E, Csendes M et al. Acute neuroendocrine changes after traumatic brain injury. Brain Spine. 2024 May 7;4:102830. doi: 10.1016/j.bas.2024.102830. DOI
19. Hasan S, Uff C. Neuroendocrine dysfunction following traumatic brain injury: Current insights and emerging perspectives. Best Pract Res Clin Endocrinol Metab. 2025 May;39(3):102006. doi: 10.1016/j.beem.2025.102006. DOI
20. Muñoz-Ballester C, Robel S. Astrocyte-mediated mechanisms contribute to traumatic brain injury pathology. WIREs Mech Dis. 2023 Sep-Oct;15(5):e1622. doi: 10.1002/wsbm.1622. DOI
21. Janigro D, Mondello S, Posti JP, Unden J. GFAP and S100B: What You Always Wanted to Know and Never Dared to Ask. Front Neurol. 2022 Mar 21;13:835597. doi: 10.3389/fneur.2022.835597. DOI

CONFLICT OF INTERESTS

The Authors declare no conflict of interest

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