Neurotransmitter Systems in a Mild Blast Traumatic Brain Injury Model: Catecholamines and Serotonin

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Abstract

Exposure to improvised explosive devices can result in a unique form of traumatic brain injury—blast-induced traumatic brain injury (bTBI). At the mild end of the spectrum (mild bTBI [mbTBI]), there are cognitive and mood disturbances. Similar symptoms have been observed in post-traumatic stress disorder caused by exposure to extreme psychological stress without physical injury. A role of the monoaminergic system in mood regulation and stress is well established but its involvement in mbTBI is not well understood. To address this gap, we used a rodent model of mbTBI and detected a decrease in immobility behavior in the forced swim test at 1 d post-exposure, coupled with an increase in climbing behavior, but not after 14 d or later, possibly indicating a transient increase in anxiety-like behavior. Using in situ hybridization, we found elevated messenger ribonucleic acid levels of both tyrosine hydroxylase and tryptophan hydroxylase 2 in the locus coeruleus and the dorsal raphe nucleus, respectively, as early as 2 h post-exposure. High-performance liquid chromatography analysis 1 d post-exposure primarily showed elevated noradrenaline levels in several forebrain regions. Taken together, we report that exposure to mild blast results in transient changes in both anxiety-like behavior and brain region–specific molecular changes, implicating the monoaminergic system in the pathobiology of mbTBI.

Key words: anxiety; mood disorder; noradrenaline; PTSD; stress

Introduction

BLAST-INDUCED TRAUMATIC BRAIN INJURY (bTBI), AND post-traumatic stress disorder (PTSD) have emerged as leading military health issues, with major long-term neuropsychiatric consequences.[1–5] Blast-induced TBI is a spectrum disorder[6] and at its mild end (mild bTBI [mbTBI]), the neurobehavioral symptoms substantially overlap with those observed in PTSD. PTSD is characterized by emotional instability, increased anxiety, and hyperarousal.[7–11] The sudden and violent nature of the blast, even without detectable physical injury, can result in an initial elevation in anxiety and lead to sustained emotional impairment.[2]

In the majority of instances, these symptoms are acute; however, for some, they can develop into a chronic state.[12,13] The lack of obvious or easily detectable structural damage and overlapping symptomology with PTSD have made the pathomechanism(s) of mbTBI difficult to identify and has hindered correct diagnosis.[11] One, therefore, has to rely on the patient's symptoms, which are underreported and often biased and/or based on subjective neuro-behavioral testing.[14]

A link between stress/mood disorders and distinct changes in the monoamine neurotransmitters have been extensively reported in the literature and include both noradrenaline (NA)[15–25] and serotonin (5-hydroxytryptamine; 5-HT).[26–33] Importantly, elevated NA levels are implicated in PTSD symptomology, associated with sleep disturbance and increased nightmare frequency, a hallmark of PTSD.[34]

NA is synthesized in the locus coeruleus (LC)[35] involving the rate limiting enzyme tyrosine hydroxylase (TH).[36,37] The LC gives rise to projections to virtually all regions of the central nervous system, including the prefrontal cortex (PFC), the dorsal and ventral hippocampal formation (dHiFo and vHiFo, respectively), and the hypothalamus.[38–40] The dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) harbor the neuronal cell bodies, giving rise to the extensive serotonergic forebrain projections.[35,41,42] The 5-HT neurones express the rate-limiting enzyme for the 5-HT synthesis, (brain) tryptophan hydroxylase 2 (TPH2).[43]

The role of the monoaminergic system in mbTBI is not well understood. One way to address this issue is to apply animal models, which have been developed to replicate the effects of bTBI.[44,45] Here, we have used a metal blast tube as previously

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described.[46] We tested exposed and sham animals for anxiety-/depression-like behavior using the forced swim test (FST)[47–50]; assessed the expression of the monoamine biosynthetic enzymes TH and TPH2 in the LC and DRN, respectively, using quantitative in situ hybridization (ISH); and quantified NA, dopamine (DA), and 5-HT, as well as some of their metabolites using high-performance liquid chromatography (HPLC).

Methods

Animals and housing conditions

A total of 78 male Sprague-Dawley rats (Taconic, Ry, Denmark), 10–12 weeks old and weighing 290–320 g at the beginning of the experiments, were used. They were housed in groups of four in Type IV MakrolonR plastic cages under standardized conditions (12 h light/dark cycle, lights on at 07:00; temperature of $22 \pm 0.5°C$; and 40–50% relative humidity). Food and water were provided ad libitum to the animals.

Experimental groups and manipulations

This study is made up of three separate experiments, and for each experiment, different animals were used. All animals were handled and given a week to habituate to their environment prior to the experiments, then randomly assigned to one of three groups: control, sham, and exposed. Control rats were kept in the animal facility without any experimental manipulations for the duration of the study. Sham rats were anesthetized by isoflurane inhalation, then injected intraperitoneally with 2.4 mL/kg of a mixture of 1 mL Dormicum® (5 mg/mL midazolam; Roche, Stockholm, Sweden), 1 mL Hypnorm® (Fentanyl/fluaniscane; Janssen, Stockholm, Sweden) and 2 mL of distilled water. Exposed rats were treated identically to shams in addition to being exposed to a single blast wave.

Experiment One was a behavior test—the FST. Animals were divided into three groups: control ($n = 6$), sham ($n = 7$), and exposed ($n = 8$). Experiment Two consisted of in situ hybridization and immunohistochemistry analysis. A total of 33 animals were terminated at four post-exposure time-points: 2 h ($n = 5$), 1 d ($n = 5$), 3 d ($n = 4$) and 7 d ($n = 8$), and a total of 11 shams were used. Experiment Three consisted of HPLC analysis. Twenty-four animals were sacrificed at 1 d = 6, 6, and 7 d = 6, 6, exposed and sham, respectively.

Exposure conditions

A validated blast tube[46,51–53] designed by Clemedson in 1955 was used for exposure.[54] Swedish army plastic explosive containing explosive m/46, 86% pentaerythritol tetranitrate and mineral oil was used with a Nonel ignition (Dyno Nobel Sweden, Nora, Sweden). Anesthetized animals were placed in a rigid metallic holder that protects all parts of the body except the head. This holder protects from lung injuries that otherwise would occur with the employed amount of explosive. The holder also prevents acceleration movements of the head relative to the rest of the body. The holder was subsequently mounted into the 1.5 m metal shock tube, with the rat placed in a transverse prone position at a distance of 1 m from the charge. Five grams of the explosive were then detonated, triggering a simple blast Friedlander-type wave at the surface of the animal with a peak pressure of 550 kPa and a duration of 0.2 msec.[46] The rat's left side faced the charge. Post-exposure, animals were monitored for 1 h, after which time they awoke, as did the shams.

Forced swim test

Anxiety-/depression-like behavior was measured using the FST[47] at 1 d pre-exposure (baseline) and 1 d, 14 d, and 35 d post-exposure. Animals were individually placed in a vertical plastic cylinder (50 cm height, 18 cm diameter) containing water to a height of 30 cm, at a temperature of $25 \pm 0.5°C$. Animals were exposed to two swimming sessions, 24 h apart: a 10-min pre-test, and a 5-min test. The total duration of immobility and climbing behavior were recorded during the second, test day.[49] Immobility was defined as floating passively in an upright position in water, with only small movements necessary to keep the head above the water surface, typically only one paw movement at a time. Climbing was defined as vigorous forepaw movements directed toward the walls of the cylinder.

In situ hybridization (ISH)

Animals were anesthetized with isoflurane, injected with 1.5–2.0 mL of pentobarbital, and then decapitated. The brains were rapidly removed and placed on dry ice and, once frozen, stored at $-70°C$ until use. Serial coronal sections were cut at a thickness of 14 μm at the level of the LC (bregma $-10.52 - -9.16$ mm), and DRN ($-8.30 - -7.30$ mm; coordinates according to Paxinos and Watson[55]) using Cryo-Star HM 560M (MICROM International GmbH, Heidelberg, Germany), and two sections were thaw-mounted per super frost slide (Thermo Scientific, Stockholm, Sweden). Three slides per animal were processed for ISH. Method of selection of slides was random.

Oligonucleotides complementary to rat TPH2 nucleotides[43]: TCC TCC GTC CAA ATG TTG TCA GGT GGA TTC AGC GTC ACA ATG GTG GTC [GENBANK ID NM_017139], and TH[37]: GCG CTG GAT ACG AGA GGC ATA GTT CCT GAG CTT GTC [GENBANK ID NM_012740] (CyberGene AB, Solna, Sweden), were labeled with deoxyadenosine 5′triphosphate α–P32 at the 3′-end using terminal deoxynucleotidyltransferase (Thermo Scientific, Waltham, MA).

The labeled oligoprobes were purified using ProbeQuant G-50 Micro Columns (Amersham Pharmacia Biotech, Piscataway, NJ). Sections were air-dried and incubated with the oligonucleotide probe for 18 h at 42°C. Post-hybridization, sections were rinsed in $1 \times SSC$, 4×30 min at 55°C followed by 1 h at room temperature (RT), dehydrated with 70%, followed by 90% and finally 100% ethanol, air-dried and dipped in liquid photo emulsion NTB2 at RT (Kodak, Rochester, NY).

The optimal exposure time was determined by exposing the slides to imaging plates (BAS-SR Fujifilm, Tokyo, Japan) for 24 h at RT, which were scanned using a phosphoimager (Fuji BAS 3000, Tokyo, Japan). Based on this data, TPH2 slides were developed after 72 h and TH after a week using D19 developer (Kodak) and AL-4 fixative (Kodak) and mounted in glycerol-phosphate.[56]

Dark field photomicrographs were captured in a microscope (Nikon Eclipse E-600; Nikon, Tokyo, Japan), connected to a digital camera (Digital Sight, U1; Nikon). The images were analyzed according to the mean gray density (MGD) of the messenger ribonucleic acid (mRNA) signal in the regions of interest (ROIs), using ImageJ 1.48 (National Institutes of Health, Bethesda, MD).

Histo- and immunohistochemistry (IHC)

Sections not used for ISH were used to evaluate nerve cell death (using Fluoro-Jade B [FJ] staining; Merck Millipore AG310, Darmstadt, Germany), β-amyloid precursor protein accumulation (APP; IHC) and leakages of blood vessels (using a secondary rat antibody, IHC). As positive control, sections from experiments with a focal penetrating injury model were used.[57]

For FJ staining, sections were air dried for 1 h before being fixed in 4% formaldehyde for 10 min, and rinsed in 0.01 M phosphate-buffered saline (PBS) 3×3 min. The sections were subsequently dipped in dH_2O for 2 min, followed by 10 min in 0.06% potassium permanganate ($KMnO_4$), then rinsed with dH_2O for 2 min. Slides

were then soaked in FJ solution for 30 min, washed in dH_2O 3×1 min, and finally placed on a hot plate 50°C for 5 min before a quick dip in xylene and thereafter mounted with Entellan (Merck).

For all other staining, slides were fixed in ice cold methanol for 5 min, then shortly dipped in ice cold acetone, and rinsed in 0.01 M PBS 2×10 min. One group was incubated in a humid chamber at 4°C for 18 h with a solution of 0.3% Triton, 5% bovine serum albumin, and 0.1% sodium azide in 0.01M PBS and a rabbit polyclonal antibody against APP (51-2700, dilution 1:400; Life Technologies, Stockholm, Sweden). The other group was not exposed to any primary antibody.

The following day, all sections were rinsed in 0.01M PBS 2×10 min and incubated for 1 h at RT with 0.01 M PBS, 0.1% sodium azide, 0.3% Triton. The first group then processed with an Alexa Fluor 488 conjugated anti-rabbit immunoglobulin G (dilution 1:400; Jackson ImmunoResearch, Suffolk, UK). The second group was incubated with CY2- conjugated donkey anti-rat antibody (Jackson ImmunoResearch, 712-225-153, dilution 1:100) to visualize blood vessel leakage.

Sections were rinsed in 0.01M PBS 2×10 min, and mounted in a mixture of glycerol and PBS (1:3), then cover-slipped. Slides were viewed in a microscope equipped with epifluorescence (Eclipse E600; Nikon).

High-performance liquid chromatography

Chemicals required for HPLC included: NA hydrochloride, DA hydrochloride, 5-HT hydrochloride, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), potassium chloride, phosphate monobasic monohydrate, sodium phosphate dibasic, sodium acetate, citric acid, octanesulfonic acid sodium salt and methanol, which were obtained from Sigma-Aldrich (St. Louis, MO). EDTA-2Na was purchased from Dojindo (Kumamoto, Japan).

The following regions were dissected using the appropriate landmarks as detailed by Paxinos and Watson[55]: PFC, hypothalamus, the dHiFo and vHiFo formation, occipital cortex (CX) and entorhinal cortex (ERC). Both ipsi-lateral (left)- and contra-lateral (right) regions were dissected, except the hypothalamus. The isolated brain regions were put into marked plastic vials and placed on dry ice. Dissected brain tissue samples (1–10 mg) were mixed at a ratio of 1:10 (weight/volume) with 0.2 M perchloric acid including 100 μM EDTA-2Na, and homogenized at 0°C in a glass-pestle micro-homogenizer. Following standing for 3 min on ice, the homogenates were centrifuged for 15 min at 12,000 g at 4°C. The supernatants were aspirated and mixed with 1 M Na-acetate buffer, pH 3 at a ratio 5:1 (volume/volume) and filtered through a 0.22 μm centrifugal filter for 4 min at 12,000 g at 4°C. The filtrates were stored at −80°C before HPLC analysis.

Concentrations of NA, DA, and 5-HT in the brain tissue samples were determined by HPLC with electrochemical detection as described elsewhere.[58] Briefly, the HPLC system consisted of a HTEC500 unit (Eicom, Kyoto, Japan), and a CMA/200 Refrigerated Microsampler (CMA Microdialysis, Stockholm, Sweden) equipped with a 20 μL loop and operating at 4°C. The potential of the glassy carbon working electrode was +450 mV versus the Ag/AgCl reference electrode. The separation was achieved on a 200×2.0 mm Eicompak CAX column (Eicom). The mobile phase was a mixture of methanol and 0.1 M phosphate buffer (pH 6.0; 30:70, volume/volume) containing 40 mM potassium chloride and 0.13 mM EDTA-2Na. The chromatograms were recorded and integrated by use of a computerized data acquisition system Clarity (DataApex, Prague, Czech Republic). The detection limit (signal-to-noise ratio=3) for NA, DA, and 5-HT was 0.05 nM (i.e., 0.75 fmoL in 15 μL injected onto the column).

Concentrations of DOPAC, HVA, and 5-HIAA were determined by a separate HPLC system with electrochemical detection (HTEC500). The potential of the glassy carbon working electrode

was +750 mV versus the Ag/AgCl reference electrode. The separation was achieved on a 150×3.0 mm Eicompak SC-5ODS column (Eicom). The mobile phase was a mixture of methanol and 0.1 M citrate/0.1 M sodium acetate buffer solution (pH 3.5; 16:84, volume/volume) and contained 210 mg/L octanesulphonic acid sodium salt and 5 mg/L EDTA-2Na. The detection limit (signal-to-noise ratio=3) for DOPAC, HVA, and 5-HIAA was 2 nM (i.e., 10 fmoL in 5 μL injected onto the column). The chromatograms were recorded and integrated by use of the computerized data acquisition system Clarity (DataApex).

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 5 (GraphPad Software, CA).

For the FST differences in the total time spent climbing and immobile during each test session (baseline and 1 d, 14 d, and 35 d post-exposure) were independently evaluated across all three groups (exposed, $n=8$; sham, $n=7$; control, $n=6$) using analysis of variance (ANOVA), repeated measures ANOVA, and Dunnett's t-test.

For ISH, the MGDs were determined for each of the ROIs (DRN, MRN, and LC) for each transcript (TH and TPH2). The MGDs of individual ROIs from each specific time-point (2 h, $n=5$; 1 d, $n=5$, 3 d, $n=4$; and 7 d, $n=8$; and a total of 11 shams) and transcript were separately compared with sham MGDs of corresponding ROIs and transcript, and analyzed using ANOVA and followed up by the Tukey-Kramer multiple comparison test, which enables comparisons across all five experimental groups. No difference between shams of the different time-points was found as evaluated by ANOVA, so the groups were collapsed. The MGD of each ROI was normalized to its corresponding sham level to clearly show increases and/or decreases in transcript level on sham levels.

For HPLC, each specific monoamine/ metabolite (NA, DA, 5-HT and DOPAC, HVA, 5-HIAA) was evaluated exclusively from one brain region against its corresponding sham region. The two terminal time-points post-exposure were evaluated separately, using an unpaired t-test, 1 d=6, 6 and 7 d=6, 6, exposed and sham, respectively. Since none of the brain regions showed a significant difference between the left and right, these groups were collapsed in the analysis.

All data are presented as the mean ± standard error of the mean (SEM). The level of significance is as follows: $*p<0.05$, $**p<0.01$, and $***p<0.001$.

Results

Anxiety-/depression-like behavior

Anxiety-/depression-like behavior was assessed in the second day of the FST, during a 5-min test session ran at 1 d pre-exposure (baseline) and 1 d, 14 d, and 35 d post-exposure (immobility, Fig. 1A; climbing, Fig. 1B). A one-way ANOVA comparing the behavioral differences across the groups revealed no differences in the three groups pre-exposure (baseline), as expected. However, after 1 d, the exposed group exhibited decreased immobility relative to the control and sham groups, whereas the values for the climbing behavior were not statistically significant (between the groups). In the remaining test sessions, even after 35 d, similar climbing and immobility behaviors were observed across the groups.

Further analysis of the data using a repeated measures ANOVA, which looks at the changes in the behavior of the individuals in each group across the different time-points, revealed a significant change in the amount of time spent climbing in the exposed group ($p=0.0472$). Thus, 1 d post-exposure climbing behavior increased versus baseline in the exposed group, but returned to baseline levels 35 d post-exposure. No change in climbing behavior was seen in the
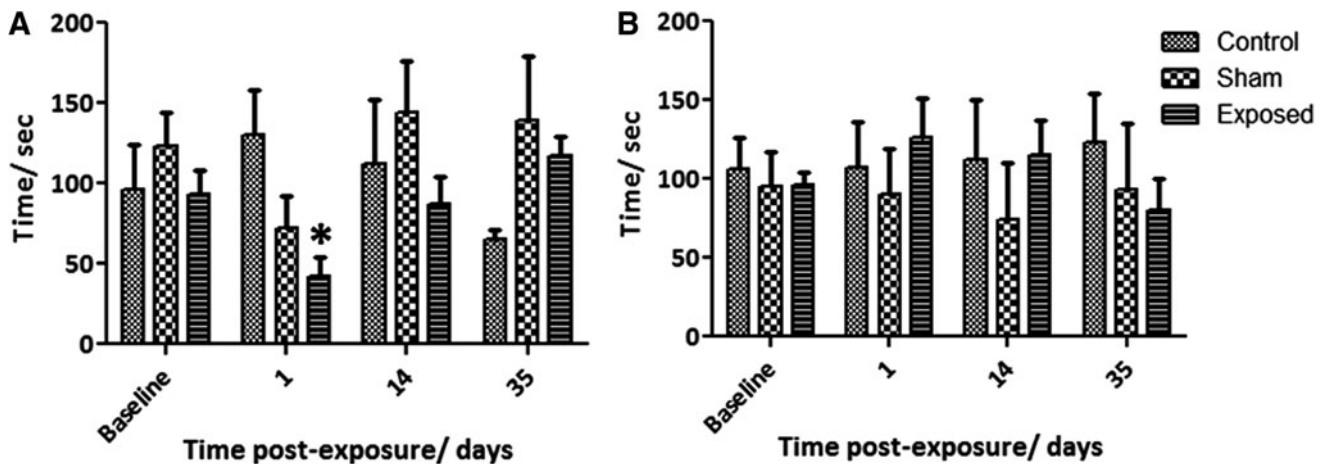
FIG. 1. Time of immobility **(A)** and climbing **(B)** in the forced swim test measured at 1 d pre-exposure (baseline), and 1 d, 14 d, and 35 d post-exposure. Immobility behavior decreased in the exposed group 1 d post-exposure relative to sham and controls (a). Data are presented as means ± standard error of the mean in the three groups; control ($n=6$), sham ($n=7$), and exposed ($n=8$). *$p < 0.05$.

control and sham group ($p=0.9691$ and 0.7075, respectively). The same statistical parameters were used to look at the immobility behavior, again no difference was seen in the control group ($p=0.3404$). Both the sham and exposed group exhibited increased immobility behavior as time progressed, this trend towards an increase in immobility was far more apparent in the exposed group as evident in the p values (0.0298 and 0.0003 for sham and exposed, respectively).

Transcript of TPH2 and TH as measured by ISH

ISH analysis of the transcript levels of the two key biosynthetic enzymes, TPH2 and TH, showed an early and significant upregulation. TH was upregulated bilaterally in the LC and TPH2 increased in the mid/caudal, but not rostral, DRN as early as 2 h post-exposure (Fig. 2 and Fig. 3). The results indicate TH transcript was distinctly sensitive to the blast exposure, remaining significantly elevated at 1 and 3 d post-exposure, and returning to sham levels after 7 d. The increase in TPH2 transcript was modest, being limited to the mid/caudal region of the DRN and returned to sham levels 3 d post-exposure. TPH2 levels in the median raphe were also measured but no changes were observed at any of the time-points.

Nerve cell death, blood vessel leakage and APP accumulation assessed by IHC

Sections from the LC, DRN and both dHiFo and vHiFo were tested for nerve cell death, blood vessel leakage and APP accumulation by IHC. No differences were seen between shams and exposed rats terminated at 1 and 7 d post-exposure. Exposed rats did not appear to have leakages of any major blood vessels in the brain stem or forebrain regions post-exposure, nor was cell death seen in any of these areas. Evaluation of the white matter tracts by staining for APP accumulation, revealed no difference to the shams (data not shown).

Monoamine levels as measured by HPLC

The HPLC analysis, performed at 1 and 7 d post-exposure, showed regional and varying changes in the levels of the neurotransmitters NA, DA, and 5-HT (Table 1A), along with their metabolites DOPAC, HVA, and 5-HIAA (Table 1B), in a number of forebrain regions (i.e., projection areas of the LC NA and DRN/MRN 5-HT neurones). No statistically significant differences were observed between left versus right sides; therefore, these groups were collapsed. The major findings were significant increases in NA levels 1 d post-exposure in CX, ERC, and dHiFo. The changes did not reach significance in the vHiFo. A significant increase in DA levels was observed in both the dHiFo and vHiFo on 1 d post-exposure. No significant differences were seen in 5-HT levels. With regard to the metabolites, a significant decrease in DOPAC was measured in the CX, and HVA levels were significantly decreased in the ERC and dHiFo. These changes occurred at 7 d post-exposure. Finally, a significant reduction in 5-HIAA levels was seen in the PFC at 1 d post-exposure. Taken together, the NA system appears most sensitive to the blast but only in the early phase (i.e., 1 d post-exposure).

Discussion

Here, we show that exposure to a single blast wave with short duration and relatively high peak pressure results in an apparent mTBI. The blast caused decreased immobility in the FST only during the first day and in parallel, an early and only transient increase in extracellular levels of NA 1 d post-exposure. The changes in TH and TPH2 transcripts occurred as early as 2 h post-exposure and remained elevated for few days (TPH2 only for one day; TH for 3 but not 7 d). Moreover, we could not detect white matter injury, blood–brain barrier disruption, or cell death. All of these findings support the involvement of the noradrenergic system originating in the LC in the mild form of blast TBI.

The interpretation of the behavioral state of our rats at this time-point is complex. Estanislau and colleagues[59] evaluated FST behavior in the elevated plus-maze[60] and found that the most anxious animals have a higher latency to immobility versus a stronger immobility behavior in less anxious animals. According to these results, our rats exhibit anxiety-like behavior, a term that we use in this discussion. It should be noted that the behavior associated with increased NA levels has often been termed hyperarousal.[61]

Except for 1 d post-exposure, the rats did not show any changes in the FST, compared with control and shams (i.e., either at 14 or 35 d post-exposure). Thus, no signs of increased immobility and decreased climbing were recorded, at the later time points, i.e.,
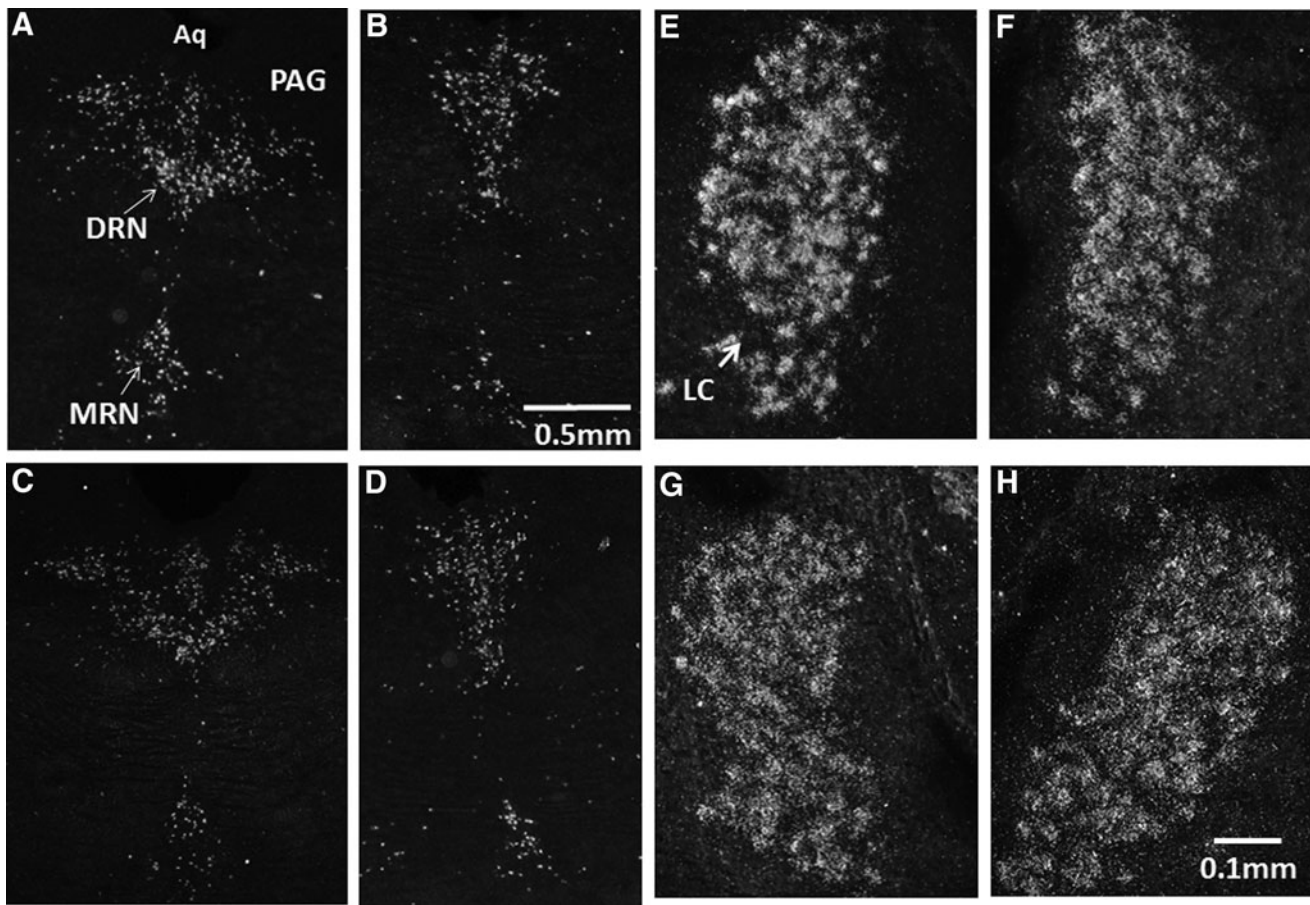
FIG. 2. Representative dark field in situ hybridization photomicrographs of emulsion-dipped sections showing the distribution of tryptophan hydroxylase 2 (TPH2; **A-D**) and tyrosine hydroxylase (TH; **E–H**), messenger ribonucleic acid (mRNA) in the DRN and LC, post-exposure to a mild blast-induced traumatic brain injury in the rat. TPH2 mRNA is found upregulated 2 h post-exposure in the mid/caudal DRN (A) but not in the rostral DRN (B), relative to shams, mid/caudal DRN (C), and rostral (D). TH mRNA is upregulated 2 h post-exposure in both the right (E) and left (F) LC, relative to sham right (G) and left (H) LC. Aq, aqueduct; PAG, periaqueductal gray; DRN, dorsal raphe nucleus; MRN, median raphe nucleus; LC, locus coeruleus.

there was no sign of behavioral despair (failure of continuing an escape-directed behavior), nor of development of learned help-lessness (passive behavior).[47,62] Failure to cope with stressful events is considered a feature of the depression syndrome and has been associated with dysfunction of monoamine systems, in particular NA, and 5-HT neurones.[63]

TBI and monoamine turnover

Our HPLC analysis shows that mild blast increases NA levels in several forebrain regions, in concert with the noradrenergic LC system being activated by stress.[9,21–25] Also, a second catechol-amine, DA, showed a significant increase 1 d post-exposure but only in the hippocampal formation (HiFi). No changes were seen in 5-HT levels; however, a decrease in the serotonin metabolite 5-HIAA was seen in the PFC 1 d post-exposure.

The increase in monoamine levels could indicate a substantial release of neurotransmitters followed by compensatory re-synthesis. This also would explain the increase in the transcript levels for the synthesizing enzymes, and the decrease in metabolite levels, as the cell tries to replenish its neurotransmitter stores. The surge in catecholamine levels in the brain also may have a relation to inflammation.[64] It has been shown by Cernak[65] that there is a

systemic inflammatory response to blast and that this includes the brain. Interestingly, no effects on monoamine transmitter levels were seen in the PFC, in spite of the fact that PFC is a key region in cognition and emotions.[63,66–68]

Monoamine turnover has previously been monitored after various types of TBI in animal experiments. Six days after unilateral, ventrolateral cortical lesions, 5-HT and NA were decreased ipsilaterally, whereas 5-HIAA was increased.[69] Unilateral, focal cortical freezing or heat lesion both decreased 5-HT levels bilaterally after 1 d, whereas 5-HIAA increased and remained elevated up to 10 ld.[70] Subsequently, this group could show an ipsilateral increase in 5-HT synthesis in the cortex, hippocampus, and DRN using the same model.[71] Finally, the monoamine system also has been monitored shortly after concussion/head injury. Thus, Tanaka and colleagues[72] found a strong, very rapid decrease in cortical NA level at 25 and 210 sec using a cortical weight drop model but no effects on DA or 5-HT, again pointing to the high reactivity and sensitivity of the noradrenergic LC system.

Brain monoamines and PTSD have also been analyzed in models not involving physical lesions (e.g., various types of stress). Using sequential restraint stress, swim stress, and halothane exposure, NA, 5-HT, and DA concentrations were elevated in the hippocampus after 7 d but not after 1 h.[73] Wilson and colleagues[74] exposed rats to a predator (cat) plus daily cage cohort changes. Using HPLC, they
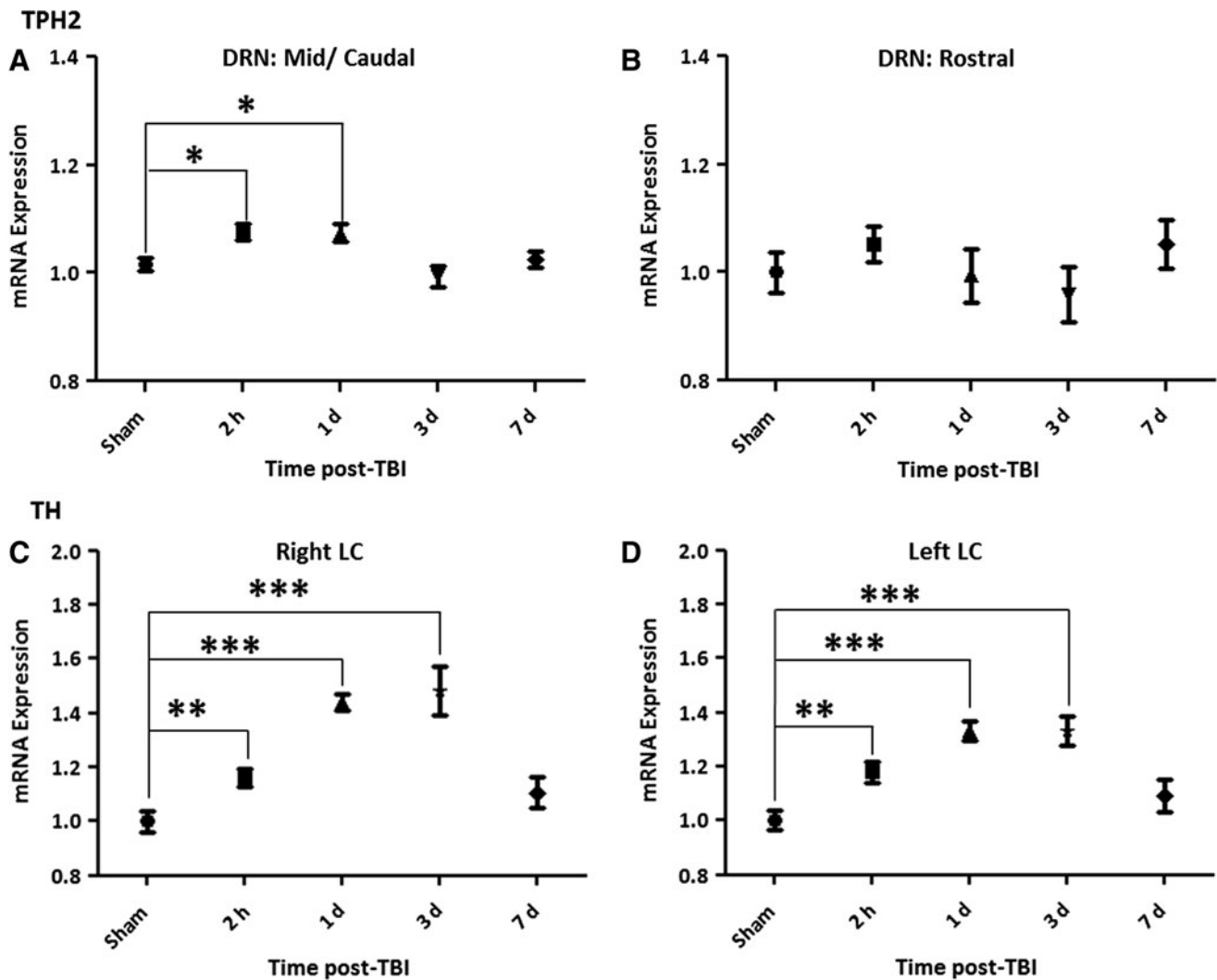
TPH2

TH

FIG. 3. Quantification of messenger ribonucleic acid (mRNA) levels of the biosynthetic enzymes tryptophan hydroxylase 2 (TPH2) in the dorsal raphe nucleus (DRN; **A-B**) and of tyrosine hydroxylase (TH) in the locus coeruleus (LC; **C-D**), at 2 h, 1 d, 3d, and 7 d post-exposure in the rat. The expression levels of the mRNA at each time-point were normalized to sham levels (shams of the different time-points showed no statistical difference and were pooled). There is an initial increase in TPH2 mRNA levels immediately post-exposure, which lasts up to 1 d, and is statistically significant in the mid/caudal part of the DRN (A). An increase in TH mRNA in both the right LC (C) and left (D) is detected as early as 2 h post-exposure continuing to rise up to 3d and returning to sham levels at 7d. Data are presented as mean ± standard error of the mean. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

observed increased levels of NA and DOPAC in the hippocampus and PFC in the stress group, whereas 5-HT was lower in the PFC.

Taken together, these studies suggest that the monoamine systems react in response to various TBIs and psychosocial stress and that the increased transmitter release represents a generalized reaction. The NA neurons appear to be particularly sensitive and rapidly activated, in agreement with our findings in the blast model.

The employed blast tube with the rigid montage of the animals represents a generic model for primary blast only, in which any rotational acceleration injury or focal impacts has been limited. No accumulation of APP was observed in white matter. Such accumulations have been reported in blast and are generally attributed to rotational acceleration rather than to primary blast.[46,75] However, effects by smoke emission and heating (quaternary blast) cannot be excluded. Gene expression in the hippocampus has been shown to be very different in this model for primary blast, com-pared with rotational injury.[46] It should therefore be of interest to analyze catecholamines and serotonin also in models for rotational acceleration.

Clinical trauma materials are generally very heterogeneous in terms of age and type of trauma. One interesting exception is the Vietnam head injury study, in which most patients were young men with a penetrating TBI.[76] In addition, the patients were tested before they were injured (i.e., at the time they entered the armed forces), and outcome data have been sampled for decades. This material has been used to analyze the relation between the TBI and PTSD[77] or other disorders. A new model for experimental penetrating TBI has been used to analyze changes in brain-derived neurotrophic factor (BDNF) and neurotrophin receptors,[78] since it was shown that BDNF polymorphism has a strong effect on outcome in the Vietnam head injury study.[79] Thus, an analysis of catecholamines and serotonin in penetrating TBI could be of interest.

TABLE 1. HPLC RAW DATA VALUES FOR MONOAMINES (A), AND THEIR METABOLITES (B), AT 1 D AND 7 D POST-EXPOSURE

(A)

Monoamines [pg/mg]

Group	NA				DA				5-HT			
Mean±SEM	Sham 1 d	Exposed 1 d	Sham 7 d	Exposed 7 d	Sham 1 d	Exposed 1 d	Sham 7 d	Exposed 7 d	Sham 1 d	Exposed 1 d	Sham 7 d	Exposed 7 d
Hypothalamus	2176±153.4	2516±282.7	1468±176.7	1236±93.1	241.4±58.0	368.0±31.01	199.5±28.2	208.2±43.4	261.5±47.1	334.9±34.3	263.2±40.2	288.8±31.3
Prefrontal cortex	322.5±12.8	320.3±13.4	291.8±23.5	288.1±30.8	78.1±7.3	87.6±8.4	154.8±47.1	120.0±24.4	237.9±24.9	242.1±11.9	186.5±15.5	180.3±16.7
Occipital cortex	238.3±10.5	293.3±8.3***	196.2±13.2	194.1±18.8	21.8±3.5	25.1±3.7	5.8±0.39	6.1±0.7	159.2±13.5	190.2±8.2	72.7±5.3	84.1±9.2
Entorhinal cortex	348.7±22.8	510.0±59.4*	325.9±21.3	327.7±28.4	193.5±38.7	335.5±82.1	54.7±9.9	46.1±9.1	519.7±46.5	526.6±59.6	183.6±22.0	204.8±22.9
Dorsal hippocampus	295.7±15.5	399.2±25.0*	232.2±21.9	234.3±19.8	17.1±1.3	26.4±3.9*	5.9±0.8	8.2±1.0	251.5±20.4	291.4±20.6	112.4±11.5	142.9±10.7
Ventral hippocampus	473.4±17.1	533.9±38.5	266.5±15.9	307.5±15.3	17.7±1.5	26.5±3.0*	7.0±0.8	7.4±0.6	390.5±31.5	435.2±39.3	125.5±9.6	130.2±9.7

(B)

Metabolites [pg/mg]

Group	DOPAC				HVA				5-HIAA			
Mean±SEM	Sham 1 d	Exposed 1 d	Sham 7 d	Exposed 7 d	Sham 1 d	Exposed 1 d	Sham 7 d	Exposed 7 d	Sham 1 d	Exposed 1 d	Sham 7 d	Exposed 7 d
Hypothalamus	115.7±11.1	88.9±9.3	88.7±5.8	83.3±9.7	45.7±3.9	36.1±6.9	54.3±7.9	49.5±6.4	760.2±31.9	665.3±42.2	427.7±21.5	418.7±26.4
Prefrontal cortex	43.7±3.5	35.8±2.6	151.0±37.1	125.3±10.2	94.9±9.7	87.8±12.3	114.0±10.7	144.9±21.7	305.1±10.9	257.9±8.0**	336.6±26.0	371.5±25.3
Occipital cortex	5.2±0.2	5.7±0.7	8.2±0.5	6.0±0.5**	6.3±0.3	7.2±0.9	7.5±0.7	7.2±0.6	172.3±5.2	179.0±6.3	198.3±8.4	184.9±6.5
Entorhinal cortex	49.9±10.7	47.0±14.0	31.1±4.3	22.1±4.3	40.9±8.9	44.0±11.2	33.2±2.3	18.1±2.5***	319.0±23.4	344.8±38.9	327.7±13.4	318.9±24.7
Dorsal hippocampus	6.3±0.5	8.8±1.5	4.0±0.8	3.7±0.4	9.5±0.9	9.0±1.0	9.9±1.1	5.9±0.5**	388.2±15.7	384.6±11.4	324.2±25.8	359.4±15.6
Ventral hippocampus	7.0±1.1	4.9±0.3	4.1±0.4	3.8±0.5	6.3±0.4	6.2±0.5	10.4±1.4	11.4±2.0	382.2±16.9	376.6±16.8	336.7±19.0	329.3±13.9

Data are presented as mean±S.E.M.

*$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

HPLC, high-performance liquid chromatography; NA, noradrenaline; DA, dopamine; 5-HT, 5-hydroxytryptamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; SEM, standard error of the mean.

TBI and monoamine synthesizing enzymes

In agreement with the HPLC results, we observed bilaterally increased transcript levels for TH in the LC and, less pronounced, for TPH2 in the DRN. There are several reports on elevation of both TH and TPH2 following exposure to various other types of stressors.[80–84] Supporting our observations, Tümer and colleagues[85] also have reported significant increases in TH protein levels in the adrenal medulla (20%) and an even higher increase in nucleus tractus solitarii of the brain stem (49%), and showed that this increase is associated with elevated plasma norepinephrine levels (23%) 6 h post-exposure to blast. The authors have linked this upregulation of the sympathetic system to elevated oxidative stress in the hypothalamus. However, our HPLC analysis of the hypothalamus did not reveal any changes in the monoamines or their metabolites.

George and colleagues[86] found differences in both the firing rate of the LC neurones and elevations of TH mRNA in a validated rat PTSD model. Associations between TPH2 genetic variants and "risk " of development of PTSD have been reported.[10] However, Wilson and colleagues[74] showed elevated TH protein levels in stressed hippocampus and PFC but reduced TPH2 levels in both regions.

Mild TBI and other trauma/stress models

Milman and colleagues[87] have analyzed concussive head trauma in mice and shown increased immobility in the FST at 7 and 90 d post-injury. Another recent study reported increased immobility in the FST of rats 3 d post-exposure in a moderate fluid percussion model.[88] Neither of these two studies examined short term consequences, as in our study, and we could not reproduce their demonstration of depression-like behavior at later time-points. These variances may in part be explained by the different injury models, species and severity of injury, such as weight drop versus blast, with the former causing a focal injury and carried out on mice;[87] while the latter resulting in a diffuse injury and on rats.[88] Even if both these injuries are considered mild, our blast model appears even less severe. In contrast, the fluid percussion model is considered moderate in severity.

PTSD in humans

Dysfunction of the monoamine system has been found in patients suffering from PTSD. NA has a prominent role in arousal, autonomic stress responses and encoding of emotional memories in humans, thus being of central importance in PTSD.[89] Studies using yohimbine, an α2 receptor antagonist, prompting increased NA release, induced flashbacks, and increased stress responses in PTSD patients.[90] Indirect evidence implicates that 5-HT plays a role in stress and PTSD[89,91] due to its extensive role in a number of mood and anxiety disorders. Importantly, 5-HT interacts with NA in the LC exerting inhibition,[89] hence possibly modulating the "fight-or-flight" response. Interestingly, in a recent genetic association analysis a risk haplotype in SLC18A2 (also known as vesicular monoamine transporter 2, VMAT2) for PTSD was identified,[92] thus reinforcing the role of monoamines in PTSD.

Limitations

The limitations of this study include modeling highly complex physical events, such as explosive blast. The short duration of the blast wave in our model is not representative for all possible types of explosive blasts occurring in various environmental situations.

An under-belly detonation of a vehicle by a road-bomb is likely to result in a much more complex waveform with reflections and longer duration. Also, the timetable of pathological changes is likely different in rats, compared with in humans. A larger number of animals and more time-points may reveal even more detailed information about the temporal pattern of changes that occur following exposure. Rat strains also may differ in their neuroendocrine responses,[93] and even the same rat strain obtained from different suppliers can have different behaviors in the FST.[50] We have not determined changes in NA metabolites in this experiment, and we only used young male rats.

Conclusions

Our present findings indicate that exposure to a single mild blast triggers rapid and transient changes in both NA levels in several forebrain brain regions, and of an NA-synthesising enzyme in the LC. Effects on the serotonin system were essentially confined to changes in the 5-HT synthesising enzyme TPH2, but they were of small magnitude with a short duration, compared with the LC system. Taken together, these findings provide novel information for the further characterization of the pathobiology of mbTBI. A further step could be to analyze biomarkers related to metabolites of NA in animal models for blast and in comparable human populations, such as breachers, which is a situation with a good control of physical parameters and possibilities for sampling of biomarkers.[94]

Acknowledgments

Author Disclosure Statement

No competing financial interests exist.

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