IKK2/NF-κB signaling protects neurons after traumatic brain injury

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ABSTRACT: Traumatic brain injury (TBI) is the leading cause of death in young adults. After the initial injury, a poorly understood secondary phase, including a strong inflammatory response determines the final outcome of TBI. The inhibitor of NF-κB kinase (IKK)/NF-κB signaling system is the key regulator of inflammation and also critically involved in regulation of neuronal survival and synaptic plasticity. We addressed the neuron-specific function of IKK2/NF-κB signaling pathway in TBI using an experimental model of closed-head injury (CHI) in combination with mouse models allowing conditional regulation of IKK/NF-κB signaling in excitatory forebrain neurons. We found that repression of IKK2/NF-κB signaling in neurons increases the acute posttraumatic mortality rate, worsens the neurological outcome, and promotes neuronal cell death by apoptosis, thus resulting in enhanced proinflammatory gene expression. As a potential mechanism, we identified elevated levels of the proapoptotic mediators Bax and Bad and enhanced expression of stress response genes. This phenotype is also observed when neuronal IKK/NF-κB activity is inhibited just before CHI. In contrast, neuron-specific activation of IKK/NF-κB signaling does not alter the TBI outcome. Thus, this study demonstrates that physiological neuronal IKK/NF-κB signaling is necessary and sufficient to protect neurons from trauma consequences.—Mettang, M., Reichel, S. N., Lattke, M., Palmer, A., Abaei, A., Rasche, V., Huber-Lang, M., Baumann, B., Wirth, T. IKK2/NF-κB signaling protects neurons after traumatic brain injury. FASEB J. 32, 1916–1932 (2018). www.fasebj.org

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Traumatic brain injury (TBI) represents a leading cause of mortality and morbidity, especially among young individuals below the age of 45 yr (1, 2). It is estimated that 10 million deaths and hospitalizations annually worldwide are direct consequences of TBI. Approximately 57 million people, currently alive, have experienced a brain injury (1). Thus, TBI represents a major global public health problem (3). This statistic translates within the United States alone to ~1.7 million annual TBI cases, and 5.3 million people are living with a TBI-related disability (4). TBI is typically triggered by a blunt- or sharp-force trauma, with most patients having a closed-head injury (CHI) (5, 6). TBI initiates a complex disease, and the pathophysiology is characterized by great heterogeneity and widely varying outcomes (7). Overall, TBI pathophysiology can be subdivided into two stages: Whereas the primary injury causes direct mechanical damage of blood vessels and parenchymal cells at the lesion site, subsequent secondary injury develops over a period from minutes to weeks and, potentially, months or years (8, 9). Secondary pathology is characterized by an early increase in glutamate and reactive oxygen species, as well as the occurrence of mitochondrial dysfunction and increased expression of cytokines and chemokines (10–12). The secondary pathology is accompanied by neuroinflammation initiated by release of damage-associated molecular patterns, which is supposed to promote delayed cell death, cerebral edema formation and blood–brain barrier damage (4, 11–14). Nevertheless, inflammation may also have beneficial effects.
by contributing to functional repair, tissue remodeling and neuroplasticity (4, 14–16).

The NF-κB family of transcription factors plays a critical role in the regulation of stress-associated and inflammatory gene expression, as well as in cell survival and neuronal differentiation in the CNS (17–20). The family of NF-κB transcription factors comprises 5 members—RelA, RelB, c-Rel, p105/p50, and p100/p52—that form homo- or heterodimers. In its inactive state, NF-κB is retained in the cytoplasm by IkB inhibitory proteins. The crucial step in NF-κB activation is the phosphorylation of IkB proteins by the inhibitor of NF-κB kinase (IKK) complex, resulting in their proteosomal degradation. This process induces nuclear translocation and transcriptional regulation of target genes. The IKK complex contains 2 protein kinases, IKK1 (IKKa) and IKK2 (IKKB), as well as the regulatory protein NF-κB essential modulator (IKKy). IKK2 is the critical kinase subunit inducing the canonical signaling pathway, essentially involved in the regulation of inflammation and cell survival (17, 21). In the CNS, a variety of stimuli, such as damage- and pathogen-associated molecular patterns, synaptic activity, oxidative stress, cytokines, chemokines, neurotransmitters, neurotrophic factors, and neurotoxins induce NF-κB (17, 22, 23). NF-κB is activated in neurons and glial cells upon experimental injury and in the context of neuropathological disorders and has been linked to both neurodegenerative and neuroprotective activities (20, 24–27). NF-κB activation in glial cells has been mainly related to inflammation (28–31), NF-κB activation in neurons is involved in differentiation, synaptic plasticity and neuronal development and survival (26, 32–35). Cytokines such as TNF and IL-1β are typically up-regulated by TBI induction. They are both NF-κB target genes, but can also activate NF-κB, resulting in a self-propagating vicious circle (17, 36). Consistently, NF-κB was elevated in rats after controlled cortical impact and fluid percussion brain injury (37, 38) as well as in biopsies of human contused brain tissue (39). Furthermore, after cortical aspiration, NF-κB is mainly activated in neuronal cells of the degenerating cortex and in astrocytes of the corpus callosum (40). The NF-κB subunit p50 has been found to be highly expressed in neurons that survive hippocampal injury, arguing for a critical role in the regulation of repair and regeneration (41). In contrast, controlled cortical impact trauma leads to increased brain injury volumes and blood–brain barrier dysfunction in transgenic mice with elevated NF-κB activity in the brain (42). These studies indicate that NF-κB signaling may have a beneficial or detrimental role, depending on the cell type where it is expressed and the nature of the trauma (43–45).

Although activation of NF-κB is a common feature of different pathologies of the CNS, including trauma, excitoxicity, and ischemia (37, 46–49), the precise role of this transcription factor in damaged neurons is still unknown. To characterize the specific role of neuronal NF-κB in the pathogenesis of TBI, we analyzed the consequences of NF-κB inhibition and activation in neurons, using corresponding neuron-specific loss-of-function and gain-of-function mouse models, respectively. We found that neuronal NF-κB repression increases the acute posttraumatic mortality rate and worsens the neurological outcome of survivors at various time points after CHI, including enhanced reactive astrogliosis and apoptosis. Ectopic neuronal NF-κB activation does not reduce the harmful effects of secondary TBI pathogenesis. Thus, our findings provide the first direct link of CHI and neuronal IKK2/NF-κB signaling, depicting a key pathway in the development of secondary TBI pathology.

MATERIAL AND METHODS

Transgenic mice

Mice were housed in a specific pathogen-free animal facility at Ulm University under standardized conditions with food and water provided ad libitum. Camk2a.tTA×luciferase-(tetO)7-DN-IKK2 (short IKK2-DNCamk2a) and Camk2a.tTA×luciferase-(tetO)7-CA-IKK2 (short IKK2-CACamk2a) were generated by crossing Camk2a.tTA mice with single transgenic mice carrying a luciferase-(tetO)−→IKK2-DN or a luciferase-(tetO)→IKK2-CA transgene, respectively (49). All mouse lines were bred on an NMRI background. Inactivation of the transgenes was achieved by administration of Dox (0.1 g/L in the drinking water containing 1% sucrose; MP Biomedicals, Eschwege, Germany) to the mothers during pregnancy until weaning (at age 4 wk) and as indicated in Figs. 6 and 7 and Supplemental Fig. S8. Transgene expression was induced by Dox withdrawal. Wild-type and single transgenic mice were used as control groups. Luciferase activity was measured in the living animals with the IVIS200 in vivo imaging system (Caliper Life Sciences, Hopkinton, MA, USA) (49, 50). All animal experiments were performed in compliance with the guidelines of the National Institutes of Health (Bethesda, MD, USA) and the German Animal Protection Act, and were approved by the Regierungspädisium Tübingen (Tübingen, Germany).

CHI model

Twelve-week-old mice were subjected to experimental CHI with a standardized weight-drop device (51, 52). In brief, the animals were anesthetized with ketamine (Pfizer Pharma, Karlsruhe, Germany), with an intraperitoneal dose of 100 mg/kg body weight, and 2% xylazine (Bayer Health Care, Monheim, Germany), with an i.p. dose of 16 mg/kg body weight. Afterward, the skull was exposed by a longitudinal incision of the skin, and a focal blunt injury was induced in the left hemisphere by dropping a 330 g metal rod on the skull from a height of 2.7 cm. Only animals with a visible imprint of the falling rod on the skull were included for further analyses. After trauma, the mice received supporting oxygenation with 100% O2, the wound was sutured, and the animals were placed into a warmed recovery cage with ad libitum access to food and water. Buprenorphine analgesia (Temgesic; Essex Pharma, Munich, Germany) was administered subcutaneously (0.05 mg/kg body weight) immediately after trauma and every 8 h thereafter until 24 h. Sham-procedure mice underwent anesthesia, scalp incision, suturing of the wound, and analgesia, but no experimental head trauma. Animals that died directly after the mechanical insult and during anesthesia (up to 2 h after TBI) were included in the acute posttraumatic mortality rate.

Neurological severity score

A 10-point neurological severity score (NSS) was used to assess the posttraumatic neurological impairments (52–54). This scoring system consists of 10 tests, including tasks to measure cognitive and motor functions (e.g., beam walk, round-stick balance, exit circle, gait pattern, and exploratory interest in new environment),
whereby 1 point is given for failure of the task and 0 points for succeeding. Thus, a maximum NSS of 10 points indicates severe neurological dysfunction, with failure of all tasks. In the present study, the NSS was assessed 2 d before and 6 h, 1 d, and 3 d after CHI.

**Rotarod, open field, and elevated-plus maze**

Motor behavior after CHI was analyzed with the ENV-575M rotarod (Med Associates, St. Albans, VT, USA). After 1 min at 4 rpm for adjustment, the cylinder accelerated within 5 min to 40 rpm. The latency until falling off the accelerating rotarod was recorded. Pretraining was assessed 2 d before injury (consisting of 4 trials) and analysis was performed at 2, 7, and 30 d post trauma.

To assess general locomotor activity and anxiety/exploratory behavior, the animals were put individually in an open field (OF) arena (50 × 50 cm) (55) and were recorded for 15 min with a video camera with the Tracking System Viewer 2 software (Biobserve, Bonn, Germany). OF analysis was performed at 2 d before injury and 1, 3, 7, and 30 d post trauma.

To further study anxiety and exploratory behavior, mice were subjected to an additional elevated-plus maze test. This maze consists of an elevated plus-shaped platform with 4 arms of equal size (30 × 5 cm), of which 2 opposing arms are surrounded by 16 cm–high walls (55). Animals were placed in the center platform of the maze facing an open arm and were video-tracked for 5 min with the Tracking System Viewer 2 software.

**Protein isolation and immunoblot analysis**

Tissue samples (cortical impact area) were snap frozen in liquid nitrogen, homogenized with a mortar and pestle and lysed in KÄ-lysis buffer [25 mM Tris-HCl, 150 mM NaCl, 25 mM sodium pyrophosphate, 50 mM β-glycerophosphate, 50 mM NaF, 2 mM EGTA, 2 mM EDTA, 1 mM DTFF, 10% glycerol, 1% Triton X-100 (pH 8.0)] supplemented with protease inhibitors (1 mM PMSF and Complete Mini Tablet; Roche Diagnostics, Mannheim, Germany). After centrifugation (30 min, 13,000 rpm), the supernatant was used as the total protein extract. Equal amounts of protein (30 μg) were separated by SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 5% nonfat dry milk in TBS buffer for 1 h at room temperature, primary antibodies (see below) were incubated in blocking solution overnight at 4°C or for 2 h at room temperature. After 3 washing steps, membranes were incubated with horseradish peroxidase–conjugated secondary antibody for 1 h at room temperature. Membranes were exposed to ECL detection reagent (Thermo Fisher Scientific) and developed by ECL.

**Histology and immunofluorescence staining**

Brains were fixed by immersion with 4% paraformaldehyde (overnight at 4°C), dehydrated, embedded in paraffin, and cut to 7 μm–thick coronal sections on a microtome (Microm HM355S; Thermo Fisher Scientific). After rehydration, heat-mediated antigen retrieval was performed with sodium citrate (10 mM, pH 6.0, 0.05% Tween 20) and the tissue sections were additionally incubated with 0.5% Triton X-100 for 30 min. Sections were washed with PBS and blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature. Incubation with primary antibodies (in 5% BSA) was performed overnight at 4°C and incubated afterwards with secondary antibodies (in 5% BSA) for 1 h at room temperature with 100 ng/ml DAPI for nuclear counterstaining. Fluorescence images were acquired with the Keyence BZ-9000 Bio-Revoo microscope (Keyence, Neu-Isenburg, Germany) with filter for DAPI, FITC/Alexa Fluor 488, and TexasRed/Alexa Fluor 568/594 and the BZ-II Viewer software (Keyence).

**Antibodies for immunoblot analysis and immunostaining**

The following antibodies were used for immunoblot analysis: rabbit anti-IKKβ [Y466] (ab32135, 1:2000; Abcam, Cambridge, United Kingdom), goat anti-Lcn2 (AF1857, 1:1000; R&D Systems, Minneapolis, MN, USA), rabbit anti-glial fibrillary acidic protein (GFAP; ab7779, 1:1000; Abcam), rabbit anti-Bax (2772, 1:1000; Cell Signaling Technology, Danvers, MA, USA), rabbit anti-Bad (9292, 1:1000; Cell Signaling Technology), rabbit anti-NF-kB p65 (C20, sc-372, 1:1000; Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-phospho NF-kB p65 (Ser536, 3033, 1:1000; Cell Signaling Technology), rabbit anti-GAPDH (FL-335, sc-27758, 1:2000; Santa Cruz Biotechnology), rabbit anti-β-tubulin (ab6046 1:10000; Abcam) and HRP-conjugated goat anti-rabbit or donkey anti-goat were obtained from Santa Cruz Biotechnology.

For immunofluorescence, the following primary antibodies were used: mouse anti-NeuN (MAB377, 1:300; Millipore-Sigma), rabbit anti-cleaved caspase 3 (ab13847, 1:400; Abcam), and mouse anti-tubulin-β3 (TUJ1) (MMS-435P, 1:500; BioLegend, San Diego, CA, USA). Corresponding Alexa Fluor-conjugated secondary antibodies were obtained from Thermo Fisher Scientific, and DAPI was purchased from Merck (Darmstadt, Germany).

**TUNEL staining**

For in situ detection of cell death, TUNEL staining was performed with 7 μm–thick coronal paraffin-embedded sections of head-injured mice (3 d after TBI) with the In Situ Cell Death Detection Kit, Fluorescein, according to the manufacturer’s protocol (Roche). Sections were evaluated with the BZ-9000 BioRevo microscope with filters for DAPI and FITC/Alexa Fluor 488.

**RNA extraction, cdNA synthesis, and quantitative RT-PCR**

RNA from the cortex (impact area) was isolated using the PeqGold Trifast Kit (Peqlab, Erlangen, Germany) as described in the manufacturer’s protocol. One microgram of total RNA was used to synthesize cDNA with the Transcriptor High Fidelity cDNA Synthesis kit (Roche) with oligo-dT-primers according to the manufacturer’s instructions. Quantitative PCR (qPCR) assays were run on the Lightcycler 480 Instrument (Roche) with primers and hydrolysis probes designed by the Roche Universal Probe Library (UPL) system. Sequences and UPLs were as follows: C3: forward (F) 5′-ACCTTACCTCGGCAAGTTTCT-3′, reverse (R) 5′-TGTAGAGCTCTGCCTAGCAG-3′, UPL 76; Ggfp: F 5′-CCAATGCAAGGCGCGTCTGA-3′, R 5′-GCTTGAAGGACTCGTTGCT-3′, UPL 109; Lcn2: F 5′-CCATCATGAGCTACAAAGAGAACAACT-3′, R 5′-CTGTAGGCTAGGCAACAGC-3′, UPL 58; Il6b: F 5′-TGTAATGAAAGAGCCGCACACC-3′, R 5′-TTAATGGAAAAGGCGCCACC-3′, UPL 78; F-Fos: F 5′-CCACGGTTTTCTTACTACATTCC-3′, R 5′-ACAGATCCTGGCCAAGTGC-3′, UPL 67; Bax: F 5′-AGTCTCTCCGGAATGGTT-3′, R 5′-ACACGTGCAAACTATCATCT-3′, UPL 56; Bad: F 5′-CAAGCCAAACAGTCTCATCAT-3′, R 5′-GCTAAGCTCCTCTTCCCATC-3′, UPL 88; F5′-CCCACCTCGTACGAGCAGATGC-3′, R 5′-TGGTCAAGATCCATTTITCCA-3′, UPL 22; Bcl2: F 5′-AGTACCTGTGACCCGCTGCT-3′, R 5′-GGGCGCATATACTTTCCACA-3′, UPL 75; Bcl-x: F 5′-TGACCACTTACGAGCCTTGA-3′, R 5′-GGCTGCTTGTCCCTGCGTAGA-3′, UPL 2; Aff3: F 5′-GGCTGGAATTCTACCCATGCT-3′, R 5′-GAGCTTCTTCCTTCCAT-3′, UPL 80; P21: F 5′-CAGATCAGGCGATATCCCA-3′, R 5′-CAGCCATCTTCCTTCCAT-3′, UPL 80; P21: F 5′-CAGATCAGGCGATATCCCA-3′, R 5′-CAGCCATCTTCCTTCCAT-3′, UPL 80;
Thromboelastometry

Rotational thromboelastometry (Rotem Delta; TEM International, Munich, Germany) analysis was performed to determine alterations in coagulation before TBI induction. Whole blood was collected via cardiac puncture from control, IKK2-DNCamk2a, and IKK2-CACamk2a mice and anticoagulated with 3.2% citrate. Immediately after blood drawing, the extrinsic coagulation pathway was determined using the EXTEM test. Clotting time, clot formation time, maximum clot firmness, and α-angle were determined.

GFAP ELISA

Whole blood was taken via decapitation and transferred to sterile plasma EDTA microtubes (Kabe Labortechnik, Nürnberg-Elsenroth, Germany). EDTA-free protease inhibitor cocktail (10%; Roche) was added, incubated for 10 min on ice, and centrifuged at 4000 rpm for 10 min at 4°C, before storage at −80°C. Plasma samples were analyzed by commercially available ELISA specific for mouse GFAP, according to the manufacturer’s instructions (LSBio, Seattle, WA, USA). Optical density was read at a wavelength of 450 nm.

Figure 1. Immediate consequences of CHI on mice with modulated neuronal NF-κB activity. A) Generation of conditional transgenic IKK2-DNCamk2a and IKK2-CACamk2a mouse models using the tetracycline-regulated gene-expression system. Expression of the IKK2-DN (loss-of-function) or IKK2-CA (gain-of-function) transgene and a luciferase reporter gene was controlled by the bidirectional tetracycline-controlled transcriptional activation (tTA)-dependent promoter (tetO7). The Camk2α.tTA module specified transgene expression to neurons with Camk2α-activity. Dox blocked transgene expression. B) Mortality of mice during CHI procedure. Immediate posttraumatic mortality rate is depicted as the percentage of animals that died. IKK2-DNCamk2a mice showed a significantly enhanced posttraumatic mortality compared to control and sham-treated animals, whereas mice with neuronal NF-κB activation (IKK2-CACamk2α) had a mortality similar to that of as control mice. (Control TBI, n = 115; Control Sham-Treated, n = 62; IKK2-DNCamk2a TBI, n = 78; IKK2-DNCamk2a Sham, n = 21; IKK2-CACamk2a TBI, n = 35; IKK2-CACamk2a Sham, n = 20). **P < 0.01, ***P < 0.001 (ns, by Fisher’s exact test). C) Quantification of hematoma formation in IKK2-DNCamk2a, IKK2-CACamk2a, and control mice after TBI. CHI induced similar distributed hematoma formation in control and IKK2-DNCamk2a animals. IKK2-CACamk2a mice were slightly more prone to hematoma development than controls. (Control TBI, n = 76; Control Sham, n = 51; IKK2-DNCamk2a TBI, n = 37; IKK2-DNCamk2a Sham, n = 18; IKK2-CACamk2a TBI, n = 51; IKK2-CACamk2a Sham, n = 20). **P < 0.01, ****P < 0.0001 (ns, by Fisher’s exact test). D) Plasma levels of GFAP after CHI. IKK2-DNCamk2a mice showed elevated levels of GFAP in the plasma 6 h after TBI. Means ± SEM (n = 6–12). **P < 0.01 (ns, by 1-way ANOVA, followed by Bonferroni’s post hoc test).
on a plate reader (Sunrise Plate Reader; Tecan, Crailsheim, Germany). As a standard curve, a fitted line of the 7-fold standard was used according to the manufacturer’s protocol.

**High-resolution MRI**

Imaging was performed before and 6 h and 1, 3, and 7 d after trauma. Measurements were performed on a 11.7 T small-animal MRI (BioSpec 117/16; Bruker Biospin, Billerica, MA, USA). All data were acquired with a cryogenically cooled 1H 2-element surface (MRI CryoProbe; Bruker BioSpin) transmit–receive coil. After initiation of anesthesia with 5% isoflurane in air, the mice were placed prone in the cradle. The anesthesia gas was administered via a facial mask, and, during scanning, the isoflurane concentration was adjusted between 1.25 and 1.5% to maintain the respiratory frequency at ~90 cycles/min. Coronal images were acquired with a T1-weighted multislice Fast Length Adjustment of Short Reads (FLASH; Johns Hopkins University, Baltimore, MD, USA; https://ccb.jhu.edu/software/FLASH/) sequence.

Acquisition parameters were as follows: echo/repetition time, 5/190 ms; flip angle, 17.5°; resolution, 65 × 65 × 500 μm³; field-of-view, 20.8 × 20.8 mm²; and bandwidth, 96 KHz, using 8-signal averages. Total acquisition time for 18 slices was 5 min 20 s.

**Statistical analysis**

Immunoblot densitometry was analyzed with Image J. Quantifications from microscopy images were performed with the BZ-II Analyzer software with the Hybrid cell-counting tool (Keyence). Statistical analyses were performed with Prism software (GraphPad, San Diego, CA, USA) and are indicated in the

**Figure 2.** IKK2-DN Camk2a mice show enhanced neurologic deficits post TBI. A, B) Assessment of injury severity, neurologic impairment and recovery of control, IKK2-DN Camk2a, IKK2-CA Camk2a, and sham-treated mice after CHI, with a standardized 10-point NSS. A) Head-injured IKK2-DN Camk2a mice showed a delayed recovery from TBI, as reflected by a significantly increased NSS, compared to head-injured littermates (Control TBI, n = 18; Control Sham, n = 14; IKK2-DN Camk2a TBI, n = 12; IKK2-DN Camk2a Sham, n = 9). B) No significant difference in NSS of head-injured IKK2-CA Camk2a and control mice was seen at any time point assessed (Control TBI, n = 17; Control Sham, n = 10; IKK2-CA Camk2a TBI, n = 19; IKK2-CA Camk2a Sham, n = 10). All data are presented as box plots with median ± interquartile range; whiskers, minimum–maximum range. *P < 0.05, **P < 0.001, ****P < 0.0001 [not significant (ns), by nonparametric Mann-Whitney U test]. C) Rotarod performance after TBI. Impaired motor coordination of IKK2-DN Camk2a mice after trauma, demonstrated in a 30 d rotarod experiment. Latency until falling off of an accelerating rotarod was reduced 2 and 30 d after TBI compared to controls. Means ± SEM (n = 7-13). *P < 0.05 (2-way-ANOVA followed by Bonferroni’s post hoc test).
IKK2-DNCamk2a mice exhibit prominent neuronal apoptosis after CHI. A) Neuronal cell loss 3 d after CHI. Significantly enhanced neuronal cell loss in the ipsilateral cortex of IKK2-DNCamk2a mice compared with control TBI and sham-treated mice. Immunofluorescent staining and quantification of NeuN+ cells. The percentage of neuronal cell loss was calculated as the ratio of neuronal cells from the injured (ipsilateral) hemisphere to the number of neurons in the uninjured (contralateral) hemisphere. Impact, TBI impact area; Cor, cortex; CC, corpus callosum. Means ± SEM (n = 4–5). *P < 0.05, **P < 0.01 (by 1-way ANOVA with (continued on next page)
specific figure legend. One- or 2-way ANOVA with Bonferroni’s correction was used to compare independent measurements at one or different time points, respectively. For the NSS analysis, the nonparametric Mann-Whitney U test was used. Differences in postinjury mortality and hematoma formation were evaluated in a contingency table with Fisher’s exact test. Statistical significance was set at \( P < 0.05 \).

RESULTS

Neuronal IKK/NF-κB inhibition induces a detrimental phenotype after CHI

To study the role of neuronal IKK/NF-κB signaling for the outcome of traumatic brain injury, we used 2 previously generated mouse models called IKK2-DN\(^{Camk2a}\) and IKK2-CA\(^{Camk2a}\) (35, 49, 56). These conditional loss-of-function and gain-of-function models express a dominant negative (IKK2-DN) or a constitutively active (IKK2-CA) allele of human IKK2, respectively. Furthermore, a luciferase reporter gene is coexpressed under the control of the neuron-specific \( Camk2a \) promoter in a tetracycline-regulated manner (Fig. 1A). To avoid any impact of the IKK2-DN and -CA transgenes on brain development, animals were bred and housed in the presence of Dox up to the age of 4 wk to block transgene expression. Thereafter transgene expression was induced by Dox withdrawal.

TBI was induced in 12-wk-old animals by using a standardized weight-drop device (51, 52). Transgene expression analyzed 6 h after TBI in the cortex of IKK2-DN\(^{Camk2a}\) and IKK2-CA\(^{Camk2a}\) mice by immunoblot analysis revealed robust IKK2 expression on both the ipsilateral and contralateral side (Supplemental Fig. S1A, B respectively). After CHI, the acute postinjury mortality rate was significantly enhanced in IKK2-DN\(^{Camk2a}\) mice (37%) compared to controls (16%) suggesting a detrimental effect of impaired IKK2/NF-κB signaling on posttraumatic survival rate (Fig. 1B). Neuronal activation of IKK2/NF-κB in the IKK2-CA\(^{Camk2a}\) animals did not significantly affect the posttraumatic mortality rate (11% for IKK2-CA\(^{Camk2a}\) vs. 16% for control mice). Since transgene expression is activated 2 mo before TBI, we asked whether neuronal IKK2/NF-κB modulation affects basic physiologic parameters critical for posttraumatic survival. However, we could not detect obvious differences in body temperature (Supplemental Fig. S2A), blood glucose levels (Supplemental Fig. S2B), and thromboelastometric function (Supplemental Fig. S2C) in IKK2-DN\(^{Camk2a}\), IKK2-CA\(^{Camk2a}\), and control mice.

CHI initiates heterogeneous secondary processes including development of cerebral edema and hematoma (6). Therefore, we analyzed the frequency of hematoma formation in the transgenic mouse models. Control and IKK2-DN\(^{Camk2a}\) mice show a similar pattern of epidural hematoma formation, whereas IKK2-CA\(^{Camk2a}\) mice are slightly more prone to induction of hematoma (Fig. 1C). Hematoma and edema formation were confirmed by MRI coronal T1*-weighted imaging (Supplemental Fig. S3). GFAP is a serum biomarker for TBI that correlates with injury severity (57, 58). The quantification of GFAP in plasma samples of head-injured mice by ELISA revealed slightly enhanced GFAP levels in IKK2-DN\(^{Camk2a}\) mice, compared to control and IKK2-CA\(^{Camk2a}\) animals (Fig. 1D). These data suggest elevated damage in mice with neuronal NF-κB repression.

Neuronal NF-κB inhibition promotes increased neurologic deficits after TBI

Functional consequences of CHI were assessed with the 10-point NSS (52–54). This analysis revealed significantly elevated median NSS levels 6 h, 1 d, and 3 d after TBI in IKK2-DN\(^{Camk2a}\) mice compared with control mice (Fig. 2A) implying an increased severity and possibly a delayed recovery from experimental CHI. In contrast, no statistically significant difference was noted in the median NSS at any time point between IKK2-CA\(^{Camk2a}\) and control littermates (Fig. 2B). To further characterize the neurologic deficits of IKK2-DN\(^{Camk2a}\) mice after TBI, we performed additional motor and anxiety behavior tests over a time course up to 30 d. In the rotarod test, IKK2-DN\(^{Camk2a}\) mice showed deficits 2 and 30 d after CHI in comparison to control littermates (Fig. 2C), indicating a mild permanent motor impairment. In the open-field test, IKK2-DN\(^{Camk2a}\) animals showed a hyperactive phenotype 30 d after TBI in comparison to control mice, determined by the length of elevated track covered (Supplemental Fig. S4A). In contrast, IKK2-DN\(^{Camk2a}\) mice did not show alterations in anxiety in the elevated plus-maze testing after TBI (Supplemental Fig. S4B).

In conclusion, neuronal NF-κB repression results in enhanced neurologic and motoric deficits.

Neuronal NF-κB inhibition increases apoptosis of neurons after CHI

Since neurologic deficits correlate with neurodegeneration we investigated the extent of neuronal cell damage after TBI in IKK2-DN\(^{Camk2a}\) and control mice. For this purpose, we performed immunofluorescence analyses using an anti-NeuN antibody as a neuron-specific cell marker.
Neuronal cell loss was evident in control animals, but significantly enhanced in IKK2-DN^{Camk2a} mice (Fig. 3A). To test whether neuronal cell loss depends on neuronal apoptosis, tissue sections were analyzed by cleaved caspase 3 costained with TUJ1, a marker for neurons. Indeed, prominent costaining was detected, and quantification revealed increased cleaved caspase 3+ cells 3 d after TBI in IKK2-DN^{Camk2a} mice, in comparison to control animals (Fig. 3B), suggesting that neuronal IKK/NF-κB inhibition sensitizes these cells for apoptosis.

**Figure 4.** IKK2-DN^{Camk2a} mice exhibit prominent up-regulation of neuroinflammatory factors after trauma. A–E) Expression of proinflammatory genes 3 d after TBI. Most proinflammatory factors were significantly up-regulated in the cortical impact area of IKK2-DN^{Camk2a} mice, but not in IKK2-CA^{Camk2a} mice vs. control littermates. These factors include the complement factor C3 (A), the astroglial marker Gfap (B), the acute phase protein Lcn2 (C), the inflammatory cytokine Il1b (D), and the neuronal activity marker c-Fos (E). Expression levels measured by qPCR are presented relative to Hprt. Means ± SEM (n = 4–6). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 [not significant (ns), 3 d after TBI between indicated groups, by 1-way-ANOVA followed by Bonferroni’s post hoc test]. F) Expression of astroglial and acute phase proteins after trauma. Representative immunoblot analysis and quantification of LCN2 and GFAP protein levels in brain lysates of 12-wk-old control and IKK2-DN^{Camk2a} mice 3 d after CHI. GAPDH was the loading control. Means ± SEM (n = 3). *P < 0.05, **P < 0.01 (by 1-way ANOVA followed by Bonferroni’s post hoc test).
Neuronal NF-kB inhibition leads to enhanced up-regulation of neuroinflammatory mediators after trauma

To address the role of inflammatory and apoptotic pathways in the regulation of neuronal survival after TBI in the IKK2-DNCamk2a model, we analyzed marker gene expression in the cortical impact area at various time points after CHI. Whereas 6 h after TBI, we did not see prominent changes in gene expression (Supplemental Fig. S5A–E), 3 d after TBI, significant up-regulation of several proinflammatory genes was seen in the ipsilateral cortex of IKK2-DNCamk2a animals compared to sham-treated animals and control littermates (Fig. 4A–E, left panels). These include the central complement factor C3, the astrogliosis marker gene Gfap, the acute phase gene Lcn2, and the inflammatory cytokine Il1b (Fig. 4A–D, left panels). In control animals, up-regulation of these genes does not reach significance after TBI, compared with sham-treated animals. Protein analysis confirmed enhanced levels of LCN2 and GFAP in IKK2-DNCamk2a animals, compared to control littermates 3 d after trauma (Fig. 4F). We also found the neuronal activity marker c-Fos elevated in IKK2-DNCamk2a animals compared to controls (Fig. 4E, left panel), suggesting enhanced neurogenesis related to elevated neurologic impairments (59). No significant differences in the expression pattern of the different genes were observed between IKK2-CAcamk2a and control littermates (Fig. 4A–E, right panels). Analysis of these proinflammatory genes 7 d after CHI did not reveal continuing significant differences between IKK2-DNCamk2a and control mice (Supplemental Fig. S6A–E).

To investigate the underlying mechanism responsible for enhanced apoptosis in the IKK2-DNCamk2a model, we performed a longitudinal gene expression profiling for pro- and anti-apoptotic genes. Three days after CHI, we detected an enhanced expression of the proapoptotic genes Bax and Bad, whereas the expression of Bak and the antiapoptotic genes Bcl2 and BclXL was not affected (Fig. 5A–E, left panels). These results were confirmed on a protein level, as IKK2-DNCamk2a animals showed increased BAX and BAD expression compared to control littermates (Fig. 5H). As a consequence of proapoptotic protein expression, we found an up-regulation of TUNEL+ cells in the ipsilateral cortex of IKK2-DNCamk2a animals compared with control littermates (Supplemental Fig. S7). In addition, stress response genes and p53 target genes, including Atf3 and p21 were up-regulated in these animals (Fig. 5F, G, left panels). Again, expression of these genes was unaltered in IKK2-CAcamk2a mice (Fig. 5A–G, right panels). Similar to the proinflammatory genes, we did not identify significant differences in the expression of these pro- and antiapoptotic genes 6 h (Supplemental Fig. S5F–L) and 7 d (Supplemental Fig. S6F–L) after CHI in the
IKK2-DN\textsuperscript{Camk2a} mouse model. These results indicate that basal NF-κB activity in neurons is necessary to protect these cells from transiently increased cellular stress resulting from traumatic injury. However, this protective effect is not further enhanced by increased NF-κB activity.

**Acute inhibition of IKK/NF-κB signaling in neurons also worsens TBI outcome**

IKK2-DN transgene expression and therefore inhibition of IKK/NF-κB signaling was induced at the age of 4 wk for a total period of ∼8 wk before TBI. Therefore,

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**Figure 5.** Neuronal NF-κB inhibition leads to up-regulation of proapoptotic factors after CHI. 

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**A**–**G** Expression of genes regulating apoptosis 3 d after TBI. Proapoptotic, but not antiapoptotic, factors were significantly elevated in the cortical impact area of IKK2-DN\textsuperscript{Camk2a} mice, but not in IKK2-CA\textsuperscript{Camk2a} mice vs. control littermates. qPCR analysis of the proapoptotic genes \textit{Bax} (A), \textit{Bad} (B), and \textit{Bak} (C); the antiapoptotic genes \textit{Bcl2} (D) and \textit{BclXL} (E); and the stress response genes in the p53 pathway \textit{Atf3} (F) and \textit{p21} (G). Expression levels measured by qPCR are presented relative to \textit{Hprt}. Means ± SEM (n = 4–6). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 [not significant (ns), at 3 d after CHI, between the indicated groups, by 1-way-ANOVA followed by Bonferroni’s post hoc test].

**H** Expression of proapoptotic proteins after trauma. Representative immunoblot analysis and quantification of \textit{BAX} and \textit{BAD} protein levels in brain lysates of 12-wk-old control and IKK2-DN\textsuperscript{Camk2a} mice. TUBB is used as loading control. Means ± SEM (n = 3). *P < 0.05, ****P < 0.001 (ns, 3 d after CHI, between the indicated groups, by 1-way-ANOVA followed by Bonferroni’s post hoc test).
we cannot exclude consequences of chronic NF-κB inhibition that predispose IKK2-DNCamk2α animals to the observed detrimental TBI outcome. To investigate, we activated transgene expression in a more acute paradigm shortly before TBI induction. As the transgene is only slowly activated after Dox withdrawal, we

Figure 5. (Continued)
Figure 6. Acute neuronal IKK2-DN transgene expression also results in detrimental TBI outcome. A) Mortality of mice with acute neuronal NF-κB inhibition after CHI. The mortality rate is depicted as the percentage of animals that died. Acute IKK2-DN Camk2a mice showed a significantly enhanced posttraumatic mortality rate compared to control littermates, similar to the chronic IKK2-DN Camk2a mouse model. (Control TBI, n = 30; Acute IKK2-DN Camk2a TBI, n = 17). **P < 0.01, vs. control, by Fisher’s exact test. B) NSS score of mice with acute neuronal NF-κB inhibition. Head-injured acute IKK2-DN Camk2a mice also showed a delayed recovery (continued on next page)
removed Dox 12 d before TBI, which resulted in transgene activation after 8 d (i.e. 4 d before TBI induction), as indicated by the detection of luciferase reporter gene activity by in vivo bioluminescence imaging (Supplemental Fig. S8A). Transgene expression was confirmed by protein immunoblot analysis 6 h after TBI (Supplemental Fig. S8B). Similar to the chronic IKK2-DN\textsuperscript{Camk2a} model, acute NF-κB repression resulted in elevated postinjury mortality (Fig. 6A), and no differences in the hemotoma formation were found (data not shown). Again, animals with acute IKK2-DN\textsuperscript{Camk2a} expression showed significantly elevated NSS levels 1 and 3 d after injury compared to controls (Fig. 6B), a tendency for enhanced neuronal cell loss in the ipsilateral cortex after TBI (Supplemental Fig. S8C) and significantly increased neuronal apoptosis measured by cleaved caspase 3 expression coatined with TUJ1 (Fig. 6C).

Next, we asked whether acute IKK2-DN\textsuperscript{Camk2a} animals would also show an up-regulation of neuroinflammatory and proapoptotic factors. Gene expression was analyzed 3 d after TBI and revealed elevated levels of C3, Gsn, Lcn2, Iliib, and c-Fos in the injured hemisphere of mice with acute neuronal IKK2-DN expression (Fig. 7A–E). We also detected higher levels of the proapoptotic genes Bax and Bak, whereas the expression of Bad and of the anti-apoptotic genes Bcl2 and Bcl\textsubscript{XL} were not affected indicating a similar expression pattern in both paradigms (Fig. 7F–J). Furthermore, Atf3 and p21 were also induced in the injured cortex of IKK2-DN\textsuperscript{Camk2a} animals compared to littermates (Fig. 7K, L).

Taken together, our findings showed that appropriate neuronal IKK/NF-κB signaling is critical for neuroprotection after traumatic brain injury.

**DISCUSSION**

IKK/NF-κB signaling plays an important role in brain development, neuronal survival, and differentiation and has been shown to be involved in the pathogenesis of various neurologic diseases (22, 26, 32, 60). However, the cell-type–specific contribution of IKK/NF-κB signaling to specific pathophysiology is less clear, and studies have suggested ambivalent roles in the pathogenesis of brain injuries (26, 61). Whereas neuronal NF-κB activation is essential for maintaining homeostasis and integrity of neurons (26, 62–64), NF-κB also controls inflammation and apoptotic cell death after nerve injury (65).

To characterize the specific function of neuronal NF-κB in the pathophysiology of TBI, we used 2 conditional mouse models—loss-of-function (IKK2-DN\textsuperscript{Camk2a}) and gain-of-function (IKK2-CA\textsuperscript{Camk2a})—and subjected the animals to experimental CHI, the most common form of head injuries observed in trauma patients (6). We show that neuronal NF-κB inhibition is detrimental for the outcome of TBI. IKK2-DN\textsuperscript{Camk2a} mice that have a higher immediate posttraumatic mortality and survivors revealed significantly enhanced neurologic deficits. Even though we focused in this study on the analysis of the cerebral cortex, secondary injury of other brain regions, like hippocampus, corpus callosum and cerebellum, can also critically influence the outcome of TBI (52, 66). Despite the well-established proinflammatory role of the NF-κB system, inhibition of neuronal NF-κB signaling in IKK2-DN\textsuperscript{Camk2a} mice unexpectedly resulted in increased expression of inflammatory mediators at the site of injury. This effect could be a consequence of elevated tissue damage, which promotes the activation of microglia and astrocytes. Both cell types are critically involved in the induction of neuroinflammatory responses via the NF-κB pathway (67) and are still able to activate NF-κB signaling in our model. A similar phenotype of enhanced secondary inflammation due to prominent tissue damage upon NF-κB inactivation was observed in models of intestinal NF-κB deficiency (68). Enhanced neuronal NF-κB activation did not alleviate the consequences of TBI, as cell death and neurologic deficits in IKK2-CA\textsuperscript{Camk2a} mice did not differ from control littermates. This finding indicates that physiologic NF-κB activation in neurons is sufficient to mediate full neuroprotection. However, it remains open whether a specific timing of activation in neurons (e.g., induction only at later phases of secondary TBI pathogenesis) is able to induce beneficial effects. In line with the hypothesis of a secondary enhancement of inflammation by increased tissue damage, IKK2-CA\textsuperscript{Camk2a} animals did not show alterations in the expression of inflammation-associated gene expression compared to control animals. These data indeed suggest that the observed proinflammatory gene expression profile is induced by glial cells. The up-regulation of the proapoptotic genes Bax and Bad and other genes linked to p53-mediated stress responses in IKK2-DN\textsuperscript{Camk2a}, but not IKK2-CA\textsuperscript{Camk2a} mice suggests that endogenous physiologic neuronal NF-κB signaling protects neurons in conditions of injury-related cellular stress, possibly by suppressing p53-mediated apoptosis via the intrinsic mitochondrial pathway (69).

In a model of controlled cortical impact, it has been shown that brain-specific NF-κB activation in Nestin-Cre IkB\textsubscript{fl/fl}−/− mice leads to impaired inflammatory responses after TBI and worsened brain damage (42). In this mouse model, NF-κB is activated because of deletion of the Ikb from TBI, as reflected by a significantly increased NSS, compared to head-injured control animals. Data are presented as box plots with median ± interquartile range; whiskers show minimum and maximum range (n = 11–22). ****P < 0.0001 [not significant (ns), by nonparametric Mann-Whitney U test]. C) Expression of apoptotic cells in mice with acute neuronal NF-κB repression. Increased posttraumatic neuronal cell death 3 d after TBI in the injured (ipsi) hemisphere of acute IKK2-DN\textsuperscript{Camk2a} mice vs. control animals and the uninjured (contra) hemisphere. Immunofluorescent staining of cleaved caspase 3\textsuperscript{+} neurons (TUJ1\textsuperscript{+} cells). Quantiﬁcation of cleaved caspase 3\textsuperscript{+} cells indicated signiﬁcantly enhanced apoptosis in mice with acute neuronal NF-κB inhibition vs. control littermates. Means ± SEM (n = 3–4). *P < 0.05, **P < 0.01, ****P < 0.0001 (by 1-way ANOVA with Bonferroni’s correction). Scale bars, 500 μm; i’, inset’: 100 μm; i”, inset”: 20 μm.
NF-κB, not only in neurons, but also in astrocytes and oligodendrocytes (70). Thus, the observation that the trauma outcome was not worsened in our neuron-specific, gain-of-function mouse model suggests that NF-κB activation in astrocytes and oligodendrocytes may determine the detrimental processes in the Nestin-Cre IκBαfl/2 model. In the setting of cerebral ischemia, a detrimental role of neuronal NF-κB was proposed, as constitutive activation of IκK2 enlarged the infarct size, whereas IκK2 inactivation resulted in neuroprotection (49). These findings...
indicate a complex role of neuronal NF-κB upon distinct experimental injuries, resulting in different outcomes dependent on the specific injury models. This result may be explained by different mechanisms of the primary injury (e.g., blunt vs. sharp mechanical injury to TBI or injury caused primarily by hypoxia and impaired supply of metabolites in case of ischemic injury). These distinct modes of injury might elicit different protective or maladaptive responses, resulting in different vulnerabilities. We have previously shown that NF-κB regulates synapse formation and spine maturation in adult hippocampal neurons via Igf2 signaling (35). Thus, defective synaptic contacts may contribute to impaired posttraumatic neuronal survival. However, we also found that constitutive IKK2 activation in neurons can generate a selective neuroinflammatory response in the dentate gyrus leading to local neurodegeneration with aging (56). Therefore, neuronal IKK/NF-κB does not exclusively induce survival-promoting genes, in line with our observation that constitutive neuronal NF-κB activation did not further improve the TBI outcome.

Other candidate mechanisms that may underlie the protective role of NF-κB in neurons include the anti-apoptotic effects mediated by inducing endogenous caspase inhibitors or by triggering the expression of antioxidant genes, such as manganese superoxide dismutase or calcium-binding protein calbindin D28k (40, 60), as NF-κB transcription factors regulate a wide range of survival-promoting target genes (65).

Prolonged inhibition of neuronal NF-κB may induce indirect structural and functional alterations in the brain, which may sensitize animals for elevated posttraumatic damage. To assess such a detrimental predisposition of IKK2-DN\textsuperscript{Camk2a} animals because of chronic NF-κB inhibition, we also analyzed acute IKK2-DN\textsuperscript{Camk2a} mice in which the NF-κB/IKK2 transgene was activated shortly before CHI induction. Upon TBI, these mice showed a similar detrimental phenotype to mice with chronic IKK2-DN\textsuperscript{Camk2a} expression, excluding that long-term structural changes are responsible for the observed sensitization of IKK2-DN\textsuperscript{Camk2a} mice for TBI.

Current clinical treatment strategies imply the administration of neuroprotective and anti-inflammatory agents; however, until today, most clinical trials have failed (11). Promising therapeutic agents for treatment of head injuries are statins, inhibitors of cholesterol biosynthesis with additional pleiotropic properties (71), as well as progesterone (72). These agents limit the production of inflammatory mediators by attenuating NF-κB, p65 and TNF expression in TBI models (11). However, they most likely target the inflammatory phenotype of M1-like microglia and not neurons (4). Indeed, NF-κB inhibition in microglia and astrocytes has been shown to induce a beneficial outcome from CNS injuries (29,73–77).

In summary, neuronal IKK2/NF-κB inhibition aggravated neurodetrimental effects on CHI, whereas additional chronic IKK2/NF-κB activation in neurons cannot convert these harmful effects into an improved recovery process. Thus, our findings indicate that basal neuronal NF-κB activity is neuroprotective for the outcome of TBI.

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AUTHOR CONTRIBUTIONS

M. Mettang was responsible for the experimental design, performed most of the experiments, analyzed the data, and wrote and edited the manuscript; S. N. Reichel performed the TBIs and critically reviewed the manuscript; M. Lattke was responsible for experimental design and critically reviewed the manuscript; A. Palmer performed thromboelastometry and GFAP ELISA, helped in establishing the TBI model, and critically reviewed the manuscript; B. Baumann generated and established the transgenic mouse models, was responsible for experimental design and critically reviewed the manuscript; A. Abaei and V. Rasche performed high-resolution MRI and critically reviewed the manuscript; M. Huber-Lang helped in establishing the TBI model, made helpful comments throughout the project, and critically reviewed the manuscript; B. Baumann generated and established the transgenic mouse models, was responsible for experimental design and data interpretation, and wrote and edited the manuscript; and T. Wirth performed experimental design and data interpretation, wrote the manuscript, and edited the document.

REFERENCES

1. Langlois, J. A., Rutland-Brown, W., and Wald, M. M. (2006) The epidemiology and impact of traumatic brain injury: a brief overview. J. Head Trauma Rehabil. 21, 375–378
2. Thompson, H. J., McCormick, W. C., and Kagan, S. H. (2006) Traumatic brain injury in older adults: epidemiology, outcomes, and future implications. J. Am. Geriatr. Soc. 54, 1500–1505
3. Roozenbeek, B., Maas, A. L., and Menon, D. K. (2013) Changing patterns in the epidemiology of traumatic brain injury. Nat. Rev. Neurol. 9, 231–236
4. Simon, D. W., McGeachy, M. J., Bayr, H., Clark, R. S., Lowe, D. J., and Kochanek, P. M. (2017) The far-reaching scope of neuroinflammation after traumatic brain injury. Nat. Rev. Neurol. 13, 171–191
5. Masson, F., Thicoipe, M., Aye, P., Mokni, T., Senjean, P., Schmitt, V., Dessalles, P. H., Cazaugade, M., and Labadens, P.; Aquitaine Group for Severe Brain Injuries Study, (2001) Epidemiology of severe brain injuries: a prospective population-based study. J. Trauma 51, 481–489
6. Namjoshi, D. R., Good, C., Cheng, W. H., Panenka, W., Richards, D., Cripton, P. A., and Wellington, C. L. (2013) Towards clinical management of traumatic brain injury: a review of models and mechanisms from a biomechanical perspective. Dis. Model. Mech. 6, 1325–1338
7. Menon, D. K., and Ercolé, A. (2017) Critical care management of traumatic brain injury. Handb. Clin. Neurol. 140, 239–274
8. Bramlett, H. M., and Dietrich, W. D. (2007) Progressive damage after brain and spinal cord injury: pathomechanisms and treatment strategies. Prog. Brain Res. 161, 125–141
9. Lozano, D., Gonzales-Portillo, G. S., Acosta, S., de la Pena, I., Tajiri, N., Kaneko, Y., and Borlongan, C. V. (2015) Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatr. Dis. Treat. 11, 97–106
10. Kabadi, S. V., and Faden, A. I. (2014) Neuroprotective strategies for traumatic brain injury: improving clinical translation. Int. J. Mol. Sci. 15, 1216–1236
11. Loane, D. J., and Faden, A. I. (2010) Neuroprotection for traumatic brain injury: translational challenges and emerging therapeutic strategies. Trends Pharmacol. Sci. 31, 596–604
12. Schmidt, O. I., Heyde, C. E., Ertel, W., and Stahel, P. F. (2005) Closed head injury: an inflammatory disease? Brain Res. Brain Res. Rev. 48, 388–399.
13. Goyenea, S., and Ransohoff, R. M. (2015) Inflammatory reaction after traumatic brain injury: therapeutic potential of targeting cell-cell communication by chemokines. Trends Pharmacol. Sci. 36, 471–480.
14. Woodcock, T., and Morganti-Kossmann, M. C. (2013) The role of markers of inflammation in traumatic brain injury. Front. Neurol. 4, 18.
15. Morganti-Kossmann, M. C., Rancan, M., Stahel, P. F., and Kossmann, T. (2002) Inflammatory response in acute traumatic brain injury: a double-edged sword. Curr. Opin. Crit. Care. 8, 101–105.
16. Quillinan, N., Herson, P. S., and Traystman, R. J. (2016) Neuropathophysiology of brain injury. Anesthesiol. Clin. 34, 453–464.
17. Hayden, M. S., and Ghosh, S. (2012) NF-κB, the first quarter-century: remarkable progress and outstanding questions. Genes Dev. 26, 205–234.
18. Kaltschmidt, B., Widera, D., and Kaltschmidt, C. (2005) Signaling via NF-κB and the cell cycle. Biochem. Soc. Trans. 42, 76–81.
19. Ledoux, A. C., and Perkins, N. D. (2014) NF-κB and the cell cycle. Biochem. Soc. Trans. 42, 76–81.
20. Mattson, M. P. (2005) NF-κBp65 in the survival and plasticity of neurons. Neuron. Rev. 30, 885–893.
21. Ockershausen, A., Hayden, M. S., and Ghosh, S. (2011) Crosstalk in NF-κB signaling. Neuron. Rev. 19, 173–181.
22. Ménét, S. (2006) NF-κB functions in the nervous system: from development to disease. Biochem. Pharmacol. 72, 1180–1195.
23. Perkins, N. D. (2007) Integrating cell-signalling pathways with NF-κB. Nature Rev. Mol. Cell Biol. 8, 57–70.
24. Helmy, A., De Simoni, M. G., Guilfoyle, M. R., Carpenter, K. L., and Hutchinson, P. J. (2011) Cytokines and innate inflammation in the pathogenesis of human traumatic brain injury. Prog. Neurobiol. 95, 352–372.
25. Kaltschmidt, B., and Kaltschmidt, C. (2015) NF-κBp65 in long-term memory and structural plasticity in the adult mammalian brain. Front. Mol. Neurosci. 8, 1–11.
26. Pennypacker, K. R., Kassed, C. A., Eidizadeh, S., Saporta, S., Sanberg, P. R., and Willing, A. E. (2001) NF-κBp50 is increased in neurons surviving hippocampal injury. Exp. Neurol. 172, 507–519.
27. Lian, H., Shim, D. J., Gadela, S. S., Rodriguez-Rivera, J., Bitner, B. R., Pautler, R. G., Robertson, C. S., and Zheng, H. (2012) IkBα deficiency in brain leads to elevated basal neuroinflammation and attenuated response following traumatic brain injury: implications for functional recovery. Mol. Neurodegener. 7, 47.
28. Mattson, M. P., and Camandola, S. (2001) NF-κBp65 in neuronal plasticity and neurodegenerative disorders. J. Clin. Invest. 107, 247–254.
29. Mincheva-Tasheva, S., and Soler, R. M. (2013) NF-κB signaling pathways: role in nervous system physiology and pathology. Trends Pharmacol. Sci. 34, 296–303.
30. Mattson, M. P., Culmsee, C., Yu, Z., and Camandola, S. (2000) Roles of NF-κB in the nervous system: closed head injury: analysis of neurological outcome, blood-brain barrier dysfunction, intracranial neuropil infiltration, and neuronal cell death in mice deficient in genes for pro-inflammatory cytokines. J. Cereb. Blood Flow Metab. 20, 630–638.
31. Braunstein, K. E., Eschbach, J., Röma-Vörös, K., Soyru, L., Mikroulli, E., Larmet, Y., René, F., Gonzalez De Aguilar, J. L., Loeffler, J. P., Müller, H. P., Bucher, S., Kaulisch, T., Niesen, H. G., Tillmanns, J., Fischer, K., Schwalenstöcker, B., Kassubek, J., Pichler, B., Stiller, D., Petersen, A., Ludolph, A. C., and Dupuis, L. (2010) A point mutation in the dynein heavy chain gene leads to striatal atrophies and neuronal nuclear outgrowth of striatal neurons. Hum. Mol. Genet. 19, 4385–4398.
Maqbool, A., Lattke, M., Wirth, T., and Baumann, B. (2013) Sustained, neuron-specific IKK/NF-κB activation generates a selective neuroinflammatory response promoting local neurodegeneration with aging. Mol. Neurodegener. 8, 40

Nylen, K., Ost, M., Caqibok, L. Z., Nilsson, I., Blennow, K., Nelligård, B., and Rosengren, L. (2006) Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. J. Neurol. Sci. 240, 85–91

Vos, P. E., Lamers, K. J., Hendriks, J. C., van Haaren, M., Beems, T., Zimmerman, C., van Geel, W., de Reus, H., Biert, J., and Verbeck, M. M. (2004) Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. Neurology 62, 1303–1310

Villasana, L. E., Westbrook, G. L., and Schnell, E. (2014) Neurologic impairment following closed head injury predicts post-traumatic neurogenesis. Exp. Neurol. 261, 156–162

Qin, Z. H., Tao, L. Y., and Chen, X. (2007) Dual roles of NF-kappaB in cell survival and implications of NF-kappaB inhibitors in neuroprotective therapy. Acta Pharmacol. Sin. 28, 1859–1872

Pizzii, M., and Spano, P. (2006) Distinct roles of diverse nuclear factor-kappaB complexes in neuropathological mechanisms. Eur. J. Pharmacol. 545, 22–28

Duckworth, E. A., Butler, T., Collier, L., Collier, S., and Pennypacker, K. R. (2006) NF-kappaB protects neurons from ischemic injury after middle cerebral artery occlusion in mice. Brain Res. 1088, 167–175

Kaltzschmidt, C., Kaltzschmidt, B., Neumann, H., Wekerle, H., and Baeuerle, P. A. (1994) Constitutive NF-kappaB activity in neurons. Mol. Cell. Biol. 14, 3981–3992

Widera, D., Kleinbe, C., Nair, D., Heidebrecher, M., Malkusch, S., Silberita, J. B., Choquet, D., Kaltzschmidt, B., Heilemann, M., and Kaltzschmidt, C. (2016) Single-particle tracking uncovers dynamics of glutamate-induced retrograde transport of NF-kappaB p65 in living neurons. Neumophotosomes 3, 041804

Yang, L., Tao, L. Y., and Chen, X. P. (2007) Roles of NF-kappaB in central nervous system damage and repair. Neurosci. Bull. 23, 307–313

Mouzon, B., Chaytow, H., Crynen, G., Bachmeier, C., Stewart, J., Mullan, M., Stewart, W., and Crawford, F. (2012) Repeated mild traumatic brain injury in a mouse model produces learning and memory deficits accompanied by histological changes. J. Neurotrauma 29, 2761–2773

Kirkley, K. S., Popichak, K. A., Afzali, M. F., Legare, M. E., and Tjalkens, R. B. (2017) Microglia amplify inflammatory activation of astrocytes in manganese neurotoxicity. J. Neuroinflammation 14, 99

Nenci, A., Becker, C., Wallaert, A., Gareus, R., van Loo, G., Danese, S., Huth, M., Nikolaev, A., Neufert, C., Madison, B., Gumucio, D., Neurath, M. F., and Pasparakis, M. (2007) Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature 446, 557–561

Yang, L. B., Kinoshita, C., Kinoshita, Y., and Morrison, R. S. (2014) p53 and mitochondrial function in neurons. Biochem. Biophys. Acts 1842, 1186–1197

Lothian, C., and Lendahl, U. (1997) An evolutionarily conserved region in the second intron of the human nestin gene directs gene expression to CNS progenitor cells and to early neural crest cells. Eur. J. Neurosci. 9, 452–462

Cucchiara, B., and Kasner, S. E. (2001) Use of statins in CNS disorders. J. Neurol. Sci. 187, 81–89

Roof, R. L., and Hall, E. D. (2000) Gender differences in acute CNS trauma and stroke: neuroprotective effects of estrogen and progesterone. J. Neurotrauma 17, 367–388

Chen, J., Zhou, Y., Mueller-Steiner, S., Chen, L. F., Koon, H., Yi, S., Mucke, L., and Gan, L. (2005) SIRT1 protects against microglia-dependent amyloid-beta toxicity through inhibiting NF-kappaB signaling. J. Biol. Chem. 280, 40364–40374

Jayakumar, A. R., Tong, X. Y., Ruiz-Cordero, R., Bregy, A., Bethea, J. R., Bramlett, H. M., and Norenberg, M. D. (2014) Activation of NF-kappaB mediates astrocyte swelling and brain edema in traumatic brain injury. J. Neurotrauma 31, 1249–1257

Khoroooshi, R., Babcock, A. A., and Owens, T. (2008) NF-kappaB-driven STAT2 and CCL2 expression in astrocytes in response to brain injury. J. Immunol. 181, 7284–7291

Lagraoui, M., Sukumar, G., Latoche, J. R., Maynard, S. K., Dalgaard, C. L., and Schaefer, B. C. (2017) Salubrate treatment following traumatic brain injury reduces inflammation and promotes a neuroprotective and neuroregenerative transcriptional response with concomitant functional recovery. Brain Behav. Immun. 61, 96–109

Yuan, Y., Zhu, F., Su, Y., Wang, D., Huang, A., Hu, X., Qin, S., Sun, X., Su, Z., and He, C. (2015) Neuroprotective effects of nitidine against traumatic CNS injury via inhibiting microglia activation. Brain Behav. Immun. 48, 287–300

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