Acid-ion sensing channel 1a deletion reduces chronic brain damage and neurological deficits after experimental traumatic brain injury

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Abstract

Traumatic brain injury (TBI) causes long-lasting neurodegeneration and cognitive impairments, however, the underlying mechanisms of these processes are not fully understood. Acid-sensing ion channels 1a (ASIC1a) are voltage-gated Na^+-and Ca^{2+}-channels shown to be involved in neuronal cell death, however, their role for chronic post-traumatic brain damage is largely unknown. To address this issue we used ASIC1a-deficient mice and investigated their outcome up to six months after TBI.

ASIC1a-deficient mice and their wild-type littermates were subjected to controlled cortical impact (CCI) or sham surgery. Brain water content was analyzed 24 h and behavioral outcome up to six months after CCI. Lesion volume was assessed longitudinally by magnetic resonance imaging and six months after injury by histology.

Brain water content was significantly reduced in ASIC1a$^{-/-}$ animals compared to wild-type controls. Over time, ASIC1a$^{-/-}$ mice showed significantly reduced lesion volume and reduced hippocampal damage. This translated into improved cognitive function and reduced depression-like behavior. Microglial activation was significantly reduced in ASIC1a$^{-/-}$ mice.

In conclusion, ASIC1a-deficiency resulted in reduced edema formation acutely after TBI and less brain damage, functional impairments, and neuroinflammation up to six months after injury. Hence, ASIC1a seems to be involved in chronic neurodegeneration after TBI.

Key words: traumatic brain injury, controlled cortical impact, brain edema, animal studies, cognitive function

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Introduction

Traumatic brain injury (TBI) is a major cause of death and disability wordlwide.[1-3] In past decades, most clinical and experimental studies concentrated on clarifying pathomechanisms occurring in the first hours and days after trauma. It is, however, increasingly recognized that pathophysiologic processes that aggravate posttraumatic brain damage thus causing progressive neurocognitive and behavioral deficits are not restricted to the first few days but may be active for months and even years after TBI.[4-9] Furthermore, a history of TBI is a risk factor for the development of neurocognitive,[10, 11] neurodegenerative,[12, 13] and psychiatric disorders,[14-16] and increases all-cause mortality.[17, 18] These clinical symptoms were linked to progressive brain atrophy and persisting inflammatory changes,[19, 20] findings corroborated by experimental studies showing that progressive histological damage occurs up to one year after experimental TBI and is accompanied by cognitive dysfunction and depressive behavior.[21-24] The exact mechanisms resulting in chronic posttraumatic brain damage are, however, not fully elucidated yet.

Acidosis is important in the development of acute posttraumatic brain damage and occurs after experimental as well as clinical TBI.[25-27] The mechanisms of acidosis-induced neuronal damage in TBI are, however, not completely understood. Recently, acid-sensing ion channels (ASICs) have been proposed as important acid-sensors[28, 29] and hypothesized to play an important role for acidosis-induced post-ischemic neuronal damage.[30, 31] ASICs are widely expressed in the central (CNS) and peripheral nervous system. [32-35] ASIC1a, one of the most prevalent isoforms in the CNS, [33, 36, 37] is permeable to Na^+ and Ca^{2+} and results in neuronal (over)excitation when activated by extracellular acidosis.[38] Based on its potential role in amplifying excitotoxicity, ASIC1a has been investigated in a variety of cerebral diseases: its pharmacological inhibition or genetic disruption reduced infarct volume after cerebral ischemia,[30, 39, 40] conferred neuroprotection in ischemic pre- and post-conditioning[41], reduced brain injury in

models of autoimmune encephalomyelitis,[42] and improved outcome in Huntington´s disease models.[43] In TBI, ASIC1a-deficient mice showed attenuated neuronal cell-death 24 hours and partially improved memory function four days after the insult.[44] So far, however, the role of ASIC1a for chronic brain damage and long-term behavioral impairments after TBI is unknown.

Based on these previous results we hypothesize that acidosis-induced pathomechanisms mediated by ASIC1a contribute to the development of chronic posttraumatic brain damage. To test this, we investigated posttraumatic neuropathological and neurobehavioral outcome in ASIC1a-deficient mice up to six months after experimental TBI.

Methods

Experimental animals

ASIC1a transgenic mice were purchased from Jackson Laboratories (strain# 013733), male homo- and heterozygous mice and their wild-type littermates were bred heterozygously[45] for experiments.

Experimental protocol

All procedures were approved by the Animal Ethics Board of the Government of Upper Bavaria. Mice of all genotypes were randomly assigned to experimental groups by drawing lots. All procedures and analyses were performed by a researcher blinded to group allocation/ genotype and reported according to the ARRIVE criteria. Mice not reaching the end of the study, i.e. 180 days after TBI, were excluded from analysis in order to avoid mortality bias.

Brain water content was assessed in 8-10 week-old male ASIC1a$^{-/-}$ and wild-type (WT) littermates (Sham: n=4/group, TBI groups: n=16/group). Long-term evaluation was performed in 8-10 week-old male ASIC1a$^{+/-}$ (n=12), ASIC1a$^{-/-}$ (n=12), and WT littermates (n=11; **Figure 1**).

Experimental TBI

Controlled Cortical Impact (CCI) was induced as previously described.[22, 46-48] After right parietal craniotomy CCI (impact depth: 1 mm, impact duration: 150 ms, impact velocity 8 m/s) was performed onto the intact dura, then the bone flap was re-implanted. Sham operation consisted in craniotomy without CCI.

Body weight

Body weight was evaluated from three days before until 180 days after surgery using a scale with movement correction (OHAUS®, Munich, Germany).

Brain water content

Brain water content was measured 24h after TBI by the wet-dry-weight-method as previously described.[22, 46, 47, 49, 50] Briefly, both hemispheres were dissected and weighed to obtain the wet weight. After drying (100°C, 24 hours) to obtain the dry weight, brain water content was calculated using the following formula: wet weight-dry weight)/wet weight × 100%. The difference between traumatized and non-traumatized hemisphere was calculated and plotted.

Beam walk test

The Beam walk test for the evaluation of motor function was performed as previously described.[22, 51, 52] The time to cross the beam and the number of missteps were assessed from three days before until 180 days after CCI.

Tail Suspension test

The Tail Suspension test is a widely used behavioral test for the evaluation of depression-like behavior in rodents.[22, 53, 54] Animals were fixed by the tail and suspended head down in a custom-made frame; movement patterns were digitally recorded for three minutes on day 60, 90, and 180 after TBI. Mobility/ immobility time were automatically analyzed (EthoVision®XT, Noldus Information Technology, Wageningen, Netherlands).

Barnes Maze test

The Barnes Maze test is a test paradigm evaluating spatial learning and memory.[22, 55, 56] The animal is placed on a brightly lit circular platform and trained to locate the home-cage placed under one of the 20 holes at the platform rim. Mice were trained twice daily for four days. On day six the time to reach the home cage (latency), distance, and speed travelled were evaluated by automated image analysis (EthoVision®XT).

Analysis of cortical and hippocampal lesion volume

Floating sections were prepared using a vibratome as previously described.[57] Briefly, 13 50 µm thick coronal brain sections were prepared at 500 µm intervals. Sections were stained according to Nissl and digitized at 12.5-fold magnification (Zeiss Axio Imager M2, Carl Zeiss, Oberkochen, Germany). The area of the non-traumatized (A) and the traumatized hemisphere (B) were determined (**Supplemental Fig. 1a**) and the lesion area (C) was calculated (A-B=C) for each level. The lesion volume was then calculated according to the following formula:

Lesion volume = 0.5 mm*(C1/2+ C2+……+ C13/2).[22]

Six sections containing the hippocampus (1.5 mm anterior to 4 mm posterior to bregma) were used to determine hippocampal damage as depicted in **Supplemental Fig. 1b**.

Evaluation of lesion volume by Magnetic Resonance Imaging (MRI)

Lesion size after TBI was assessed longitudinally by T2-weighted (T2W) imaging using a 3 Tesla Nanoscan PET/MRI scanner (Mediso Medical Imaging Systems, Budapest, Hungary) on day 14, 60, 90, and 180 following TBI as previously described.[22] On 19 coronal slices, both hemispheres were manually segmented (**Supplemental Fig. 1c**) and lesion volume was analyzed using an image analysis software (InterView™ FUSION, Mediso Medical Imaging Systems, Budapest, Hungary) and calculated as described above.

Immunohistochemistry

Sections were incubated overnight with the respective primary antibody in 1% bovine albumin, 0.1% gelatin, 0.5% Triton X-100 in 0.01 M PBS before addition of the secondary antibody (1:200, 2h, Alexa Fluor® 594, Jackson, Pennsylvania, USA). The following primary antibodies were used: iba-1 (Wako, #019-19741, 1:200), CD68 (Milipore, #MAB1435, 1:200), NeuN (Synaptic Systems, #266004).

To quantify iba-1 and CD68 positive cells, maximum projection Z-stacks were obtained at bregma level with a 40-x oil immersion objective using a confocal microscope (Zeiss LSM810, Zeiss, Oberkochen, Germany) as previously described. [57] Quantitative image analysis was performed by an investigator blinded to the genotype (ImageJ software; NIH, Bethesda, MD). Quantitative image analysis was performed by an investigator blinded to the genotype (ImageJ software; NIH, Bethesda, MD). Three corresponding ROIs (x:354 µm, y:354 µm, z:25 µm) in the ipsi- as well as the contralateral hemisphere were analyzed; after background correction, all iba-1 or CD68 positive signals/pixels were subjected to automated threshold processing and subsequently counted.

Statistical analysis

Statistical analysis was performed using Sigma plot 13.0 (Systat, Erkrath, Germany). Sample size calculations were performed with the following parameters: alpha-error=0.05, beta-error=0.2, standard deviation 15 - 20% (depending on the parameter investigated), and a biologically relevant difference of 30%. For comparing two groups the Mann–Whitney test was used, while the Kruskal–Wallis followed by Dunn's post-hoc test was used for multiple-group comparisons. For correlation analysis, the Pearson test was used. Survival rate was analyzed by the Log Rank test. Differences between groups were considered significant at $P<0.05$. All data is expressed as mean ± standard deviation (SD).

Results

ASIC1a-deficiency does not affect mortality and body weight after TBI

Two animals in the WT group, three in the ASIC1a$^{+/-}$ group, and three in the ASIC1a$^{-/-}$ group died within seven days after trauma. All other mice survived until at least 180 days after TBI. After three more WT and two more ASIC1a$^{+/-}$ mice and one more ASIC1a$^{-/-}$ mouse died around 190 days after trauma, the study was terminated and all animals were sacrificed. There was no significant difference between groups regarding acute or delayed mortality (**Fig. 2a**). Body weight of all mice decreased by approximately 13% one day after TBI, then gradually recovered back to baseline. There was no significant difference between genotypes (**Fig. 2b**).

ASIC1a knock-out reduces brain edema formation 24h after TBI

In wild-type littermates TBI induced a significant increase in brain water content 24 hours after injury (+2.5 ± 2.0%). In ASIC1a$^{-/-}$ animals, this increase was significantly reduced by almost 75% (0.6 ± 1.4%, $p<0.01$), indicating a reduction of brain edema formation in these animals (**Fig. 3**).

ASIC1a-deficiency has no effect on motor function after TBI

Three days before TBI, all animals crossed the beam in about 10 seconds, and without missteps. One day after CCI, motor function significantly deteriorated in all investigated groups as indicated by a prolonged crossing time (70-100s; $p<0.01$ vs baseline, **Fig. 4a**) and an increase in the number of missteps (**Fig. 4b**, $p<0.01$). Both parameters partially recovered within 7 days, but did not return to pre-trauma values. There was no difference between groups at any time point.

ASIC1a-deficiency reduces depression-like behavior after TBI long-term

When hanging head down by the tail mice usually try to right themselves up for most of the observation time, i.e. 180 seconds. In wild-type and heterozygous mice TBI reduced the time the animals tried to right themselves up (mobility time) to about 100 seconds. Homozygous ASIC1a-deficient animals, however, showed a significantly higher mobility time of about 140 seconds at all time points (**Fig. 5**; $p<0.01$). These

findings strongly suggest that ASIC1a-deficiency improves depression-like behavior after TBI.

ASIC1a-deficiency improves long-term memory function after TBI

Following TBI, WT and ASIC1a$^{+/-}$ mice had difficulties finding their home cage as evidenced by representative heat map analysis (**Fig. 6a**). When quantifying memory function, latency to goal as well the distance travelled improved over time in all groups (**Fig. 6b and c**), however, ASIC1a$^{-/-}$ mice found their home cage twice as fast (**Fig. 6b**; $p<0.05$) and covered half of the distance compared to their wild-type littermates at the end of the observation time (**Fig. 6c**; $p<0.05$). These results suggest that ASIC1a-deficiency preserved memory function after TBI.

ASIC1a-deficiency protects the brain against TBI-induced neurodegeneration

In order to evaluate lesion volume in the same animals over time, we performed longitudinal T2-weighted MR-imaging up to six months after injury (**Fig. 7a**). In wild-type mice lesion volume steadily increased from 14 until 180 days after TBI (**Fig. 7b**; open circles; $p<0.01$ vs 14 days after TBI). In heterozygous and homozygous ASIC1a-deficient animals, however, this increase was not observed (**Fig. 7b**, closed circles). Despite similar lesion volumes 14 days after brain injury, there was no obvious longitudinal lesion progression in these animals. 60, 90, and 180 days after TBI heterozygous and homozygous ASIC1a-deficient animals had significantly smaller lesion volumes than their wild-type littermates (**Fig. 7b**). There was, however, no difference between homozygous and heterozygous ASIC1a-deficient mice. At the end of the observation time, lesion volume was also determined by histomorphometry (**Fig. 7c**). Also this analysis revealed that WT mice had significantly larger lesion volumes (37.0 ± 5.1 mm^3) than ASIC1a$^{+/-}$ (26.9 ± 3.3 mm^3; $p<0.01$ vs WT) and ASIC1a$^{-/-}$ mice (25.8 ± 3.9 mm^3; $p<0.01$ vs WT; **Fig. 7d**). Volumes obtained by histology strongly correlated with those measured by MRI ($r=0.8266$, $P<0.0001$, **Fig. 7e**), indicating that MRI measurements are a reliable way to quantify posttraumatic brain damage.

TBI caused significant hippocampal damage in the traumatized hemisphere (**Fig. 8a**). WT animals lost over 80% of their ipsilateral hippocampal volume 180 days after injury (26.3±4.0% of hippocampal volume preserved vs. non-traumatized side). In homozygous and heterozygous ASIC1a-deficient mice hippocampal volume loss was significantly reduced from 80 to about 55% (ASIC1a$^{+/-}$: 44.2 ± 13.1%, ASIC1a$^{-/-}$: 45.4 ± 4.8%, **Fig. 8b**).

ASIC1a-deficiency results in less neuroinflammation after TBI

In order to examine neuroinflammation, we assessed microgliosis by iba-1-staining and microglial activation by CD68-staining near the rim of the contusion and in the same area of the contralateral hemisphere in WT and homozygous ASIC1a-deficient mice six months after TBI (**Fig. 9a**). Even at this very late time point after injury, the traumatic contusion was surrounded by a large number of iba-1- (red fluorescence) and CD68-positive (green fluorescence) cells, suggesting massive microglia proliferation and activation (**Fig. 9a**; ipsi WT). No such changes were observed in the contralateral hemisphere (**Fig. 9a**; contra WT). In homozygous ASIC1a- deficient mice much less iba-1- and CD68-positive cells were observed and most cells were located in the immediate proximity of the lesion boarder (**Fig. 9a**; ipsi ASIC1a$^{-/-}$). Again no activated microglia were observed in the contralateral hemisphere (**Fig. 9a**; contra ASIC1a$^{-/-}$). When quantifying iba-1 and CD68-fluorescence signals by integrated density analysis, we found the same amount of iba-1-fluorescence in the contralateral hemisphere of both genotypes, suggesting that our approach was sensitive enough to detect quiescent microglia in healthy brain tissue (**Fig. 9b**; open and closed bars). After TBI, the iba-1 signal increased by more than nine-fold in WT mice, but only 3.5-fold in homozygous ASIC1a-deficient mice (**Fig. 9b**; striped bars; $p<0.01$ vs. contralateral and vs. WT). Regarding CD68, a marker for microglia activation, as expected, almost no CD68 signal was detected in healthy brain, i.e. in the contralateral hemisphere (**Fig. 9c**; open and closed bars). After TBI, however, the CD68 signal in WT mice increased

by more than 25-fold (**Fig. 9c**; striped open bar; $p<0.001$). This massive activation of microglial cells was significantly reduced in homozygous ASIC1a-deficient mice ($p<0.01$); in these animals, the CD68 signal increased only three-fold (**Fig. 9c**; striped closed bar; $p<0.001$), suggesting that ASIC1a may play a significant role for microglial activation and maintenance of neuroinflammation following TBI.

Discussion

Here, we investigated the effect of ASIC1a-deficiency on long-term structural and functional outcome after experimental traumatic brain injury. We observed a significant reduction of cortical and hippocampal brain damage accompanied by improvement of cognitive and behavioral deficits as compared to littermate controls up to six months after TBI. The improved outcome seems to be mainly caused by reduction of hippocampal damage as the extent of posttraumatic hippocampal atrophy has been shown to correlate to cognitive deficits and mood disorders.[58-61] Heterozygous knock-out of ASIC1a led to a significant reduction of structural brain damage, comparable to that seen in homozygous knock-out mice, however, behavioral testing yielded variable results, i.e. there was a trend towards reduced depression like behavior and towards worse performance in learning and memory function. However, these changes were not significantly different as compared to wild type animals and may be may be caused by varying ASIC1a activity levels in heterozygous animals. Nevertheless, to our knowledge this is the first report of a gene-dose effect in ASIC1a mediated neuronal damage. The results in heterozygous animals may have implications for a future putative pharmacological treatment strategy with possibly incomplete ASIC1a inhibition.

Acidosis is considered a pivotal factor in the pathophysiology of secondary brain injury after cerebral insults.[62-64] After TBI, a drop in extracellular pH occurs and seems to correlate with injury severity and adverse outcome.[65-68] Acidosis is most commonly

caused by posttraumatic cerebral ischemia which is a major pathomechanism of secondary brain damage after traumatic brain injury and can be detected very early on after TBI, [25, 69-72] but has also been described to occur and persist at later time points, e. g. in association with delayed phenomena like posttraumatic vasospasm,[73, 74] and cortical spreading depolarizations.[75-77] Acidosis may, however, also develop without obvious ischemia, most probably due to metabolic derangement, dysfunctional autoregulatory mechanisms, or a mismatch between cerebral blood flow and energy demand after TBI.[78, 79] While it is well established that acidosis induces and exacerbates neuronal injury[64] e.g. by protein denaturation,[80] induction of cell swelling,[81-84] or impeding mitochondrial energy metabolism,[85] the exact damage pathways are not entirely clear yet.

Acid-ion sensing channels 1a (ASIC1a) are pH-dependent sodium and (to a lesser degree) calcium-channels expressed on the postsynaptic membrane of central and peripheral neurons.[86] ASIC1a facilitate excitatory neurotransmission, thereby mediating synaptic plasticity and memory functions under physiological conditions.[28, 34, 87] It is increasingly recognized, that ASICs also may mediate acidosis-associated damage after cerebral insults by inducing glutamate-independent neuronal cell death.[29, 40, 88] They also seem to be involved in necroptotic neuronal cell death,[89, 90] a caspase-independent form of cell death that is mediated by receptor-interacting protein (RIP) 1 and 3.[91, 92] ASIC1a has been shown to play a role in the development of neuronal damage in the acute phase after TBI.[44] In the present study, this was corroborated by our finding that ASIC1a-deficiency significantly decreased brain edema formation 24h after TBI. This acute protection has previously been reported to partially improve memory function in the first days after fluid percussion TBI.[44] However, ASIC1a-effects on structural or functional outcome in the chronic posttraumatic phase have not been previously investigated. Thus, it remained unclear whether ASIC1a-mediated protection was only short-lived or covered also clinically

relevant time windows, i.e. three or six months. Here, we show that ASIC1a-deficiency notably attenuates progression of lesion volume and hippocampal tissue loss for up to six months after trauma. Furthermore, it ameliorated depressive behavior and cognitive dysfunction at six months post-TBI, while not affecting motor deficits.

Chronic changes after a single traumatic brain injury are characterized by progressive global, hippocampal, and gray matter atrophy, as well as ventricular enlargement.[21, 22, 93-96] These long-term progressive posttraumatic changes are thought to be caused by chronic neuroinflammation[96, 97] and to persist for years and even decades after a single TBI.[94, 98] Anti-inflammatory strategies have been shown to reduce chronic posttraumatic neuroinflammation, thereby improving neurocognitive deficits long-term.[24, 99] Chronic inflammation promotes low tissue pH and may thus activate ASICs. Whether this is a pathway of importance when it comes to chronic posttraumatic changes, however, has previously not been investigated. ASIC1a has been shown to contribute to inflammasome activation,[100] cytosolic multi-protein complexes which play an important role for neuroinflammation after TBI.[101-104] Furthermore, in dorsal root ganglia, inflammatory cytokines increased ASIC1a-activity.[105] Our data support the notion that acidosis-related ASIC1a-activation is (at least) partially responsible for the chronic inflammatory reaction after TBI, since ASIC1a-deficiency resulted in reduced microglia activation in the present investigation. Our results therefore indicate that ASIC1a-mediated inflammatory pathways play an important role for chronic lesion progression after traumatic brain injury. Acidosis-induced brain damage mediated by ASIC1a is a novel and previously not considered factor in the pathogenesis of chronic posttraumatic brain damage and may therefore be a suitable target to reduce long-term sequelae of traumatic brain injury.

Conclusion

Acidosis-associated mechanisms seem to play an important role in the development of long-term consequences of TBI. ASIC1a-deficiency led to an improvement of

structural and functional outcome, most probably by attenuating posttraumatic inflammatory changes. Targeting ASIC1a-channels may therefore be a pharmacological treatment strategy for chronic posttraumatic brain damage.

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Authorship confirmation statement

Conception or design of the study: SC, NP, NAT, MW, FR

Acquisition, analysis, or interpretation of the data: SC, XM, XL, AW, SH, UM, IK, NP, NAT

Drafting the paper or revising it critically for important intellectual content: all authors.

All authors gave final approval of the current manuscript version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Author disclosure statement

The authors declare no conflict of interest

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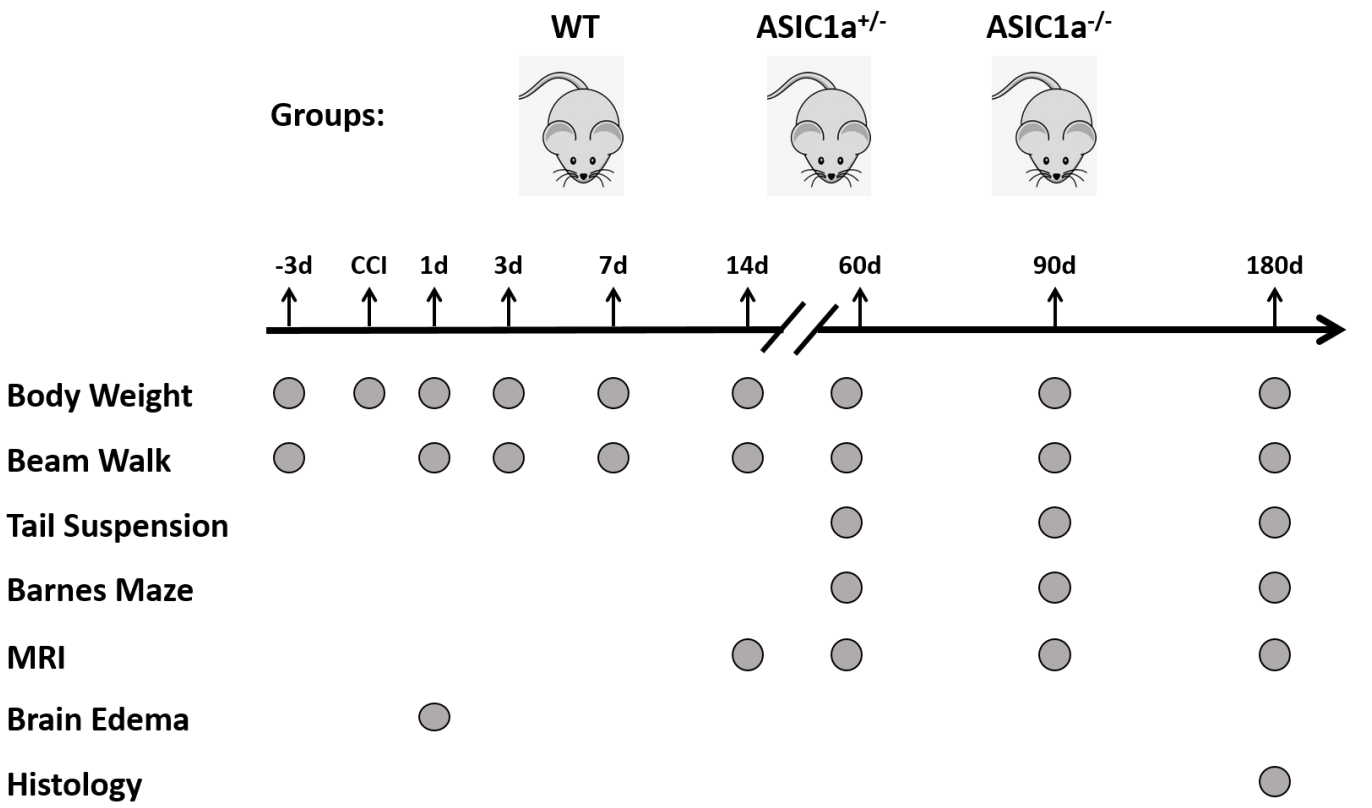
Figure 1


WT
ASIC1a+/-
ASIC1a-/-
Groups:
-3d
CCI
1d
3d
7d
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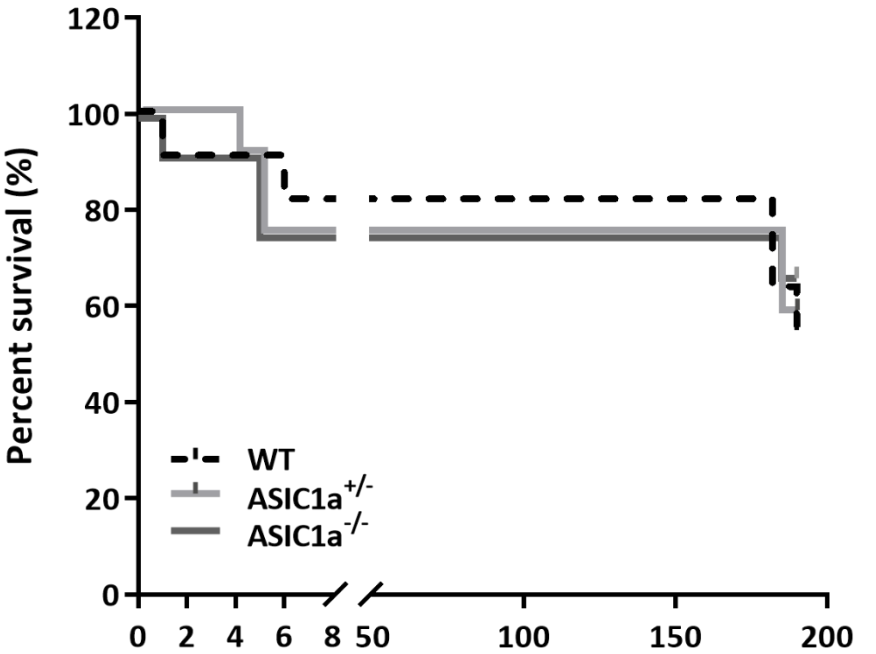
Body Weight
Beam Walk
Tail Suspension
Barnes Maze
MRI
Brain Edema
Histology

Figure 2

a


120
100
80
60
40
20
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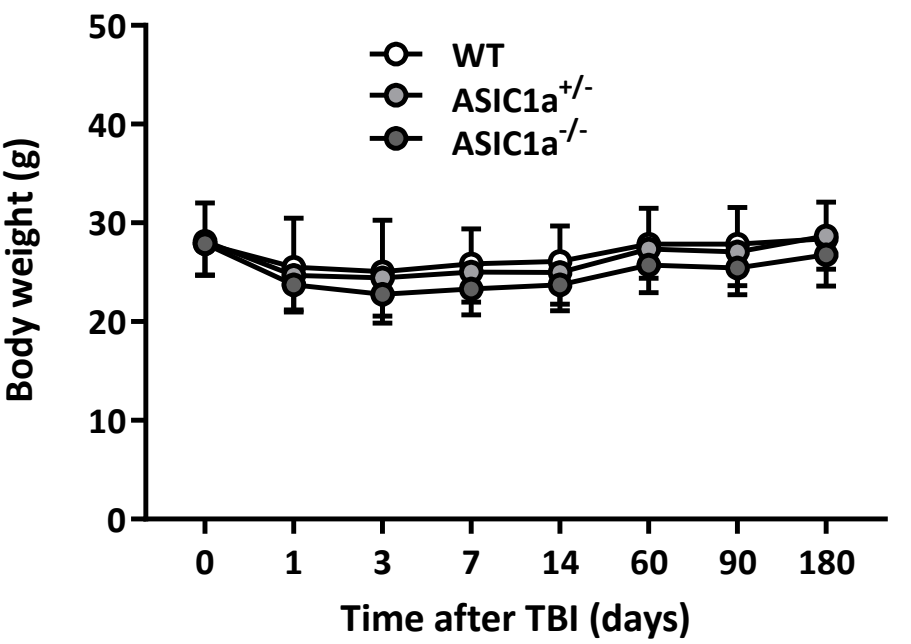
Percent survival (%)
WT
ASIC1a+/-
ASIC1a-/-
0
2
4
6
8
50
100
150
200

b


50
40
30
20
10
0
Body weight (g)
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ASIC1a+/-
ASIC1a-/-
0
1
3
7
14
60
90
180
Time after TBI (days)

Figure 3

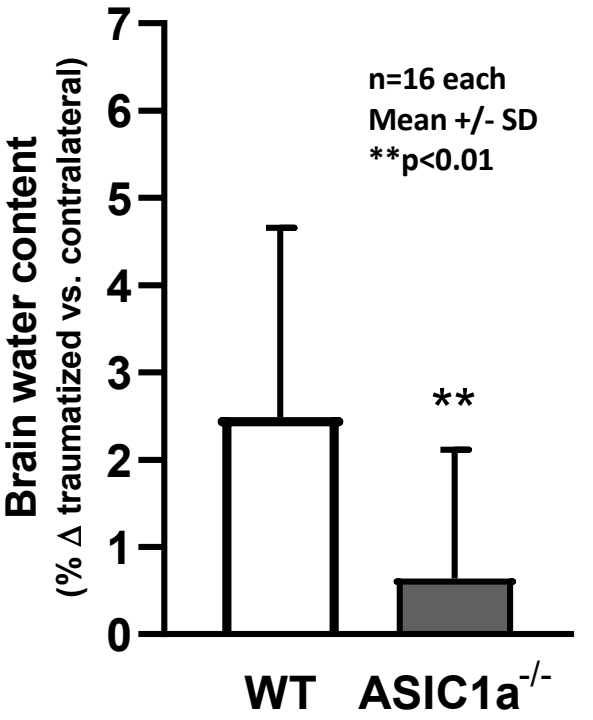
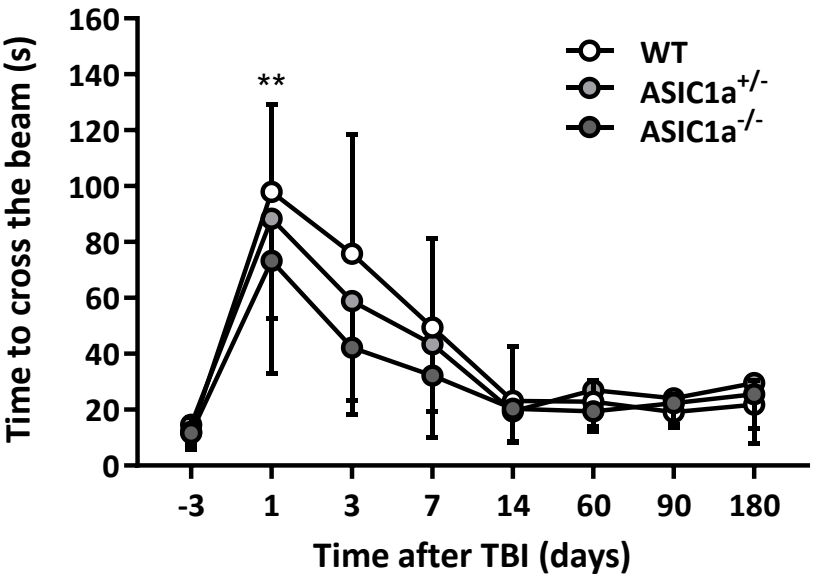

Brain water content
(% Δ traumatized vs. contralateral)
7
6
5
4
3
2
1
0
n=16 each
Mean +/- SD
**p<0.01
**
WT
ASIC1a-/-

Figure 4

a


Time to cross the beam (s)
160
140
120
100
80
60
40
20
0
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WT
ASIC1a+/-
ASIC1a-/-
-3
1
3
7
14
60
90
180
Time after TBI (days)

b

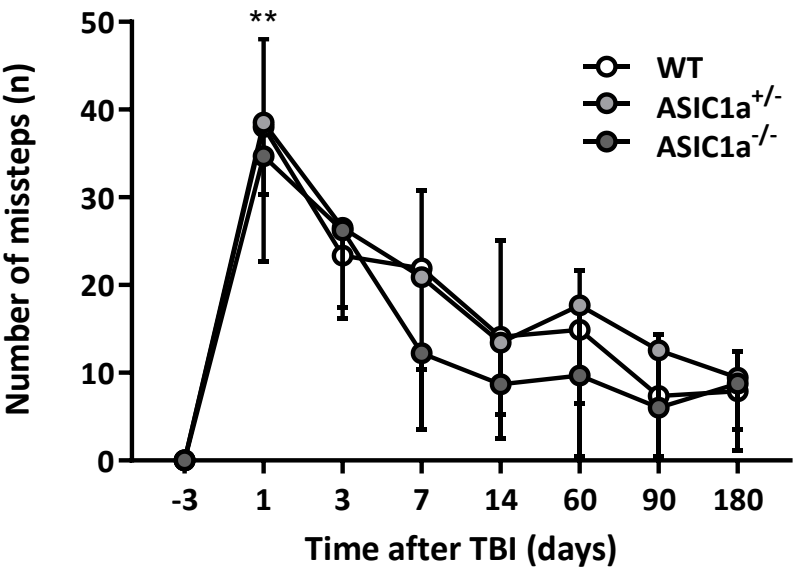

Number of missteps (n)
50
40
30
20
10
0
**
WT
ASIC1a+/-
ASIC1a-/-
-3
1
3
7
14
60
90
180
Time after TBI (days)

Figure 5

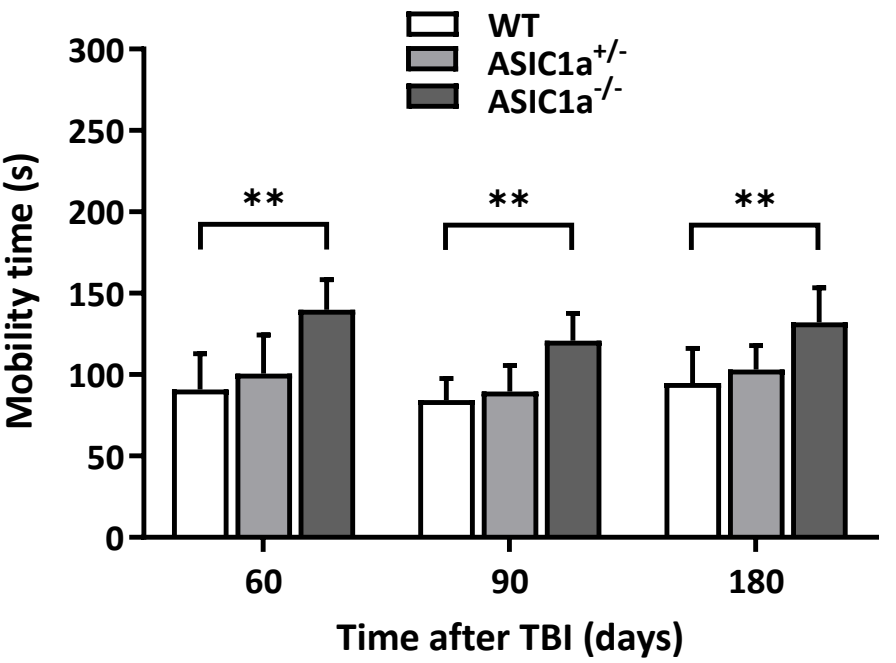

WT
ASIC1a+/-
ASIC1a-/-
Mobility time (s)
300
250
200
150
100
50
0
**
**
**
60
90
180
Time after TBI (days)

Figure 6

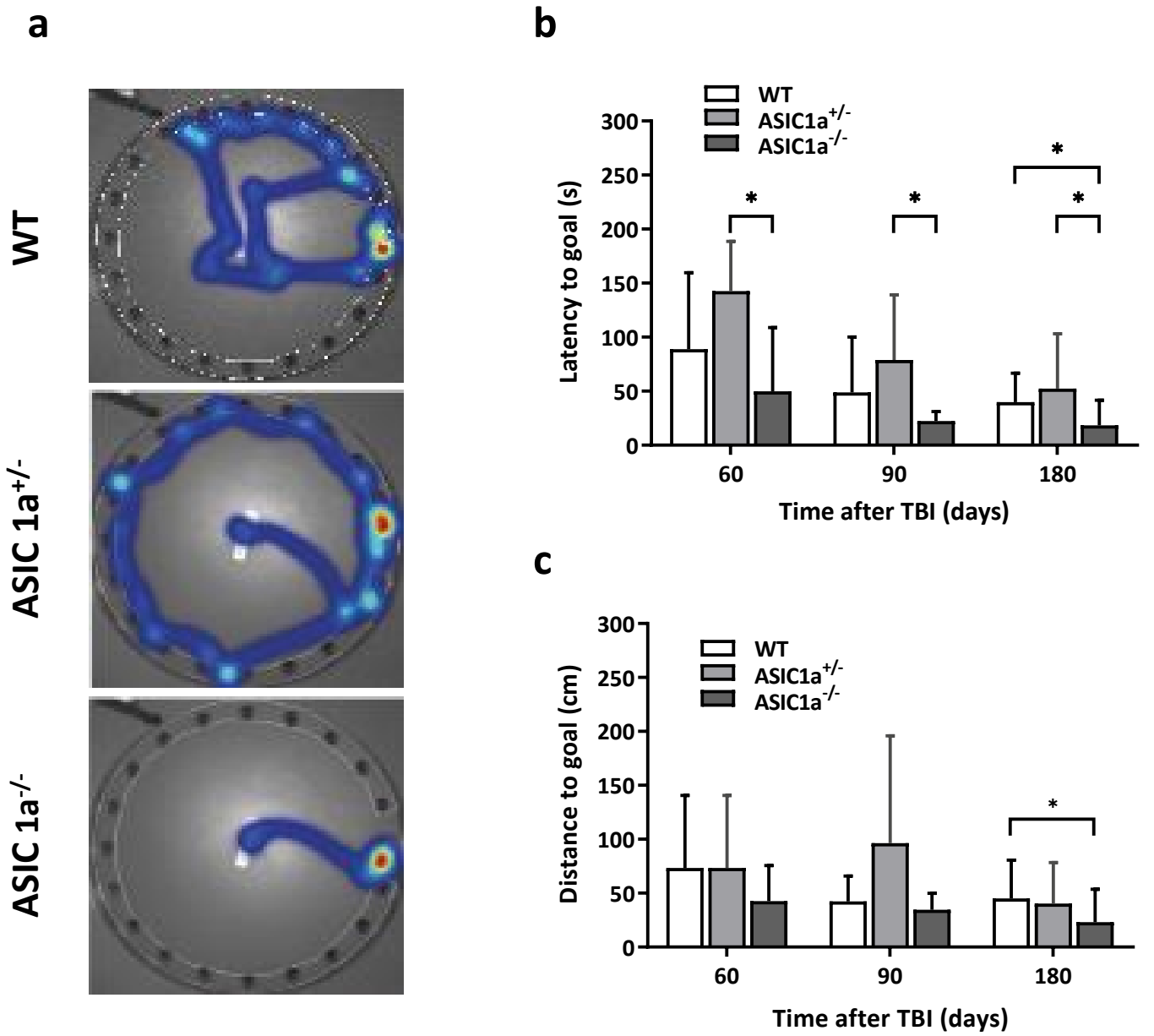

a
WT
ASIC 1a+/-
ASIC 1a-/-
b
WT
ASIC1a+/-
ASIC1a-/-
Latency to goal (s)
300
250
200
150
100
50
0
60
90
180
Time after TBI (days)
*
*
*
*
c
WT
ASIC1a+/-
ASIC1a-/-
Distance to goal (cm)
300
250
200
150
100
50
0
60
90
180
Time after TBI (days)
*

Figure 7

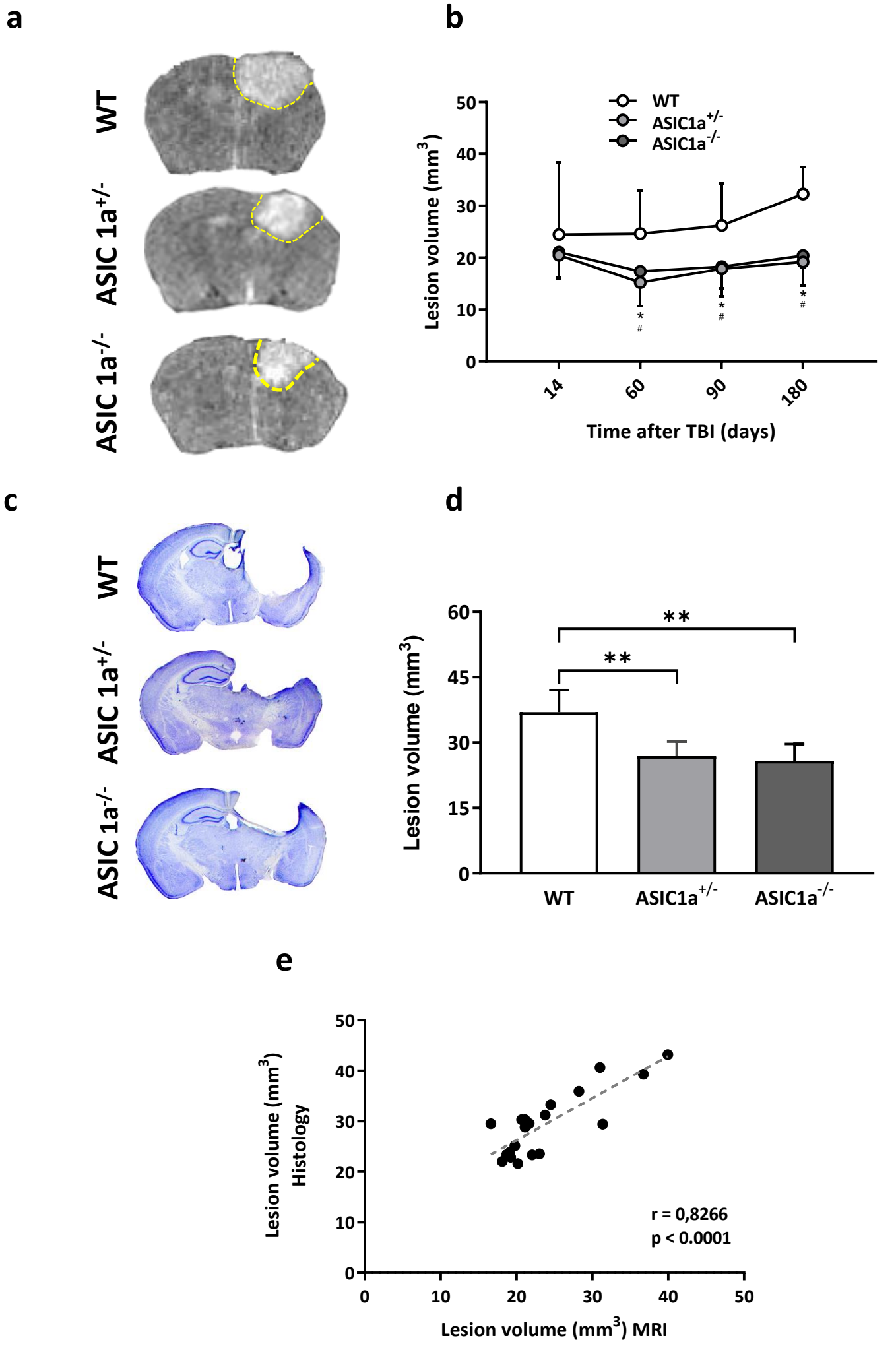
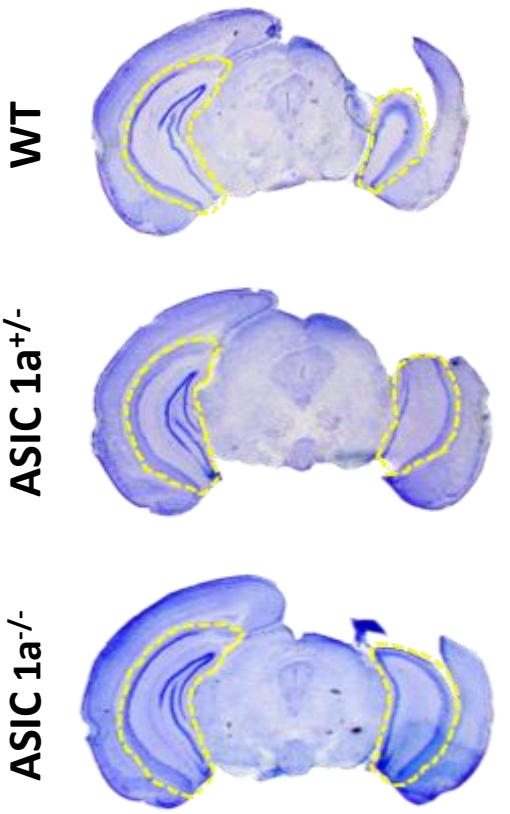

a
WT
ASIC 1a+/-
ASIC 1a-/-
b
WT
ASIC1a+/-
ASIC1a-/-
Lesion volume (mm3)
Time after TBI (days)
14
60
90
180
c
WT
ASIC 1a+/-
ASIC 1a-/-
d
Lesion volume (mm3)
**
**
WT
ASIC1a+/-
ASIC1a-/-
e
Lesion volume (mm3) Histology
Lesion volume (mm3) MRI
r = 0,8266
p < 0.0001

Figure 8

a


WT
ASIC 1a+/-
ASIC 1a-/-

b

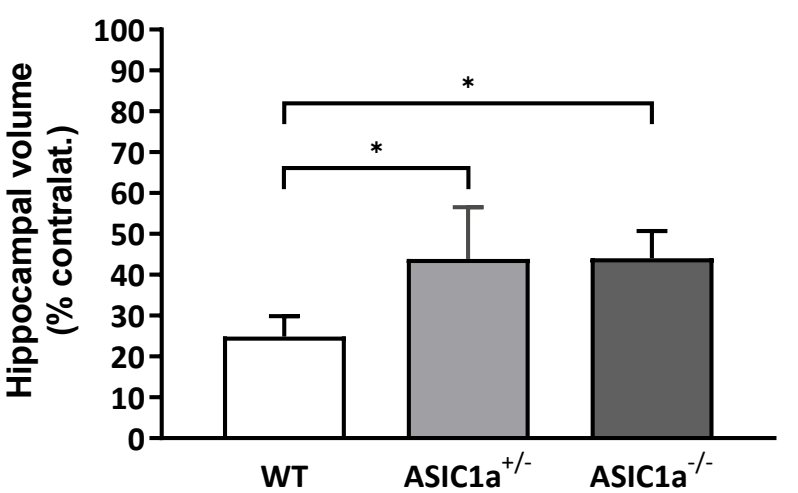
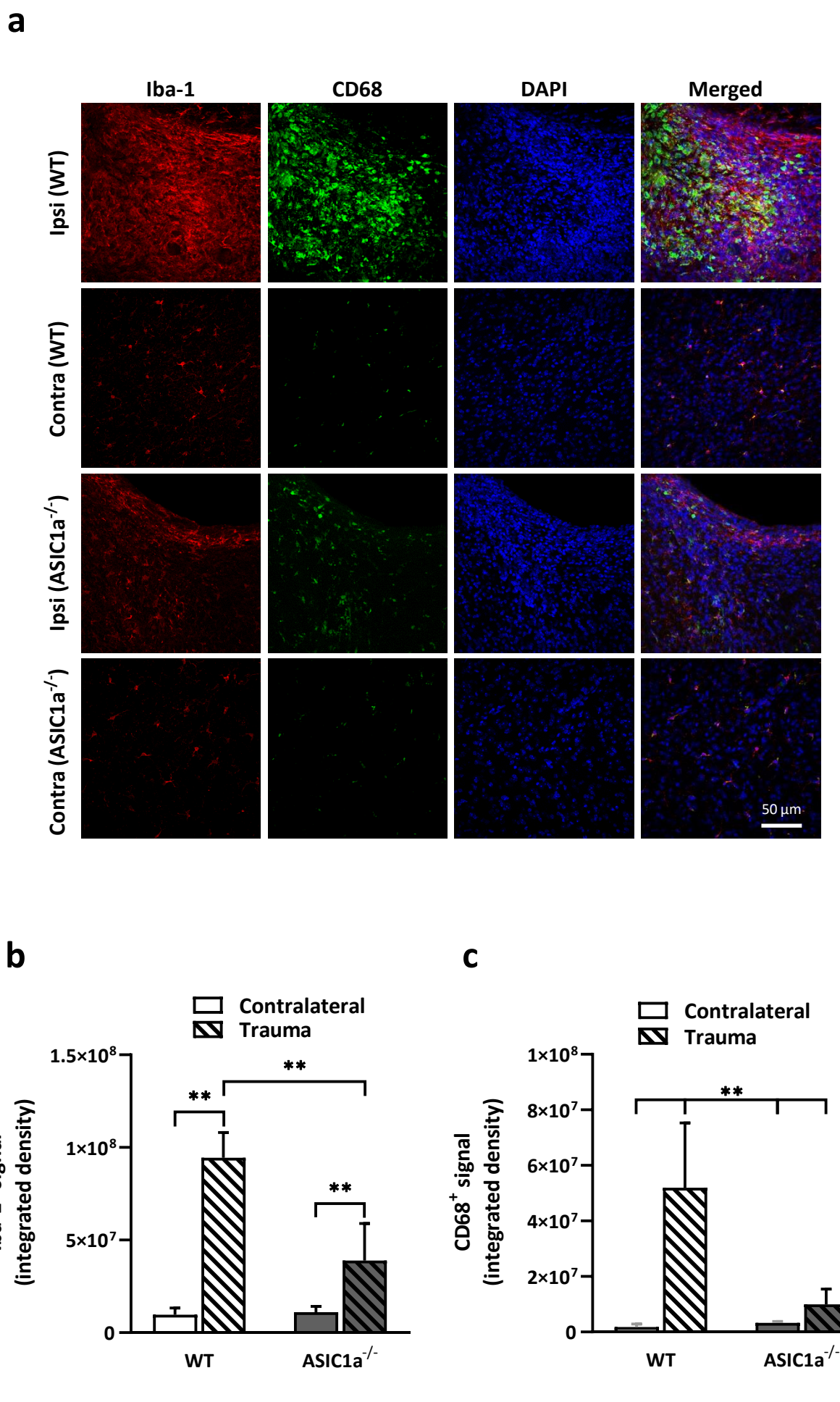
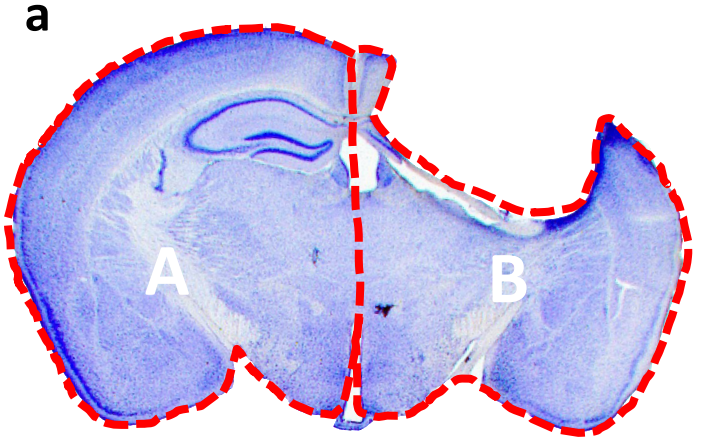

Hippocampal volume
(% contralat.)
100
90
80
70
60
50
40
30
20
10
0
*
*
WT
ASIC1a+/-
ASIC1a-/-

Figure 9

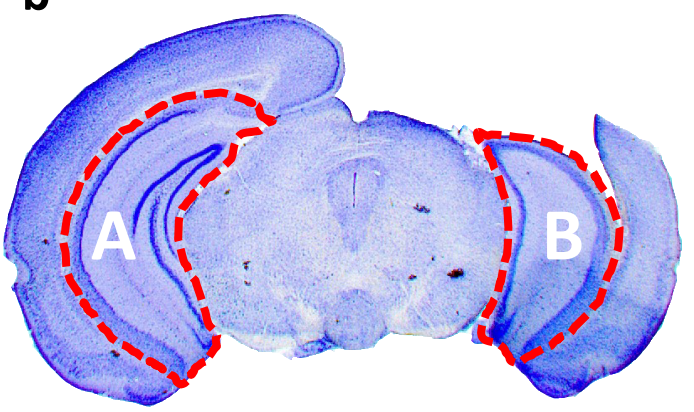

a
Iba-1
CD68
DAPI
Merged
Ipsi (WT)
Contra (WT)
Ipsi (ASIC1a-/-)
Contra (ASIC1a-/-)
50 µm
b
Contralateral
Trauma
Iba-1+ signal (integrated density)
1.5×10^8
1×10^8
5×10^7
0
**
**
**
WT
ASIC1a-/-
c
Contralateral
Trauma
CD68+ signal (integrated density)
1×10^8
8×10^7
6×10^7
4×10^7
2×10^7
0
**
WT
ASIC1a-/-

Supplemental Figure 1

a



b



Non-traumatic hemisphere hippocampus volume (E) = 0.5 mm*(A1/2+…+An/2)

Traumatic hemisphere hippocampus volume (F) = 0.5 mm*(B1/2+…+Bn/2)

Hippocampus volume ratio (ipsilateral/contralateral volume) = F/E*100

c