Efficacy of inhaled Argon administered acutely after traumatic brain injury in mice

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ABSTRACT

Background: While supportive treatment for traumatic brain injury (TBI) has progressed, specific neuroprotective interventions are still lacking. Models of ischaemic heart and brain injury show a therapeutic potential for Argon gas, but it is still not known whether inhaled Argon (iAr) is protective in TBI. We tested the effects of iAr administered acutely to TBI mice on brain oedema, tissue microenvironmental changes, neurological functions and structural outcome.

Methods: Anaesthetized adult C57BL/6J mice were subjected to severe TBI by controlled cortical impact. Ten minutes after TBI, mice were randomized to 24h treatment with iAr 70%-O_2 30% or air (iCtr). Sensorimotor deficits were evaluated up to six weeks post-TBI by three independent tests. Cognitive function was evaluated by Barnes maze test at four weeks. Magnetic resonance imaging (MRI) was done to examine brain oedema at three days and white matter damages at five weeks. Microglia/macrophage activation and functional commitment was evaluated at one week after TBI by immunohistochemistry.

Results: iAr significantly accelerated sensorimotor recovery and improved cognitive deficits one month after TBI, with less white matter damage in the ipsilateral fimbria and body of the corpus callosum. Early changes underpinning protection included a reduction of pericontusional vasogenic oedema and action on the inflammatory response. iAr significantly reduced microglial activation with increases in ramified cells and the M2-like marker YM1.

Conclusion: iAr accelerates recovery of sensorimotor function and improves cognitive and structural outcome one-month after severe TBI in mice. Early effects include a reduction of brain oedema and neuroinflammation in the contused tissue.

Introduction

Supportive treatment for the management of traumatic brain injury (TBI) has progressed over the past 20 years, but specific neuroprotective strategies are still lacking.[1–3] There is an urgent need for new therapeutic interventions.

Noble gases including argon and xenon have emerged as promising neuroprotective agents in different models of acute brain injury.[4-7] Advantages of inhaled argon (iAr) over xenon seem numerous and include the possibility to use it in open-flow ventilation, the absence of anaesthetic properties under normobaric conditions, low costs (3 cents L^{-1}) and – most importantly – the absence of cerebral vasodilatory effects[6], a key limiting factor in TBI patients at risk of intracranial hypertension.[8] Neuroprotective effects of iAr were first observed in an *in vitro* model of hypoxic-ischaemic injury by oxygen-glucose deprivation in neuronal culture[9] and have since been confirmed *in vivo* after brain ischaemia,[10] hypoxia ,[11,12] and subarachnoid haemorrhage.[7] However, no data are available on TBI *in vivo* models. Preliminary evidence of protection from biomechanical damage were obtained in an *ex vivo* model of hippocampal slices subjected to stylus drop and treated immediately after injury with 50% Ar enriched culture medium. The reduction in injury varied, ranging from ~30 to 80% at 72 hours after trauma in two different studies.[9,13] This suggests that 50% Ar is the lower limit for neuroprotection, thus leading to variable results. Further studies to address the neuroprotective effects of higher doses of Ar are needed.

iAr exerts neuroprotection after experimental hypoxic/ischaemic insults [7,11,14,15] by reducing inflammatory and pro-apoptotic signaling molecules and attenuating cellular stress.[14,16,17] Oxidative stress, apoptotic processes and neuroinflammation are key modulators of secondary damage after TBI as well.[18] Thus, hypothetically, iAr might exert neuroprotection and induce lasting functional and structural benefits after TBI.

The aim of the study was to test whether a 70% concentration of iAr administered acutely for 24 hours, 10 minutes after severe TBI in mice, exerted neuroprotection.

Materials and methods

Study design

Figure 1 shows the time line of the experimental plan. We ran a first experiment to evaluate the effects of iAr administered for 24h, 10 minutes after injury, on sensorimotor deficits, brain oedema and inflammatory changes within a week from TBI in mice (Exp. 1). In a second independent experiment we did confirmatory analyses on the early effects on sensorimotor deficits and tested whether there was lasting protection against functional and structural outcomes by analysing sensorimotor and cognitive functions, with magnetic resonance imaging (MRI) up to six weeks after TBI (Exp. 2).

Animals

Male C57BL/6J mice (9 weeks of age) from Envigo were used. They were housed in a pathogen-free vivarium with a constant temperature (21 [1]°C) and relative humidity (60 [5]%) with a 12h light–dark cycle and free access to pellet food and water.

All animal experiments were designed in accordance with the ARRIVE guidelines,[19] with a commitment to refinement, reduction and replacement, minimizing the numbers of mice, and using biostatistics to optimize mouse numbers (as in our previous work with the mouse TBI model[20,21]). For statistical validity, ten mice were assigned to the iAr or control group using randomization lists (http://www. random.org/list) for Exp. 1 and 2. Treatment, behavioural, imaging and histological assessments were done by researchers blinded to the experimental groups.

Ethics statement

Procedures involving animals and their care were conducted in conformity with the institutional guidelines at the Istituto di Ricerche Farmacologiche Mario Negri IRCCS (IRFMN) which adheres to the principles set out in the following laws, regulations, and policies governing the care and use of laboratory animals: Italian Governing Law (D.lgs 26/2014; Authorization no.19/2008-A issued March 6, 2008 by Ministry of Health); IRFMN Institutional Regulations and Policies providing internal authorization for persons conducting animal experiments (Quality Management System Certificate – UNI EN ISO 9001:2008–Reg. no 6121); the EU directives and guidelines (EEC Council

Directive 2010/63/UE). They were reviewed and approved by the IRFMN Animal Care and Use Committee, which includes ad hoc members for ethical issues, and by the Italian Ministry of Health (Decreto no. D/07/2013-B and 301/2017-PR, authorization no. 801/2018-PR). Animal facilities meet international standards and are regularly checked by a certified veterinarian who is responsible for health monitoring, animal welfare supervision, experimental protocols and review of procedures.

Traumatic brain injury

Mice were anaesthetized with isoflurane inhalation (induction 3%; maintenance 1.5%) in an N_2O/O_2 (70%/30%) mixture and placed in a stereotaxic frame. Rectal temperature was maintained at 37°C. Mice were then subjected to craniectomy as previously described.[22,23] Controlled cortical impact (CCI) brain injury was induced using a 3 mm diameter rigid impactor driven by an electromagnetic controlled impact device (ImpactOne, Leica) rigidly mounted at an angle of 20° to the vertical plane and applied vertically to the exposed dura mater, between bregma and lambda, over the left parieto-temporal cortex (antero-posteriority -2.5 mm, laterality -2.5 mm), at impactor velocity 5 m s^{-1} and deformation depth 2 mm, resulting in a severe TBI.[24] The craniotomy was then covered with a cranioplasty and the scalp sutured.

Treatment

Ten minutes after TBI an independent operator randomly assigned the mice to the experimental groups. Argon 70%-O_2 30% (premixed gas from SIAD, Bergamo, Italy), N_2 70%-O_2 30% or room air were delivered through a 200-L chamber prefilled with the gas (temperature 21 [1]°C, standard light-dark cycle, in which the mice were kept in their home cage with food/water available) for 24h (Supplementary Fig S1A). Total gas flow was measured with a flowmeter and was the same for all treatment groups (saturation 10L min^{-1}, maintenance 1L min^{-1}). FiO_2 was monitored at several times inside the chamber by a portable oxygen analyser (Handy+, Maxtec), showing that FiO_2 levels in the chamber were constantly in the range of normoxia (Supplementary Fig S1B). Soda lime was added as a CO_2 scavenger. Core body temperature measured at the end of the treatment showed that all animals were normothermic (Supplementary Fig S1C).

In a preliminary experiment we compared the effects on sensorimotor functions and neuroinflammation of iAr or room air or N_2 70%-O_2 30% exposed TBI mice. Room air and N_2 70%-O_2 30% TBI mice behaved similarly and both had significantly worse deficits than iAr mice when tested at 24h or 7d (Supplementary Fig. S2A,B) and a comparable increase in IBA1 stained area in the ipsilateral(il)-cortex and il-hippocampus (Supplementary Fig. S2C,D). After confirming the comparable main outcomes of air or 30% FiO_2 exposed TBI mice, we used room air as control for subsequent experiments and referred to it as iCtr.

Behavioural tests

Sensorimotor deficits were rated with the neuroscore,[23] Simple Neuroassessment of Asymmetric Impairment (SNAP)[25] and beam walk tests[23] as previously described, at the times indicated in Fig. 1. Cognitive deficits including learning and memory were evaluated in the Barnes maze.[26] Methodological details related to behavioural assessments can be found in Supplementary Methods.

MRI acquisitions and analysis

Anaesthetized mice (isoflurane 1.5-2% in a N_2O/O_2 (70/30%) were positioned in the magnet at the times indicated in Fig. 1. Respiratory frequency was monitored throughout the experiment and body temperature was maintained at 37°C with a water heating pad. Brain imaging was done on a 7 T small-bore animal scanner (Bruker Biospec, Germany) running ParaVision 6.01 and equipped with a quadrature 1H cryo probe surface coil as transmitter and receiver.

Diffusion weighted imaging (DWI), diffusion tension imaging (DTI) and T2 weighted imaging (T2WI) were obtained to quantify oedema, white matter damage and contusional volume,[27] respectively as detailed, in Supplementary Methods.

Tissue processing for histopathological analysis

Mice were euthanized by deep anaesthesia (ketamine 1 mg kg^{-1} and medetomidine 0.1 mg kg^{-1}, i.p.) and perfused transcardially with 20 mL of phosphate-buffered saline (PBS) 0.1 mol L^{-1}, pH 7.4, followed by 50 mL of chilled paraformaldehyde (4%, PFA) in PBS. The brains were removed from the skull and post-fixed overnight at 4°C, then dehydrated with 30% sucrose (Sigma-Aldrich) in 0.1

mol L^{-1} PBS for 24h at 4°C, then frozen in n-pentane for 3 min at -45°C and stored at -80°C until use. Serial coronal brain sections (20 μm thick) were cut on a cryostat (+1 to -4 mm from bregma) at 200 μm intervals for histological analysis. The il-cortex was analysed over an area 200 μm deep from the edge of the contusion containing the sensorimotor cortex. Il-hippocampus (il-Hipp), il-body of the corpus callosum (il-CCB) and il-fimbria (il-Fi) were selected manually (as detailed in Supplementary Fig. S3A) and quantified on a single slice (-1.6 mm from bregma). Images were analyzed using Fiji software. Neuronal cell loss was evaluated on Nissl-stained sections, while white matter damage on luxol fast blue (LFB) stained sections using standard histological protocols. [21,23]

For immunohistochemistry we used anti-IBA1 (1:200; Wako, Neuss, Germany) and anti-YM1 (1:400; Stem cell technologies, Vancouver, Canada) to detect microglia/macrophage activation and polarization. Immunoreactivity was tested using 3,3'-diaminobenzidine (Vector Laboratories, Burlingame, CA, USA), as previously described.[22] Histopathological analysis was done on three 20-μm thick brain coronal sections per mouse, -0.4, -1.6, and -2.8 mm from bregma. The brain sections were acquired at 20x with an Olympus BX-61-VS microscope. YM1 positive cells were expressed as total number of cells within the defined boundries over the three sections considered.

Microglia/macrophage morphology was analyzed as previously described.[28] A size threshold of >25 μm^2 was used to select cells to be analysed for area and aspect ratio. Mean single cell values for each parameter were used for statistics.

For immunofluorescence we used anti-CD11b (1:200, BioRad) and anti-YM1 (1:400; Stem Cell Technologies). The fluoro-conjugated secondary antibodies were Alexa 488 antirat and Alexa 594 antirabbit (all 1:500; Invitrogen, Carlsbad, CA, USA), as previously described.[29] Brain sections were acquired at 40x by a Nikon A1 confocal scan unit managed by NIS elements software with 10% overlapping for stitching, z-axis 10 μm.

Statistical analysis

Data are presented as means (standard error of the mean [SEM]). Differences between groups over time (group-by-time interaction) for continuous variables were tested using two-way analysis of

variance (ANOVA) for repeated measurements (time points) followed by Tukey's post-hoc test. When two groups were compared the unpaired t-test was used; p-values <0.05 were considered significant. Assumptions of normality were checked using the Kolmogorov-Smirnov test. A standard software package was used (GraphPad Prism version 6.00, La Jolla, CA, USA).

Results

There was no mortality associated with the model or iAr treatment. iAr gave measurable neuroprotective effects both in neurobehavioural and hystological outcomes.

iAr accelerated recovery of sensorimotor functions

Three independent tests showed significant improvement of sensorimotor deficits within the first week after injury (Fig. 2A-B, Exp 1 and 2, SNAP: $p<0.001$ and Neuroscore: $p<0.01$; beam walk: $p<0.05$, and Supplementary Fig. S4). When long-term sensorimotor deficits were evaluated (Exp. 2), SNAP showed lower deficits in iAr treated mice up to two weeks ($p<0.05$), but no difference at later times (Supplementary Fig. S5A,B).

iAr improved memory performance after TBI

Four weeks after TBI we performed the Barnes maze test to evaluate spatial learning and memory function. All animals were able to learn, as reflected by decreasing latencies to find the escape box over the three-day test period (Supplementary Fig. S5). On the probe trial, iAr TBI mice performed significantly better than iCtr (mean latency 14.1 [2.1] and 32.4 [5.9] s respectively, $p<0.05$), indicating improvement in spatial memory (Fig. 2C,D).

iAr reduced acute brain oedema and attenuated delayed white matter damage

Three days after TBI apparent diffusion coefficient (ADC) maps were obtained by DWI-MRI (Fig. 3A-B). The maps were significantly lower in iAr than iCtr mice both in terms of total volume (6.3 [0.4] and 9.6 [0.5] mm^3, $p<0.001$, Fig. 3B) and mean ADC (0.68 [0.04] and 0.76 [0.01] $mm^2\ ms^{-1}$, $p<0.05$, Supplementary Fig. S6A) reflecting a reduction of vasogenic oedema three days post-TBI. Quantification of T2WI showed that iAr and iCtr TBI mice had comparable contusion volumes both at three days (14.7 [1.3] and 17.3 [1.2] mm^3) and six weeks (16.8 [1.0] and 18.1 [1.1] mm^3) after TBI (Supplementary Fig. S6D).

Five weeks after TBI, DTI analysis showed a reduction of white matter (WM) damage in the il-Fi and il-CCB (Fig. 3C-E). Compared to iCtr TBI mice, iAr treated mice had increased in fractional anisotropy (FA) in the il-Fi and il-CCB ($p<0.05$, Fig. 3D-E) with a concomitant reduction of mean

diffusivity (MD) in the same WM tracts ($p<0.05$, Supplementary Fig. S6B-C). In regions more distal to the biomechanical impact (i.e. splenu and genu of corpus callosum, external and internal capsule, optic tract and anterior commissure) no differences were found (data not shown).

iAr modulated microglial/macrophage activation

One week post TBI we found a marked reduction of the IBA1 stained area at the contusional edge, corresponding to the somatosensory cortex (8.12 [0.47] and 10.37 [0.71]% stained area, $p<0.05$), il-Hipp (9.92 [0.80] and 7.18 [0.91]% stained area, $p<0.05$) and il-CCB (17.09 [1.39] and 13.23 [1.26]% stained area, $p<0.05$), in iAr compared to iCtr TBI mice with a tendency towards a reduction in the il-Fi (13.46 [1.90] and 9.65 [0.77]% stained area, $p=0.08$), Fig. 4A-E). Quantification of shape parameters for IBA1 positive cells in the somatosensory cortex indicated a reduction in the area ($p<0.01$) and an increase in the aspect ratio in iAr versus iCtr TBI mice ($p<0.05$, Fig. 4F-G) suggesting a less toxic phenotype of myeloid cells.[28] Analysis of the M2-like marker YM1 in the contusional cortex showed significantly more positive cells in iAr mice ($p<0.001$, Fig. 5A-C).

Discussion

The present study found that iAr 70% administered 10 min after TBI for 24h: *i)* accelerated the recovery of sensorimotor function and induced memory improvements up to four weeks after TBI, *ii)* reduced brain oedema (three days after TBI) and reduced white matter damage (five weeks after TBI), as shown by MRI, *iii)* ameliorated the neuroinflammatory microenvironment in the contusional cortex, il-Hipp and il-CCB by reducing microglia/macrophage activation, with pericontusional inflammatory cells displaying markers and morphological parameters of M2-like activated microglia/macrophages.

We characterized the sensorimotor functional impairment up to six weeks after TBI. iAr clearly accelerated the recovery of sensorimotor deficits with improvements in the SNAP and neuroscore already immediately after treatment and lasting up to two weeks. Thereafter the difference between the two groups were no longer clear though there was a tendency to better performance in iAr treated mice only in the SNAP test.

As previously reported, mice showed spontaneous partial recovery of sensorimotor functions between one and six weeks from TBI[21,30], so it is difficult to detect a treatment effect after the acute phase. However, the data also suggest that the effect on sensorimotor functions may be potentiated, so follow-on studies may now be useful to test multiple or longer exposure to iAr. Memory performance at one month post-TBI was improved in iAr treated mice. The ability of iAr to improve cognition after brain ischaemia was shown in pigs three days after resuscitation from cardiac arrest.[11] Our results now show that iAr also improves cognition in TBI mice up to one month after injury.

Brain oedema is a hallmark of TBI, and it usually aggravates secondary injuries due to tissue compression around the contusion. While clinically oedema volumes are not routinely utilized, in animal models the extent of oedema is useful to monitor the progression of an intervention.[31,32] Here we used DWI to reconstruct ADC maps and quantify brain oedema. Hyperintense areas in ADC maps are associated with increased movement of water molecules outside the cells, reflecting vasogenic oedema.[33] We found a clear reduction of vasogenic oedema around the contusional area in iAr

compared to iCtr TBI mice, with no overt effect on contusion volume, indicating that despite a similar degree of tissue loss induced by the biomechanical impact, the blood-brain barrier integrity was preserved better in the surrounding parenchyma in iAr treated mice.

The effects of iAr on brain water content as an indicator of oedema was previously measured up to three days post-injury in subarachnoid haemorrhage rats.[7] The authors found no effect of iAr (50% for 1h exposure). While pathogenic differences related to the primary injury might explain these contrasting results it can also be hypothesized that prolonged iAr exposure and/or a higher iAr concentration – as in our study – may be needed to induce greater neuroprotection after acute brain injury.

iAr may mitigate neuroinflammation after brain ischaemia.[10,16] Argon exposure of cortical neuronal cultures subjected to oxygen-glucose deprivation reduced the expression of TNF-α and IL-6.[34] More recently, Liu et al[35] showed that short treatment with iAr (50%) had no real effect on microglia/macrophage overall activation but significantly increased the M2-like marker arginase 1 at the inner boundary of the infarction seven days after reperfusion in an *in vivo* model of ischaemia in rats. Our data now indicate that in TBI prolonged exposure to iAr leads to a more robust effect on microglia/macrophages, with an overall reduction of IBA1 positive cells in the contusional cortex, ipsilateral hippocampus and corpus callosum. At this stage post TBI myeloid cells display an amoeboid phenotype with enlarged soma and retracted processes.[28] iAr clearly induced morphological changes in IBA1-positive cells in the injured somatosensory cortex, towards a more ramified phenotype. Importantly, this morphological switch was also associated with a significant increase of the M2-like marker YM1, supporting a role of iAr in mitigating the neuroinflammatory environment, switching the cell polarization towards a pro-resolving and tissue repair action[36] and quite likely contributing to the improvement in sensorimotor function.

Imaging studies were also conducted five weeks after TBI to evaluate the evolution of contusion volumes and white matter damage. In line with the acute assessments, iAr had no effect on contusion volume. However, white matter tracts in proximity to the contusion edges including the il-CCB and

il-Fi had higher FA and lower MD values than in iCtr. The fimbria is the main connection to the hippocampal formation and is vital in spatial memory.[37] Its greater integrity in iAr treated mice probably explains their better performance in the Barnes maze test, compared to controls.

FA is a marker of white matter damage[38] and an increase in MD is associated with axonal degeneration or demyelination.[39] DTI analysis indicated that iAr reduced the markers of white matter damage close to the contusion. A trend towards greater white matter integrity in iAr mice was already detectable by luxol fast blue in the il-CCB one week after injury. All white matter regions showed lower microglia activation in iAr treated mice at this time point. There is evidence from studies in mice[23] and humans[40] that persistent inflammation may underpinn white matter degeneration later on. Thus, the mitigation of white matter inflammation early on may explain the reduction of later white matter damage, and the better functional outcome despite no overt effect on the contusion volume.

Limitations of the study and future perspectives

The control arm was treated with air (21% FiO_2) while the Argon group received 30% FiO_2. This potential confound is mitigated by our initial experiment, where sensorimotor deficits and inflammatory changes were similar in animals treated with air and oxygen-nitrogen mixture at 30% FIO_2. Most importantly, O_2 levels in the chamber remained in the range of normoxia (i.e. from 21-30%) in all conditions and we excluded others confounders such as hypothermia. Protective effects of small FiO_2 changes (<10%) in the range of normoxia are not reported in TBI, making it unlikely that the benefit in the treatment group was simply due to the 30% FiO_2 used in iAr treated mice.

iAr is easy to administer and transport, so treatment at the scene of the primary injury, enroute to emergency care, can be envisaged with no delay to therapy. Our data provide a proof of principle that Argon can be exploited for therapy in TBI. However, we only tested acute administration, ten minutes after TBI. This is a limit and future studies are needed to define the therapeutic window and long-term outcome of iAr in TBI. Defining the patient population that may benefit from this proposed treatment is an additional key preclinical step, and the effects of iAr at lower concentrations (40-60%) should be explored since TBI patients with concomitant pulmonary injury often require high FiO_2.

Conclusions

Our study shows that early adminstration of iAr after experimental TBI for 24h has anti-oedematous properties, promotes early recovery of sensorimotor function and persistent improvement of memory with white matter preservation.

iAr attenuats maladaptive facets of microglia/macrophage activation in the contused tissue. Thus the study yields new understanding of iAr-neuroprotective properties and its potential use in TBI.

Authors' contributions

Study design/planning: FM, FFo, AM, GR, ERZ.

Study conduct: FM, FFo, AM, RP, FB, DT, EM, ES.

Data analysis: FM, FFo, RP, ES, FB, ERZ.

Data interpretation: FM, FFo, AM, GC, NS, FFu, SM, RL, GR, and ERZ

Drafting the paper: FM, FFo, ERZ.

All authors contributed to discussion, data analysis, and the final draft of the paper.

All authors take responsibility for the paper is contents.

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Declaration of interests

The authors declare no conflicts of interest.

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Figure legends

Fig. 1

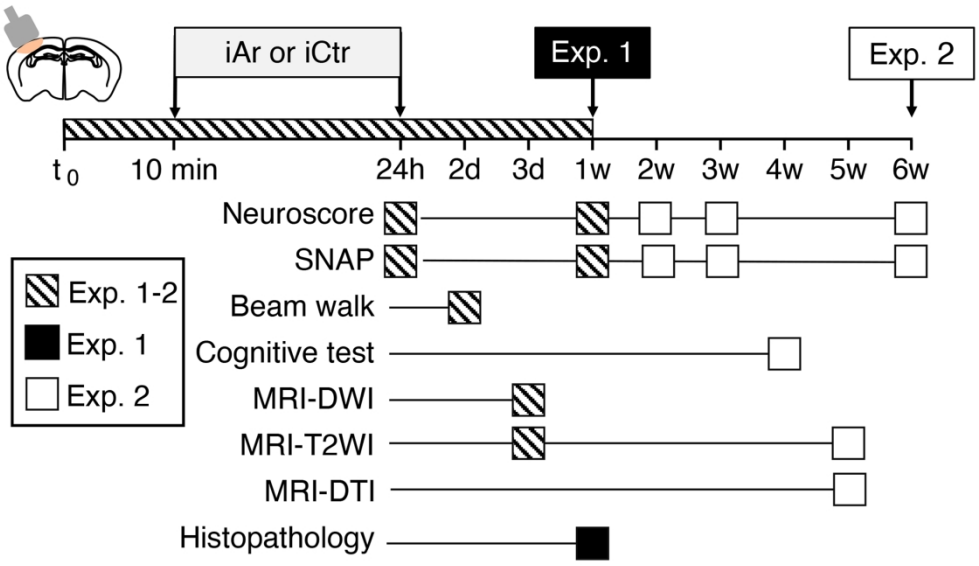

Figure 1. Experimental design. Mice were assigned to two separate groups (Exp. 1 and 2). TBI was induced by controlled cortical impact on the left parieto-temporal cortex in anesthetized mice. Ten minutes after injury, animals were treated with argon 70% - O_2 30% (iAr) or room air (iCtr) for 24h in a ventilated chamber. The black box indicates measurements in Exp. 1, and the white box in Exp. 2; dashed boxes stand for both (Exp. 1-2).

Fig. 2

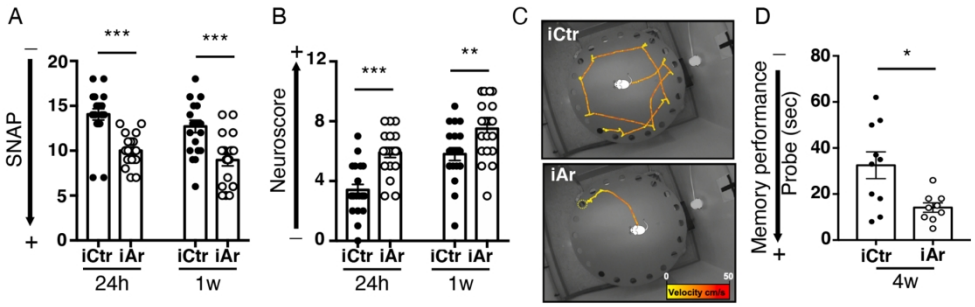

Figure 2. Behavioral deficits. Sensorimotor deficits were rated with the Simple Neuroassessment of Asymmetric impairment (SNAP) and Neuroscore one and seven days post-TBI in Exp. 1 and 2. iAr-

treated mice had a significantly better SNAP score (A) and neuroscore (B) on both days. Spatial learning and memory were assessed in the Barnes maze four weeks post-TBI. Barnes maze tracings show the memory performance of a representative animal in the iCtr and iAr groups (C). Memory performance in the probe trial was better in iAr than iCtr TBI mice (D). Data are mean±SEM, n = 20 (pooled data from Epx 1 and 2). SNAP, Neuroscore were analysed by two-way RM-ANOVA followed by Tukey's post-hoc test; probe trial in the Barnes maze by unpaired t-test. *p<0.05, **p<0.01, ***p<0.001.

Fig. 3

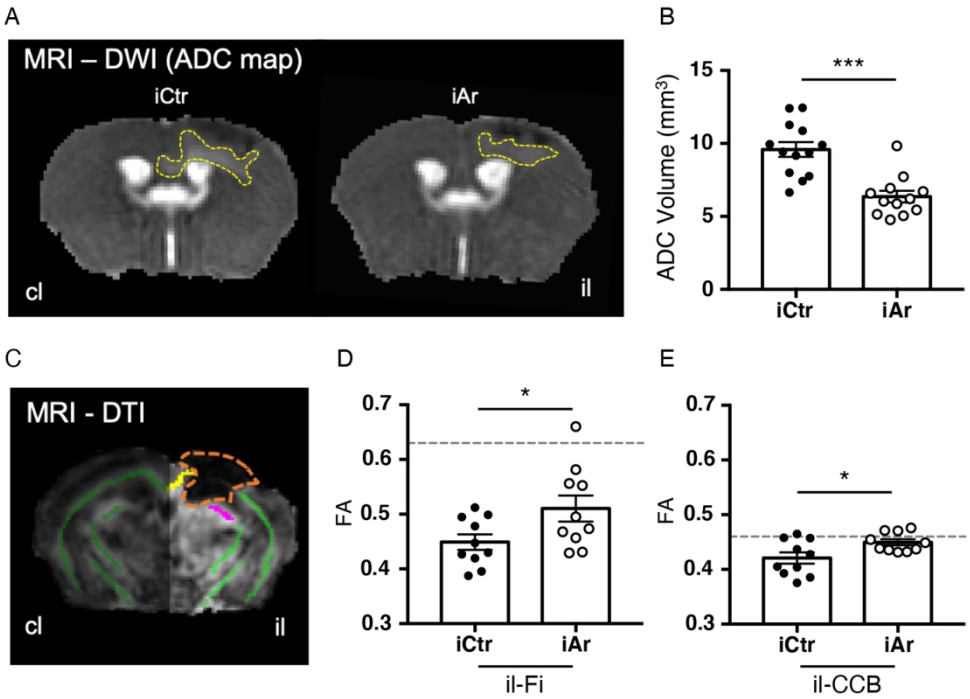

Figure 3. MRI analysis. DWI-MRI was done three days after TBI. Representative apparent diffusion coefficient (ADC) maps show a smaller hyperintense region reflecting unrestricted water diffusion (vasogenic oedema) around the contusion edge of iAr (A, right) compared to iCtr (A, left) TBI mice. iAr reduced vasogenic oedema (B). DTI-MRI was done five weeks after TBI. Representative brain

coronal (C) section shows ipsilateral (il) white matter tracts (highlighted in pink, yellow and green), analysed by diffusion tensor imaging (DTI). DTI metrics at five weeks post-TBI show a reduction of white matter damage in the ipsilateral fimbria (il-Fi, pink) and in the body of the corpus callosum (il-CCB, yellow) but not in more distal white mater tracks (in green, data not shown). The dotted line indicates the level of the corresponding contralateral white matter region. Data are mean±SEM, ADC volume: n = 13 (pooled data from Exp 1 and 2), FA: n = 10 (Exp 2). Unpaired t-test. *p<0.05, ***p<0.001.

Fig. 4

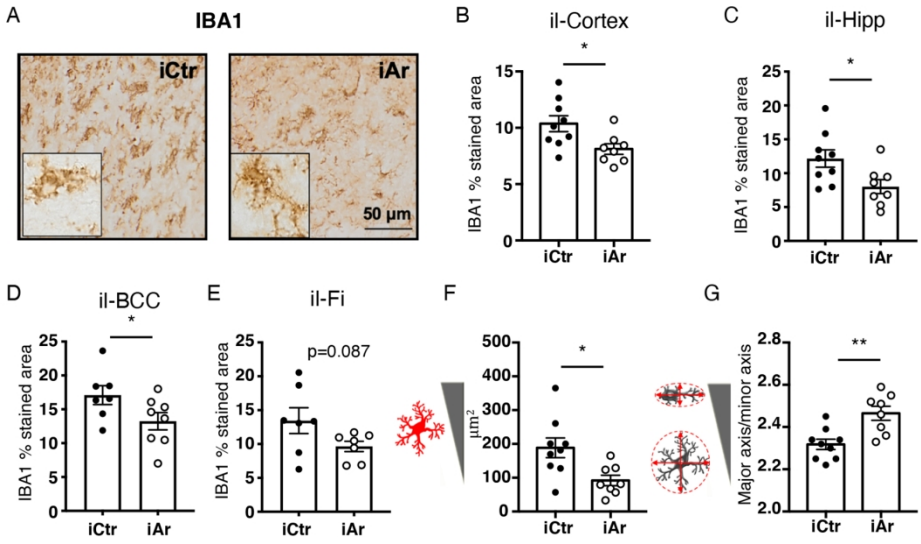

Figure 4. Representative micrographs of IBA1 immunostainings (A) and their quantifications in the contusional cortex (B) ipsilateral hippocampus (il-Hipp) (C), body of the corpus callosum (il-CCB) (D) and fimbria (il-Fi) (E) seven days after TBI. The IBA1 stained area was significantly smaller in iAr than iCtr TBI mice (B-D) with a trend toword a reduction in the il-Fi (E). Shape descriptor parameters including area and aspect ratio were quantified in the il-Cortex on IBA1 positive cells (F-G). These cells had smaller area (C) with a concomitant increase in the aspect ratio (D) in iAr-treated mice. Data are mean±SEM. Unpaired t-test. *p<0.05, **p<0.01. Bar 50 µm.

Fig. 5

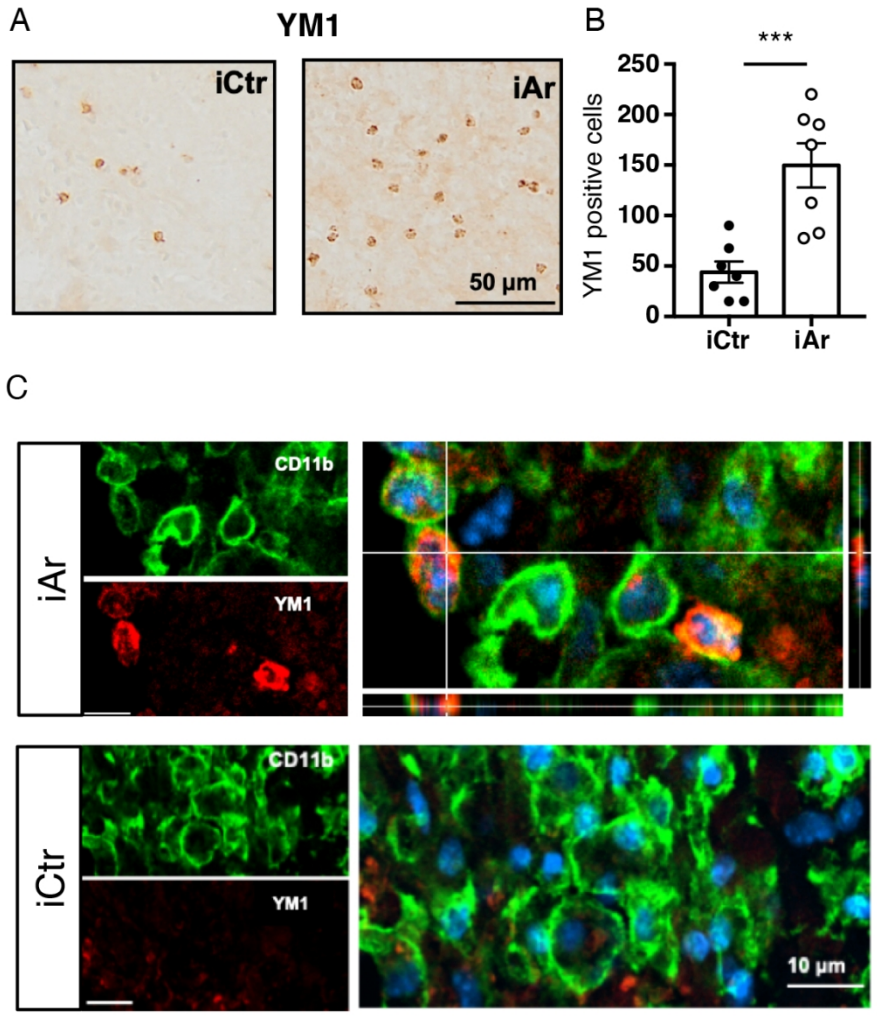

Figure 5. Representative YM1 immunostaining (A) and their quantification in the contusional cortex (B) seven days after TBI. YM1 positive cells increased in iAr-treated mice (B). Microphotographs show coexpression of CD11b (green) and YM1 (red) markers in the contusional cortex of iAr TBI mice seven days post-injury (C). Data are mean±SEM. Unpaired t-test. *p<0.05, **p<0.01, ***p<0.001. Bars: 50 µm (A) and 10 µm (C).