Global brain atrophy after unilateral parietal lesion and its prevention by erythropoietin

Anna-Leena Sirén,1,4 Konstantin Radyushkin,1,2 Susann Boretius,3 Daniel Kämmer,1 Claas-Christian Riechers,1 Oliver Natt,3 Derya Sargin,1 Takashi Watanabe,3 Swetlana Sperling,1 Thomas Michaelis,3 Jack Price,5 Barbara Meyer,2 Jens Frahm3 and Hannelore Ehrenreich1

1Division of Clinical Neuroscience, Max Planck Institute of Experimental Medicine and 2Department of Molecular Cell Biology and 3Biomedizinische NMR Forschungs GmbH, Max Planck Institute for Biophysical Chemistry, Göttingen, 4Section of Experimental Neurosurgery, Department of Neurosurgery, University of Würzburg, Würzburg, Germany and 5Institute of Psychiatry, King's College London, London, UK

Correspondence to: Prof. Hannelore Ehrenreich, MD, DVM, Division of Clinical Neuroscience, Max Planck Institute of Experimental Medicine, Hermann-Rein Strasse 3, 37075 Göttingen, Germany

E-mail: ehrenreich@em.mpg.de

In humans, neurotrauma is suspected to cause brain atrophy and accelerate slowly progressive neurodegenerative disorders, such as Alzheimer’s disease or schizophrenia. However, a direct link between brain injury and subsequent delayed global neurodegeneration has remained elusive. Here we show that juvenile (4-week-old) mice that are given a discrete unilateral lesion of the parietal cortex, develop to adulthood without obvious clinical symptoms. However, when monitored 3 and 9 months after lesioning, using high-resolution three-dimensional MRI and behavioural testing, the same mice display global neurodegenerative changes. Surprisingly, erythropoietin, a haematopoietic growth factor with potent neuroprotective activity, prevents behavioural abnormalities, cognitive dysfunction and brain atrophy when given for 2 weeks after acute brain injury. This demonstrates that a localized brain lesion is a primary cause of delayed global neurodegeneration that can be efficiently counteracted by neuroprotection.

Keywords: EPO; MRI; neuroprotection; neurodegeneration; neurotrauma; schizophrenia

Abbreviations: BBB = blood-brain barrier; BrdU = bromodeoxyuridine; EPO = erythropoietin

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Introduction

In humans, mild and moderate traumatic brain injury has been suspected to be the cause of whole-brain atrophy and to accelerate slowly progressive neurodegenerative disorders, including Alzheimer’s disease and schizophrenia (Parker and Rosenblum, 1996; Smith et al., 1997; McAllister, 1998; Nemetz et al., 1999; Malaspina et al., 2001; Koponen et al., 2002; MacKenzie et al., 2002; AbdelMalik et al., 2003; Max et al., 2004). However, a direct link between brain injury and subsequent delayed global neurodegeneration has never been demonstrated. Altered functioning within the parietal cortex (induced exogenously, e.g. by an injury, or endogenously, by a developmental abnormality) may play a central role as a starting point in this slowly progressing process. The parietal cortex is critically involved in sensorimotor and cognitive tasks (Kolb and Whishaw, 1985; Olesen et al., 2004; Sirigu et al., 2004) and is the first to display signs of progressive grey matter loss in early-onset schizophrenia, a neuropsychiatric disease characterized by a strong cognitive decay: ‘dementia precox’ (Thompson et al., 2001). For obvious technical and ethical limitations, providing a direct link between human brain trauma, the immediate cellular response and putative delayed neurodegeneration has remained impossible. Understanding brain injury as a risk factor of neurodegeneration in general and of schizophrenia in particular, is a major public health challenge. The issue we have addressed here is (i) whether or not a small localized lesion in the developing parietal cortex is sufficient to trigger global morphological changes in the mature brain, (ii) what the behavioural consequences of such a cascade might be and (iii) whether a novel neuroprotective strategy involving recombinant erythropoietin (EPO) might prevent these changes.

Materials and methods

All experiments were approved by and conducted in accordance with the regulations of the local Animal Care and Use Committee.
Erythropoietin prevents neurodegeneration

Surgery
Four-week-old male C57BL6 mice were anaesthetized with an intraperitoneal (i.p.) injection of 0.25% tribromoethanol (Avertin) (0.125 mg/g). The parietal skull was exposed through a scalp incision and a freezing lesion was placed on the right parietal cortex (unilateral lesions) or on both parietal cortices (bilateral lesions) (coordinates from bregma: 1.5 mm posterior, 1.5 mm lateral). For lesions on the left occipital cortex, the following coordinates from bregma were used: 3.0 mm posterior, 1.5 mm lateral. A cone-shaped copper cylinder with tip diameter of 1 mm was cooled with liquid nitrogen (−183°C). Its tip was stereotaxically placed in direct contact with the exposed parietal/occipital skull and kept in place for 60 s. Sham-operated animals went through the same procedure without cooling the metal cone. We injected EPO (epoetin-α, Janssen-Cilag, Neuss, Germany) or placebo (diluent for EPO, 0.01 ml/g, i.p.) immediately after setting of the lesion and every other day for 14 days thereafter. In a separate group of mice, bromodeoxyuridine (BrdU, Chemicon, Temecula, CA, USA) was injected immediately following the lesion and the brains were removed after 30 min or 24 h.

Histology and immunohistochemistry
Extravasation of albumin-bound Evans-Blue (0.3 ml/mouse of a 2% solution in 0.9% NaCl, Sigma, Deisenhofen, Germany) through the blood–brain barrier (BBB) was evaluated after 30 min and 24 h. We used parafomaldehyde-fixed paraffin-embedded tissue for all histology and immunohistochemistry. Tissue-sections were boiled in citrate buffer, washed in phosphate-buffered saline (PBS), blocked in 10% horse serum and exposed overnight at 4°C to mouse anti-bromodeoxyuridine (BrdU; 10 μg/ml, i.p.) immediately after setting of the lesion and every other day for 14 days thereafter. In a separate group of mice, bromodeoxyuridine (BrdU; 10 μg/ml, i.p. Boehringer, Mannheim, Germany) was injected immediately following the lesion and the brains were removed after 30 min or 24 h.

MRI
Upon completion of the behavioural analysis, the mice were anaesthesitized with ketamine/xylazine (0.1 mg/0.015 mg/g, i.p.), intubated and kept under anaesthesia with 1–1.5% halothane in 70:30 N₂/O₂. In vivo brain volumetry was performed by MRI at a field strength of 2.35 T (Bruker Biospin, Ettlingen, Germany) using a T₁-weighted 3D FLASH sequence as previously described (Natt et al., 2002). We determined the total brain volume (excluding bulbus olfactorius, cerebellum and brainstem) (T) and the size of the lateral ventricles (V) by manually drawing respective regions-of-interest on up to 50 contiguous horizontal MRI sections (117 μm thickness). Brain matter (M) is calculated by subtraction of ventricle volume from total brain volume (M = T − V). The analysis was blinded for sham-operated, lesioned and EPO-treated animals.

Behavioural testing
Animals were tested in a battery of behavioural tests including hole board, elevated plus maze, Morris water maze, Rota-Rod and open field at 3 months (n = 30) and 9 months (n = 30) after setting of the cortical lesion. In addition, prepulse inhibition and hole board test were performed 3 months after parietal or occipital cortical lesioning (n = 7–13). All testing was done in a sound-attenuated room.

Hole board test
Each mouse was placed in the centre of the hole board (21 × 21 × 36 cm transparent Perspex chamber with non-transparent floor raised 5 cm above the bottom of the chamber with 12 equally spaced holes, 2 cm in diameter) and allowed to explore the chamber for 3 min. The distance travelled and number of holes explored (± head dips) were monitored by two layers of infrared photo beams connected to a computer equipped with the ‘AKS’ software (TSE, Bad Homburg, Germany).

Elevated plus maze
The animal was placed in the central platform facing an open arm of the plus-maze (made of grey Perspex with a central 5 × 5 cm central platform, 2 open arms, 30 × 5 cm, 2 enclosed arms, 30 × 5 × 15 cm, illumination ~300 lux). Behaviour was recorded by an overhead video camera and a PC equipped with ‘VideoMot 2’ (TSE) software to calculate the time each animal spent on open or closed arms. The proportion of time spent in open arms was used for estimation of open arm aversion (fear equivalent). The frequency of open arm entries was determined as a measure of motor activity.

Morris water maze
Spatial learning and memory was assessed in a water maze (Morris, 1984) consisting of a large circular tank (diameter 1.2 m, depth 0.4 m) containing opaque water (25 ± 1°C, depth 0.3 m). To escape from water, mice had to find a hidden platform (9 × 9 cm) submerged ~1 cm below the surface. The pool was located on an elevated platform 50 cm above the floor in a special well-lit room with white walls. Swim paths were monitored by a PC linked video camera mounted on the ceiling. The coordinates of the animal’s position were sampled in real-time by ‘VideoMot 2’ software (TSE) that provided measures of escape latency, swim speed and path length during acquisition and, during the probe trial, the number of visits to each quadrant of the maze. In the visible platform task, the platform was cued by means of a 15 cm high black flag mounted on it and placed pseudo-randomly in different locations across trials. The cued training was performed in the room without prominent extra-maze cues on the walls. Mice had four trials per day for 2 days and escape latency was measured. For spatial training (hidden platform), extra-maze cues were mounted on the walls. The platform was located at the centre of one of the four quadrants of the pool. Mice were trained to find the hidden escape platform, which remained in a fixed location throughout testing. They received four trials per day for 8 days, with an intertrial interval of ~5 min. The mice were placed into the pool facing the side wall randomly at one of four start locations and allowed to swim until they found the platform, or for a maximum of 90 s. Any mouse that failed to find the platform within 90 s was guided to the platform. The animal then remained on the platform for 20 s before being removed from the pool. On the day after completion of the spatial training, a probe trial was conducted to
determine the extent to which the mice had learned to find the location of the platform. The platform was removed from the pool and the mice were allowed to swim freely for 90 s. The number of visits to each quadrant of the maze was recorded.

**Rota-Rod**

Rota-Rod is a test for motor function, balance and coordination and comprises a rotating drum which is accelerated from 4 to 40 revolutions per minute over the course of 5 min. Mice were placed individually on the revolving drum (Ugo Basile, Comerio, Varese, Italy). Once they were balanced, the drum was accelerated. The time in seconds at which each animal fell from the drum was recorded using a stop-watch. Each animal received three consecutive trials, the longest time on the drum being used for analysis.

**Open field**

Spontaneous activity in open field was tested in a grey Perspex arena (120 cm in diameter, 25 cm high). The animal was placed in the centre of the open field and was allowed to explore it for 5 min. The behaviour was recorded by a PC-linked overhead video camera. ‘VideoMot 2’ (TSE) software was used to calculate the distance travelled and the time spent in the central, intermediate or the peripheral zones of the open field.

**Prepulse inhibition**

To measure the startle reactivity, mice were placed in small metal cages (90 × 40 × 40 mm) which restrict major movements and exploratory behaviour. The cages are equipped with a movable platform floor attached to a sensor recording vertical movements of the floor. The cages are placed in four sound-attenuating isolation cabinets (TSE). A startle reflex is evoked by acoustic stimuli delivered from a loudspeaker suspended above the cage and connected to an acoustic generator. The startle reaction of a mouse to the acoustic stimuli evokes a movement of the platform. The transient force resulting from this movement of the platform is recorded on a personal computer during a recording window of 260 ms and stored in the computer for further evaluation. The recording window is measured from the onset of the acoustic stimuli. An experimental session consisted of a 2 min habituation to the 65 dB background white noise (continuous throughout the session), followed by a baseline recording for 1 min at background noise. After baseline recording, 6 pulse alone trials using the startle stimuli of 120 dB intensity and 40 ms duration were applied in order to decrease influence of within-session habituation. These data were not included in the analysis of the prepulse inhibition. For tests of prepulse inhibition, the 120 dB/40 ms startle pulse was applied either alone or preceded by a prepulse stimulus of 70, 75 and 80 dB intensity and 20 ms duration. An interval of 100 ms with background white noise was employed between each prepulse and pulse stimulus. The trials were presented in a pseudorandom order with an interval ranging from 8 to 22 s. Amplitude of the startle response (expressed in mV) was defined as a difference between the maximum force detected during a recording window and the force measured immediately before the stimulus onset. Amplitudes were averaged for each individual animal, separately for both types of trials (stimulus alone, stimulus preceded by a prepulse). Prepulse inhibition was calculated as a percentage of the startle response using the formula: % prepulse inhibition = 100 – [((startle amplitude after prepulse – pulse pair)/ (startle amplitude after pulse only) × 100].

**Statistical analysis**

Data expressed as mean ± SEM in figures and text were compared by analysis of variance (ANOVA) with post hoc planned comparisons, or Kruskal–Wallis ANOVA with Mann–Whitney U-test using STATISTICA (StatSoft Inc., Tulsa, OK, USA) software. A P-value ≤ 0.05 was considered significant.

**Results and discussion**

To model discrete brain injury that may predispose to late neurodegenerative changes, we developed a cryolesion procedure of the parietal cortex in juvenile (4-week-old) mice. We induced the lesion by freezing through the skull bone with a liquid nitrogen-cooled copper cone with a tip diameter of 1 mm (Fig. 1A). We discovered that freezing for 60 s produces a highly reproducible lesion that evolves over time, reaching a maximum diameter 24 h after freezing (Fig. 1B and C). This lesion is replaced over time by a fibrous scar in the parietal grey matter (Fig. 1D), which is barely visible by high-resolution three-dimensional MRI at 3 months (Fig. 1E and F, arrows). Transient leakage of the BBB most likely contributes to the neurodegenerative process. This leakage is maximal 24 h after setting of the lesion and extends over the entire hemisphere to the contralateral white matter (illustrated in Fig. 1G–J). The primary cortical lesion, however, is always restricted to the parietal cortex, with apoptotic cells within and surrounding the lesion during its acute evolution (Fig. 1K and L).

We next addressed whether this discrete parietal lesion had more global effects on brain anatomy. In previous studies we provided evidence for a profound microglial activation and strong inflammatory cytokine expression that were not restricted to the lesion perimeter in the right parietal cortex but were seen bilaterally in the hippocampus hours to days after unilateral cryolesion (Knerlich et al., 1999; Sire´n et al., 2000). The hippocampal dentate gyrus harbours progenitor cells that are capable of proliferation and neuronal differentiation and respond to brain injury (Arvidsson et al., 2002; Yoshimura et al., 2003; Wang et al., 2004). Neuroinflammation in the dentate inhibits proliferation of progenitor cells (Monje et al., 2003). Disruption of adult neurogenesis in the dentate, in turn, prevents some forms of associative learning (Shors et al., 2001) and the level of dentate neurogenesis seems to correlate with spatial memory performance in aged rats (Drapeau et al., 2003). We hypothesized that the capacity of cellular self-renewal in the dentate hilus is crucial for long-term functional recovery after lesioning. Therefore, acute changes in the ratio of cell proliferation to cell death in the dentate were monitored after the lesion. As early as 24 h after placing the unilateral cortical lesion, we observed a bilateral reduction in the ratio cell proliferation to cell death (quantified here as a ‘BrdU/cleaved caspase-3’ ratio) in the hilar region of the dentate (Fig. 2A–C).

Haematopoietic growth factors that pass the BBB have shown striking effects on proliferation and survival of newly generated neurons (Shingo et al., 2001; Yoshimura et al., 2003). However, a recent investigation in our laboratory failed to show any effect of haematopoietic growth factors on cell proliferation in the dentate following cryolesion. In that study, the effect of haematopoietic growth factors was assessed using 5-bromo-2′-deoxyuridine (BrdU) incorporated into the DNA and cell proliferation as determined by BrdU incorporation. We induced cryolesions on both sides of the brains of juvenile mice and monitored cell proliferation in the dentate hilus from 24 h to 7 days after cryolesion using the BrdU method. We observed a significant reduction in the ratio of BrdU-positive cells compared to controls at all time points. This reduction was not affected by the presence of haematopoietic growth factors, suggesting that the reduction in cell proliferation following cryolesion is not due to a decrease in the capacity of progenitor cells to divide.
et al., 2003; Wang et al., 2004; Ehrenreich et al., 2005). We therefore expected a peripheral application of recombinant erythropoietin (EPO) to modulate the cellular response to brain injury. EPO is a haematopoietic growth factor with a 20-year history of safe clinical application and has been identified as a potent neuroprotective agent in vitro and in vivo (Sakanaka et al., 1998; Bernaudin et al., 1999; Brines et al., 2000; Sirén et al., 2001; Ehrenreich, 2004; Ehrenreich et al., 2004). Its neuroprotective profile is unusually complex with antiapoptotic, antioxidant, anti-inflammatory, stem cell modulatory, neurotrophic and angiogenic properties, that collectively reflect a non-haematopoietic function of EPO in brain development, presumably during phases of physiological hypoxia (Shingo et al., 2001; Sirén et al.,

Fig. 1 Characterization of the lesion model and evolution of the cortical lesion. (A) Lesion maker with a tip diameter of 1 mm. (B–D) Evolution of lesion on haematoxylin–eosin stained sections, at 24 h (B and C) and 9 months (D) after lesioning (lesion depicted by arrows, hippocampal CA1 region with arrow heads). (E and F) Lesion is visible at 3 months in MRI as a small indentation on the cortical surface. (G–J) Illustration of Evans-Blue albumin leakage (as indicator of BBB breakdown) 30 min (G and H) and 24 h (I and J) after lesion. Ongoing apoptosis at 24 h is evident as cleaved caspase-3 (K) and TUNEL positive (L) cells surrounding the lesion.

Fig. 2 Ratio of cell proliferation to cell death in the dentate 24 h after unilateral cortical lesion. Many bromodeoxyuridine (BrdU) (A, A1) and few cleaved caspase-3 (B, B1) positive cells (arrows) in the contralateral dentate of an EPO-treated animal at 24 h after lesion. (C) BrdU/cleaved caspase-3 ratio calculated using bilateral dentate cell counts 24 h after lesion; n = 5–7.
These properties may explain its powerful effect in entirely different neurological disease models, ranging from acute stroke to chronic conditions like experimental autoimmune encephalitis or diabetic neuropathy (Sakanaka et al., 1998; Bernaudin et al., 1999; Brines et al., 2000; Agnello et al., 2002; Ehrenreich et al., 2002; Bianchi et al., 2004; Diem et al., 2004). At the cellular level, EPO binds to a specific receptor of the cytokine type I receptor superfamily, which in the brain may consist of heteromeric subunits (Brines and Cerami, 2005). Molecular mechanisms of action of EPO in the nervous system include phosphorylation of Akt, ERK1/2, Stat5 and activation of NFκB (Digicaylioglu and Lipton, 2001; Sirén et al., 2001; Brines and Cerami, 2005).

When recombinant human EPO (rhEPO, 5 U/g intraperitoneally) was given only once, i.e. immediately after setting of the parietal lesion in mice, the detected ‘imbalance’ of cell proliferation and cell death, as measured after 24 h in the dentate gyrus, was fully prevented (Fig. 2C). In agreement
with these findings, increased neurogenesis by EPO during brain development and after injury has been demonstrated by several independent groups (Shingo et al., 2001; Yu et al., 2002; Wang et al., 2004). Apart from a direct stimulation of neurogenesis, the antiapoptotic and anti-inflammatory (Siren et al., 2001; Agnello et al., 2002; Brines and Cerami, 2005) properties of EPO most probably contributed to its favourable effect on the cell proliferation/cell death ratio. In fact, caspase inhibitors increased survival of newly formed BrdU-labelled cells in the dentate gyrus following epileptic seizures (Ekdahl et al., 2001) and anti-inflammation can be expected to partly restore neurogenesis in the dentate after injury (Magavi et al., 2000; Arvidsson et al., 2002; Monje et al., 2002, 2003).

To explore whether a similar reduction of neurons in the dentate hilar region could still be observed at later time points, we determined the spatial density of neurons in this area several months after a unilateral parietal cortex lesion. In this experiment, mice were treated with rhEPO (5 U/g intraperitoneally) every other day, for a total of 14 days and their brains were analysed after 3 months. As summarized in Fig. 3, dentate hilus neurons were significantly reduced in number in both hemispheres of the lesioned mice and this bilateral cell loss was again efficiently prevented by prior administration of rhEPO.

As shown in this study, a unilateral parietal lesion reduces via as yet unknown mechanisms also the capacity of the contralateral dentate for renewal of cells. Assuming that a minimum amount of trophic factors and the generation of new cells is necessary to maintain the normal dimensions of the cortex, any disturbance of this equilibrium, along with post-traumatic acceleration of neurodegenerative processes, may contribute to global cortical atrophy. In fact, grey matter reduction and ventricular enlargement (revealed by MRI)
take place after traumatic brain injury and correlate with human cognitive and functional outcome (Parker and Rosenblum, 1996; MacKenzie et al., 2002). We thus tested the hypothesis, that brain atrophy would be seen several months after applying a parietal cortical lesion and that a neuroprotective treatment with EPO prevents brain tissue loss, similar to EPO’s ability to reduce cell loss in the dentate hilar region. Definition of global brain atrophy both in mice and man includes decreased total brain matter volume and increased ventricular size which can be visualized and quantified by MRI (MacKenzie et al., 2002; Redwine et al., 2003; Resnick et al., 2003; Rusinek et al., 2003; Delatour et al., 2005; Schott et al., 2005). Indeed, when monitored by three-dimensional MRI, a global reduction in brain matter and a bilateral enlargement of brain ventricles was evident at both 3 and 9 months after parietal lesion (Fig. 4A–C).

Symmetry, magnitude and localization of these volume changes had features of ‘premature brain aging’ (Nemetz et al., 1999; Resnick et al., 2003; Rusinek et al., 2003). The tissue loss may well be additive to the normal ageing effect that could be independently demonstrated in this experiment by a comparison of sham-operated mice at the 3 and 9 months time points (Fig. 4A and B). The magnitude of ventricular enlargement and brain matter loss after parietal lesion in

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**Fig. 6** Performance in Morris water maze 3 and 9 months after unilateral parietal cortical lesion in mice (n = 10–19). Depicted in A and B are the latency to find the hidden platform and, as insert, the number of visits to the target site in the probe trial (platform removed) on day 9 in sham, lesion and lesion + EPO groups; *P ≤ 0.05 lesion compared to sham, +P ≤ 0.05 lesion compared to lesion + EPO. (C and D) The latency to find the visible platform of these mice.
our study is comparable with the age-dependent brain atrophy in APP/PS1 transgenic mice, a mouse model of Alzheimer’s disease (Delatour et al., 2005).

Importantly, early EPO treatment for 14 days, beginning immediately after lesioning, prevented brain atrophy monitored 9 months later. Brain matter volume and ventricle size in the EPO-treated mice after 9 months survival time was nearly identical to that of sham-operated animals, but significantly different from lesioned placebo-treated mice (Fig. 4A–C).

In humans, functional consequences of brain atrophy are cognitive impairments, behavioural alterations and emotional deficits (Parker and Rosenblum, 1996; Rusinek et al., 2003; Benedict et al., 2004). We tested mice in all experimental groups using an extensive behavioural test battery over 2 weeks to explore whether morphological signs of neurodegeneration are translated into functional impairments. As expected from the discrete parietal localization and small size of the lesion (Fig. 1), no impairments of motor performance in Rota-Rod (data not shown) or open field (Fig. 5A and B) were detected at any time point after lesion. Thus, neither the selective changes in exploration- and activity-related behaviour (hole board, elevated plus maze), detectable months after parietal lesion (Fig. 5C and D), nor the impaired Morris water maze performance at 9 months (Fig. 6A and B) were related to an underlying motor disability of performing these tests.

Specifically, we observed a significantly increased exploratory behaviour in the hole board test at 3 months after unilateral cortical lesion, generally considered a test of exploratory behaviour and (hyper)activity in mice. This abnormal test result showed a spontaneous partial recovery after 9 months (Fig. 5C). A contralateral hemispheric compensation in this task is likely to explain this recuperation. Indeed, when both parietal cortices were lesioned simultaneously, the number of abnormally increased head dips persisted (with 15 ± 2 head dips in bilaterally lesioned mice as compared to 7 ± 1 and 9 ± 2 head dips in sham-operated and unilaterally lesioned mice, n = 10–19, respectively).

Open arm entries in the elevated plus maze (a measure of motor activity) were distinctly increased after 3 months in lesioned mice. In contrast, the time spent in open arms (a measure of fear-related behaviour) was not different in lesioned and sham-operated mice (61 ± 7 and 50 ± 6%, respectively, n = 10, P = 0.2). At 9 months, entries were highly reduced in all experimental groups (Fig. 5D). In contrast, a significantly impaired spatial learning ability of lesioned mice in Morris water maze did not become evident before 9 months after brain injury (Fig. 6A and B). Therefore, age-related degeneration/grey matter loss obviously added to the lesion-induced degeneration, resulting in clear spatial learning deficits which are still compensated for in younger animals. However, in the visual platform paradigm, a simple target recognition test, all groups at a respective time point performed equally (Fig. 6C and D).

Prepulse inhibition, a test of sensorimotor gating, considered to be affected in schizophrenia (Braff et al., 2001), showed a distinct reduction in lesioned mice (Fig. 7A). Inasmuch as this test is non-specific for schizophrenia but found impaired in many other conditions (Braff et al., 2001), we saw it not only reduced after right parietal but also after left occipital lesion (Fig. 7B). In contrast, neither hole board performance nor ventricular size are affected by lesioning the occipital cortex (Fig. 7C).
We next tested whether, similar to the morphological consequences, the behavioural alterations set forth by the unilateral parietal cortex lesion were preventable by early neuroprotective therapy with EPO. We discovered that both the lesion-induced atypical behaviour in hole board and elevated plus-maze as well as the only later evident spatial learning deficit in Morris water maze at 9 months were abolished by EPO, as was the reduced prepulse inhibition (Figs 5–7). Thus, a circumscribed parietal lesion in the young mouse leads to significant alterations in behaviour in the mature animal and these changes can be blocked by the neuroprotective agent, EPO, delivered in the intervening period.

Interesting and yet unclear is the reduction in head dips in lesioned, EPO-treated mice as compared to sham-operated mice ($P < 0.01$). The test measures exploration behaviour and (hyper)activity. It could potentially be influenced by the level of anxiety. In our mice, however, elevated plus-maze (time spent in open arms—see above) and open field behaviour (Fig. 5A and B) point against this possibility.

We note again that overall motor performance (Rota-Rod, open field) of EPO-treated mice was not different from that of control mice at any time point tested. There were also no differences in blood haematocrit levels in the EPO-treated mice compared to placebo-treated or sham-operated mice at the time of testing at 3 months after setting of the lesion ($41 \pm 2, 42 \pm 2$ and $40 \pm 2\%$, $n = 8$, respectively). As expected, blood haematocrit was elevated immediately after 14 days of EPO injections ($57 \pm 3\%$, $n = 5$) as compared to placebo-treated mice ($38 \pm 1\%$, $n = 5$, $P < 0.05$) but returned to normal levels already within 4-weeks after cessation of EPO therapy ($38 \pm 2$ versus $40 \pm 2\%$, $n = 5$, not significant). As a general health indication, body weight was not different in sham, lesion and lesion + EPO groups (Fig. 8A and B).

Taken together, in this report, we made the surprising observation that a discrete lesion to the parietal cortex of juvenile mice, i.e. during brain maturation, is by itself the primary cause of a global neurodegeneration, with significant changes in brain morphology and function upon long-term follow-up. Such exogenously evoked neurodegeneration resembles ‘premature’ aging and might help to explain how degenerative processes that begin in the parietal lobe (as found in childhood onset schizophrenia (Thompson et al., 2001) can spread over the entire brain. Our data also indicate that brain trauma is likely to accelerate, or even to induce, those neuropsychiatric disorders that have a progressive degenerative component, such as Alzheimer’s disease or schizophrenia. Although the molecular details of the underlying spreading mechanisms need to be defined, we provide proof of principle that the critical downstream events of experimental brain trauma can be efficiently prevented by EPO-mediated neuroprotection. These findings suggest that prophylactic therapies should be considered to prevent some of the late consequences of brain injury.

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**References**


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Delatorre B, Guegan M, Volk A, Dhenain M. Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. Proc Natl Acad Sci USA 2003; 100: 14385–90.


