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Persistent region-dependent neuroinflammation, NMDA receptor loss and atrophy in an animal model of penetrating brain injury

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Abstract

Dynamic changes in neuroinflammation and glutamate NMDA receptors (NMDAR) have been noted in traumatic and ischemic brain injury.

Aim—Here we investigate the time course and regional distribution of these changes and their relationship with atrophy in a rat model of penetrating brain injury.

Materials & methods—Quantitative autoradiography, with the neuroinflammation marker [³H]PK11195 and the NMDAR antagonist [¹²⁵I]iodoMK801, was performed on brains of animals subjected to a unilateral wireknife injury at the level of striatum and killed 3 – 60 days later. Regional atrophy was measured by morphometry.

Results—The injury produced large increases in [³H]PK11195 binding density in cortical and septal regions adjacent to the knife track by day 7, with modest increases in the striatum. [¹²⁵I]iodoMK801 binding was reduced in cortical and hippocampal regions showing marked neuroinflammation, which showed marked atrophy at subsequent time points.

Conclusion—These results indicate that neuroinflammation and loss of NMDAR precede and predict tissue atrophy in cortical and hippocampal regions.

Keywords

brain atrophy; brain injury; neuroinflammation; NMDA receptors; peripheral benzodiazepine receptors; TSPO

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interests disclosure

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Delayed cerebral atrophy is a prominent feature of brain injury resulting from blunt trauma as well as intracranial surgical models and interventions [1–5]. Although the importance of atrophy for the neurobehavioral outcome of human brain injury has been recognized for decades [6], the mechanisms underlying this phenomenon are not entirely clear.

Acute brain injury is strongly associated with neuroinflammation in animals as well as humans and the regional intensity of the neuroinflammatory response is often quantified by autoradiography or PET using radiolabeled PK11195, a ligand for the translocator protein TSPO (previously named peripheral benzodiazepine receptor). TSPO density was shown to be an excellent quantitative marker for microglial activation and microlesions in the brain [7–13], recently reviewed by Chen and Guilarte [14], and Papadopoulos and Lecanu [15].

We have previously reported that increased [³H]PK11195 binding occurs in a distinct regional pattern and coincides with decreased NMDA receptor (NMDAR) availability following intracisternal injection of the endotoxin *Escherichia coli* lipopolysaccharide (LPS), a rat model of acute meningitis [16], after closed head injury in mice [17] and in a rat stroke model [18]. Regions involved in cognitive function (frontal and temporal cortex and the hippocampus) that also contain high densities of NMDAR were especially vulnerable [16]. The present study was designed to test the hypothesis that acute penetrating brain injury causes regional tissue loss that is related to the regional intensity of neuroninflammation and loss of NMDAR.

Experimental procedures

Animals & tissue preparation

Male Sasco Sprague Dawley rats were used in these studies. All experimental protocols followed NIH guidelines and were approved by the Institutional Animal Care and Use Committee. Rats aged 11 weeks old receiving penetrating brain lesions were anesthetized with a ketamine and xylazine cocktail, placed in a stereotaxic apparatus and the skull opened with a dental drill. A thin wireknife was lowered to the base of the brain, 0.8 mm left of the midline at anterior–posterior (AP), –1.4 mm from bregma, drawn forward to AP +0.4 mm from bregma and then withdrawn. The skull incision was filled with Gelfoam, the scalp sutured, and the animals were returned to their home cages after treatment with the analgesic Buprenorphine. Groups of four to six lesioned rats were sacrificed for autoradiographic analysis at 3, 7, 15, 30 and 60 days postlesion. Intact age-matched controls (n = 3–4 per group) were sacrificed at 0, 30 and 60 days to control for any maturational changes during the post-lesion interval. The rats were sacrificed under isoflurane anesthesia and the brains quickly removed after decapitation. Brains were frozen in powdered dried ice and stored at –70°C until sectioned. Four consecutive series of coronal cryostat sections were collected at 100 µm intervals (4 × 10 µm consecutive sections thaw-mounted onto four coated glass slides, six sections discarded and the cycle repeated) from AP+1 (anterior striatum) to AP-8 (midbrain).

In vitro autoradiography

On the day of the assay, sections were removed from the –70°C freezer and allowed to reach room temperature. TSPO was labeled with [³H]PK11195 (Perkin-Elmer, SA ~80Ci/mmol) using a methodology adapted from the literature [11,19] as previously described [16]. Briefly, sections were preincubated in 50 mM Tris HCl, pH 7.4 for 15 min at room temperature, followed by 30 min incubation at room temperature with the radioactive ligand. Total binding was determined with 1 nM [³H] PK11195. Nonspecific binding was determined on consecutive sections in the presence of excess (20 µM) unlabeled PK11195. Sections were then washed 2 × 6 min in ice-cold (4°C) 50 mM Tris HCl and dipped in 4°C

deionized water prior to drying to remove buffer salts. The dried sections were exposed to tritium-sensitive film for 33 days alongside calibrated tritium scales (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA). The films were developed in Kodak D-19, fixed and dried.

Labeling of NMDAR: Consecutive sections to those labeled with TSPO were labeled with the neuronal NMDA receptor marker ^{125}I -iodoMK801 as previously described [20]. Briefly, sections were incubated with 0.2 nM of the labeled ligand, washed and apposed to film for 6 h.

Histology

After film development, sections were stained with cresyl violet for anatomical verification with reference to a rat brain atlas [21].

Quantitative image analysis

The films were scanned and digitized using PhotoShop and a large bed Umax scanner; and saved in TIFF format so as to be accessible to NIH Image software. Using NIH Image routines, the standard curve was measured and used to calibrate regional brain measurements. The severity and spread of the neuroinflammatory response were assessed through regional density measurements of anatomically defined structures. Brain regions were identified on the images with reference to the histologically stained sections and a rat brain atlas [21].

For morphometric analysis, the brains were divided into five consecutive anatomical levels from anterior to posterior: level 1 included anterior frontal, motor, cingulate, parietal and piriform cortex, lateral septum, corpus callosum, lateral ventricles and striatum, anterior to the lesion. Level 2 included, in addition, the globus pallidus, perirhinal cortex and anterior thalamus. Level 3 included the dorsal hippocampus (CA1, CA3 and dentate gyrus) and amygdala. Level 4 included temporal and perirhinal cortex, ventral hippocampus, substantia nigra and medial mammillary nucleus. Level 5 included occipital and entorhinal cortex, posterior subiculum, substantia nigra and superior colliculus. Overall, [^3H] PK11195 and NMDAR binding was measured in 26 distinct anatomic regions. All of these regions were sampled bilaterally. Calibrated nonspecific binding was subtracted from total binding at each level to generate specific binding, expressed in nCi/mg.

Regional areas were measured from the NMDAR labeled sections with reference to the histology in the five coronal levels described above using the area tool in the NIH Image software package. The NMDAR autoradiograms were chosen for assessment of tissue loss rather than the TSPO or cresyl violet stained sections, since they were darker, had higher white-gray contrast and thus were the most amenable to semi-automatic segmentation along regional boundaries. All area measurements were performed bilaterally in intact and injured rats killed 30 or 60 days after injury. The measured regions included the striatum, dorsal hippocampus, ventral hippocampus, anterior cortex (extending from the cingulate to the piriform cortex, at the coronal level of the lesion, level 1) and the posterior basal cortex (level 4, 5; extending from the rhinal fissure to the ventral aspect of the entorhinal cortex).

Statistical analysis

Results were pasted into a Microsoft Excel worksheet and imported into Statview (Version 5.0; Abacus, Inc., Berkeley, CA, USA), for statistical analysis. The effects of the lesion on regional TSPO and NMDAR and cross-sectional areas were examined by analysis of variance as described in the relevant result sections. *Post hoc* comparisons were performed using the Fisher protected least significant difference test. Alpha was preset at $p < 0.05$.

Results

Effect of the lesion on regional TSPO & NMDAR density

PK11195 binding was significantly increased in all brain regions close to the lesion by day 3 and was maximal 7 days after the lesion. The time course is illustrated in Figure 1, which shows the results of quantitative autoradiographic analysis of the lateral septum. TSPO densities on day 7 in all of the brain regions sampled are shown in Table 1. MK801 binding was significantly decreased at the same time and in the same regions where a large increase in PK11195 binding was observed (Figure 2). However, there were no significant changes in NMDAR in those brain regions where TSPO increases were relatively small, although statistically significant (Figure 3). On day 15 we observed a decline of mean regional TSPO binding and increased NMDAR binding, culminating in values close to those measured in control animals in most regions by 30 days, although small areas of intense TSPO labeling were still found in all animals even 60 days after the surgery (Figures 1 & 2). The extent of injury-induced increases in TSPO binding at 7 days was highly dependent on region. Among the regions ipsilateral and at close proximity to the knife track, the largest increases (>5-fold) were observed (from dorsal to ventral) in the motor cortex and lateral septum. The striatum exhibited a remarkable relative resistance to the lesion compared to equidistant cortex and septum, with a TSPO increase of about twofold, and no significant change in NMDAR (Figures 1 & 3; Table 1). Conversely, relatively large increases in TSPO were found in the hippocampus and overlying cortex located posteriorly and laterally to the lesion (Figure 4). These were accompanied by significant decreases in NMDAR density (Figure 4).

Effect of the lesion on brain atrophy in specific brain regions

To examine region-specific atrophy (Figure 5), we measured the area of two regions at the coronal level of the lesion (anterior cortex and striatum) and two regions posterior to the lesion (hippocampus and postero-ventral cortex). Among regions at close proximity to the knife track, the largest decreases in brain area were observed in the anterior cortex, which extends from the anterior cingulate dorsally to the piriform cortex ventrally. The cross-sectional area of the left (ipsilateral) anterior cortex in lesioned animals was significantly smaller compared with the right side ($p = 0.015$) (Table 2). Moreover, the cross-sectional area of the anterior cortex in lesioned animals, was significantly reduced compared with intact animals ($p = 0.002$) (Table 2). The area of the striatum exhibited a remarkable relative resistance to the lesion. The striatum area of the lesioned animals was not significantly different from the area in intact animals ($p = 0.1$) and there was no significant difference between ipsilateral and contralateral striatal areas in lesioned animals ($p = 0.32$).

The mean hippocampal cross-sectional area in the lesioned animals demonstrated a significant atrophy in this structure (Table 2). Visual inspection of brain sections of the lesioned animals suggested a much stronger effect of the lesion on the dorsal (anterior) hippocampus compared to the ventral (posterior) hippocampus. To examine this quantitatively, we divided the hippocampus into anterior (dorsal) hippocampus (just posterior to the lesion) and posterior (ventral) hippocampus (i.e., remote from the lesion). The ipsilateral (left) anterior hippocampal area was significantly smaller compared with the contralateral (right) hippocampus ($p = 0.0001$) (Table 2). By contrast, the difference between the ipsilateral and contralateral sides of the ventral hippocampus was not significant ($p = 0.48$) (Table 2).

The posterior basal cortex measured, which extended from the rhinal fissure ventrally to the base of the entorhinal cortex, also did not show significant atrophy.

Discussion

The results of this study show that intracranial knife injury produces time- and region-specific microgliosis, reduction in NMDAR density and atrophy. While brain injury-related atrophy, neuroinflammation and NMDAR have been investigated in the past in both humans and animal models, this is the first study to examine the temporal and neuroanatomical profile of all three parameters in the same brains over a prolonged (60 days) survival period.

Like in previous studies, we found that penetrating brain injury – in the current study, wireknife deafferentation from the cortical surface to the supraoptic nucleus – resulted in a significant, wide-spread and long-lasting increase in TSPO binding, as reported in conjunction with neuroinflammation and neuronal death in a large number of human pathologies and animal models [7,10–13,16,17,22–29]. We chose this surgical procedure, which does not involve the hippocampus directly (the knife traverses the cortex, lateral septum/medial striatum and lateral hypothalamus) to rule out direct disruption of hippocampal tissue. The time course of changes in TSPO that we report, with peak increases at 7 days, is similar to that reported in published autoradiographic studies of TSPO after cortical impact or stab injury [8,11]; however, these studies reported measurements from fewer time points and regions. Although some increase in TSPO binding was observed in many brain regions, the effect was not uniform throughout the brain. The most sensitive regions (five- to seven-fold increases) were the cortex, lateral septum and dorsal hippocampus. Unlike the dorsal cortical surface and the lateral septum, which were traversed or found at close proximity to the knife track, the hippocampus was posterior to the lesion and the large increases in this structure (specifically in CA1) imply that it is inherently sensitive to inflammatory signals arriving from other regions. This observation is supported by our results [16,30] and those of Hauss-Wegrzyniaka *et al.* [31], reporting large increases in TSPO binding, microglial number and microglial reactivity in the hippocampus, subiculum, entorhinal and piriform cortices following acute or chronic intracisternal or intraventricular infusion of endotoxin (LPS).

The striatum, on the other hand, exhibits remarkable resistance to the proinflammatory effects of the injury. Despite the fact that the structure was adjacent to the knife track, it exhibited relatively minor (though statistically significant) increases in TSPO. This observation is also similar to our findings with intracisternal LPS, whereby the striatum was among the least responsive regions to the inflammogen [16,30].

TSPO levels in most regions of the control animals did not show significant changes with age over the 2-month follow-up period in this study. However, we did observe a sustained reduction of TSPO binding in some regions contralateral to the lesion 30 days or more after surgery, possibly signifying depletion of microglia that migrated to the ipsilateral side or death of the contralaterally activated microglia [32]. Future studies localizing the changes in TSPO to specific cell types, including microglia, astrocytes, macrophages and neurons, may help clarify the interplay between these cells in the acute as well as chronic phase after brain injury [32]. The apparent decrease in ipsilateral TSPO signal intensity over time probably does not signify overall recovery but rather may reflect cell and tissue loss, since the brain regions with large (>threefold) increases in TSPO at 7 days postlesion show marked atrophy at 30 and 60 days.

Significant reductions in NMDAR binding were found in the cortical and hippocampal regions that showed the highest increases in TSPO. These are also the brain regions containing the highest density of NMDAR in the brain of healthy rats and humans [33]. The striatum, which had a relatively low density of NMDAR at baseline and showed a small, though statistically significant, increase in TSPO, did not show significant decreases in

NMDAR binding at any time point. In general, NMDAR loss was more restricted, confined to regions with the highest increases in TSPO and highest baseline levels of NMDAR. These results are similar to those we previously reported in animals treated with intracisternal LPS and killed 7 days later [16]. Region-selective loss of NMDAR was also reported by us and others in animal models of stroke and closed head injury [18,20,34,35]. At the later time points (30 and 60 days postinjury), which were not previously investigated, we noted a delayed bilateral decrease in NMDARs in some regions, although there was no delayed increase in TSPO. This decrease probably reflects loss of NMDA receptive neurons due to deafferentation related to the transection of fiber tracts in our model.

In view of previous reports showing global and region-selective atrophy in human and animal brain injury [1–5,36], we have postulated that penetrating brain injury will also result in delayed, region-specific brain atrophy. Furthermore, the growing literature showing that activated glia secrete glutamate, which may cause excitotoxicity, and other neurotoxic molecules [37–40] led us to predict that atrophy will be related in space and time to the intensity of microglial activation as well as changes in NMDAR expression. The results of the present study support this hypothesis, since significant tissue loss was found 30–60 days after the lesion in brain regions that exhibited the highest increase in microglial activations 7 days after the procedure, which also contain high densities of NMDAR (i.e., cortical and hippocampal regions). Similar observations were made by Cagnin *et al.* in a longitudinal study of viral encephalitis in humans, where the authors showed that regions with high levels of neuroinflammation (evidenced by increased [^{11}C]PK11195 density on PET) at an earlier time point were lost in follow-up MRI studies a few months later [12].

The severe atrophy in the hippocampus, more specifically the dorsal part (equivalent to the hippocampal head in humans), parallel reports from human traumatic brain injury, which also documented correlations between loss of hippocampal volume and specific neuropsychological deficits [1]. The vulnerability of the neocortex and hippocampus to various insults is likely to be related to the preponderance of cognitive deficits in the aftermath of surgical, traumatic and ischemic brain injuries in human subjects as well as in animal models [41–43]. It appears that cognitive abilities are especially vulnerable to brain injury, such that long-lasting cognitive deficits are observed not only after severe brain injury [20,41] but also after moderate [44] and even minimal, mild concussion models that do not involve appreciable neuronal cell loss [45]. Importantly, cortical and hippocampal NMDAR play a pivotal role in memory formation and brain plasticity [46–48].

Future perspective

Taken together, our results and the above observations suggest that [^{11}C]PK11195 imaging with PET [9,10] within a few days or weeks of injury may be used in the future as a prognostic marker of subsequent long-term atrophy and neuropsychological deficits in human subjects. Furthermore, microgliosis, increases in TSPO and loss of NMDAR in the subacute period after brain injury may present an attractive treatment target for the prevention of brain atrophy and cognitive decline in the aftermath of accidental blunt or penetrating brain injury, as well as planned neurosurgical procedures [15,20,49,50], using pharmacological agents already approved for human use, such as minocycline for neuroinflammation and D-cycloserine, an indirect agonist at NMDAR to counteract NMDAR hypofunction.

References

Papers of special note have been highlighted as:

- of interest

■ of considerable interest

1. Ariza M, Serra-Grabulosa JM, Junqué C, et al. Hippocampal head atrophy after traumatic brain injury. *Neuropsychologia*. 2006; 44:1956–1961. [PubMed: 16352320]
2. Isoniemi H, Kurki T, Tenovuo O, Kairisto V, Portin R. Hippocampal volume, brain atrophy, and APOE genotype after traumatic brain injury. *Neurology*. 2006; 67:756–760. [PubMed: 16966535]
3. Siren AL, Radyushkin K, Boretius S, et al. Global brain atrophy after unilateral parietal lesion and its prevention by erythropoietin. *Brain*. 2006; 129:480–489. [PubMed: 16339796]
4. Bendel P, Koivisto T, Niskanen E, et al. Brain atrophy and neuropsychological outcome after treatment of ruptured anterior cerebral artery aneurysms: a voxel-based morphometric study. *Neuroradiology*. 2009; 51(11):711–722. [PubMed: 19568738]
5. Warner MA, Youn TS, Davis T, et al. Regionally selective atrophy after traumatic axonal injury. *Arch Neurol*. 2010; 67:1336–1344. [PubMed: 20625067]
6. Bigler ED. The clinical significance of cerebral atrophy in traumatic brain injury. *Arch Clin Neuropsychol*. 1987; 2:293–304. [PubMed: 14589621]
7. Dubois A, Bénavidès J, Peny B, et al. Imaging of primary and remote ischaemic and excitotoxic brain lesions. An autoradiographic study of peripheral type benzodiazepine binding sites in the rat and cat. *Brain Res*. 1988; 445:77–90. [PubMed: 2835123]
8. Miyazawa N, Diksic M, Yamamoto Y. Chronological study of peripheral benzodiazepine binding sites in the rat brain stab wounds using [³H] PK-11195 as a marker for gliosis. *Acta Neurochir*. 1995; 137:207–216. [PubMed: 8789663]
9. Banati RB, Myers R, Kreutzberg GW. PK ('peripheral benzodiazepine') – binding sites in the CNS indicate early and discrete brain lesions: microautoradiographic detection of [³H]PK11195 binding to activated microglia. *J Neurocytol*. 1997; 26:77–82. [PubMed: 9181482]
10. Banati RB, Newcombe J, Gunn RN, et al. The peripheral benzodiazepine binding site in the brain in multiple sclerosis: quantitative *in vivo* imaging of microglia as a measure of disease activity. *Brain*. 2000; 123:2321–2337. [PubMed: 11050032]
11. Raghavendra Rao VL, Dogan A, Bowen KK, Dempsey RJ. Traumatic brain injury leads to increased expression of peripheral-type benzodiazepine receptors, neuronal death, and activation of astrocytes and microglia in rat thalamus. *Exp Neurol*. 2000; 161:102–114. [PubMed: 10683277]
12. Cagnin A, Myers R, Gunn RN, et al. *In vivo* visualization of activated glia by [11C] (R)-PK11195-PET following herpes encephalitis reveals projected neuronal damage beyond the primary focal lesion. *Brain*. 2001; 124:2014–2027. [PubMed: 11571219]
13. Cagnin A, Brooks DJ, Kennedy AM, et al. *In-vivo* measurement of activated microglia in dementia. *Lancet*. 2001; 358:436–437. [PubMed: 11513903]
14. Chen MK, Guilarde TR. Translocator protein 18 kDa (TSPO): molecular sensor of brain injury and repair. *Pharmacol Ther*. 2008; 118(1):1–17. [PubMed: 18374421]
15. Papadopoulos V, Lecanu L. Translocator protein (18 kDa) TSPO: an emerging therapeutic target in neurotrauma. *Exp Neurol*. 2009; 219:53–57. [PubMed: 19409385]
- 16■. Biegon A, Alvarado M, Budinger TF, et al. Region selective effects of neuroinflammation and antioxidant treatment on peripheral benzodiazepine receptors and NMDA receptors in the rat brain. *J Neurochem*. 2002; 82:1–11. First report of reciprocal relationship between neuroinflammation and NMDA receptors. [PubMed: 12091459]
17. Grossman R, Shohami E, Alexandrovich A, Yatsiv I, Kloog A, Biegon A. Increase in peripheral benzodiazepine receptors and loss of glutamate NMDA receptors in a mouse model of closed head injury: a quantitative autoradiographic study. *Neuroimage*. 2003; 20:1971–1981. [PubMed: 14683703]
18. Dhawan J, Benveniste H, Nawrocky M, Smith SD, Biegon A. Transient focal ischemia results in persistent and widespread neuroinflammation and loss of glutamate NMDA receptors. *Neuroimage*. 2010; 51:599–605. [PubMed: 20206701]
- 19■. Guilarde TR, Kuhlmann AC, O'Callaghan JP, Miceli RC. Enhanced expression of peripheral benzodiazepine receptors in trimethyltin-exposed rat brain: A biomarker of neurotoxicity. *Neurotoxicology*. 1995; 16:441–450. Early documentation of the regional specificity of neuroinflammation owing to a systemic insult. [PubMed: 8584276]

20. Biegon A, Fry PA, Paden CM, et al. Dynamic changes in NMDA receptors after closed head injury in mice: implications for treatment of neurological and cognitive deficits. *Proc Natl Acad Sci USA*. 2004; 101:5117–5122. [PubMed: 15044697]
21. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. 4. Academic Press; CA, USA: 1998.
22. McGeer EG, Singh EA, McGeer PL. Peripheral-type benzodiazepine binding in Alzheimer's disease. *Alzheimer Dis Assoc Disord*. 1988; 2:331–336. [PubMed: 2848549]
23. Lavoie J, Layrargues GP, Butterworth RF. Increased densities of "peripheral-type" benzodiazepine receptors in autopsied brain tissue from cirrhotic patients with hepatic encephalopathy. *Hepatology*. 1990; 11:874–878.
24. Leong DK, Le O, Oliva L, Butterworth RF. Increased densities of binding sites for the "peripheral-type" benzodiazepine receptor ligand [³H]PK11195 in vulnerable regions of the rat brain in thiamine deficiency encephalopathy. *J Cereb Blood Flow Metab*. 1994; 14:100–105. [PubMed: 8263044]
25. Stephenson DT, Schober DA, Smalstig EB, Mincy RE, Gehlert DR, Clemens JA. Peripheral benzodiazepine receptors are colocalized with activated microglia following transient global forebrain ischemia in the rat. *J Neurosci*. 1995; 15:5263–5274. [PubMed: 7623150]
26. Lacor P, Benavides J, Ferzaz B. Enhanced expression of the peripheral benzodiazepine receptor (TSPO) and its endogenous ligand octadecaneuropeptide (ODN) in the regenerating adult rat sciatic nerve. *Neurosci Lett*. 1996; 220:61–65. [PubMed: 8977149]
27. Vowinckel E, Reutens D, Becher B, et al. PK11195 binding to the peripheral benzodiazepine receptor as a marker of microglia activation in multiple sclerosis and experimental autoimmune encephalomyelitis. *J Neurosci Res*. 1997; 50:345–353. [PubMed: 9373043]
28. Banati RB, Goerres GW, Myers R, et al. [¹¹C](R)-PK11195 positron emission tomography imaging of activated microglia *in vivo* in Rasmussen's encephalitis. *Neurology*. 1999; 53:2199–2203. [PubMed: 10599809]
29. Hirshler YK, Polat U, Biegon A. Intracranial electrode implantation produces regional neuroinflammation and memory deficits in rats. *Exp Neurol*. 2010; 222:42–50. [PubMed: 20026042]
30. Liraz-Zaltsman S, Alexandrovich AG, Trembovler V, et al. Regional sensitivity to neuroinflammation: *in vivo* and *in vitro* studies. *Synapse*. 2011; 65:634–642. [PubMed: 21108236]
31. Hauss-Wegrzyniaka B, Willard LB, Del Soldato P, Pepeu G, Wenk GL. Peripheral administration of novel anti-inflammatories can attenuate the effects of chronic inflammation within the CNS. *Brain Res*. 1999; 815:36–43. [PubMed: 9974120]
32. Maeda J, Higuchi M, Inaji M, et al. Phase-dependent roles of reactive microglia and astrocytes in nervous system injury as delineated by imaging of peripheral benzodiazepine. *Brain Res*. 2007; 1157:100–111. [PubMed: 17540348]
33. Young, AB.; Greenamyre, JT. Autoradiographic analysis of ³H-glutamate receptors in mammalian brain. In: Boast, CA.; Snowhill, EW.; Altar, CA., editors. *Quantitative Receptor Autoradiography*. Liss; NY, USA: 1986. p. 79-101.
34. Miller LP, Lyeth BG, Jenkins LW, et al. Excitatory amino acid receptor subtype binding following traumatic brain injury. *Brain Res*. 1990; 526:103–107. [PubMed: 1964103]
35. Ogawa N, Haba K, Mizukawa K, Asanuma M, Hirata H, Mori A. Loss of *N*-methyl-D-aspartate (NMDA) receptor binding in rat hippocampal areas at the chronic stage after transient forebrain ischemia: histological and NMDA receptor binding studies. *Neurochem Res*. 1991; 16(5):519–524. [PubMed: 1836545]
36. Tomaiuolo F, Bivona U, Lerch JP, et al. Memory and anatomical change in severe non missile traumatic brain injury: ~1 vs. ~8 years follow-up. *Brain Res Bull*. 2012; 87(4–5):373–382. [PubMed: 22289841]
37. Arvin B, Neville LF, Barone FC, Feuerstein GZ. The role of inflammation and cytokines in brain injury. *Neurosci Biobehav Rev*. 1996; 20:445–452. [PubMed: 8880734]
38. Gourin CG, Shackford SR. Production of tumor necrosis factor alpha and interleukin-1 beta by human cerebral microvascular endothelium after percussive trauma. *J Trauma*. 1997; 42:1101–1107. [PubMed: 9210549]

39. Bal-Price A, Brown GC. Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. *J Neurosci*. 2001; 21:6480–6491. [PubMed: 11517237]
40. Bezzi P, Domercq M, Brambilla L, et al. CXCR4-activated astrocyte glutamate release via TNF α : amplification by microglia triggers neurotoxicity. *Nat Neurosci*. 2001; 4:702–710. [PubMed: 11426226]
41. Chen Y, Constantini S, Trembovler V, Weinstock M, Shohami E. An experimental model of closed head injury in mice: pathophysiology, histopathology, and cognitive deficits. *J Neurotrauma*. 1996; 13:557–568. [PubMed: 8915907]
42. Courtiere A, Hardouin J, Locatelli V, et al. Selective effects of partial striatal 6-OHDA lesions on information processing in the rat. *Eur J Neurosci*. 2005; 21:1973–1983. [PubMed: 15869490]
43. Engstad T, Almkvist O, Viitanen M, Arnesen E. Impaired motor speed, visuospatial episodic memory and verbal fluency characterize cognition in long-term stroke survivors: the Tromsø study. *Neuroepidemiology*. 2003; 22:326–331. [PubMed: 14557682]
44. Piot-Grosjean O, Wahl F, Gobbo O, Stutzmann JM. Assessment of sensorimotor and cognitive deficits induced by a moderate traumatic injury in the right parietal cortex of the rat. *Neurobiol Dis*. 2001; 8:1082–1093. [PubMed: 11741403]
45. Zohar O, Schreiber S, Getslev V, Schwartz JP, Mullins PG, Pick CG. Closed-head minimal traumatic brain injury produces long-term cognitive deficits in mice. *Neuroscience*. 2003; 118:949–955. [PubMed: 12732240]
46. Baker KB, Kim JJ. Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. *Learn Mem*. 2002; 9:58–65. [PubMed: 11992016]
47. Broersen LM. Attentional processes and learning and memory in rats: the prefrontal cortex and hippocampus compared. *Prog Brain Res*. 2000; 126:79–94. [PubMed: 11105641]
48. Kossel AH, Williams CV, Schweizer M, Kater SB. Afferent innervation influences the development of dendritic branches and spines via both activity-dependent and non-activity-dependent mechanisms. *J Neurosci*. 1997; 17:6314–6324. [PubMed: 9236241]
49. Sanchez Mejia RO, Ona VO, Li M, Friedlander RM. Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage, and neurological dysfunction. *Neurosurgery*. 2001; 48:1393–1399. [PubMed: 11383749]
50. Yaka R, Biegon A, Grigoriadis N, et al. D-cycloserine improves functional recovery and reinstates long-term potentiation (LTP) in a mouse model of closed head injury. *FASEB J*. 2007; 21:2033–2041. [PubMed: 17351125]

Executive summary

Effect of wireknife lesion on regional TSPO density

- Penetrating brain injury produced large increases in TSPO density in septum, cortex and hippocampus regions adjacent to the knife track peaking on day 7 postsurgery, while the striatum showed only modest increases.
- TSPO density was back to normal values within 30 days of the surgery in most but not all brain regions.

Effect of wireknife lesion on regional NMDA receptor density

- Significant reductions in NMDA receptor binding were found in the regions and time points showing the largest increases in TSPO, including cortical and hippocampal regions.

Effect of the lesion on brain atrophy in specific brain regions

- Penetrating brain injury resulted in significant hippocampal and cortical, but not striatal atrophy in the ipsilateral hemisphere, evident 30–60 days after the surgical procedure.

Relationship between neuroinflammation, NMDA receptor loss & atrophy

- Penetrating brain injury leads to time and region-specific neuroinflammation and loss of NMDA receptors, which precede and predict tissue atrophy in cortical and hippocampal regions.

Figure 1. Time course of changes in TSPO binding following penetrating brain injury

Animals were sacrificed 3, 7, 15, 30 or 60 days after penetrating knife injury. Brain sections were labeled with the neuroinflammation marker [^3H]PK11195. Autoradiograms were pseudo-colored using the rainbow spectrum, such that the highest densities of radioactivity are depicted in red and the lowest levels in purple/black. **(A)** Representative autoradiograms from individual animals and time points. A coronal section from an intact 7-week-old animal is on the left, showing the normal distribution of TSPO at the level of the injury. Subsequent sections (from left to right) were taken from animals killed 3, 7 or 15 days after the lesion. Nonspecific binding (not shown) was low and uniform in all brain regions and experimental groups. **(B)** Mean specific binding of [^3H]PK11195, expressed in nCi/mg, in the lateral septum of brain-injured animals (4–6/time point) and age-matched controls. Nonspecific binding was subtracted from all readings. Statistical analysis of the binding data in the lateral septum ipsilateral to the lesion by one-way analysis of variance revealed a significant effect of treatment (time after lesion) with $F = 8.8$ and $p < 0.0001$. Subsequent *post hoc* analysis resulted in the following salient differences: lesion, 3 days compared to intact, $p = 0.0002$; lesion, 7 days compared to intact, $p < 0.0001$; lesion, 15 days compared to intact, $p = 0.0007$; lesion, 30 days compared to intact, $p = 0.35$ (not significant); lesion, 60 days compared to intact, $p = 0.18$ (not significant). The values at 7 days postsurgery were also significantly higher than 30 or 60 days postsurgery ($p = 0.0006$ and $p = 0.0014$, respectively). The contralateral septum data were analyzed in a similar fashion. To avoid multiple symbols and highlight salient comparisons, a single asterisk was used to denote any significant differences between lesioned and intact that reached significance at the preset

alpha ($p < 0.05$). (C) Regional neuroinflammation and tissue loss 30 days after penetrating brain injury. Autoradiograms from one control and three individual animals killed 30 days after injury show small regions of intense neuroinflammation as well as remarkable loss of tissue in the ipsilateral hemisphere.

* $p < 0.05$ compared to mean intact control values in the same hemisphere, Fisher's protected least significant difference *post hoc*.

Figure 2. Reciprocal changes in NMDA receptor and TSPO binding following brain injury
 Top: (A) Mean specific TSPO and (B) NMDA receptor binding in motor cortex of injured animals (4–6/time point) and age-matched controls. Bottom: pseudocolored autoradiograms from individual controls and injured animals killed three (top row) 30 (middle row) or 60 (bottom row) days after injury, depicting TSPO density (C) and NMDA receptor density (D) in consecutive sections from the same animals. Red: injured animals, ipsilateral hemisphere. Statistical analysis was performed as described for Figure 1.

* $p < 0.05$ compared with mean control values in the same hemisphere, Fisher's protected least significant difference *post hoc* comparison following significant main effects of side, region and treatment in three-way analysis of variance.

Figure 3. Injury causes changes in TSPO but not NMDA receptor in moderately inflamed regions

Top: time-dependent changes in NMDA receptor-specific binding in (A) the olfactory tubercle and (B) lateral striatum of the same animals processed for TSPO. Bottom: time-dependent changes in TSPO specific binding in (C) the olfactory tubercle and (D) lateral striatum.

* $p < 0.05$ compared to mean control values in the same hemisphere, Fisher's protected least significant difference *post hoc* comparison.

Figure 4. Pattern of changes in TSPO and NMDA receptor in the hippocampus

(A) Mean specific NMDA receptor (top) and TSPO (bottom) binding in the dorsal hippocampal CA1 field in control animals and animals killed 3–60 days after penetrating brain injury. (B) Pseudo-colored autoradiograms illustrating NMDA receptor (top) and TSPO (bottom) distribution 7 days after injury at the level of anterior hippocampus, just posterior to the lesion, dominated by CA3 and dentate gyrus. (C) Pseudo-colored autoradiograms illustrating NMDA receptor (top) and TSPO (bottom) distribution 7 days after injury at the level of dorsal hippocampus posterior to (B), including CA1–4, dentate gyrus and subiculum.

* $p < 0.05$ compared to mean control values in the same hemisphere, Fisher's protected least significant difference *post hoc* comparison.

Figure 5. Brain atrophy following a transhemispheric lesion

Animals were sacrificed 30 days after penetrating knife injury. Brains were removed, sectioned and labeled with the NMDA receptor/neuronal marker [^{125}I] iodoMK801. The images illustrate representative autoradiograms from intact (left column) and lesioned (right column) animal brains from anterior (level 2, top) to posterior (level 5, bottom) levels >4 weeks after surgery. Note the cavitation near the knife track as well as the marked reduction in cortical thickness, septum and anterior hippocampus size on the side ipsilateral to the lesion and the apparent sparing of the striatum.

Table 1

Regional-specific binding of [³H]PK11195 7 days after injury.

Region	Contralateral (right)			Ipsilateral (left)		
	Control	Lesion	p-value	Control	Lesion	p-value
Motor cortex	15.3 ± 1.6	24.2 ± 2.9	0.006	15.3 ± 2.3	76 ± 24.8	0.0026
Lateral septum	11.4 ± 3.1	34.3 ± 4.1	<0.0001	11.4 ± 2.8	83.2 ± 14.9	<0.0001
Medial striatum	10.8 ± 2.4	14.4 ± 1.0	NS	10.7 ± 2.0	25.4 ± 3.9	0.0045
Medial preoptic area	14.3 ± 2.1	18.3 ± 1.1	NS	15.6 ± 1.8	51.9 ± 10.8	0.0013
Olfactory tubercle	13.7 ± 2.7	24.4 ± 1.4	0.0045	15.6 ± 1.9	26.5 ± 1.7	0.0036
Lateral striatum	10.7 ± 2.7	15 ± 1.2	NS	10.9 ± 2.1	19.6 ± 2.3	0.0026
Sensory cortex	14.9 ± 2.1	19.1 ± 2.0	NS	14.7 ± 1.8	44.9 ± 14.3	0.008
Insular cortex	14.9 ± 2.9	18.2 ± 1.7	NS	15.4 ± 2.2	39.3 ± 10.0	0.006
Piriform cortex	16 ± 3.1	17.1 ± 1.4	NS	14.8 ± 1.5	27.9 ± 3.8	0.004
Anterior thalamus	15.7 ± 2.4	17.5 ± 0.8	NS	17.1 ± 2.1	38.4 ± 4.4	0.002
Lateral hypothalamus	11 ± 2.3	17.5 ± 3.9	NS	12.4 ± 1.0	40.4 ± 7.7	0.0018
Medial hypothalamus	13 ± 1.2	17.2 ± 0.9	NS	13.4 ± 1.0	43.6 ± 9.0	0.002
Substantia innominata	9.5 ± 1.0	13.4 ± 0.6	NS	11.9 ± 1.0	17.7 ± 2.2	NS
Supraoptic nucleus	31.3 ± 10.3	35.8 ± 4.4	NS	39 ± 19.8	33.9 ± 3.0	NS
Amygdala	10.6 ± 1.6	11.2 ± 0.9	NS	9.9 ± 1.7	20.9 ± 3.6	0.035
Hippocampus, CA3	9.8 ± 1.7	14.5 ± 1.7	NS	9.7 ± 2.6	23.9 ± 7.6	NS
Hippocampus, CA1	12.6 ± 2.2	27.1 ± 3.5	0.0006	9.8 ± 2.8	46.9 ± 8.5	0.0002
Centromedian thalamus	11.7 ± 2.5	16.7 ± 0.9	NS	12.3 ± 2.3	25.2 ± 2.6	NS

Region	Contralateral (right)			Ipsilateral (left)		
	Control	Lesion	p-value	Control	Lesion	p-value
Hippocampus, dentate	11.2 ± 3.1	19 ± 1.5	NS	11.6 ± 3.4	30.5 ± 4.0	0.035
Perirhinal cortex	12.7 ± 2.5	14 ± 1.2	NS	12.8 ± 2.6	17.5 ± 7.9	NS
Corpus callosum	4.3 ± 0.8	9.1 ± 2.8	NS	3.9 ± 0.2	14.3 ± 4.3	0.03
Posterior cingulate	6 ± 1.0	6 ± 1.0	NS	6.4 ± 1.0	12.7 ± 2.0	0.04
Substantia nigra	8 ± 1.3	7.5 ± 1.2	NS	6.6 ± 0.8	6.9 ± 1.4	NS
Temporal cortex	8.7 ± 1.1	5.9 ± 0.9	NS	7 ± 1.0	12.5 ± 3.3	NS
Ventral hippocampus	6.2 ± 0.5	5.3 ± 0.9	NS	6.3 ± 0.7	8.6 ± 0.8	NS
Entorhinal cortex	7 ± 1.1	5.1 ± 1.0	NS	7.5 ± 2.2	10.8 ± 0.7	NS
Mammillary body	8.5 ± 1.7	9.9 ± 1.5	NS	8 ± 1.5	10.8 ± 1.8	NS
Occipital cortex	6.5 ± 0.5	5.7 ± 1.0	NS	6.4 ± 0.8	10.8 ± 2.5	NS

Results are means ± standard error of the mean specific binding (in nCi/mg) of three to four intact controls and four to six lesioned rats/region.

Three-way analysis of variance by region, surgery and side revealed highly significant main effects for all three variables ($F > 30$, $p < 0.0001$) and significant interaction terms (side × region, $p = 0.015$, side × group × region, $p = 0.03$). The large majority of ipsilateral regions were significantly increased relative to the same hemisphere in control animals, shown by *post hoc* Fisher's protected least significant difference (p-values reported in the table). A smaller number of regions on the contralateral side were increased relative to controls as well, including the septum and dorsal hippocampus.

NS: Not significant.

Table 2

Effects of lesion on regional atrophy.

Side	Anterior cortex		Posterior cortex		Striatum		Anterior hippocampus		Posterior hippocampus	
	Intact	Lesion	Intact	Lesion	Intact	Lesion	Intact	Lesion	Intact	Lesion
Ipsilateral	42.8 ± 2.3	34.8 ± 0.82 *	8.7 ± 0.4	8 ± 0.5	24 ± 1.0	20 ± 0.6	5.8 ± 0.3	4.5 ± 0.4 *	19.7 ± 1.3	18.9 ± 0.7
Contralateral	42.9 ± 1.9	40.8 ± 1.2	9.2 ± 0.4	9.5 ± 0.6	22 ± 0.9	22 ± 0.4	6.3 ± 0.4	6.8 ± 0.3	19.9 ± 1.3	20 ± 0.7

Results are mean ± standard error of the mean of the cross-sectional area (in mm²) of the specified regions from 11 animals with lesions (30 and 60 days post-penetrating knife injury) and seven age-matched intact animals. Results for each region were first analyzed by two-way analysis of variance (side and lesion). Data from regions in which there was a significant effect of lesion, side and a significant side by lesion interaction term were subjected to further *post hoc* analysis. By way of illustration, there was a significant effect of side ($F = 13.8$; $p = 0.0008$), a nonsignificant effect or lesion ($F = 1.09$; $p = 0.3$) and a significant side × lesion interaction ($F = 6.1$; $p = 0.018$) in the anterior hippocampus, permitting subsequent *post hoc* comparison of the ipsilateral and contralateral sides within and across the two groups. In lesioned animals there was a highly significant difference ($F = 22$; $p = 0.0001$) between the sides. The difference between right and left in intact animals was not significant. These comparisons were not performed in the striatum, posterior cortex and posterior hippocampus where the results of the initial analysis of variance were not significant (e.g., posterior hippocampus analysis of variance, effect of side: $F = 0.51$, $p = 0.48$, affect of lesion: $F = 0.08$, $p = 0.77$, side × lesion interaction: $F = 0.28$, $p = 0.6$).

* $p < 0.05$ compared to intact by Fisher's protected least significant difference *post hoc* test.