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Transient focal ischemia results in persistent and widespread neuroinflammation and loss of glutamate NMDA receptors

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Abstract

Stroke is accompanied by neuroinflammation in humans and animal models. To examine the temporal and anatomical profile of neuroinflammation and NMDA receptors (NMDAR) in a stroke model, rats (N=17) were subjected to 90 minutes occlusion of the middle cerebral artery (MCAO) and compared to sham (N=5) and intact (N=4) controls. Striatal and partial cortical infarction was confirmed by MRI 24 hr after reperfusion. Animals were killed 14 or 30–40 days later and consecutive coronal cryostat sections processed for quantitative autoradiography with the neuroinflammation marker [³H]PK11195 and the NMDAR antagonist [³H]MK801. Significantly increased specific binding of [³H]PK11195 relative to non-ischemic controls was observed in the ipsilateral striatum (>3 fold, p<0.0001), substantia innominata (>2 fold) with smaller (20%–80%) but statistically significant (p=0.002–0.04) ipsilateral increases in other regions partially involved in the infarct such as the parietal and piriform cortex, and in the lateral septum, which was not involved in the infarct. Trends for increases in PBR density were also observed in the contralateral hemisphere. . In the same animals, NMDAR specific binding was significantly decreased bilaterally in the septum, substantia innominata and ventral pallidum. Significant decreases were also seen in the ipsilateral striatum, accumbens, frontal and parietal cortex. The different anatomical distribution of the two phenomena suggests that neuroinflammation does not cause the observed reduction in NMDAR, though loss of NMDAR may be locally augmented in ipsilateral regions with intense neuroinflammation. . Persistent, bilateral loss of NMDAR, probably reflecting receptor down regulation and internalization, may be responsible for some of the effects of stroke on cognitive function which can not be explained by infarction alone.

Keywords

Stroke; Autoradiography; β -imager; [³H] PK11195; [³H] MK801; Microglia; Neuroinflammation

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Introduction

Stroke is the third leading cause of mortality in the US and each year, about 780,000 people experience a new or recurrent stroke (Rosamond et al., 2008). On average, every 40 second someone in United States has a stroke. The middle cerebral artery and its territory are commonly involved (Wang et al., 2000), and the hallmark of ischemic stroke is a focal cerebral infarction in the affected vessel core territory. However, acute and chronic cognitive and other neurological deficits in stroke victims may be in part related to brain regions which are quite remote from the infarction (including contralateral regions), a phenomenon referred to as “diaschisis” (Fiorelli et al., 1991, Hochstenbach et al., 1998; Sunderland et al., 1999; Sobesky et al., 2005). The mechanisms underlying this phenomenon are not entirely clear.

The most popular animal model of ischemic stroke involves transient or permanent occlusion of the middle cerebral artery (MCAO). MCAO results in an infarction of the striatum and overlying cortex which can be visualized *in vivo* by magnetic resonance imaging (Benveniste et al., 1991; Haefelin et al., 2000; Ryck et al., 2000; Dijkhuizen et al., 2003; Rossini et al., 2003; Weber et al., 2006; Wu et al., 2007) as well as neuroinflammation (Candelario et al., 2005; Wiart et al., 2007; Rojas et al., 2007). Stroke - related neuroinflammation has been studied in by in vitro autoradiography with the selective peripheral benzodiazepine receptor (PBR) ligand [³H]PK11195 (Benavides et al., 1983; Dubois et al., 1988; Myers et al., 1991a, b; Rojas et al., 2007) and by in vivo PET imaging using the ¹¹C labeled form of the same tracer (Sette et al., 1993, Ramsay et al., 1992; Gerhard et al., 2000, 2005; Pappata et al., 2000; Price et al., 2006), showing significant increases in PBR density in the infarcted area (mainly striatum) as well as in regions at a considerable distance from the infarct, such as the thalamus. Neuroinflammation is also a prominent feature in several other acute and chronic neuropathologies including global forebrain ischemia, traumatic brain injury, Alzheimer’s disease and meningitis, all of which are accompanied by cognitive deficits and loss of glutamate NMDA receptors, known to play a major role in memory formation (e.g. Bieganski et al., 2002, 2004; Dalkara et al., 1996, Grossman et al., 2003; Izquierdo, 1991; Liu et al., 2007, McGeer et al., 2010. Miller et al., 1990; Ogawa et al., 1991, Penney et al., 1990, Shohami et al., 2003, Sihver et al., 2001). The present study was designed to test the hypothesis that like the above mentioned pathologies, focal ischemia results in persistent loss of NMDAR which is augmented by neuroinflammation and may be relevant to long-term cognitive deficits in stroke victims.

Materials and Methods

Animals

Twenty six female Sprague-Dawley rats (220–250g) from Taconic Farms, Long Island NY), were kept under controlled light and dark conditions and given food and water ad libitum. The experiments were approved by the institutional animal care and use committee.

Surgery

MACO was performed using adaptation of published methods ((Koizumi et al., 1986; Longa et al., 1989) on 17 rats. Animals were anesthetized with isofluorane (0.75–1.0%) mixed in O₂ – enriched air flow during the surgery. Body temperature was continuously monitored with rectal probe and maintained at 37.0 °C with heating pad. A midline incision was made and left common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were exposed under operating microscope. A 4-0 silicone rubber coated monofilament (Doccot corp) was inserted through the ECA into the lumen of the ICA until the resistance was felt approximately 18–19 mm until resistance was encountered, ensuring

that the intraluminal suture has blocked the origin of the MCA. The incision was tightly closed with 0–6 silk suture, leaving 1 cm of the monofilament protruding so it could be withdrawn to allow reperfusion. The silicon rubber coated monofilament was allowed to remain in place for 90 minutes and then retracted so as to allow reperfusion of the ischemic region. Sham surgery (N=5) consisted of anesthesia, incision and exposure of the arteries. Intact animals (N=4) did not receive any intervention.

Imaging

Magnetic resonance images (MRI) were first acquired 24 hrs after MCAO, using a superconducting 9.4T/210 horizontal bore magnet (Bruker BioSpin AVANCE II, Magnex) with gradient strength of 950 mT/m equipped with an actively shielded 11.6-cm gradient set capable of providing 20 G/cm (Bruker). A birdcage coil (inner diameter 72 mm) was used to transmit and a 30-mm diameter surface RF coil secured above the head of the rat was used to receive the MR signal.

Images were acquired in the axial plane using a T_2 -weighted (T_2W) RARE spin echo sequence. The acquisition parameters were: TR = 2500 ms, TE = 9.8 ms, slice thickness = 0.7 mm, slice gap = 0.1 mm, matrix size = 256× 256, field of view (FOV) = 3.00 cm, no of averages = 4, no of slices = 29, RARE factor = 8, spatial resolution = 0.117mm and total experiment time = 5 min 20 sec.

Diffusion weighted (DW) images were acquired using TR = 877.2 ms, TE = 20 ms, no of averages = 2, slice thickness = 1.4 mm and spatial resolution = 0.1mm.

The RARE sequence was repeated on the last day of follow-up (14 or 30). Animals with no discernible infarcts were excluded from the experiment.

Post surgical monitoring

Rats were examined for spontaneous locomotion, posture, forelimb placing, body weight and general appearance daily after surgery. Animals showing signs of morbidity (e.g. severe weight loss) were euthanized.

In-Vitro quantitative autoradiography

Fourteen or 30–40 days after MCAO rats (N=4 and 13 respectively) were decapitated and brains were quickly removed, rinsed with 0.9% saline, and frozen in powdered dry ice. Long-term storage of brains was at -70°C . Frozen brains were sectioned in a cryostat (Jencons, OTF 5000) at -15°C in the coronal plane. Consecutive series of coronal brain sections (20 μ) were collected at 200 μ m intervals from frontal cortex ($\sim$ 3.7mm from Bregma) to the posterior striatum ($\sim$ 1.4 from Bregma), thus covering the expected maximal extent of the lesion in consecutive series of 24 evenly spaced sections/ligand. Regions of interest (ROIs) were chosen to represent anatomical regions in and around the expected infarction and contralaterally, previously shown to exhibit variable sensitivity to neuroinflammation and loss of NMDAR in other models (Biegon et al., 2002; Grossman et al., 2003; Biegon et al., 2004) and included the frontal, cingulate, parietal and piriform cortices, striatum, accumbens, lateral septum, medial preoptic area, substantia innominata and ventral pallidum, all measured bilaterally. ROIs were drawn on the autoradiograms around the whole extent of each region on all sections where the region could be clearly distinguished on the histologically stained section. Thus the size of the quantitation fields was maximal and proportional to the actual size of the various regions, and included a minimum of 3 measurements/side (6/animal) for the smallest regions and a larger number for the bigger regions.

[³H]PK11195 Autoradiography—On the day of the assay, sections were removed from the −70 °C freezer and allowed to reach room temperature. PBR autoradiography was performed with [³H] PK-11195 (specific activity 83.5 Ci/mmol, PerkinElmer Life Sciences) as previously described (Biegon et al., 2002). Sections were first pre-incubated in 50mM Tris–HCl buffer (pH7.4) for 15min at room temperature, followed by 30min incubation at room temperature with the radioactive ligand. Total binding was determined with 1nM [³H] PK-11195. Non-specific binding was determined on consecutive sections in the presence of excess (20μM) unlabeled PK-11195 (sigma). Sections were then washed two times for 6 min in 4°C 50mM Tris–HCl and dipped in 4°C deionized water prior to drying to remove buffer salts. Sections were dried on a slide warmer at 60°C. Slides were scanned and digital autoradiograms were obtained using a β-imager (Biospace, Paris, France).

[³H]MK801 Autoradiography—NMDA receptor autoradiography was performed as previously reported (Biegon et al., 2002; Grossman et al., 2003). Briefly, after a 30-min prewash in 50 mM Tris-acetate buffer at pH 7.4, the sections were incubated for 3h at room temperature, in 50mM Tris-acetate buffer at pH7.4 containing 10nM [³H]MK801, 30μM glutamate, and 10μM glycine (200μL per section). Nonspecific binding was determined in the presence of excess (100μM) unlabeled MK801. At the end of the incubation, the sections were dipped for 5sec in ice-cold buffer and then washed for 90min in cold, fresh buffer, followed by a dip in ice-cold distilled water. Sections were dried on a slide warmer at 60°C. Slides were scanned and digital autoradiograms were obtained using a β-imager (Biospace, Paris, France). Sections used for autoradiography as well as one consecutive series of unused sections were stained with cresyl violet for final verification of tissue morphology and histopathology.

Quantitative image analysis

One series of sections from each animal was stained with cresyl violet for histological confirmation of the brain anatomy and lesion location. Brain regions were identified from the histologically stained sections using a rat brain atlas (Paxinos and Watson, 1986). Regional quantitative image analysis was performed using Betavision software (Biospace, Paris, France/Capintec, USA) and manual Regions of Interest (ROI's) were drawn on the left and right side of the brain. ROIs were measured bilaterally, with a minimum of 6 measurements/ROI and a mean value calculated for each animal/side. Non-specific binding was subtracted from total binding to yield regional specific binding values.

Statistical analysis

The effect of ischemia on regional density of PBR and NMDAR were analyzed by ANOVA with Fisher's PLSD post-hoc tests or paired t-tests, using the Statview software package. p-Values smaller than <0.05 were considered significant.

Results

Stroke validation - Magnetic Resonance Imaging and neurological examination

As expected, T₂-weighted MRI of 0.7mm-thick brain slices showed unilateral hyperintensity in the MCA territory, involving the striatum and some adjacent cortical regions. Cortical hyperintensity was noted most frequently in the parietal cortex (N=4) and piriform cortex (N=4). The frontal cortex was intact in most animals, with only 2 rats showing frontal cortical hyperintensity (Fig. 1A). DWI at this time point (24 h post surgery) produced virtually identical results (Fig 1B).

The procedure also resulted in impairment of placing of the contralateral (right) forelimb. This sensorimotor dysfunction was maximal in the first few days after the stroke onset and improved gradually over the follow-up period.

Neuroinflammation

Autoradiography with [^3H]PK11195 showed increased PBR density, representing microglial activation, in all MCAO animals both 14 and 30 or more days after surgery. The increase was largest in and around the infarct zone but smaller increases were observed in additional regions both ipsi- and contralateral to the lesion (Fig 2C, 3C). Sections from control animals showed the known distribution of PBR in the brain, with high density in ependymal cell layers associated with ventricles (Fig 2F) and relatively low and uniform levels in most brain regions.

Statistical analysis of the PBR specific binding density by 3 way ANOVA revealed highly significant effects of region, stroke and side ($p < 0.0001$) and significant interaction terms. Comparison of the 14 and 30 day time points within the stroke group (2-way ANOVA by time and region) revealed no significant effect of time within this range, so the results of the 14 and 30 day rats were pooled for the final analysis (Table 1). Regional Posthoc analysis demonstrated a large (>3 fold, $p < 0.0001$) increase in the ipsilateral striatum relative to control. Smaller (20%–120%) but statistically significant ($p = 0.002$ – 0.04) ipsilateral increases were also observed in the accumbens, lateral septum, parietal cortex, piriform cortex, substantia innominata, and ventral pallidum. The cingulate cortex and medial preoptic area did not show significant increases in PBR (Table 1). Trends towards contralateral increases were seen in several regions, most notably in the accumbens, frontal cortex and septum, but not in the substantia innominata and ventral pallidum (Table 1). The relatively larger increases in the ipsilateral hemisphere are also expressed in the ratio of ipsilateral to contralateral binding in the MCAO animals, which was larger than 1 in most regions and highest in the striatum and substantia innominata (Table 1).

NMDA receptors

MCAO rats showed a widespread bilateral decrease in specific binding of [^3H]MK801 to NMDAR receptors at 14 as well as 30 days after surgery (Fig 2C and 3C). Quantitative analysis of the autoradiograms followed by 3 way ANOVA revealed highly significant main effects of surgery ($p < 0.0001$) and region ($p < 0.0001$) and a significant region X surgery interaction ($p < 0.0001$) but no effect of side. Comparison of the 14 and 30 day time points within the stroke group (2 way ANOVA by time and region) revealed no significant effect of time within this range, so the results of the 14 and 30 day rats were pooled for the final analysis (Table 2). Regional analysis confirmed decreases in NMDAR density ranging from 10% to 45% relative to non-ischemic controls. The largest decreases were seen in the ipsilateral striatum and in the substantia innominata bilaterally. Other regions showing significant decreases or strong trends ($p < 0.1$) in the same direction included the bilateral accumbens, frontal cortex, septum, parietal cortex, and ventral pallidum, with no change in the preoptic area and cingulate cortex. The ratio of ipsilateral to contralateral binding in most regions was close to 1 and subtle differences between the hemispheres were revealed only by paired t-test analysis of the MCAO animals, where significantly lower values were found in the striatum, frontal and parietal cortex but not in the lateral septum, substantia innominata and ventral pallidum (Table 2).

Discussion

Using a common model of transient (90 min.) focal ischemia which results in reliable infarction but minimal mortality (Memezawa et al., 1992a,b; Spratt et al. 2006; Tsubokawa

et al., 2006; Tsuchiya et al., 2003) in rats, we show here for the first time that MCAO is accompanied not only by long lasting neuroinflammation but also by loss of NMDAR in brain regions extending far beyond the infarct area, including regions contralateral to the lesion. Inflammation is well recognized as an important feature of subacute ischemic stroke in humans (Emsley et al., 2008). The hallmark of neuroinflammation is activation of glial cells (e.g. Price et al., 2006) which are known to release toxic substances including glutamate, reactive oxygen species (Peters et al., 1998) and pro inflammatory cytokines (Pera et al., 2004; Emsley et al., 2008). Activated microglia are responsible for increased [^3H]PK11195 binding in lesions where the Blood Brain Barrier (BBB) is intact (Banati et al., 1997). Our observation of long lasting and widespread increases in [^3H]PK11195 binding are in accord with in vivo studies performed in stroke patients (Gerhard et al. 2000, 2005). Using [^{11}C]PK11195 and Positron Emission Tomography (PET) in patients with ischemic stroke 5–53 days after infarction, these groups reported the prolonged presence of activated microglia in MCA as well as PCA territory infarctions. Similarly, Price et al. (2006) demonstrated elevated [^{11}C]PK11195 binding potential in patients scanned 7 to 14 and 25 to 30 days after stroke, with only a small increase in the first 3 days post ictus. The first study to bridge the gap between human PET studies and in vitro autoradiography in animal models was performed by Rojas et al. (2007), who have used [^{11}C]PK11195 and [^3H]PK11195 in rats 4 and 7 days after MCAO with PET and autoradiography. Both techniques detected a similar pattern of PBR overexpression, mostly limited to the infarcted area, although even at these relatively short times some remote increases were noted. Using longer survival times and a fully quantitative approach, we have been able to show significant increases in PBR in regions not involved in the infarct area as defined on MRI and histology. These results, too, are in line with the relevant human stroke literature showing the increased PK11195 signal spreading to remote and contralateral regions in the subacute, rather than acute phase after ischemia (Pappata et al., 2000; Gerhard et al. 2000, 2005). Unlike neuroinflammation, the effect of focal ischemia on NMDA receptors has not been investigated in either humans or animal models, although bilateral reductions in NMDAR density and gene expression were documented in the aftermath of global forebrain ischemia (Ogawa et al., 1991; Dalkara et al., 1996; Liu et al., 2007) and traumatic brain injury (Miller et al., 1990; Sihver et al., 2001; Biegon et al., 2004).

Although neuroinflammation and loss of NMDAR show similar persistence over time in this model, the differences in relative regional intensity and lateralization between the two phenomena suggests that neuroinflammation does not cause the reduction in NMDAR, although the loss of NMDAR may be locally augmented by neuroinflammation. The mechanisms underlying loss of NMDAR after MCAO are not fully understood, but the regional characteristics of this phenomenon as well as published observations from other models of brain injury may offer some clues. In the vicinity of the lesions as well as in remote non-infarcted regions where no cell loss occurs, most notably the contralateral hemisphere, receptor down regulation and reduced gene expression are likely to occur in response to the high levels of agonist (glutamate) released during ischemia in the terminal fields of damaged or hyper-excited neurons (e.g. Biegon et al., 2004; Ogawa et al., 1991). Another possible mechanism for loss of NMDAR binding is NMDAR internalization in response to amyloid beta release, which was reported in MCAO rats (Li et al., 2009; Snyder et al., 2005). This process may be augmented by neuroinflammation in regions where neuroinflammation and NMDAR coexist, since activated glia are known to release glutamate in addition to other mediators (Bezzi et al., 2001). In fact, we have found that pure neuroinflammation (injection of LPS into the cisterna magna) results in large reductions of NMDAR density in many brain regions involved in cognitive function (Biegon et al., 2002) with no apparent cell loss. Down regulation of NMDAR by subtoxic levels of NMDA may even function as a neuroprotective response against glutamate neurotoxicity in regions where no cell loss occurs (Zhu et al., 2005). While possibly protecting neurons from

excitotoxic death, this mechanism may still result in functional deficits in the performance of tasks requiring NMDAR activation.

In infarcted regions, loss of cells expressing NMDAR due to direct excitotoxicity augmented by neuroinflammatory cytokines and reactive oxygen species is highly likely to contribute further to loss of NMDAR (Bal-Price et al., 2001; Bezzi et al., 2001; Peters et al., 1998; Schroeter et al., 2009). Such an additive effect may be responsible for the greater loss of NMDAR in the ipsilateral striatum when compared by paired t-test to the contralateral striatum of the same MCAO animals, as reported here.

We found NMDA receptor density decreased bilaterally in several regions which are important for cognitive function. These results are similar to prior findings reported by us and others, demonstrating bilateral NMDAR reduction in response to unilateral head injury (Biegion et al. 2004; Miller et al., 1990; Sihver et al., 2001). These findings support the notion that persistent neuroinflammation and even more importantly, persistent and widespread loss of NMDAR may contribute to cognitive and other neurological deficits in stroke patients which can not be localized to the site of infarction (diaschisis). Further experiments are needed to explore the relationship between NMDAR loss in the reported and additional brain regions (e.g. hippocampus) and cognitive performance in MCAO rats; as well as the possible effect of pharmacological manipulation of NMDAR (Yaka et al., 2007) and the involvement of changes in NMDAR subunit composition.

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Fig. 1.

MR images of a rat brain 24 hrs after transient focal ischemia

A = T₂ Weighted (T₂W) and B = Diffusion Weighted (DW) magnetic resonance images of 0.77mm thick slices, from the most anterior (left) to most posterior (right) aspect of the lesion (hyperintense area). CG = Cingulate, FR = Frontal Cortex, ACC = Accumbens, PIR = Piriform Cortex, PAR = Parietal Cortex, STR = Striatum, VP = Ventral Pallidum, SI = Substantia Innominata

Fig. 2.

Ischemia, neuroinflammation and NMDAR distribution 14 days after MCAO.

A = Infarction (hyperintense area) shown by T₂W-MRI, B = cresyl violet stained section C = Distribution of PBR labeled with [³H] PK11195, D = Distribution of NMDAR labeled with [³H] MK801, E = Non-specific (NS) binding of [³H] PK11195 in the presence of excess unlabeled PK11195, same anatomical level as A-D; F = Distribution of PBR labeled with [³H] PK11195 in a control animal, G = Distribution of NMDAR labeled with [³H] MK801 in a control animal.

Non-specific binding of [³H] MK801 (not shown) in control and stroked animals was low and homogeneous, similar in appearance to the non specific binding of [³H] PK11195. Autoradiograms were pseudocolored using the rainbow spectrum, Max = Maximum and MIN = Minimum. Note the intense but variable increase in PBR in and around the infarct and the relatively symmetrical decrease in NMDAR.

Fig. 3.

Ischemia, neuroinflammation and NMDAR distribution 30 days after MCAO.

A = Infarction (hyperintense area) shown by T₂W-MRI, B = cresyl violet stained section C = Distribution of PBR labeled with [³H] PK11195, D = Distribution of NMDAR labeled with [³H] MK801, E = Non-specific binding of [³H] MK801 in the presence of excess unlabeled MK801, same anatomical level as A-D. Autoradiograms were pseudocolored using the rainbow spectrum, Max = Maximum and MIN = Minimum.

Normal distribution patterns of PBR and NMDAR are illustrated in figure 2 above. Note the presence of atrophy in the ipsilateral hemisphere at this late time point (enlarged ventricle and smaller area of the ipsilateral hemisphere), with a persistent intense increase in PBR and persistent decrease in NMDAR which is most pronounced in areas of the ipsilateral cortex where PBR density is highest.

Table 1

Brain Regions	Control			Stroke			Ratio L/R Stroke
	Left	Right	Left (Ipsi)	Right (Contra)	Ratio L/R Control	Ratio L/R Stroke	
Accumbens	1.85 ± 0.40	1.85 ± 0.28	2.43 ± 0.8*	2.12 ± 0.5	0.99 ± 0.1	1.14 ± 0.2	
Caudate-Putamen	1.35 ± 0.40	1.31 ± 0.4	4.86 ± 2.94 ^a	1.56 ± 0.37	1.02 ± 0.06	3.2 ± 2.0	
Cingulate	3.47 ± 0.51	3.5 ± 0.40	3.84 ± 0.59	3.77 ± 0.52	0.99 ± 0.05	1.02 ± 0.05	
Frontal Cortex	2.4 ± 0.30	2.37 ± 0.19	2.72 ± 0.46 [#]	2.66 ± 0.44 [#]	1.01 ± 0.06	1.02 ± 0.04	
Lateral Septum	2.22 ± 0.72	2.28 ± 0.55	3.09 ± 0.86 ^a	2.69 ± 0.64	0.96 ± 0.1	1.15 ± 0.15	
Medial Preoptic Area	2.76 ± 1.5	2.56 ± 1.11	3.35 ± 1.40	2.78 ± 1.08	1.11 ± 0.3	1.2 ± 0.14	
Parietal Cortex	2.6 ± 0.46	2.63 ± 0.37	3.94 ± 2.09 ^a	2.9 ± 0.45	0.98 ± 0.05	1.35 ± 0.65	
Piriform Cortex	1.97 ± 0.62	1.88 ± 0.41	3.15 ± 1.3 *	2.13 ± 0.50	1.03 ± 0.12	1.48 ± 0.49	
Substantia Innominata	1.66 ± 0.68	1.44 ± 0.66	3.8 ± 2.68*	1.48 ± 0.98	1.26 ± 0.4	3.28 ± 2.2	
Ventral Pallidum	1.83 ± 0.76	1.81 ± 0.73	2.88 ± 1.13*	1.79 ± 0.52	1.02 ± 0.1	1.63 ± 0.5	

Effect of MCAO on regional specific binding of the microglial marker [³H] PK11195

Results are Mean ± SD of PBR specific binding, expressed in cpm/mm², measured in 9 control and 17 MCAO animals killed 2 weeks (N=4) or 30-40 days (N=13) after occlusion. Non-specific binding assessed in the presence of excess unlabeled PK11195 was subtracted from all measurements. Values from regions involved in MCAO-related infarction are in bold. Left = Left hemisphere, Right = Right Hemisphere, Ipsi = ipsilateral to lesion, Contra = Contralateral, L/R=ratio of left hemisphere value to right hemisphere value. A three way ANOVA revealed highly significant effects of region, surgery and side (p<0.0001) and significant interaction terms. Subsequent post hoc analyses were performed using Fisher's PLSD test.

* p<0.05 compared to control (mean of both hemispheres).

[#] 0.05< p<0.1 compared to control (mean of both

^a p<0.05 compared to contralateral hemisphere hemispheres)

Table 2

Brain Regions	Control		Stroke		Ratio L/R Control	Ratio L/R Stroke
	Left	Right	Left (Ipsi)	Right (Contra)		
Accumbens	1.41±0.39	1.53±0.44	1.09±0.44 [*]	1.17±0.32 [#]	0.92 ± 0.04	0.92 ± 0.18
Caudate-Putamen	1.32±0.31	1.37±0.34	1.01±0.22[*]	1.16±0.20 ^{#,a}	0.97 ± 0.05	0.88± 0.14
Cingulate	2.5±0.3	2.48±0.3	2.36±0.31	2.34±0.34	1.0 ± 0.04	1.01 ± 0.04
Frontal Cortex	2.3±0.27	2.31±0.3	1.98±0.41[*]	2.07±0.35 ^a	0.99 ± 0.04	0.95± 0.06
Lateral Septum	1.6±0.63	1.63±0.6	1.16±0.26 [*]	1.15±0.28 [*]	0.98 ± 0.09	1.02 ± 0.12
Medial Preoptic Area	0.67±0.19	0.59±0.2	0.48±0.25	0.49±0.26	1.2 ± 0.24	0.98 ± 0.23
Parietal Cortex	2.38±0.28	2.42±0.3	1.89±0.50[*]	2.07±0.3 ^{#,a}	0.99 ± 0.09	0.91 ± 0.13
Piriform Cortex	1.55±0.15	1.55±0.2	1.38±0.40	1.43±0.38	1.0 ± 0.12	0.96 ± 0.08
Substantia Innominata	0.9±0.3	0.85±0.4	0.5±0.23 [*]	0.51±0.27 [*]	1.2 ± 0.6	1.02 ± 0.3
Ventral Pallidum	0.98±0.4	0.98±0.4	0.68±0.36 [#]	0.63±0.33 [*]	1.03 ± 0.24	1.13 ± 0.3

Effect of MCAO on regional specific binding of [³H] MK801 to NMDA receptors

Results are Mean ± SD of [³H]MK801 specific binding, expressed in cpm/mm², measured in 9 control and 17 MCAO animals killed 2 weeks (N=4) or 30–40 days (N=13) after occlusion. Non-specific binding assessed in the presence of excess unlabeled MK801 was subtracted from all measurements. Values from regions involved in MCAO-related infarction are in bold. Left = Left hemisphere, Right = Right Hemisphere, Ipsi = ipsilateral to lesion, Contra = Contralateral, L/R=ratio of left hemisphere value to right hemisphere value. A three way ANOVA revealed highly significant effects of region and surgery (p<0.0001) but no effect of side and no region x side interaction. . Subsequent post hoc analyses were performed using Fisher's PLSD test and paired t-test.

^{*} p<0.05 compared to control.

[#] 0.05< p<0.1 compared to control

^a p<0.05 compared to ipsilateral hemisphere by paired t-test.