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Focal stroke in the barrel cortex of rats enhances ipsilateral response to vibrissal input

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Short communication

Abstract. Brain injury triggers spontaneous plasticity, often resulting in considerable restoration of function. To investigate mechanisms of this compensatory plasticity we followed changes in the brain's pattern of activation evoked by stimulation of vibrissae, after a focal cortical stroke which destroyed the cortical representation of vibrissae, the barrel cortex. The pattern of brain activation was visualized with [^{14}C]-2-doexyglucose (2DG) autoradiography in rats 7 days after photothrombotic stroke. During isotope incorporation, vibrissae contralateral to stroke were stimulated. In control rats this stimulation activates the barrel cortex and the second somatosensory cortex in the contralateral hemisphere. Seven days after stroke in the barrel cortex, significant increases in activation were found in ipsilateral, uninjured hemisphere in the barrel cortex and anterior vibrissae representation, and also in regions not specifically connected to vibrissae stimulation, such as motor and auditory cortex. Shortly after cortical stroke, the intact hemisphere shows higher metabolic activation in several cortical regions, possibly due to abnormal interactions with the injured hemisphere.

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In most cases of stroke-induced lesions and other brain injuries some spontaneous recovery of impaired function can be observed. This recovery is due to compensatory plasticity; however, mechanisms of this plasticity are still not well understood and some data are conflicting. Human brain imaging data from patients with stroke in sensorimotor region showed ipsilateral activation after stimulation of paretic limbs (see Cramer 2004). In most cases, activation of the uninjured hemisphere was seen shortly after stroke (Binkofski and Seitz 2004), but cases in which it was much longer lasting were also reported (Feydy et al. 2002). In an animal miniPET investigation, alterations of metabolism in the uninjured hemisphere were not observed (Carmichael et al. 2004). Rema and Ebner (2003) lesioned barrel cortex in one hemisphere in rats and found a long lasting decrease of responsiveness and susceptibility to plastic changes in the contralateral barrel cortex. The changes in activity of the intact hemisphere may be involved in the recovery processes. Another factor in the process of recovery is altered responsiveness of the periinfarct area. Electrophysiological experiments on animals showed that considerable remapping of receptive fields occurs in somatosensory and motor cortex after lesions damaging part of the sensory or motor body map. After the focal infarct in the motor cortex a shift of hand representation in monkeys was observed with the use of microstimulation mapping at long post-stroke delays (Nudo and Milliken 1996). The electrophysiologically registered enlargements of receptive fields in the spared somatosensory representation in the neighborhood of the damaged cortex were observed in hand representation in the monkey (Xerri et al. 1998) and hind paw representation in the rat (Coq and Xerri 1999). Seven days after parietal cortex thrombosis in rats the somatosensory receptive field enlargement appeared in both injured and uninjured hemisphere (Reinecke et al. 2003).

We reexamined the question of effects of stroke in the barrel cortex of rats upon the pattern of functional activation of somatosensory cortex by stimulation of vibrissae. The vibrissae-to-cortical barrels pathway of rats is a convenient model for plasticity research and also for studies investigating the results of cortical stroke (Carmichael et al. 2001, 2004, Dietrich et al. 1986a,b, 1994a,b). Decreases in glucose metabolism after unilateral infarct in the somatosensory barrel cortex were observed around the site of the lesion, in the striatum, thalamus and trigeminal nuclei, and also in the frontal, cingulated and auditory cortex, and the hippocampus, within the first 5 days after stroke in rats (Carmichael et al. 2004, Dietrich et al. 1986a). Decreases of response to sensory stimulation of vibrissae were noted throughout the trigeminal pathway (Dietrich et al. 1986b) after fluid percussion injury of parietal cortex or transient forebrain ischemia. However, a detailed neuroanatomically identified pattern of activation of cortical regions after focal stroke with damage restricted to the barrel cortex was not investigated. In the present study we examined with [14C]2-deoxyglucose autoradiography the metabolic pattern of brain activation in response to unilateral stimulation of vibrissae after focal stroke damage in their cortical representation, the barrel field. Maps of cortical somatotopy, obtained with cytochrome oxidase (CO) histochemistry were compared with 2DG autoradiograms to obtain exact localization of the metabolically active regions.

Male Wistar rats weighing about 230 g were used in the experiment. There were 5 control and 4 experimental rats. Throughout the whole experiment animals were housed separately in plastic cages in 12 h light-dark cycle at a temperature approximately 20°C with free access to food and water. All experimental protocols were approved by the Ethics Commission of the government of Poland and were in accordance with The European Communities Directive (86/609/EEC).

Photothrombotic stroke was conducted under 3% isoflurane/air mixture anesthesia. A catheter was inserted into the rat's femoral vein and animals were then placed in a stereotaxic frame. An incision was made down the midline of the head and the skull surface exposed and cleaned. The right temporalis muscle was detached from the bone crest and the crest removed. A fiber-optic bundle with a 3 mm aperture was placed stereotaxically with its light center 2.5 mm posterior to the bregma and 5.5 mm lateral to the midline, and at an angle of 30° over the right barrel field. Bengal Rose in a concentration of 10 mg/ml was injected intraveneously through the catheter and the skull surface was illuminated for 20 min. Afterwards the catheter was removed and all postsurgical wounds were stitched.

Seven days after the stroke a catheter was placed in the femoral vein under isoflurane narcosis. Animals were restrained by taping to a block and the whiskers

ipsilateral to the stroke were cut close to the skin. About 30 minutes after awakening [^{14}C]2-deoxyglucose (7 μCi/100 g) was injected through the catheter. Whiskers contralateral to the stroke were manually stimulated with a brush for 45 minutes at a frequency of about 2 Hz. After whiskers stimulation rats were killed by intravenous Vetbutal injection and perfused with 4% paraformaldehyde for about 5 minutes. Then the brain was removed and the two hemispheres flattened between glass slides and frozen in heptane at -70°C and stored at that temperature.

Hemispheres were cut into 40 mm tangential slices on a cryostat at -20°C. Sections were collected alternately on specimen slides and coverslips. Sections on coverslips were immediately dried and exposed to X-ray film for three weeks with a set of [^{14}C] standards. After the films were developed, the sections were Nissl stained for identification of barrels and cortical layers. Remaining sections were stained for cytochrome oxidase activity.

Autoradiograms were analyzed by a computer image analysis program (MCID). We measured local optical density (LOD) in the barrel field (BF), hind paw representation area (HP), anterior vibrissae representation (AV) secondary somatosensory cortex (SII), the auditory cortex (Au), motor cortex (MI) and frontal cortex (FR) (Fig. 1). Fibers surrounding the hippocampal formation, taken from the last sections of each hemisphere were used as expressed as an indexed ratio reference structure. The results are expressed as an indexed ratio of the examined area LOD to a reference structure LOD. The statistical significance of differences between the groups was tested using Mann–Whitney nonparametric two-tailed test and the difference between hemispheres was tested with paired student t test.

Histological verification of stroke – induced lesion was based on cytochrome oxidase and Nissl staining and precisely established the extent of cortical damage. Lesion in the barrel cortex damaged all cortical layers without touching the underlying white matter. There was some variability in the size and position of the lesion. The mean areal extent of the lesion was 3.5 mm^2, with the range from 3.0 to 4.6 mm^2. One to five barrels were usually spared in random position at the edges of the barrel field (Fig. 1A).

In the control rats unilateral stimulation of all mystacial vibrissae resulted in an 88 ± 30% increase of 2DG uptake in the contralateral barrel cortex ($P \leq 0.01$)

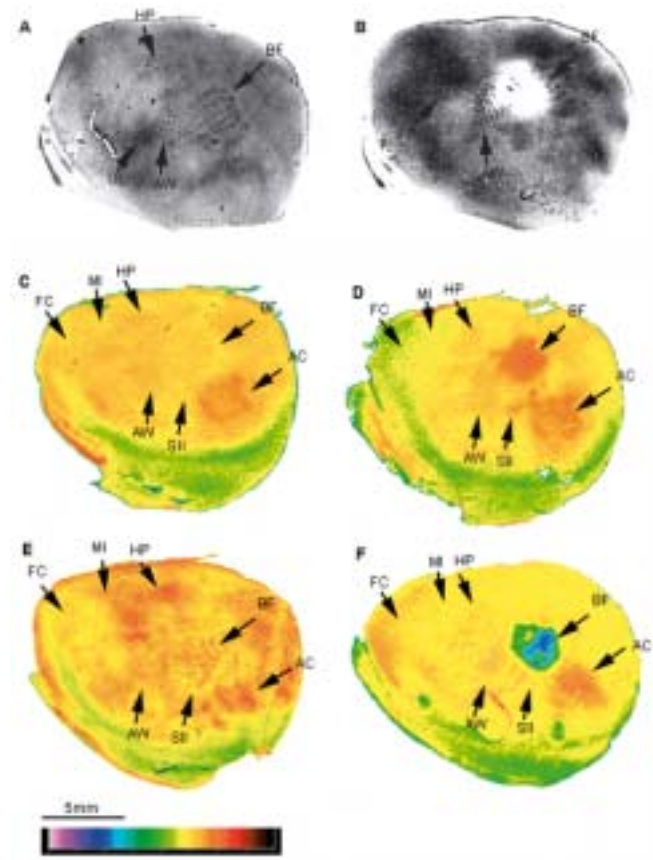

Fig.1. (A) Localization of cortical body representation on section across layer IV of rat brain stained for CO histochemistry; (B) stroke-induce lesion in the barrel field; (C–F) pseudo-colored autoradiograms of 2DG incorporation into brains of control and stroked rats. Section across layer IV. Red = high 2DG uptake. (C) control rat, autoradiogram from hemisphere ipsilateral to stimulated vibrissae. Activation visible in AC. Note low activation of the barrel field. (D) control rat, autoradiogram from hemisphere contralateral to stimulated vibrissae. Activation in BF, SII, AC. (E) Rat 7 days after stroke, intact hemisphere ipsilateral to stimulated vibrissae. Activation of BF, AV, AC, MI. (F) Rat 7 days after stroke, hemisphere with stroke contralateral to stimulated vibrissae. Activation of AC. (G) Autoradiogram with white matter taken as a reference structure (RS).
Abbreviations: (BF) Barrel field; (AC) auditory cortex; (SII) second somatosensory cortex; (AV) anterior vibrissae; (FP) front paw; (HP) hind paw; (UL) upper lip; (FC) frontal cortex; (MI) motor cortex; (RS) reference structure.

and a 52 ± 25% increase in the secondary somatosensory whiskers representation ($P \leq 0.01$) as compared to the homologous region in the ipsilateral hemisphere. No differences in activation of the other examined areas (anterior vibrissae, hind paw representation,

auditory cortex) between the two hemispheres were observed (Fig. 1B).

One week after the stroke we observed a statistically significant increase in 2DG uptake in several areas of the intact hemisphere, compared to the same areas in intact control rats (barrel field +147 ± 22%, $P<0.05$, anterior vibrissae +132 ± 15%, $P<0.05$, auditory cortex +113 ± 11%, $P<0.05$, motor cortex +139 ± 11%, $P<0.05$). In the stroked hemisphere the 2DG uptake was greatly decreased at the site of the stroke, (Fig. 1C), but did not change significantly in other examined areas, which had the same 2DG uptake level as in the control rats (Fig. 2).

Our results demonstrate the changed pattern of brain activation 7 days after a focal stroke in the barrel cortex in rats. We compared 2-deoxyglucose (2DG) incorporation in the selected regions of somatosensory cortex (the barrel cortex, anterior whiskers, hind paw and secondary somatosensory representations) and in the control auditory motor and frontal cortex during stimulation of the whiskers providing afferent input to the damaged barrel field. The 2DG incorporation is proportional to metabolic processing of the brain and thus to its activation. We provide evidence that the lesion in one primary somatosensory area results in increased activation of sensory and motor regions of the undamaged hemisphere.

These results expand the previously reported data on ipsilateral activation of cortex after stimulation of paretic limbs (Cramer 2004). Several studies on stroke patients find that early after stroke the intact, contralateral MI cortex shows activation, which subsides with longer post stroke time (e.g. Jaillard et al. 2005, Nelles et al. 1999). There is also data showing that ipsilateral activation may be persistent (Feydy et al. 2002). Experimental animal studies give detailed information only for short post-stroke survival times and bring conflicting results. Metabolic mapping after focal cortical damage in the parietal cortex produced by photochemical lesion in rats showed that somatosensory cortex of the intact hemisphere had decreased resting glucose utilization for 3 days after stroke (Dietrich et al. 1986a) and returned to normal values at day 5. This deficit was not observed after focal cortical lesion by artery ligation, probably because of methodological problems (animal PET). Cortical damage by fluid percussion found no changes in stimulus-induced glucose uptake in the uninjured hemisphere 24 hours after injury (Dietrich et al. 1994b). In fMRI investigations, the spread of activation was observed during electrical stimulation of ipsilateral forelimb in the hemisphere contralateral to the infarct 3 and 14 days after electrocoagulation of the middle cerebral artery (Dijkhuizen et al. 2001).

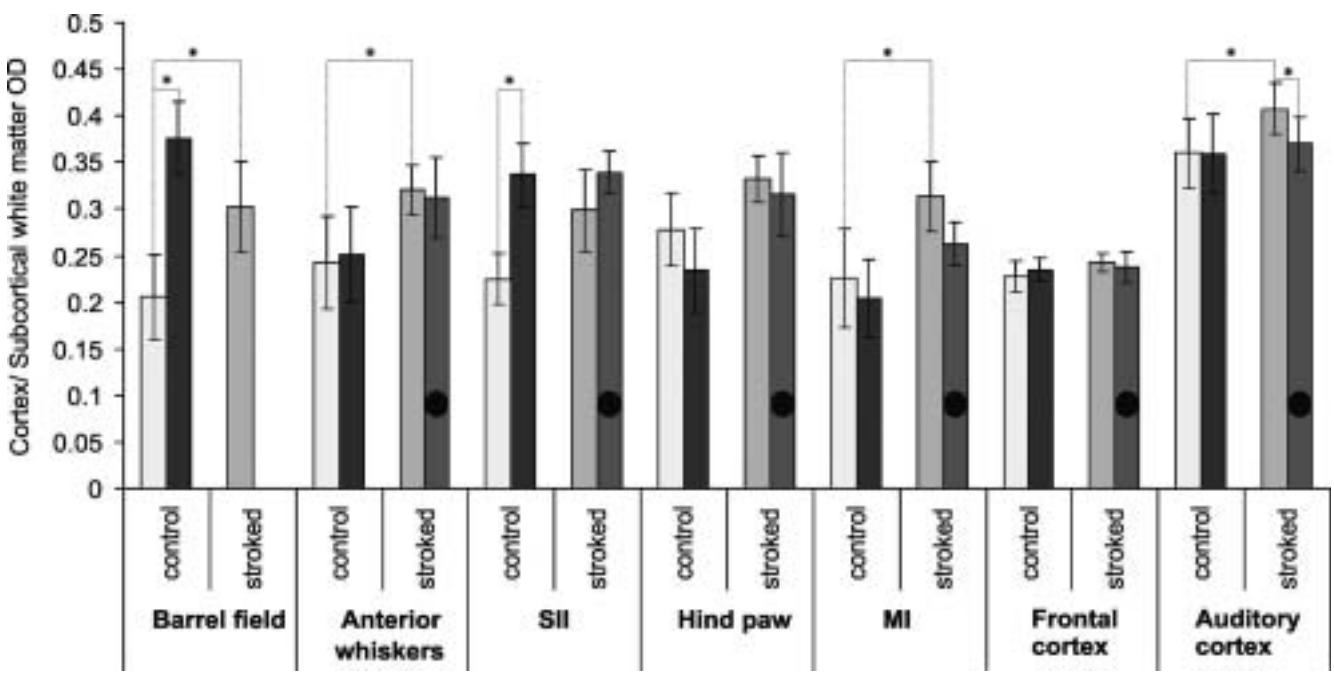

Fig.2. 2DG uptake into cortical areas of control and stroked brains. Results expressed as an indexed ratio of cortical structure/subcortical white matter optical density. Error bars show SD; *$P<0.05$.

In our study, the increased activation of the barrel field in the uninjured hemisphere may be due to input from the parts of the damaged barrel field spared by the stroke. In normal rats, weak activation of ipsilateral barrel cortex, mediated by fibers of the corpus callosum, is observed electrophysiologically (Pidoux and Verley 1979), but not with 2DG mapping (see Fig. 1B). Under conditions of brain injury, this ipsilateral input may operate more efficiently. Interestingly, significantly increased 2DG uptake was also seen in the cortical representation of anterior vibrissae, which were not stimulated during 2DG mapping. Activation of other examined somatosensory areas, such as hind paw projection or SII, also showed some tendency to increase (Fig. 2) as if unspecific effects of lesion in the other hemisphere upregulated their activation level.

We found that 7 days after stroke the 2DG incorporation in the barrel cortex of contralesional hemisphere was 47% higher compared to barrel fields of control rats, three times as much as in the area of auditory cortex of these brains. This result contradicts the data of Rema and Ebner (2003) who found that unilateral lesions of the barrel cortex depress responses to whisker stimulation and spontaneous activity in the intact barrel field, tested 8 days after surgery. In their experiments, however, vibrissae ipsilateral to the lesion were stimulated while we activated the contralateral vibrissae. The use of a different technique does not explain the discrepancy in results. 2DG uptake reflects primarily subthreshold activity of brain tissue, but subthreshold activity results in spiking, and good correlations between 2DG uptake and spiking activity were demonstrated in several studies (Juliano and Whitsel 1987). However, under special conditions created by brain injury, this probably involves rerouting of afferent sensory information in the absence of their primary cortical targets and activation of long polysynaptic pathways and this correlation may be different. Also, in the Rema and Ebner study (2003) the rats were anesthetized, and anesthesia strongly affects the polysynaptic and subthreshold activity.

Increased activation of the uninjured hemisphere was observed not only in somatosensory cortex, but also in motor and auditory cortex. Similar activations of cortex not directly affected by stroke were observed by Binkofski and Seitz (2004). This contralesional general neuronal hypersensitivity could be a result of weakened inhibition from the lesioned hemisphere due to injury and waves of spreading depression triggered by the stroke (Dietrich et al. 1994a). The cortical area remote from the stroke, the frontal cortex, did not show enhanced activation.

In conclusion, one week after focal cortical stroke, a pronounced imbalance between activation of the brain hemispheres was observed, with the activation of the uninjured hemisphere being greater than in the control situation and dominating over the hemisphere damaged by the stroke.

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