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## Post-Ischemic Housing Conditions Influence On Gene Transcription And Translation After Permanent Focal Brain Ischemia In Rats

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POST-ISCHEMIC HOUSING CONDITIONS INFLUENCE  
ON GENE TRANSCRIPTION AND TRANSLATION AFTER  
PERMANENT FOCAL BRAIN ISCHEMIA IN RATS

By

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Lund, 2004



**LUND**  
UNIVERSITY

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*To my family  
I dedicate this thesis*

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## SUMMARY

Enriched environment (EE) housing significantly ameliorates neurological deficits induced by cortical brain ischemia without changing infarction size, suggesting that EE-related functional benefits are associated with neuronal plasticity events in the remaining tissue. Brain-derived neurotrophic factor (BDNF), nerve growth factor-induced gene A (NGF-A) and corticosteroid receptors (mineralocorticoid receptor, MR; glucocorticoid receptor, GR) have been demonstrated to be involved in brain plasticity. The purpose of this thesis was to determine if post-ischemic housing conditions had a significant effect on transcription and/or translation of BDNF, NGF-A and corticosteroid receptors. We found that BDNF gene was down regulated in EE-housed rats when compared to the rats housed in standard cages at 2~12 days after cortical brain ischemia in peri-infarct cortex, contralateral cortex and bilateral hippocampus. The protein level of BDNF in the ipsilateral frontal cortex was lower in the EE-housed rats than the standard environment (SE)-housed rats at 12d postischemia. The mRNA expression of NGF-A showed a similar pattern of BDNF except for an increase at 30 d after induction of brain ischemia in EE-housed rats. Ischemia-induced reduction of GR was prevented in the rats housed in EE condition. There was no difference between EE- and SE-housed animals in MR gene expression. Gene expressions, however, are very complex in housing conditions and postischemia. Further studies are needed to determine whether the EE-related gene transcription and/or translation reported in this thesis are linked with EE-induced functional benefits.

## ORIGINAL PAPERS

This thesis is based on the following papers and their Roman Numerals will refer to the papers in the text:

- I. Zhao LR, Mattsson B, Johansson BB. Environmental influence on brain-derived neurotrophic factor messenger RNA expression after middle cerebral artery occlusion in spontaneously hypertensive rats. *Neuroscience*. 2000;97:177-184.
- II Zhao LR, Risedal A, Wojcik A, Hejzlar J, Johansson BB, Kokaia Z. Enriched environment influences brain-derived neurotrophic factor levels in rat forebrain after focal stroke. *Neurosci Lett*. 2001; 305: 169-172.
- III. Dahlqvist P, Zhao L, Johansson IM, Mattsson B, Johansson BB, Seckl JR, Olsson T. Environmental enrichment alters nerve growth factor-induced gene A and glucocorticoid receptor messenger RNA expression after middle cerebral artery occlusion in rats. *Neuroscience*.1999; 93: 527-535.

## ABBREVIATIONS

ANOVA	analysis of variance
bFGF	basic fibroblast growth factor
BDNF	brain-derived neurotrophic factor
CA	cornu ammonis
CaMKII	calcium/calmodulin-dependent protein kinase II
CNS	central nervous system
DG	dentate gyrus
cDNA	complementary deoxyribonucleic acid
cRNA	complementary ribonucleic acid
EE	enriched environment
ELISA	enzyme-linked immunosorbent assay
fMRI	functional magnetic resonance imaging
FDA	food and drug administration
GABA	$\gamma$ -aminobutyric acid
GAP	growth associated protein
GDNF	glial cell line-derived neurotrophic factor
GR	glucocorticoid receptor
IEGs	immediate early genes
IGF	insulin-like growth factor
LDP	long-term depression
LTP	long-term potentiation
MCAo	middle cerebral artery occlusion
MR	mineralocorticoid receptor
mRNA	messenger ribonucleic acid
NGF	nerve growth factor
NGFI-A	nerve growth factor-induced gene A
NMDA	N-methyl-D-aspartate
NT-3	neurotrophin-3
NT-4/5	neurotrophin-4/5
NT-6	neurotrophin-6
PBS	phosphate buffered saline
PET	positron emission tomography
rtPA	recombinant tissue plasminogen activator
SE	standard environment
SGZ	subgranular zone
SHR	spontaneously hypertensive rat
SVZ	subventricular zone
TMS	transcranial magnetic stimulation
Trk B	tropomyosine related kinase B



# INTRODUCTION

## Stroke

Stroke remains a leading cause of disability and the third cause of death worldwide [179]. In the Caucasian population about 80% of strokes are diagnosed as ischemic strokes [179]. In the United States, each year about 700,000 people suffer a stroke, resulting in nursing care costs of more than \$ 50 billion [6]. In 1996, the US Food and Drug Administration (FDA) approved a drug for thrombolysis called recombinant tissue plasminogen activator (rtPA). This has been the only FDA-approved treatment for stroke patients in the acute phase [68]. Although intravenous administration of rtPA has shown to be of benefit with a minimum or no disability after a 3-month interval of the stroke onset the treatment window is as narrow as 3 hours after stroke symptom onset, and the treatment risks hemorrhage and potential neurotoxin effects [68,140]. Many stroke patients have already missed the critical time—first 3 h after stroke onset when they are transferred to the hospital and are diagnosed. Physiotherapy and occupational therapy may be of some help to obtain a limited degree of functional improvement; however, nearly half of stroke survivors suffer from disability and become dependent [179].

## Spontaneous functional recovery after stroke

Despite permanent damage in the brain, the spontaneous functional recovery occurs days, weeks, months and years after stroke onset [87]. Motor recovery in stroke patients happens predominantly in the initial weeks to the first three months; thereafter, it continues at a slow pace throughout the first year [26]. In the first hours to the first few days, the recovery may be due to resolution of cerebral edema and restoring neuron function in the ischemic penumbra by reperfusion. Later, beyond this acute phase, responsibility for spontaneous functional recovery is likely due to brain plasticity [60, 26, 179]. In the past decades, numerous clinical studies supported the notion of functional reorganization in stroke patients. Using noninvasive brain imaging techniques such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), transcranial magnetic stimulation (TMS) and magnetoencephalography (MEG), investigators found that functional activities were captured in the intact tissue of both the affected and nonaffected hemispheres, suggesting that damaged neuronal function was taken over by intact neurons. This may be the case for functional recovery in stroke patients [60, 80,58].

## Brain plasticity

Brain plasticity is an inherent capability of the brain for functional and structural changes and adjustments in response to external stimuli and internal injury. The brain can be plastically modified throughout the life span.

Half a century ago, Donald Hebb proposed a concept that neuronal connections could be remodeled by experience [65]. Since then, a vast amount of evidence has demonstrated that cortical maps, neuroanatomy and neurochemistry are dynamically changed by environment stimulation, training, learning, experience and neuron system injury [18, 80, 157, 181]. Using

functional magnetic resonance imaging (fMRI) technique, Karni and co-workers [85, 86] reported that training the non-dominant hand to do finger-to-thumb opposition movement in adult humans increased the area of primary motor cortex. After amputation, somatosensory representation of the digit underwent translocation in adult monkeys. The representations of adjacent intact digits and palmar surfaces expanded topographically to occupy the deafferented cortex area [118]. Housing intact rats in an enriched environment increased cortical acetylcholinesterase activity [12], and also increased nerve growth factor, brain-derived neurotrophic factor and neurotrophic factor-3 in the cerebral cortex and in the basal forebrain [71].

Brain plasticity may also include neurogenesis [139]. It has been documented that neurogenesis occurs in the adult brain including in humans throughout life [42, 50]. In rodents, neurogenesis has been documented in two neurogenetic regions with high-density cell division: the subventricular zone (SVZ) [150, 151], and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus [133]. Recently accumulating evidence has shown that the proliferation and differentiation of neural progenitor cells in the subgranular zone (SGZ) and subventricular zone (SVZ) respond to environmental changes, learning and memory, and brain damage. It has been reported that more neurons are observed in the brains of songbirds when learning songs [54] and when marsh tits store and retrieve food [136]. Nilsson and co-workers observed an increase of neurogenesis in the intact rats housed in EE condition, which accompanied with an improved spatial learning and memory [124]. In intact animals, environmental enrichment housing condition reduces spontaneous apoptotic cell death in the rat hippocampus [187] and increases survival rate of newborn neurons in the dentate gyrus SGZ [90]. Accumulating evidence shows that brain ischemia induces neural progenitor cell proliferation in SGZ and SVZ, and that most of dividing cells differentiate into neurons [74, 8, 74, 165, 170]. Ischemia-induced neurogenesis has been proposed to be associated with functional restoration [8].

### **Brain plasticity: possible mechanisms for spontaneous functional recovery after stroke**

The fact that functional improvement is possible despite permanent tissue damage indicates that changes must take place in the intact remaining brain [15]. The cellular mechanism underlying brain plastically changing in response to stroke is currently uncertain. However, several possibilities have been suggested.

#### Diaschisis

Diaschisis is the phenomenon that presents immediate depressions of neuronal function in the brain regions remote from, but anatomically connected to the damage areas due to the loss of the excitatory afferent inputs. Von Monakow, a Russian neurologist, first described Diaschisis in 1914 [75]. The beneficial effects of the drugs that increase noradrenergic activity [45] or block GABAergic activity [64] after brain lesions support the concept of remote functional depression. Combinations of amphetamine and physical therapy have shown better functional recovery than physical therapy alone [60]; however, there is no functional effect when amphetamine is administered to the stroke rats that are housed in an enriched environment [78]. Interestingly, enriched environment-housed rats that were treated with diazepam, a GABAergic agonist, did not worsen functional outcome [Zhao and Johansson unpublished observation]. Does diaschisis just mean remote functional depression? However, in a time course study of transhemispheric

diaschisis, Andrews [7] found a different observation. The electrical activity in the contralateral intact hemisphere was increased at the late stages after unilateral brain infarction. Therefore, Andrews hypothesized that diaschisis might present a loss of remote inhibition rather than a loss of remote excitation. Other evidence further confirms the concept of remote functional excitation. Qu et al., [143] reported that hyperexcitability existed in the ipsilateral (lesioned) cortex surrounding to the infarction. This was observed at one to three days after focal brain ischemia and peaked at 28 d after ischemia. Lesion induced-hyperexcitability has also been found in the contralateral (intact) hemisphere after brain ischemia [16, 149]. Redecker et al., [148] reported that GABA<sub>A</sub> receptor subunits were bilaterally reduced in the cortex, hippocampus and thalamus after photothrombotic cortical infarction. The reduction was prevented by administration of MK 801, a NMDA receptor antagonist. Taken together, the concept of diaschisis may be presented as the brain areas that remote but anatomically connect to the lesion areas are undergoing functional depression and/or hyperexcitability. A brain damage event results in remote function of either depression or hyperexcitability. Which of these occurs depends upon how much time has passed after the brain injury, as well as the size, the location and the type of the lesion [7]. Based on this view, any pharmacological interventions may give positive or negative results, depending on the timing of administration and the situation of the lesion's size, location and type [80]. However, functional restoration may be partially related to overcoming a diaschisis and a re-balance of the abnormality between excitation and inhibition.

#### *Physiological and anatomical changes in neuronal network*

Some of the processes of functional reorganization after brain ischemia can happen very quickly, while other processes occur more slowly. It has been reported that anatomical modifications in synapses are extremely rapid and it happens within seconds [46]. It has been proposed that unmasking previously existing but functionally inactive pathways is relative to rapid changes in cortical maps [73]. Some redundancy of neuronal circuits with parallel pathways performing similar functions may take over the function from the damaged area [26]. A reinforcing of existing neuronal network may also play a role in the recovery process. Although it has been suggested that main functional recovery occurs in the damaged hemisphere [180,49], substantial evidence has shown that ipsilateral pathways in the intact hemisphere may also play a role in functional rehabilitation in stroke patients [60]. Changing excitability of neuronal membrane [61] and strengthening the existing synapses in process as long-term potentiation (LTP) or weakening the existing synaptic transmission as long-term depression (LDP) [66,67] have all been proposed as participants in functional restoration as well. Other processes to consider are anatomical alterations including sprouting new axon terminals and the formation of new synapses [176]. It has been reported that after cortical brain ischemia, an increased density and distribution of growth associated protein-43 (GAP-43) immunoreactivity were observed in the ipsilateral (lesion side) cortex 3~14 days post-ischemia. Synaptophysin immunoreactivity was increased in peri-infarct cortex and the contralateral cortex 14~60 days after induction of cortical brain ischemia. The changes of GAP-43 and synaptophysin were relative to functional recovery after brain ischemia [169]. Finally, substantial evidence shows that astrocytes may also play an important role in synaptic plasticity [82, 177].

However, these recovery processes mentioned above may be operated at different time, and the processes may overlap or be followed by another serially.

Although the spontaneous recovery is often incomplete, it implies that functional reorganization

happens in the intact brain area. Therefore, to facilitate the functional reorganization events may greatly improve functional recovery after brain ischemia [80].

## **Enriched environment and functional improvement**

### *Enriched environment and intact animals*

The experimental concept of an enriched environment was originally conceived by Hebb, who let laboratory rats run free in his house, and found that the problem-solving abilities of the home-housed rats were far advanced when compared to the problem-solving abilities of the littermates that had remained in the laboratory [65]. Housing rats in a large cage with various “play-things” was first designed by Hebb’s students [47], and various modifications have been named as environmental complexity and training [101], enriched environment [156] and enriched condition [157] by Rosenzweig and his colleagues.

As early as 1960, Rosenzweig and co-workers [101] reported that enriched environment induced an up-regulation of cortical acetylcholinesterase activity. Thereafter, many studies have demonstrated that housing intact animals in an enriched environment shows increases in thickness of cortex [39], synaptic strength [48], neurotrophic factors [71], dendritic branching [57], dendritic spines [53], glial cells [5] and neurogenesis in hippocampus [90].

Overall, housing intact animals in an enriched environment increases plastically changing in the brain. Therefore, enriched environment induced-brain changing will probably be of benefit to functional recovery after brain ischemia.

### *Enriched environment and brain ischemic animals*

In 1995, Ohlsson and Johansson reported that postoperative housing in an enriched environment facilitated functional recovery in rats after focal brain ischemia [128]. A later study showed that the functional outcome of social-housed rats (a group of rats housed in a large cage with no equipment) was better than individually housed animals with free access to a running wheel; enriched environment housing showed the best functional recovery of all tested housing conditions [76]. Enriched environment-induced functional benefits also appeared with delayed timing such as 15 days after brain ischemia [77]. Interestingly, the infarction size of the ischemic rats that were housed in standard condition correlated to their neurological deficits; furthermore, the functional benefits of enriched environment were not dependent on their infarction size (56, 76, 77, 128). In agreement with earlier studies, the rats that were tested repeatedly in a number of behavioral tests during 13 post-operative weeks, clearly demonstrated that rats housed in standard environment showed a correlation between lesion size and functional outcome but there was no correlation between lesion and functional outcome in the rats housed in an enriched environment at any tested time points [56]. This is what would be expected environmental enrichment might lead to stimulating effects in the intact remaining tissue. In a series of studies that combine EE and transplantation, grafted fetal cortical tissue in the infarct cavity survived and received afferent neuronal connections from host brain; the functional outcome was better in the rats that received transplantation and were housed in EE than the grafted rats that were housed in standard cages but the functional outcome was not better than the rats housed in EE alone when the transplantation was performed at 3 weeks after brain ischemia [80]. However, when

transplantation was performed at one week after brain ischemia, the rats from an EE with graft resulted in a less thalamic atrophy and exhibited a better functional performance than EE alone [115], although there was no clear afferent in host to graft connections. It was, therefore, proposed that the enriched environment might lead to the production of more trophic factors in the grafts. Consequently, We thought it might be worth to study gene activation in infarcted rats housed in different environments.

At the time when the studies for this thesis were performed, it was already known that brain ischemia was a robust inducer for gene expression during the 24 h after induction of brain ischemia (see paper I). Several earlier papers from the laboratory had shown that environmental enrichment could significantly influence functional outcome [76,77,128]. In addition, previous studies reported above have shown that environmental housing can alter gene expression in intact animals. Therefore, it was reasonable to assume that functional improvement after cortical infarcts would be associated with activations of the genes proposed to be of importance for brain plasticity. The maturation of infarcts has been proposed to be completed 48 hour after ischemia [189], and the functional benefits of EE are not dependent on the infarction size. Based upon these deductions we have focused this thesis on precisely what genes were altered by housing conditions a couple of days after cortical brain ischemia.

### **Brain-derived neurotrophic factor and brain plasticity**

Brain-derived neurotrophic factor (BDNF) was first purified and characterized by Barde and co-workers [9]. Although it is less well known BDNF is an immediate early gene [70], and also belongs to the neurotrophin family that includes nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5) and neurotrophin-6 (NT-6) [106]. As other neurotrophins, BDNF binds to two types of transmembrane glycoproteins: one is the high-affinity tyrosine kinase receptor, tropomyosine related kinase B (trk B), and the other is the low-affinity receptor, p75 [25], which is a common receptor for all neurotrophins. It has been well documented that BDNF promotes neuron differentiation, survival and growth during the development of the nervous system [36]; however, the function of BDNF in adult brain has not been fully understood. BDNF mRNA expression was markedly and transiently up-regulated by various brain lesions such as seizure, traumatic injury, hypoglycemic coma and brain ischemia in adult brain, suggesting a neuroprotective effect of BDNF [107, 108]. Two hour-transient brain ischemia induced an up-regulation of BDNF mRNA expression has been reported to be accompanied by its protein level [95,97]. BDNF that was administered before induction of brain ischemia showed a reduction of infarction size and had a protective effect on CA1 neurons [10,159]. Larsson and co-workers [103] reported that endogenous BDNF protected neurons from global ischemia. In addition to the neuroprotective effect, BDNF has also been proposed to play a role in neuronal plasticity. It has been shown that BDNF increases axonal branching [31], enhances the length and complexity of dendrites of cortical pyramidal neurons [116], and regulates the neural network [24]. Thoenen [174] and Lindholm [106] suggested that BDNF is involved in the neuronal plasticity base of the activity-dependent manner of BDNF synthesis and release. Sensory input regulates BDNF gene expression in the visual cortex [23], and BDNF mRNA expression is up-regulated in somatosensory cortex by mechanical stimulation of whiskers [155]. It has been demonstrated

that BDNF is up-regulated by glutamate via NMDA and non-NMDA receptors, and down-regulated by  $\gamma$ -aminobutyric acid (GABA) via GABA<sub>A</sub> receptors [174]. Interestingly, BDNF increases intracellular calcium concentration, and enhances release of classical neurotransmitters, which is important for the events related to the neuronal plasticity [106]. It has been studied that BDNF enhances the release of glutamate [135]. Long-term potentiation (LTP), a long-lasting enhancement of synaptic transmission following brief repetitive stimulations, is thought to be important for the development of neuronal connections and memory formation. Kang and Schuman [84] reported that BDNF increases the synaptic transmission in adult hippocampus. Hippocampal LTP is impaired in BDNF-knock out mice [99], and restored by recombinant BDNF [137]. It has been documented that BDNF plays a role in unmasking silent synapses [72]. BDNF increases the dendritic branching and enhances synaptogenesis [113, 117, 182]. In intact brain, BDNF triggers neural progenitor cells from proliferation to differentiation [27]. However, in ischemic brain, BDNF has been evidenced to decrease neuronal differentiation [59, 104]. Enriched environmental housing condition increases BDNF gene and protein expression in the intact animals [43, 71]. However, there is much less information available for BDNF in response to environmental enrichment housing after experiment stroke.

### **Never growth factor-induced gene A and corticosteroid receptor in brain plasticity**

Never growth factor-induced gene A (NGFI-A) has been discovered as a transcription factor that rapidly induced by NGF [138]. It is also known as *zif/268*, *Krox24*, *Egr-1* and *TIS8* [152]. NGFI-A belongs to an immediate early gene transcription factor family. Because of the zinc finger motifs in the DNA binding domain, NGFI-A and NGFI-B, NGFI-C, *Egr-2*, *Egr-3* as well as *Nurr1* are all termed zinc finger immediate early genes, in an effort to distinguish these from the *fos/jun* immediate early genes [69]. NGFI-A preferentially binds to the GC-rich sequence 5'-GCGGGGGCG-3' of the genome DNA [20, 28], as a third messenger, links extracellular stimuli to long-term responses by acting target gene expression [173]. The biological function of NGFI-A has been characterized as controlling synaptic plasticity [83], neurite outgrowth [63], wound repair [92], as well as growth control and apoptosis [110]. Synapsin I and synapsin II involved in the docking of synaptic vesicles, neurite outgrowth and synapse maturation are regulated by NGFI-A. [138,172]. LTP is insufficient in NGFI-A heterozygous knock out mice [13]. NGFI-A mediated neural plasticity is dependent on NMDA receptor activity [29]. LTP formation and stabilization are associated with elevated NGFI-A [1]. NMDA receptor antagonist blocks both LTP and NGFI-A mRNA in the hippocampal neurons [29]. NGFI-A gene expression in the visual cortex is visual active dependent [185]. Enrich environment increases the levels of NGFI-A mRNA and protein, which is suggested NGFI-A plays a role in experience-induced neuronal plasticity [131, 141]. Brain ischemia rapidly and transiently increases NGFI-A gene expression in the cortex, hippocampus, striatum and thalamus [69]. Prolonged expression of NGFI-A following brain ischemia leads to apoptotic death [69]. However, it is not known whether NGFI-A gene expression is linked with EE-induced functional recovery after brain ischemia.

Corticosteroid receptors including mineralocorticoid receptor (type I, MR) and glucocorticoid receptor (type II, GR) have been shown to play roles in the learning and memory process [164]. Previous studies revealed that the two receptors co-expressing with NGFI-A, showed an association with anti-depressive drug-related and EE-induced neuronal plasticity in intact rats [121,123, 131]. However, further studies are needed to determine the relationship between the

gene expressions of MR and GR and environmental housing conditions following induction of brain ischemia.

### **Some candidate factors for brain plasticity not included in this thesis**

It has been demonstrated that nerve growth factor (NGF), neurotrophin-3 (NT-3), glial cell line-derived neurotrophic factor (GDNF), insulin-like growth factor (IGF-I), basic fibroblast growth factor (bFGF, FGF-2), calcium/calmodulin-dependent protein kinase II (CaMKII) and growth-associated protein-43 (GAP-43) participate in brain plasticity.

Housing intact rats in EE condition increased protein levels of NGF and NT-3 [71]. IGF-I has a direct proliferative effect in adult hippocampal progenitor cells [3]. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus [2]. Exogenous administration of IGF-1 and GDNF facilitated progenitor cell proliferation in the ipsilateral dentate gyrus after transient brain ischemia [38]. It has been proposed that IGF-1 receptor co-localized with Estrogen receptor plays a role in the regulation of neuronal development and neural plasticity [21]. FGF-2 acts as a mitogen to stimulate neural stem cells or progenitor cells in SVZ or SGZ proliferation and differentiation [102], as well as to stimulate non-neurogenetic tissue such as neocortex, [134] septum, striatum [132] and spinal cord [166] to give rise to neurons and glial cells. Basic FGF enhances neurite outgrowth [145]. Exogenous administration of bFGF 24h after brain ischemia reduced thalamic atrophy and improved functional recovery after induction of brain ischemia [186]. The functional benefit of bFGF was documented to associate with neurite outgrowth [88,89]. GAP-43 has been suggested as a key molecule to initiate axon growth [168], and can be regulated by NMDA receptor [19]. CaMKII is important for LTP induction [109].

### **Animal models of stroke**

Rodent models of stroke are more commonly used animal models for the following reasons: low cost, a well-studied brain anatomy and chemistry, and their cerebrovascular anatomy and physiology close to higher species [14, 52]. Brain ischemia is classified into global cerebral ischemia and focal cerebral ischemia. The global ischemia is a useful model for studying selective neuronal death or as a model of cardiac arrest whereas focal ischemia is a model of clinical brain infarcts as seen in clinical stroke. There are several models for focal brain ischemia such as proximal ligation of the middle cerebral artery (MCA), distal ligation of MCA, tandem ligation of MCA and the common carotid artery, photochemically induced focal cerebral thrombosis and injection of blood clots into the carotid artery [52].

Two focal brain ischemia models are commonly used today. One is intraluminal model of arterial occlusion and the other is ligation or electrocoagulation of the middle cerebral artery. In the intraluminal model, a suture is inserted into the internal carotid artery, further advanced and occluded the initial of the middle cerebral artery [93, 111]. Since the suture can be withdrawn at any given time for reperfusion, this model is usually used for pharmaceutical interventions for protecting neuron death from ischemia/reperfusion damage. Infarction induced by this model is manifested in the striatum and/or cortex. Although intraluminal model is less invasive than the model of MCA ligation, the disadvantages of intraluminal model have been concerned such as inconsistent infarction size, subarachnoid hemorrhage, intraluminal thrombus formation and

intraischemic and postischemic hyperthermia [11, 162]. Moreover, the hypothalamus is usually damaged by this model [190]. Therefore, the survival of the animals may be jeopardized due to a reduction in the intake of food and drink. Proximal ligation of the middle cerebral artery [171] gives different results depending on the strain used; however, the most consistent results are obtained with hypertensive rats [40,55]. Because hypertension in rats as well as in humans lead to structural adaptation with increased vessel wall to lumen ratio and increased peripheral vascular resistance; thus, reduced collateral circulation distal to a vascular occlusion results in a large and consistent infarction. The model of permanent ligation of middle cerebral artery distal to the striatal branch, which was used for the thesis, was first described by Coyle [33]. This model induces a consistent infarction in the cortex of spontaneously hypertensive rats [56].



## **AIMS OF THE STUDY**

The general goals of this thesis were to determine whether post-ischemic housing conditions influenced the transcription and/or translation of the genes that were associated with brain plasticity. The specific aims for the thesis were:

1. To test the hypothesis that post-ischemic environmental enrichment stimulates BDNF gene expression.
2. To determine whether the pattern of BDNF mRNA expression corresponds to BDNF protein levels in an enriched environment after brain ischemia.
3. To investigate if postoperative housing conditions influence the gene expressions of NGFI-A and corticosteroid receptors (MR and GR) in a similar or different way to BDNF.

## GENERAL METHODS

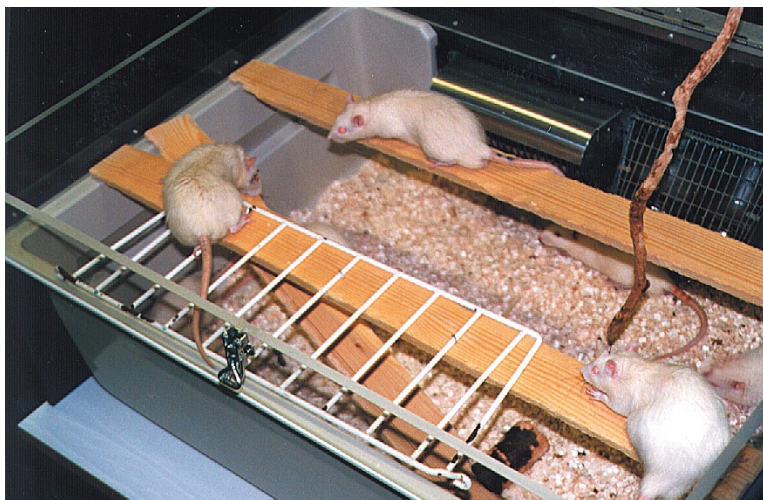
All procedures involving animals were conducted with permission from the animal ethical committee of Lund University. For details on the determination of infarct volume, tissue preparation and probes used for in situ hybridization and quantification of in situ hybridization (image analysis) see papers I and III. Details on sampling and homogenization of tissue, enzyme immunoassay for BDNF determination and quantification of the protein see paper II.

### Experimental animals

Male, three-month-old spontaneously hypertensive rats (SHR, Møllegaard Breeding Center A/S, Denmark) were used for the studies. This strain was initiated from Wistar-Kyoto (WKY) rats with elevated blood pressure and developed by brother-sister inbreeding [127]. It is the only strain used in which a selected occlusion of the middle cerebral artery distal to the striatal branches induces consistent cortical brain infarctions. A further advantage with the method used is a short operation time and rapid awakening from anesthesia (methohexital sodium, 50mg/kg, i.p.). For details see the original papers. The same procedure has been used as in earlier studies on evaluation of neurological deficits and the functional effects of an enriched environment [76, 128]. It is important to avoid unnecessary operative procedures such as catheterization and intubation that prolong the operation time and reduce the early mobilization of the animals. The animals were allowed free access to food and water prior to and after the surgery.

### Housing environments

The studies included in this thesis employed enriched environment (EE) and standard environment (SE) for housing animals.



Enriched environment: Eight to ten rats were housed together in a large cage (820 x 610 x 450 mm) furnished with horizontal and inclined boards and containing various items like wooden blocks, a chain, a swing board, a ladder, a tunnel and balls. Spaced between the boards were altered and some objects removed and other added three time per week.

Standard environment: three or four rats were reared in standard laboratory cages (550 x 350 x 200 cm) with no equipment. To be noticed is that the control of enriched environment used in the thesis is three or four rats in each cage, whereas most other studies (e.g.156, 187, 71,154) have used rats individually housed rats, usually considered deprived or impoverished housing likely to accentuate the difference between standard and enriched housing.

## **Experimental design**

Before the operation for making the stroke model, all the rats were housed in standard condition. In order not to interfere with the early gene expression, which might influence the gene expression at a later phase, all the animals used for this thesis were housed individually during the first 30 h after the ligation of the middle cerebral artery. They were then randomly either returned to standard housing or transferred to enriched environment. Paper I and III include two experiments; the first was the rats killed 2-12 day, and the second was the rats killed 20 and 30 days after the ligation. The second series were added because of the marked difference between the groups that was still significant 12 days after the lesion.

The rats were not subjected any tests for evaluation of neurological deficits because it could have influenced the gene expression. Falkenberg and co-workers reported that BDNF gene expression was increased in EE-housed rats with water maze tests but not in the rats housed in SE [43]. In addition, considering BDNF and NGFI-A belong to immediate early genes, they are rapidly induced by stimuli including stress [34]. Euthanasia procedure has also been considered to influence mRNA levels of immediate early genes [35]. Therefore, the rats were gently handed during euthanasia, and the decapitation and removal of the brains were performed very rapidly within 3~4min.

## SUMMARY AND COMMENTS OF THE RESULTS

### **Infarction size and post-ischemic housing conditions (paper I and III)**

In agreement with previous studies [56, 76, 128, 76], the infarction size was not different between EE housing rats and the rats housed in SE following cortical brain ischemia. There was no correlation between gene expression and infarction volume.

### **Gene transcription in post-ischemic housing conditions (paper I and III)**

#### *Gene expression 2~12 days post-ischemia*

In contrast to our hypothesis, BDNF and NGFI-A mRNA were downregulated in the early state in the rats housed in an enriched environment compared to standard rats with significant difference between the groups at day 2,3,12 for BDNF and at day 12 for NGFI-A at the peri-infarct frontoparietal regions and the contralateral homotopic cortex. In NGFI-A gene expression, a marked increase from day 7 to day 12 was noted in SE-housed rats. Ischemia-induced reduction of NGFI-A mRNA expression was detected at two and three days after brain ischemia in both housing conditions, which remained lower than unlesioned controls (baseline).

Hippocampal regions including dentate gyrus showed a similar pattern for BDNF in the cortex with significant difference between EE-housed and SE-housed rats at all times except day 7 on both sides. The only group difference for NGFI-A was observed at CA1, where a significantly higher values in standard rats than EE-housed rat at day 12 was shown. In dentate gyrus, the GR mRNA expression in SE-housed ischemic rats was significantly lower than unlesioned controls at all times except day 3, and in CA1 similar patterns were noted at day 2 and 7. In CA3, however, GR gene expression was higher in EE-housed lesioned rats than unlesioned control at d12.

The gene expression of BDNF in the thalamus was not influenced by housing conditions but by ischemic lesion. All of the lesioned animals showed significantly higher gene expression in the ipsilateral than the contralateral thalamus at d 2 and d7. However, 12 days after the arterial ligation all infarcted rats independent of housing conditions exhibited significantly higher BDNF mRNA values in the thalamus compared to unlesioned controls, suggesting that these changes were related to the lesion and not to the postischemic environments. Neuropathological changes have been observed in the thalamus in the ipsilateral thalamus in association with the spread of edema fluid 7 days after a proximal ligation of the MCA [125].

These results clearly show that post-ischemic events during the first 12 post-ischemic days trigger the changes in BDNF and NGFI-A mRNA in the cortex and hippocampus, and that the gene expression can be influenced by housing conditions. Detailed discussions on possible implications are presented in the original papers.

Since we did not have intact controls at each time point during the early stage, the statistical difference between lesioned and intact controls was not evaluated for BDNF gene expression. However, BDNF mRNA expression exhibited a clear trend for SE to be above and EE to be below baseline levels, which was contrary to our hypothesis. In paper I we presented the hypothesis that

it might be advantageous to reduce the early hyperexcitability that was described by others in the early stage after an ischemic lesion [16,142, 143]. However, whether or not there is a difference in hyperexcitability between the enriched and standard ischemic rats remains a hypothesis that needs to be tested with neurophysiological methods.

For MR mRNA expression, there was neither difference between the housing environments at any time points nor any differences to controls in any subregions studied. Although previous study showed a similar expression pattern between NGFI-A and MR [79] in unlesioned rats, the signal pathways might be more complicated after brain ischemia.

### Gene expression 20 and 30 days postischemia

Twenty days after the lesion, BDNF mRNA was low in both groups with significant difference to standard unlesioned controls in the bilateral cortex and hippocampus. However, the lower level of BDNF gene expression than baseline remained in periinfarct cortex and hippocampal regions in the enriched group at 30 days. In the bilateral cortex, the level of NGFI-A gene expression in SE-housed ischemic rats was at baseline at d20 while the NGFI-A mRNA level of EE-housed rats was still significantly lower than baseline. However, 30 days after brain ischemia, NGFI-A gene expression in the lesioned enriched group was significantly higher than baseline and lesioned SE-housed rats in the contralateral cortex and CA1. An upregulated level of NGFI-A in EE-housed rats was also found in the peri-infarct cortex compared to unlesioned controls. Overall, EE-housed rats showed a significantly higher level of NGFI-A mRNA when compared with the rats housed in SE at 30 d after brain ischemia.

There are two recent studies that investigated gene expressions of BDNF, NGFI-A and NGFI-B in different housing conditions [35, 154]. In those studies, the ischemic rats were randomly assigned into four housing conditions: individually housing, individually housing with a running wheel, social housing (rats housed together with no equipment) and enriched environment. One month after the ischemic event, rats in all groups showed similar levels of BDNF mRNA as sham-operated rats in the peri-infarct cortex and the contralateral cortex [154], whereas the gene expressions of NGFI-A and NGFI-B in the social and enriched groups did not differ from sham although the levels of NGFI-A and NGFI-B gene expression in EE-housed and social-housed rats were higher than individually housed rats with and without a running wheel in the bilateral cortex outside infarct and CA1. The individually housed rats, however, displayed a lower gene expression than those of sham-operated rats [35].

### **BDNF protein determination (paper II)**

In this study, 12 days were chosen because the BDNF mRNA gene expression was significant all the time up to that time point and the protein values were expected to be representative of that period.

In the ipsilateral frontal cortex surrounding to the infarct, the BDNF protein level was significantly reduced in the ischemic rats housed in EE when compared to ischemic rats housed in SE, which was in agreement with the data on gene expression. No other difference was seen between rats housed in standard and enriched environment, nor were there any side differences in the enriched group. However, the standard group had higher BDNF in the hippocampus and peri-infarct sensorimotor cortex on the lesioned hemisphere than on the intact side.

BDNF mRNA expressions and BDNF protein levels were reduced in the rats housed in EE when compared to SE-housed animals 2-12d after brain ischemia. However, for the BDNF gene expression, the differences were found in the ipsilateral frontal cortex, the contralateral cortex and bilateral hippocampus, while the BDNF protein levels that were observed a difference between the two housing conditions were evident only in the ipsilateral cortex. Comparing the BDNF mRNA expression, the less expression of its protein may be relative to the regulation of translation and/or post-translation for the production of BDNF, and cellular transportation. The variation between the relative regional distribution of BDNF protein and its mRNA in several brain structures has been reported previously [122]. Accordingly BDNF protein level has also been found only partly parallel to BDNF mRNA expression in a rat model of global brain ischemia [96].

## DISCUSSION

### **The complexity of gene activations after brain ischemia**

Microarrays/gene chip analysis, one of the multiplex techniques for gene expression, is recently being developed. This method provides the possibility to analyze thousands of gene expressions from the same sample. Using the microarray technique, about 3~4% (approximately 300) of tested genes were triggered by brain ischemia. The expressed genes were immediate early genes, heat shock proteins, anti-oxidative enzymes, trophic factors, genes relative to RNA metabolism, inflammation and cell signaling, about half of the expressed genes have not been reported in stroke [112, 163]. The patterns of ischemia-induced gene expression were complicated. The variations of the expressed genes are dependent on the methodology of gene identification and the model of brain ischemia [62, 91, 112, 144, 147, 163]. The pattern of gene expression may be influenced by the ischemic model used. Using in situ hybridization method, Hara and co-workers [62] reported that the areas of cerebral protein synthesis/ATP mismatch and the patterns of c-fos, junB, hsp70 and NSE gene expression were quite different between permanent and transient focal brain ischemia.

It has been postulated that early gene expressions regulate cell survival and death, and later expressed genes are associated with tissue repair, brain plasticity and functional recovery [112,147]. However, the situations are clearly very complex; moreover, growth inhibitory factors are also activated in the post-ischemic phase [188].

### **Enriched environment housing and gene expression at recovery stage**

In a gene chip study, Rampon and co-workers [146] reported that housing intact mice in an EE resulted in a large number of genes changing. The genes changed at late stage (2d and 14 d after an EE housing) were not the same as the genes changed at early (3h and 6h after an EE housing). A fluctuated change was observed in the gene expression. For example, one of the neuronal excitability genes, a 78-kDa glucose-regulated protein, was increased during the early time but dramatically down-regulated at 14 d after EE housing.

Most of the genes altered at late stage were the genes participated in neuronal transmission and structural changes. For example, cAMP-dependent protein kinase, a factor damaging LTP, was reduced 2.5 fold after 14 d EE housing. A number of genes related to the function of NMDA receptor like postsynaptic density 95 (PSD-95), and the molecules downstream of the NMDA receptor such as calmodulin were up-regulated 2 and 14 d after EE housing. The genes associated with neuronal growth and synaptogenesis like cytoskeletal protein dynactin and cortactin were increased at the late stage. In addition, cell death 1 (DAD1), a gene that controls apoptosis during the development, was up-regulated, suggesting an anti-apoptosis effect on long-term housing in an EE.

In this thesis, we found postischemic housing conditions led to changes of neural plastic candidate genes: BDNF, NGFI-A and GR gene transcription and/or translation in remote intact brain regions at late rehabilitation stage (2-30d post-ischemia). Downregulation of BDNF and NGFI-A in an EE housing condition during recovery stage may be associated with reduction of ischemia-induced hyperexcitability in remote areas. This reduction may be related to restoring

neuronal transmission system, protecting neurons from secondary damage and establishing new functional neuron circuits. It is worthwhile to note that during the recovery stage (2~20d) after brain ischemia, dendritic spines in the contralateral cortex layer II/III were increased in EE-housed rats [81], functional recovery was significant in EE-housed animals [128], BDNF and NGFI-A gene expression in the rats housed in EE were reduced [this thesis] and growth inhibitory factor was reported to be increased [188]. As mentioned above, based on the data of gene chips, hundreds genes were changed by brain ischemia and housing environments. Functional recovery is a result of the interactions of many genes. The alterations of the genes are dynamically orchestrated by cell context. Therefore, it is hard to draw up the entire process with some single genes at some selected times. However, to clarify the role of BDNF and NGFI-A in EE-related functional recovery more studies need to be conducted.

### **Gene transcription and/or translation of BDNF and NGFI-A outside infarction areas**

In the present studies, BDNF gene and protein, and NGFI-A gene were expressed in wide distribution regions in the brain 2-30d after unilateral cortical brain ischemia, including ipsilateral and contralateral cortex, hippocampus and thalamus. First, it may be due to diaschisis. It has been proposed that after brain ischemia, blood circulation and cell metabolism are changed in the parts of remote regions that functionally connect to the infarct area. This phenomenon is caused by denervation [7, 44] termed diaschisis, a word introduced by von Monakow in 1914 [75]. Second, it may be relative to adaptive compensation events. Keyvani and co-workers [91] reported that the mRNA expressions of transcription factors, IEGs, neuronal signaling, neuronal growth and structure-associated factors, ion channels, transport proteins, mediators of metabolic pathways were changed in both ipsilateral and contralateral motor and somatosensory cortex 10 d after brain ischemia. Cortical maps can be modified by changes of sensory input as well as focal brain ischemia [80]. The cortex adjacent to a small lesion in the motor and somatosensory cortex is modified by training [126]. After a cortical brain ischemia in rats, increased density and distribution of GAP-43 immunoreactivity were observed in the ipsilateral cortex 3~14 days after brain ischemia and of synaptophysin immunoreactivity was detected both in the peri-infarct cortex and in the contralateral cortex at postischemic days 14~60. The functional recovery was found relative to the alterations of GAP 43 and synaptophysin in the rats housed in standard cage [169]. Using transcranial magnetic stimulation (TMS), positron emission tomography (PET) technique and functional magnetic resonance imaging (f MRI), clinical studies have shown functional reorganization in the contralateral and ipsilateral cortex of stroke patients [80, 60, 158]. Taken together, the changes of BDNF and NGFI-A transcription and/or translation 2-30d after brain ischemia are most likely part of the processes for brain repair and functional restoration.

### **Is down-regulation of BDNF beneficial to functional recovery after brain ischemia?**

#### *BDNF gene expression during 2-12d post-ischemia*

We had hypothesized that BDNF could be up-regulated in the ischemic rats housed in EE. However, the data were opposite to our hypothesis that BDNF gene and protein were reduced in EE-housed stroke rats.



BDNF synthesis and release are activity-dependent [106, 175], and can be blocked by NMDA receptor antagonist [107,174]. For a positive feedback, BDNF enhances the release of glutamate [100, 114, 135]. It has been demonstrated that focal brain ischemia induces hyperexcitability in the cortex surrounding the infarction and the contralateral cortex in standard housing rodents. In the mouse, hyperexcitability was detected in the cortex next to the infarction at 1~3 d after ligation of the middle cerebral artery, and peaked at 28 d post-ischemia [143]. Not only in the peri-infarct cortex, hyperexcitability was also observed in the contralateral cortex one week after induction of cortical photothrombosis [16] or middle cerebral artery occlusion [161]. Brain ischemia-induced hyperexcitability is caused by an imbalance between excitatory system and inhibitory system, which is an up-regulation of NMDA receptor-mediated excitation and down-regulation of GABAergic inhibition [4, 143]. Post-ischemic hyperexcitability has been suggested to play both a detrimental role, which develops epileptic activity and disturbs the incoming information process, and a beneficial role that promotes plasticity in functional recovery [16, 17, 161]. However, hyperexcitability increases expression of BDNF [41, 94], and BDNF potentiates excitatory synaptic transmission [22]. Intrahippocampal infusion of BDNF enhances seizure activity [160]. Although glutamate and its receptor, NMDA, are important for neuronal plasticity in unlesioned animals [51], in ischemic animals could be different story. Lesion-induced hyperexcitability has been proposed to interfere functional recovery [184]. The improved functional restoration may link with a re-balance of excitatory and inhibitory neuronal activity. Down-regulation of BDNF during the early period after brain ischemia in EE-housed animals may imply a decrease of excitation and increase of inhibition and restore a new balance. We have in unpublished data from the same brains that have been analyzed in this thesis that neither CaMKII nor bFGF shows a corresponding down-regulation in EE-housed animals. However, to determine whether the EE-induced reduction of BDNF suppresses the lesion-induced hyperexcitability neurophysiological studies are needed.

BDNF may have both positive and negative effects on brain injury. It may be dependent on the size of the lesion and the dose of BDNF. It has been proposed a small lesion or mild insult in the brain may be protected by BDNF [107]. However, continuously producing high amount of BDNF by a BDNF recombinant adeno-associated viral transduction in vivo exaggerated ischemia-induced excitatory damage and resulted in an increase of neuronal death in ipsilateral striatum [58]. Accordingly, in an in vitro study, pretreatment with BDNF caused necrotic cell death when the cortical neurons exposed to oxygen-glucose deprivation [98]. It has been studied that brain ischemia induces neurogenesis in SGZ and SVZ [8, 74, 165, 170]. The newborn neurons after brain ischemia have been proposed to play a role in self-repair [8]. It has been reported that BDNF induces neuronal differentiation in intact brain [27], However, in the case of global brain ischemia, BDNF inhibited neuronal differentiation. Larsson and co-workers [104] reported that long-term delivery of BDNF in hippocampus led to a decrease of neurogenesis in dentate gyrus. Scavenging endogenous BDNF by intraventricular infusion of TrkB-Fc increased ischemia-induced neurogenesis in dentate gyrus [59]. Taken together, EE-mediated down-regulation of BDNF at the early recovery stage may be neuroprotection from excitotoxic injury and may be beneficial to brain repair after brain ischemia.

It has been studied that BDNF can be reduced by glucocorticoid, chronic stress, and immobilization stress [167]. Acute stress from the induction of brain ischemia resulted in a transient increase of BDNF at 2 h after perfusion, and returned to control level within 24 h after reperfusion [30, 95, 97]. However, all the lesioned animals were housed individually during the first 30-hour after brain ischemia. Therefore, different BDNF levels are not associated with the

acute stroke stress. Are the rats housed in EE condition more stressed than the rats housed in SE? As discussed in paper I, we have no indication that animals exposed to an EE condition were stressed. Moreover, acute stroke in both man and animals is associated with a transient high level of glucocorticoid in the circulation blood [120, 129, 130]. Although the high glucocorticoid levels have been proposed to have a negative effect on outcome there is no experimental or clinical evidence indicating that blocking the stress response after focal brain ischemia is beneficial for final outcome. An alternative interpretation of the fact that higher glucocorticoid levels have been associated with more dementia and depression [129, 130] is that those sequels are related to more severe lesions. In fact blocking the production of steroids during the 24 h following an ischemia in the same experimental model used as in this thesis increased infarction size and worsened functional outcome [153].

Glucocorticoid receptor (GR) in hippocampus can reduce glucocorticoid in the blood [164]. In the rats housed in SE, GR mRNA expression in hippocampus was reduced during 2~12d after brain ischemia; however, the reduction of GR gene expression was prevented in the rats housed in EE (paper III). Taken together, reduced level of BDNF in the EE-housed rats is unlikely due to stress. Down-regulation of BDNF in the rats housed in EE is probably related to more complicated mediation pathways that turnover or breakdown the expression of BDNF to comprise a better functional reorganization. However, conditional knock out or overexpression BDNF during 2-12d after brain ischemia needs further studies to determine whether BDNF dose in fact play a role in functional recovery.

#### *BDNF gene expression at 20d and 30d post-ischemia*

The levels of BDNF mRNA expression during 20-30d after brain ischemia remained below baseline in both EE- and SE-housed stroke rats; there was no difference between the two housing conditions. This suggests that the low levels of BDNF gene expression were lesion-induced. Since BDNF gene expression is neuronal activity dependent, reduction of BDNF gene expression may be due to less afferent inputs and further loss of neuronal connections between two hemispheres, and loss of cortex-cortex horizontal connections. However, in a later study comparing several housing conditions, enriched, social, individual housing with and without a running wheel, none of the groups differed from sham operated rats and there was no difference among the enriched and non-enriched groups at 30 days after ischemia for BDNF gene expression, although the rotating beam test showed a better outcome in enriched-and social-housed rats than individually housed [154]. Therefore, BDNF in the later recovery stage may be not related to EE-induced functional benefits.

#### **Changes of mRNA expressions of NGFI-A and its target genes influenced by post-ischemic housing conditions.**

NGFI-A gene expression was dynamically regulated in the ipsilateral cortex surrounding to the infarction, the contralateral cortex and hippocampal CA1 by housing conditions after brain ischemia.

The decrease of NGFI-A gene expression at two and three days after the induction of cortical brain ischemia was probably relative to ischemia-induced diaschisis in the ipsilateral and contralateral cortex and hippocampus. While the reduction of NGFI-A gene expression in EE-

housed animals during 7~20 days post-ischemia was unexpected. However, the sections from the same animals were used for both NGFI-A and BDNF gene expression studies. Interestingly, both of them were down-regulated in the peri-infarct cortex, contralateral cortex and hippocampus when compared with SE-housed rats during 2~12 d after the induction of brain ischemia. Similar to BDNF, NGFI-A gene expression is associated to activity-plasticity and regulated by NMDA receptor [29]. It has been reported that NGFI-A gene expression in the visual cortex is dependent on visual activity [185]. Antagonists of NMDA receptor blocks both NGFI-A induction and LTP formation [183]. Could it be true that down-regulation of NMDA receptor activity and/or up-regulation of GABAergic activity during this early period (2~12d post-ischemia) is critical for functional restoration? It has been also reported that prolonged gene expression of NGFI-A post-ischemia is related to slow neuronal degeneration [69] and apoptosis [32]. Therefore, in contrast to the increase of NGFI-A gene expression in the rats housed in SE condition during the early stage, EE-induced reduction of NGFI-A may be neuroprotective. There are more complex signal pathways involving in EE-mediated functional recovery after brain ischemia. However, according to the data we reported here, EE-induced down-regulation of BDNF and NGFI-A during the early phase (2~12d) after brain ischemia may suggest a process of down-regulation of neuronal excitability and upregulation of neuronal inhibition taking place in the remote areas to the infarction in EE- housed rats.

Regarding up-regulation of NGFI-A gene expression in EE-housed animals at 30d after brain ischemia, it may be associated with EE-induced synaptic plasticity. A recent study [35] showed that NGFI-A and NGFI-B were correlated to functional recovery at 30 day after cortical brain ischemia. Thirty days after brain ischemia, however, the levels of NGFI-A and -B in social- or EE-housed rats reached the same level as sham-operated rats, and the group-housed rats showed a better functional outcome. In contrast, the rats housed individually exhibited a worse functional outcome compared to EE- or social-housed ones; moreover, the levels of NGFI-A and -B for those rats were lower than sham-operated rats and than the group-housed rats (social and EE). Several lines of evidence have shown NGFI-A is involved in neuronal plasticity. Synapsin I and synapsin II, synaptic vesicle proteins, participating in the docking of synaptic vesicles, neurite outgrowth and synapse maturation have been regulated by NGFI-A [138, 172]. NGFI-A heterozygous knock out mice show insufficient in LTP [13]. LTP formation and stabilization are associated with elevated NGFI-A, NMDA receptor antagonist blocks both LTP and NGFI-A mRNA in the dentate gyrus [1, 29, 183]. Moreover, NGF-induced neurite outgrowth was blocked by inhibition of NGFI-A [105]. The translation of NGFI-A gene has not been investigated in the present study; the mRNA expression, however, can be regulated by translation or post-translation. Nevertheless, studies on the levels of NGFI-A mRNA and protein expression have shown a corresponding relationship [35, 141, 185].

It has been shown that there is a different expression pattern for NGFI-A in unlesioned animals in response to exposure of EE. Wallace et al., [178] reported that NGFI-A was increased in the cortex where related to neuronal plasticity in the rats housed in EE for two to four days. In agreement with the present study, Olsson and co-workers [131] found an up-regulation of NGFI-A gene expression in the hippocampus when the rats were housed in EE for 30 days. The data we presented in the current study showed that the gene expression of NGFI-A was reduced at 20 d after housing in an EE for unlesioned animals, which have not been studied previously. Could NGFI-A mRNA also be changed dynamically in the intact animals after housing in an EE? The strain of the animals and the methodological difference may also influence the outcome. Nevertheless, to elucidate this question, a time-course study needs to be performed in the future.

However, up-regulation of NGFI-A gene expression exhibited at 30 d after housing in an EE in both lesioned and intact animals, suggests a synaptic modification induced by an EE housing condition. This effect may be related to the targets of NGFI-A gene binding such as synapsin I and II [138, 172].

*The expressions of GR and MR, the target genes of NGFI-A, had exhibited different patterns from NGFI-A.*

There is a potential binding site for NGFI-A in the GR promoter [37]. In a previous study, the pattern of GR gene expression in the hippocampus was similar to NGFI-A gene expression in intact rats after an EE housing stimulation [131]. However, the gene expression patterns of NGFI-A and GR in the present study did not correspond to each other, suggesting some other target genes of NGFI-A may be involved in the regulation after brain ischemia. Alternatively post-ischemic events are more complex than intact situation.

In paper III, we found that GR mRNA was reduced in the rats housed in SE during 2~12d after brain ischemia, and then restored at 20 and 30 days. The early decrease of GR gene expression in SE-housed rats was prevented in the rats housed in EE, possibly suggesting ischemia-induced disturbance of HPA axis was stabilized and normalized by EE housing condition.

Mineralocorticoid (MR), another corticosteroid receptor, has been reported to be associated with neuronal plasticity and learning and memory [164]. MR gene expression, however, was not influenced by the postoperative housing conditions.

## **Concluding remarks**

Our studies show that post-ischemic events trigger a number of gene activations, which can be influenced by postischemic housing during the rehabilitation stage after brain ischemia. Neuronal functions and neuronal structures in the ipsilateral and contralateral hemispheric areas and subcortical areas outside the lesion are reorganized to compensate and/or restore the function damaged by infarction. The electrophysiological and morphological changes can happen in minutes, hours, weeks, month and years after induction of brain ischemia. Gene expression is very complex after brain ischemia. Ischemic injury is a robust stimulation triggering the expressions of hundreds genes. Both EE and brain ischemia can induce the expressions of hundreds genes. The alterations (upregulation or downregulation) of the gene expression are very complex. The location of the infarction, the size of the infarction, timing after brain ischemia, methodology, technology and manipulation of the experiments could influence the results of certain gene expression. Therefore, it is not a simple endeavor to evaluate the role of a single gene expression after brain ischemia. However, this thesis reports that post ischemic housing conditions influence BDNF, NGFI-A and GR gene transcription and/or translation. Further studies need to be conducted to determine whether or not those genes are changed by an enriched environment related to EE-mediated functional recovery. Conditional knock out or overexpression BDNF and NGFI-A or functional interrupting endogenous gene expression may provide more evidence for understanding how BDNF and NGFI-A participate in EE-induced functional recovery after experimental stroke.

## CONCLUSIONS

1. Environmental enrichment changed gene expression after brain ischemia with different patterns and time courses for the individual genes that were studied in this thesis.
2. Enriched environment housing condition induced a down-regulation of BDNF gene transcription widespread in the ipsilateral and contralateral cortex and hippocampus during early rehabilitation stage after cortical brain ischemia.
3. Corresponding to its gene expression, post-ischemic housing conditions resulted in a decrease of BDNF translation in the ipsilateral frontoparietal cortex in enriched environment-housed animals 12d after induction of cortical brain ischemia.
4. NGFI-A mRNA expression was decreased during the early stage but up-regulated at later time after brain ischemia in enriched environment-housed animals. Ischemia-induced down-regulation of glucocorticoid receptor (GR) gene expression in hippocampus was prevented by an environmental enrichment housing during the early recovery stage.

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## *Appendix*

