Neurogenic and angiogenic actions of electroconvulsive seizures in adult rat brain

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Abstract

In the current thesis, the neurogenic and angiogenic response to electroconvulsive seizure (ECS)-treatment was investigated in the adult rat brain. ECS-treatment is an animal model for the antidepressant treatment electroconvulsive therapy (ECT), which is considered to be the most effective antidepressant treatment modality today, however with not yet fully understood modes of action. Depression, which is a common and devastating illness has recently been proposed to be caused by a decreased hippocampal neurogenesis and cellular plasticity in general, possibly due to elevated levels of the stress hormone cortisol, manifesting itself as a reduction in hippocampal volume. In the current thesis, ECS-treatment was shown to be able to oppose stress hormone-induced decrease in hippocampal neurogenesis and also induce proliferation of non-neuronal cells. A large majority of these cells were identified as being endothelial cells, and neurogenesis and angiogenesis in response to ECS-treatment seemingly occurred in concert. In addition to neurogenesis, ECS-treatment induced strong neuronal activation in the hypothalamus, co-localising with a strong angiogenic response. Endothelial cells have been shown to influence neuronal and glial function and we hypothesise that the increase in hypothalamic endothelial cell proliferation could for example influence neuroendocrine signaling. Besides possibly influencing neuronal and glial function, endothelial cells are building blocks of blood vessels. We detect a strong angiogenic response in the hippocampus, which in fact results in a 16% increase in vessel length in the molecular layer of the dentate gyrus. This finding has important implications for the trophic actions of ECS-treatment. In addition to counteracting decreases in neurogenesis, ECS-treatment increase the vascularization of a structure that has been shown to be vulnerable to stress and decrease in size in depressed patients. Understanding this angiogenic response and possibly being able to stimulate it by other means than ECS-treatment could possibly lead to the development of new and more effective antidepressant treatments.

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Till Ingemar

Efter en stund hoppade Dartanjangs morfar ner på axeln på Dartanjang och började viska med honom.
”Va, kan han prata” sa Loranga. ”Vad säger han?”
”Han säger att du borde skaffa dig ett arbete”, sa Dartanjang.
”Va, börjar han sticka upp!” skrek Loranga. ”Ta ett arbete och förstöra sina bästa år!”
”Nu säger han att alla andra har arbeten”, sa Dartanjang.
”Men jag måste ju vara hemma och leka med Masarin”, sa Loranga. ”Och vem ska höra på popmusiken om inte jag är hemma, jag bara frågar.”

Barbro Lindgren
”Loranga, Masarin & Dartanjang”

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ORIGINAL ARTICLES

This thesis is based on the following papers, which will be referred to by their roman numerals:

I: Hellsten J, Wennström M, Mohapel P, Ekdahl CT, Bengzon J, Tingström A (2002): Electroconvulsive seizures increase hippocampal neurogenesis after chronic corticosterone treatment. *Eur J Neurosci* 16:283-90.

II: Hellsten J, Wennström M, Bengzon J, Mohapel P, Tingström A (2004): Electroconvulsive seizures induce endothelial cell proliferation in adult rat hippocampus. *Biol Psychiatry* 55:420-7.

III: Ekstrand J, Hellsten J, Wennström M and Tingström A (2005): Inhibition of endothelial cell proliferation does not prevent electroconvulsive seizure-induced neural precursor proliferation in adult rat hippocampus. (manuscript in preparation)

IV: Hellsten J, West MJ, Arvidsson A, Ekstrand J, Jansson L, Wennström M and Tingström A (2005): Electroconvulsive seizures induce angiogenesis in adult rat hippocampus. *Biol Psychiatry* (in press)

V: Jansson L, Hellsten J and Tingström A (2005): Region specific hypothalamic neuronal activation and endothelial cell proliferation in response to electroconvulsive seizures. (submitted)

ABBREVIATIONS

5-HT	serotonin
AVP	vasopressin
BrdU	bromodeoxyuridine
CORT	corticosterone
cPARP	caspase-cleaved poly (ADP-ribose) polymerase
CRH	corticotropin releasing hormone
DAB	3,3"-diaminobenzidine
DG	dentate gyrus
DSM-IV	diagnostic and statistical manual of mental disorder, 4th edition
ECS	electroconvulsive seizure
ECT	electroconvulsive therapy
EEG	electroencephalogram
GCL	granule cell layer
HPA	hypothalamic-pituitary-adrenal
KPBS	potassium phosphate-buffered saline
MAOI	monoamine oxidase inhibitor
ML	molecular layer
MRI	magnetic resonance imaging
NA	noradrenaline
NeuN	neuronal specific nuclei
PVN	paraventricular nucleus
RECA-1	rat endothelial cell antigen-1
SE	status epilepticus
SGZ	subgranular zone
SON	supraoptic nucleus
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
VMH	ventromedial hypothalamic nucleus

SUMMARY

Depression is a serious and common disease, estimated to be affecting every tenth woman and every twentieth man, every year. Repeated episodes are common and in severe depressive episodes the risk for suicide is high. The most common avenues of treatment include psychotherapy, antidepressant medication and in severe depressive episodes, electroconvulsive therapy (ECT). An explanation for the disease has long been sought and a well known theory states that the cause of the disease is alterations in serotonergic and noradrenergic neurotransmission.

Lately it has been shown that depressed patients display structural brain changes detectable with magnetic resonance imaging. It appears as if the hippocampus, a structure located in the temporal lobes, of importance for memory functions, is smaller in depressed patients compared to healthy controls. The longer the time with depression, the smaller the hippocampus is. New nerve cells (neurons) are generated in the hippocampus, even in the adult brain. It is not known what the function of this neurogenesis is, but it has been speculated that the new neurons are important for memory functions, in which depressed patients often display disturbances. In experiment animals, stress and depression-like states cause decreased neurogenesis and a new theory regarding the cause of depression postulates that it is due to a decreased hippocampal neurogenesis, manifesting itself as a detectable reduction in hippocampus size. The theory has gained some support since it has been shown that antidepressant medication and ECT increase the formation of new neurons and that this neurogenesis appears to be necessary for a treatment-induced behavioural change in animals in depression models.

I have in my thesis work investigated how electroconvulsive seizure (ECS)-treatment, an animal model for ECT, affects the formation of different celltypes in hippocampus and hypothalamus of the rat. In paper number one I investigated whether it was possible or not to influence the formation of new cells with ECS-treatment in rats that were chronically stressed. The rats had been treated with the stress-hormone corticosterone for a long time, something which reduced their rate of neurogenesis, and where then given ECS-treatment. It became apparent that ECS-treatment was capable of counteracting the detrimental effects of the stress-hormone on neurogenesis, but also that other, non-neuronal celltypes that normally divide in response to ECS-treatment, still, despite ECS-treatment, proliferated at a lower rate when corticosterone was present.

In paper number two I identified a large proportion of these non-neuronal cells as being endothelial cells, which are cells that are the building blocks of blood vessels. In control animals very few dividing endothelial cells were found. However, in response to ECS-treatment there was a very dramatic increase in the number of dividing endothelial cells in all of the dentate gyrus, the brain region where the subgranular zone, the site for hippocampal neurogenesis, is located.

In addition, it has been shown that in the subgranular zone, endothelial cells and neurons are located closely to each other and are thought to be influencing each other with common growth factors. It has been proposed that neurogenesis

takes place in an "angiogenic niche" in close proximity to dividing endothelial cells. I show in paper number two that the endothelial cell proliferation and the neurogenesis in response to ECS-treatment seem to occur in a coordinated fashion. We asked ourselves if it is necessary with a preserved endothelial cell proliferation in order to achieve a normal neurogenesis. In order to investigate this we, in paper number three, studied how endothelial cells and neural progenitors in the subgranular zone behave when rats are short-term-treated with stress-hormone and at the same time receive ECS-treatment.

It turned out that the endothelial cells in the angiogenic niche also are sensitive to the effects of the stress-hormone, and that the neural progenitors, despite the inhibition of endothelial cell proliferation, still divide in a normal manner in response to ECS-treatment. The results indicate that dividing endothelial cells are not a prerequisite for neural progenitors to be able to divide in response to ECS-treatment. In addition we showed that the basal endothelial cell proliferation was affected by the stress-hormone. Could it be that chronic stress can influence the vascular system and possibly cause a rarefaction of the capillary system in hippocampus? Could that be an explanation to the decreases in hippocampus size noted in depressed patients?

I now wanted to investigate if the strong increase in endothelial cell proliferation in response to ECS-treatment could lead to an increased vascularization in the hippocampus. After ten ECS-treatments I detected 30 percent more endothelial cells and 16 percent longer blood vessels. Thus it seems plausible that ECS-treatment can oppose volume changes of the hippocampus, not only by stimulating neurogenesis but also by inducing angiogenesis.

Finally, in paper number five we investigated the possible ECS-induced activation of hypothalamus, an area involved in the regulation of sleep/wakefulness, appetite and sexual drive, functions that often are disturbed in the depressed patient. We detected a strong neural activation in a number of specific hypothalamic nuclei and in the same nuclei we also detected a strong increase in endothelial cell proliferation.

Taken together, the results from the five papers in this thesis show that apart from inducing neurogenesis, ECS-treatment is also capable of inducing endothelial cell proliferation in brain structures of great significance for depression. The complete functional significance of this angiogenic response is not known. One could imagine that the endothelial cells could influence surrounding cells, for example by releasing growth factors and/or influencing hormone secretion. One thing is however evident; the angiogenic response leads to an enlargement of the vascular tree in the hippocampus, a brain structure that has been shown to be smaller in depressed patients.

If this angiogenic response is a phenomenon exclusive to ECS-treatment, it could perhaps explain the efficacy to this treatment modality compared to antidepressant medication. Further studies of angiogenic mechanisms in conjunction with antidepressant treatment could possibly lead to the development of new and more effective therapies for depression.

SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING

Depression är en allvarlig och vanligt förekommande sjukdom som har beräknats att varje år drabba var tionde kvinna och var tjugonde man. Det är vanligt med upprepade insjuknanden och vid djupa depressioner är risken för självmord hög. De vanligaste behandlingsmetoderna är psykoterapi, antidepressiva läkemedel och vid djupa depressioner, elektrokonvulsiv terapi (ECT), vanligen kallad elbehandling. Man har sökt efter en förklaring till sjukdomens uppkomst och en allmänt accepterad teori är att den beror på störningar i hjärnans signalsubstanser, framför allt serotonin och noradrenalin.

På senare år har man visat att deprimerade patienter kan få förändringar i hjärnan vilka man kan mäta med magnetröntgen. Bland annat visar det sig att hippokampus, en struktur som ligger i tinningloberna och är av betydelse för minnesfunktioner, har mindre storlek hos deprimerade patienter. Ju längre man har varit deprimerad, desto mindre är hippokampus. I hippokampus bildas det nya nervceller, även i vuxen ålder. Man vet inte vad denna nybildning av nervceller har för betydelse, men det verkar som om de nya nervcellerna är viktiga för minnesfunktioner, vilka just deprimerade patienter ofta har störningar i. Stress och depressionsliknande tillstånd hos försöksdjur leder till minskad nervcellsnybildning och en ny teori angående depressionssjukdomens uppkomst säger att den beror på en minskning av nervcellsnybildningen i hippokampus, vilken visar sig som en storleksminskning av hippokampus. Teorin har fått stöd genom att det har visats att antidepressiva läkemedel och elbehandling leder till ökad nervcellsnybildning, och att nervcellsnybildningen verkar vara nödvändig för att man ska se en förändring av beteendet hos försöksdjur i depressionsmodeller.

Jag har i mitt avhandlingsarbete undersökt hur elektrokonvulsiv stimulering (ECS), en djurmodell för elbehandling, påverkar nybildningen av olika celltyper i hippokampus och hypothalamus hos råtta. I artikel nummer ett undersökte jag om det var möjligt att med ECS påverka nervcellsnybildningen i råttor som var kroniskt stressade. Råttorna hade behandlats med stresshormonet kortikosteron under lång tid, vilket sänkte deras nervcellsnybildningstakt, och fick sedan ECS. Det visade sig att ECS kan motverka stresshormonets negativa effekter på nervcellsnybildningen men att andra icke-neuronala celler som delar sig som svar på ECS fortfarande delade sig i lägre omfattning i närvaro av stresshormon. I arbete två fann jag att en stor del av dessa icke-neuronala celler är endotelceller, det vill säga celler som bygger upp blodkärl. I obehandlade djur fanns det få delande endotelceller. Som svar på ECS skedde dock en väldigt kraftig ökning av endotelcellsnybildningen i hela gyrus dentatus, den region av hippokampus där den subgranulära zonen där nervcellsnybildningen sker är belägen. I den subgranulära zonen ligger dessutom endotelcellerna och nervcellerna nära varandra och tros kunna påverka varandra med gemensamma tillväxtfaktorer. Man har talat om att nervcellsnybildningen sker i en ”angiogen nisch” nära delande endotelceller. Jag visar i artikel två att endotelcells- och nervcellsnybildningen efter ECS verkar ske på ett samordnat sätt. Frågan är om det är nödvändigt med bibehållen

endotelcellsproliferation för att få en normal nervcellsnybildning. För att ta reda på detta studerade vi i artikel nummer tre hur endotelceller och nervcellsprogenitorer i den subgranulära zonen beter sig när man korttidsbehandlar råttor med stresshormon och samtidigt ger dem ECS.

Det visade sig att endotelcellerna i den angiogena nischen också är känsliga för stresshormon men att nervcellsprogenitorerna trots detta delar sig lika bra som svar på ECS. Resultaten visar att det inte är nödvändigt med delande endotelceller för att nybildning av nervcellsprogenitorer ska ske som svar på ECS. Vi visade dessutom i artikel nummer tre att den basala endotelcellsproliferationen påverkas av stresshormon. Kanske kan långvarig stress leda till att blodkärlssystemet påverkas och att kärlträdet i hippokampus blir glesare? Kan det i så fall vara en bidragande orsak till storleksminskningen av hippokampus som man ser hos deprimerade patienter? Jag ville nu i artikel nummer fyra ta reda på om den kraftiga endotelcellsproliferationen som ECS inducerar leder till ett större kärlträd i hippokampus. Efter tio ECS fann jag att det fanns trettio procent fler endotelceller och att blodkärlen var sexton procent längre. Det finns således anledning att tro att ECS skulle kunna motverka storleksminskningen av hippokampus hos deprimerade, inte bara genom att öka nervcellsnybildningen, utan också genom att göra kärlträdet större. Avslutningsvis undersökte vi i arbete nummer fem hur hypothalamus, ett område som reglerar bland annat sömn/vakenhet, aptit, och sexdrift, funktioner som ofta är störda hos deprimerade, aktiveras av ECS. Det skedde en kraftig neuronal aktivering i några specifika kärnor i hypothalamus som svar på ECS och på samma ställen detekterade vi också en kraftig endotelcellsproliferation.

Sammanfattningsvis visar resultaten i de fem artiklar som ingår i denna avhandling att förutom att inducera nervcellsnybildning i hippokampus så leder ECS till en nybildning av endotelceller i hjärnstrukturer av stort intresse ur ett depressionsperspektiv. Den fullständiga funktionella betydelsen av denna endotelcellsnybildning är inte känd. Man kan tänka sig att endotelcellerna kan påverka omgivande celler genom att frisätta tillväxtfaktorer eller exempelvis reglera hormonutsöndring. Klart är dock att endotelcellsnybildningen leder till ett större kärlträd i hippokampus, en struktur som har visats benägen att minska i storlek hos deprimerade patienter.

Om denna angiogena respons är något som endast sker i samband med ECS skulle det kunna vara en förklaring till denna behandlingsmetods effektivitet jämfört med antidepressiva mediciner. Vidare studier av angiogena mekanismer i samband med depressionsbehandling skulle också kunna leda till utvecklandet av nya och effektivare läkemedel mot depression.

INTRODUCTION

Major depression

Major depressive disorder is a very common, complex and serious disease characterized by a depressed mood and/or the loss of interest or pleasure in nearly all activities. Additional symptoms include appetite disturbances, impaired sleep, psychomotor agitation or retardation, loss of energy, a decreased ability to think or concentrate and feelings of worthlessness and suicidal ideation (DSM-IV) (APA 1994). The Global Burden of Disease-estimates from 2000 by the World Health Organisation (WHO) state that in a 12-month period, depression is estimated to be affecting around 10% of all women and 5% of all men (WHO 2001). The disease has a tendency to recur and if left untreated, depression is a life threatening disease with high mortality in suicide. According to the WHO, major depression will in 2020 be the second largest cause of disability worldwide, second only to ischemic heart disease, and in the developed regions it will constitute the highest ranking cause of burden of disease (WHO 2001).

Treatment strategies

The treatments available for depression today include psychological treatments i.e. psychotherapy and physical treatments which include pharmacological treatment with antidepressant drugs and electroconvulsive therapy (ECT).

Antidepressant drugs

Antidepressant drug treatment commonly constitutes the first-line choice for treatment of depression. All major, clinically used, antidepressant drugs share the common effect of enhancing monoaminergic, particularly serotonergic and noradrenergic, neurotransmission in the brain. The first modern antidepressants, which were the tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs), became available in the late 1950s (Paykel and Scott 2000). TCAs increase the availability of serotonin (5-HT) and/or noradrenaline (NA) by blocking the reuptake of 5-HT and NA and thereby prolonging the time that these monoamines act in the synaptic cleft (Siegel et al 1999). The enzyme monoamine oxidase (MAO) degrades 5-HT, NA, adrenaline and dopamine and MAOIs thus increase the levels of monoamines in the brain (Siegel et al 1999).

Newer classes of antidepressant drugs involve selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Fontex) and citalopram (Cipramil) and the selective noradrenaline reuptake inhibitor (NARI) reboxetine (Edronax). Also so called serotonin and noradrenaline reuptake inhibitors (SNRIs), for example the commonly used venlafaxine (Efexor) are available (Paykel and Scott 2000).

Typically there is a delay in the therapeutic effect by antidepressant drugs for three to four weeks. Side effects, most notable during the first weeks of treatment and more frequent with older types of antidepressants, commonly include anxiety, dry mouth, blurred vision, and nausea. Medication should be continued up to six weeks before the treatment can be considered ineffective and a successful

treatment should continue for at least six months after response (Paykel and Scott 2000).

Electroconvulsive therapy (ECT)

ECT is one of the oldest treatments available in psychiatry today and still considered the most effective therapy for severe depression (for review see Paykel (1989)). It is sometimes used a first choice treatment in patients with severe depression and high risk for suicide, but perhaps more often if the patient does not respond to antidepressants (Paykel and Scott 2000). Regarding the mechanisms of action of ECT, which still are largely unknown, ECT has been described as being a broad spectrum therapy with more effects on the activity of the monoaminergic system compared to antidepressant drug therapy (Ottosson 2000). The principle behind ECT consists of the delivery of a controlled, electrically induced, grand mal seizure. Since its introduction in 1938 the treatment procedures have been refined and today patients receiving ECT are anesthetized and oxygenated (Fink 2000). Typically the treatment is carried out in the morning with the patient fasting overnight. An i.v. cannula is placed in the arm allowing for administration of an anaesthetic drug, often sodium pentothal and a muscle relaxant, succinylcholine. Oxygen is administered via face mask prior to the onset of the seizure, blood pressure is monitored before and after the treatment and oxygen saturation and EEG are monitored throughout the procedure. The seizure is induced via electrodes placed on the head while EEG activity is monitored in order to determine the length and the quality of the seizure. Normally the grand mal seizure last for 30 to 60 seconds. Assisted breathing via face mask with delivery of oxygen is continued as soon as possible after the onset of the seizure. After the treatment the patient is allowed to wake up in a calm environment where he or she is monitored. Usually outpatients can leave the ward and return home within an hour after treatment cessation. An improvement of the depressive state can occasionally be noted as early as after one treatment, but usually it takes longer before any improvement is noticeable (Fink 2000). Typically ECT is given three times a week and a normal course runs from six to 20 treatments. Sometimes continuation ECT is given, with one treatment every second week for several months (Fink 2000). Common side effects of ECT include headache immediately after the session and retrograde and/or anterograde amnesia that can persist for weeks after a course of treatment (Weeks et al 1980).

Patophysiology of depression

The monoamine theory of depression

One of the most well-known hypotheses concerning the underlying pathology of depression advocates that disturbances in the monoaminergic neurotransmission, hence the name "monoamine theory of depression", are causing the disease. Support for this hypothesis came from experiments in which monoaminergic function was inhibited by the drug reserpine with a subsequent appearance of depressive symptoms, while MAOIs and TCAs, drugs that increased noradrenergic

activity instead had the ability to relieve depressive symptoms (Schildkraut 1965). Subsequently further studies of then available antidepressants showed that 5-HT also was involved in the regulation of mood (Carlsson et al 1969; Coppen 1967). However, no direct support for the original monoamine hypothesis of depression has arisen from clinical studies, and instead it has been proposed that the monoamine systems are modulating other neurobiological systems with a more primary role in depression (Heninger et al 1996).

Neuroendocrine disturbances

Neuroendocrine disturbances have long been recognised in association with depression. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis causing hypersecretion of cortisol is observed in approximately 50% of all patients with depression (Goodwin 2000) and was described already in the 1950s (Board et al 1956). Further support for a dysfunctional HPA-axis comes from studies showing elevated levels of corticotropin-releasing hormone (CRH) in depressed patients, which however tend to normalize after antidepressant treatment (Nemeroff et al 1991; Nemeroff et al 1984), increased numbers of CRH-producing neurons in limbic regions and changes in CRH binding sites (Nemeroff et al 1988; Raadsheer et al 1994) and an enlargement of the pituitary and adrenal glands in depressed patients (Axelson et al 1992; Krishnan et al 1991; Nemeroff et al 1992; Rubin et al 1995). It has been proposed that patients with a genetic predisposition for depression have alterations in the signaling of corticosteroid receptors, affecting CRH gene expression in the brain (for review see Holsboer (2000)). Also vasopressin (AVP), which acts in synergy with CRH in controlling the release of adrenocorticotropic hormone (ACTH), has been implicated in the clinical manifestations of major depression (for review see Tichomirowa et al (2005)).

In addition to alterations in the HPA-axis, dysregulation of the hypothalamic-pituitary-growth hormone (HPGH), hypothalamic-pituitary-thyroid (HPT) and hypothalamic-pituitary-gonadal (HPGn) axes as well as alterations in neurosteroid and leptin levels has been described in depressed patients. The space is limited here but the interested reader is recommended to seek out the recent publication by Tichomirowa et al (2005) for an excellent review.

Neuroanatomical changes in depression

Until quite recently, major depression was described as a "functional illness", i.e. an illness where no structural brain pathology can be detected. However in recent years a substantial amount of research has provided evidence for depression-associated structural changes. Volume reduction has been reported in the frontal cortex (Coffey et al 1993; Drevets et al 1997), with evidence of neuronal and glial cell pathology (Rajkowska et al 1999). Glial cell pathology has also been described in the amygdala (Bowley et al 2002). Results describing volume changes in the amygdala have been inconsistent with reports of both decreases (Sheline et al 1998; von Gunten et al 2000) and increases in volume (Bremner et al 2000; Frodl et al 2002). The hippocampus has been extensively studied and many reports have

described significantly reduced hippocampal volume in depression in the range of 8-19% (Bell-McGinty et al 2002; Bremner et al 2000; MacQueen et al 2003; Shah et al 1998; Sheline et al 1999; Sheline et al 1996). Reports presenting negative data have also been published (Ashtari et al 1999; Axelson et al 1993; Vakili et al 2000). However, a recent meta-analysis of a large number of magnetic resonance imaging (MRI) studies come to the conclusion hippocampal volume changes are readily detected if the hippocampus is measured as a discrete structure, not including the amygdala (Campbell et al 2004). The hippocampal volume has been correlated to depression severity (Shah et al 1998; Vakili et al 2000) and to the length of time the depressive illness went untreated (Sheline et al 2003). In the latter report no correlation between hippocampal volume and lifetime exposure to antidepressants was found, and the authors argued that antidepressants might protect against hippocampal volume loss associated with repeated episodes of depression.

Potential mechanisms for neuroanatomical changes in depression

Several mechanisms could potentially account for the observed volume loss in depression: dendritic atrophies, decreases in neuro- and gliogenesis and neuronal and glial loss. Preclinical and clinical studies suggest that the glucocorticoid cortisol may be involved in these mechanisms. As already presented, hypercortisolemia is a common feature in major depression. According to the glucocorticoid cascade hypothesis (Sapolsky et al 1986), high levels of glucocorticoids, resulting from impaired negative feedback regulation of the HPA-axis can be neurotoxic (for review see Sapolsky (2000)). Support for a damaging effect of high levels of glucocorticoids come from studies of Cushing's disease in which patients displayed reduced hippocampal volume which returned to normal after successful treatment and normalization of the glucocorticoid levels (Bourdeau et al 2002; Starkman et al 1992). Patients with Cushing's disease interestingly also show high incidence of depression (Sonino and Fava 2001). Long-term corticosteroid treatment is also associated with lower hippocampal volume and deficits in hippocampal functioning (Brown et al 2004). Furthermore, a correlation between cortisol levels and hippocampal volume loss has been described in aged, non-depressed subjects (Lupien et al 1998). Preclinical studies have revealed that rats exposed to corticosterone (the cortisol homologue in rat) show depressive behavior (Gregus et al 2005; Kalynchuk et al 2004) and significantly reduced hippocampal volume (Sousa et al 1998). In a similar manner, psychosocial stress, which causes elevated glucocorticoid levels, has been shown to lead to a non-significant trend towards reduction in hippocampal volume in tree shrews, which can be reversed by antidepressant treatment (Czeh et al 2001). On a cellular level, elevated levels of glucocorticoids cause reversible dendritic atrophies in the CA3 subfield of the hippocampus (for review see McEwen (1999)), reduced proliferation of NG2-positive oligodendrocyte progenitors (Alonso 2000) and reduced proliferation and neurogenesis in the dentate gyrus (Cameron and Gould 1994; Cameron et al 1998; Gould et al 1997; Tanapat et al 1998).

Neurogenesis in the adult brain

Neurogenesis, that is, the generation of new neurons, was first described in the adult rat brain in the 1960s (Altman and Das 1965). Later this finding was replicated in the adult human brain (Eriksson et al 1998). There are two neurogenic regions in the adult brain; the subgranular zone (SGZ) at the border between the granule cell layer (GCL) and hilus in the dentate gyrus (DG), and the subventricular zone (SVZ), lining the lateral ventricles. These two regions differ somewhat in their composition in that the progenitors in the SGZ are lineage restricted while the progenitors in SVZ are multipotent neural stem cells (Seaberg and van der Kooy 2002). The neuronal progenitors in SGZ progressively develop a mature neuronal phenotype and migrate from the SGZ into the GCL (Cameron et al 1993) and send out axons to the CA3 region (Markakis and Gage 1999; Stanfield and Trice 1988). These newly formed neurons integrate into the GCL, form functional connections and acquire electrophysiological properties resembling those of mature granule cells (van Praag et al 2002). It has been estimated that in the rodent brain, 250000 new neurons are born every month, representing 6% of the total size of the granule cell population, and 60% and 30% of the size of the afferent and efferent populations, respectively (Cameron and McKay 2001).

What is the function of these newly formed neurons? It has been speculated that neurogenesis is important for cognition and brain repair. Exercise, which is known to increase neurogenesis, has been associated with improved long term potentiation and memory function (van Praag et al 1999). Spatial learning enhances neurogenesis (Ambrogini et al 2000; Gould et al 1999), and some studies show that neurogenesis may directly be involved in hippocampal dependent learning (Shors et al 2001; Shors et al 2002) while others fail to do so (Merrill et al 2003). Insults to the adult brain like epileptic seizures (Bengzon et al 1997; Parent et al 1997), ischemia (Liu et al 1998) and traumatic brain injury (Dash et al 2001) causing hippocampal damage also increase neurogenesis, implicating a role for adult neurogenesis in brain repair. As previously described, stress, via elevated levels of glucocorticoids on the other hand decrease neurogenesis and is also causing learning deficits (Lemaire et al 2000). The glucocorticoid-induced decrease in neurogenesis has been shown to depend on glutamatergic signaling since blockade of the N-methyl D-aspartate (NMDA) receptor abolishes the glucocorticoid induced decrease in neurogenesis (Cameron et al 1998). Also NA and 5-HT seem to be involved in the proliferation of SGZ progenitors since depletion of these two neurotransmitters cause decreases in proliferation and neurogenesis (Brezun and Daszuta 1999; Kulkarni et al 2002).

Adult neurogenesis has been described as occurring in an "angiogenic niche" with dividing capillary endothelial cells in close contact with dividing neural (neuronal and glial) precursors (Palmer et al 2000). A number of growth factors influence neurogenesis as well as angiogenesis, indicating a possible co-regulation of neurogenesis and angiogenesis in the adult brain. Fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF) are well known endothelial cell mitogens that also have neurogenic actions (Ghosh and Greenberg

1995; Jin et al 2002). Brain-derived neurotrophic factor (BDNF) which is essential for the survival of proliferating cells within the SVZ and GCL and induce neurogenesis in the olfactory bulb, striatum, septum, thalamus and hypothalamus (Pencea et al 2001; Zigova et al 1998) can both enhance endothelial cell survival and induce vascular sprouting (Kim et al 2004). A causal relationship between angiogenesis and neurogenesis has been proposed in the avian brain where endothelium-derived BDNF promotes neuronal recruitment into the higher vocal center (HVC) from the HVC ventricular zone (Louissaint et al 2002) and endothelial cells have been shown to be a critical part of the stem cell niche, producing factors that directly enhance the neurogenic potential of neural stem cells (Shen et al 2004).

Angiogenesis in the adult brain

Angiogenesis in the adult nervous system is often recognized as a phenomenon correlated to pathology, for example in association with tumour growth (Takano et al 2004), in diabetic retinopathy (Wegewitz et al 2005) and in response to stroke (Greenberg 1998). In ischemic insults such as stroke where regional blood supply to the brain is shut down, angiogenesis provides a protective mechanism, triggered by the hypoxia sensitive transcription factor hypoxia inducible factor-1 (HIF-1) which in turn elicits VEGF-signaling and subsequent angiogenesis (Marti et al 2000). Similarly, hypoxia induces increased capillary density which however is reversible upon restoration of ambient oxygen levels (for review see LaManna et al (2004)). Oxygen sensing systems thus seem essential for angiogenesis to occur (Risau 1997).

In non-pathological situations in which the balance between oxygen availability and metabolic demand is altered, angiogenesis can occur too. Increased capillary density has been observed in cerebellum (Black et al 1990; Isaacs et al 1992), visual cortex (Black et al 1987; Black et al 1991), motor cortex (Swain et al 2003) and striatum (Ding et al 2004) after sensory stimulation and exercise.

Cerebrovascular dysfunction is associated with cognitive impairment, as seen for example in vascular dementia (for review see Jellinger (2005)). A link between cerebrovascular disease and late-life depression has been proposed (for reviews see Kales et al (2005; Thomas et al (2004)), but as yet no risk factors for vascular disease correlating with decreased hippocampal volume has been identified (Hickie et al 2005). It is reasonable to assume that alterations in angiogenic processes could be of importance for the development of cerebrovascular dysfunction.

The cellular plasticity hypothesis of depression

A novel hypothesis states that major depression partly could be explained by reductions in and/or failure of adult neurogenesis (Duman et al 2000; Jacobs 2002; Jacobs et al 2000; Kempermann 2002a). There are a number of lines of reasoning upon which this hypothesis is based and from which it has gained support. Firstly, although under debate, major depression has been described as being causally related to stressful events (Kendler et al 1999; Patton et al 2003) and animal stress

models are commonly used to screen for antidepressant drug actions (for review see Willner (1997)). Different types of stress cause reductions in proliferation and neurogenesis in a variety of experimental animals, for example marmosets (Gould et al 1998), tree shrews (Czeh et al 2001; Gould et al 1997), rats (Malberg and Duman 2003; Tanapat et al 1998; Tanapat et al 2001) and mice (Alonso et al 2004). Exogenous administration of glucocorticoids decreases proliferation and neurogenesis and thus mimics the effects of environmental stress (Cameron and Gould 1994; Cameron et al 1998; Gould et al 1992). Secondly, antidepressant medication, ECT and the mood stabilising drug lithium increase neurogenesis in the adult hippocampus (Chen et al 2000; Madsen et al 2000; Malberg et al 2000; Scott et al 2000). Chronic but not acute treatment with antidepressant drugs increases neurogenesis with approximately 30-50%, while both acute and chronic electroconvulsive seizure (ECS)-treatment, an animal model for ECT, cause a profound increase in neurogenesis in the range of 300-500%. Thirdly, antidepressants are able to counteract stress-induced decreases in hippocampal cell proliferation (Czeh et al 2001; Lee et al 2001; Malberg and Duman 2003; van der Hart et al 2002) and last but not least, neurogenesis has been shown to be necessary for the antidepressant response in two separate behavioural models for depression (Santarelli et al 2003). In the latter study mice were subjected to two behavioural paradigms; novelty suppressed feeding and chronic unpredictable stress. Antidepressant medication normalized the feeding and grooming behaviour, respectively, of mice in these two models and increased neurogenesis. However, following irradiation of the hippocampus, both the neurogenic and the behavioural response was abolished, indicating the importance of preserved neurogenesis for the antidepressant effect.

Irradiation does not only stop neurons from dividing but also glial and endothelial cells are likely to be affected. Indeed, an irradiation-induced disruption of the angiogenic niche for neurogenesis along with microgliosis has been described by Monje et al (2002). Santarelli et al (2003) could neither find evidence of gross morphological alterations of the dentate gyrus following irradiation, nor alterations in synaptic plasticity. Still, stating that an inhibition of neurogenesis is the sole reason for the blockade of the behavioural responses to antidepressants is probably somewhat of an under-appreciation of the importance of other celltypes in the cellular network of the brain.

Kempermann and Kronenberg (2003) states that it is unlikely that altered adult neurogenesis alone can be the cause of major depression, not least given the complexity of the disorder and the number of brain structures that are involved. The authors propose that the hypothesis concerning the role of neurogenesis in major depression is broadened to encompass cellular plasticity in general. It is an understatement to say that research concerning cellular plasticity in relation to major depression and the treatment thereof is warranted.

AIMS OF THE THESIS

The main objective of this thesis has been to investigate the proliferative response to electroconvulsive seizure (ECS)-treatment in the adult rat brain. The specific aims were:

1. To investigate the potential for ECS-induced neurogenesis in an animal model mimicking features of major depression.

2. To characterize the ECS-induced non-neuronal cell proliferation, its dose dependence and temporal characteristics, in relation to ECS-induced neurogenesis.

3. To investigate the role of ECS-induced endothelial cell proliferation in relation to ECS-induced neural precursor proliferation.

4. To investigate whether ECS-induced endothelial cell proliferation leads to the formation of new blood vessels.

5. To investigate ECS-induced endothelial cell proliferation in the hypothalamus in relation to neuronal activation.

MATERIAL AND METHODS

Experimental animals

Adult male Wistar rats (Møllegaard breeding centre, Denmark), weighing 180-200 g at the beginning of the studies were used. Rats were housed 3 per cage and kept on a 12 h light-dark cycle with *ad libitum* access to food and water. Experiments were carried out according to guidelines set by the Malmö-Lund Ethical Committee for the use and care of laboratory animals.

Animal treatments

Electroconvulsive seizure (ECS)-treatment

Bilateral ECS-treatment was delivered via ear clips (50 mA, 0.5 s and 50 Hz unidirectional square wave pulses). Rats were monitored after ECS-treatment to ensure that clonic movements of the face and forelimbs (indicative of limbic motor seizures) occurred for a minimum of 20-30 s. Sham-treated rats were handled identically to the ECS-treated rats except that no current was passed.

Bromodeoxyuridine-treatment

Bromodeoxyuridine (BrdU) (B5002; Sigma-Aldrich, St Louis, MO, USA) was dissolved in phosphate buffered saline (PBS) and administered intraperitoneally. Rats received 10 injections of BrdU (50 mg/kg) in 12 h intervals over 5 days (papers I-V) or four injections of BrdU (100 mg/kg) in 2 h intervals over 6 hrs (time-course study, paper II)

Corticosterone-treatment (papers I and III)

A stock emulsion of corticosterone (CORT) (C2505; Sigma-Aldrich, St Louis, MO, USA) at a concentration of 33.3 mg/ml was prepared daily by vortexing CORT in sesame oil (Sigma-Aldrich) for 10 min, followed by 60 min of sonication. Prior to every injection, the emulsion was vortexed briefly and injections were made subcutaneously in the neck region (40 mg/kg) every 24 h. Control rats received only sesame oil injections.

Blood gas measurements and oxygenation procedures (paper IV)

In order to collect blood samples and assess the effect of ECS-treatment on blood gas levels all rats were cannulated in the tail artery under halothane anaesthesia 1 h before the onset of the experimental procedures. For the oxygenation procedures, rats were given 100% O_2 in a plexi-glass chamber for 12 min followed by administration of a single ECS while 100% O_2 was given via mask and subsequent recovery in 100% O_2 in the plexi-glass chamber for 10 min. Arterial blood samples were collected immediately before and 50 s after the onset of the seizure.

Hypoxiprobe™-1-treatment (paper IV)

Rats were injected intraperitoneally with Hypoxyprobe™-1 (pimonidazole hydrochloride) (Chemicon, Temecula, CA, USA) (80 mg/kg) for detection of local

tissue hypoxia in response to a single ECS. Injections were done 30 min before ECS or sham-treatments and rats were decapitated 50 s after the ECS or sham-treatment, their brains dissected out and frozen on powdered dry ice.

Histological procedures

Tissue processing

With the exception of the rats in the Hypoxyprobe™-1 study (paper IV), rats were anaestisized with sodium pentobarbital and in the absence of nociceptive reflexes, transcardially perfused with 0.9% saline, followed by 4% paraformaldehyde (PFA). The brain was removed from the skull and postfixed at 4°C overnight, followed by cryoprotection in 30% sucrose in phosphate buffered saline (PBS). Coronal 40 μm sections (papers I-V) and/or horizontal 100 μm sections (paper IV, stereology) were cut on a freezing microtome and stored in antifreeze cryoprotectant solution at -20°C until the immunohistochemical procedure was performed. Fresh frozen brains from the Hypoxyprobe™-1 study (paper IV) were sectioned at 14 μm on a cryostat and mounted on glass slides.

Immunohistochemistry

Detailed protocols for the immunohistochemistry procedures can be found in papers I-V and a complete list of primary antibodies used in the studies can be found in Table 1. Fresh frozen, slide mounted sections, to be stained for Hypoxyprobe™-1, were initially fixated in ice cold acetone for 15 min. Free floating sections were washed in KPBS before staining procedures began. In the case of the cPARP staining (paper IV) sections were mounted on glass slides after initial washing in KPBS. Sections to be developed with horseradish peroxidase catalysed 3,3"-diaminobenzidine (DAB) conversion were pretreated with 3% H_2O_2 in 10% methanol in order to quench endogenous peroxidase activity (papers I, II, IV). cPARP stained mounted sections were subjected to quenching immediately before the peroxidase detection step. Sections to be stained for BrdU were pretreated with 1 M hydrochloric acid for 30 min in 65°C to achieve DNA denaturation. Following these initial procedures, sections were preincubated in blocking solution containing normal sera from animals in which the secondary antibodies were raised and subsequently incubated with one or two primary antibodies in blocking solution. The primary antibody in the cPARP staining was incubated in blocking solution containing bovine serum albumine. For DAB stainings, sections were then incubated with biotinylated secondary antibodies dissolved in blocking solution, followed by incubation with avidin-biotin-peroxidase complex. Staining was developed with DAB and H_2O_2. For fluorescent stainings, fluorochrome conjugated secondary antibodies and/or biotinylated secondary antibodies followed by streptavidin conjugated tertiary fluorochrome antibodies were used. Regularly used fluorochromes were Alexa 488 and Cy3/Cy5. In order to avoid destruction of epitopes by HCl, double/triple stainings with BrdU and RECA-1 and/or Laminin were performed in sequence, starting with RECA-1 and/or Laminin followed by fixation in 4% PFA for 10 min before

pretreatment with HCl and subsequent BrdU staining. DAB stained sections were counter stained with cresyl violet and fluorescent sections were in one case (assessment of sprouting angiogenesis, paper IV) counter stained with 4',6-diamidino-2-phenylindole (DAPI).

Table 1

Antibody	Working dilution	Incubation time	Source	Stains
Monoclonal mouse anti-NeuN	1:100	40 h at 4°C	Chemicon	Post mitotic neurons
Monoclonal mouse anti-RECA-1	1:25/1:100	Over night-72 h at 4°C	Serotec	Rat endothelial cells
Monoclonal mouse anti-EBA	1:1000	Over night at 4°C	Sternberger Monoclonals	Endothelial cells with intact blood-brain barrier
Monoclonal mouse anti-HP-1	1:25	24 h at 4°C	Chemicon	Hypoxyprobe™-1 adducts
Polyclonal rabbit anti-human c-fos	1:2000	40 h -72 h at 4°C	Sigma-Aldrich	Activated cells expressing the immediate early gene c-fos
Polyclonal rabbit anti-rat cPARP	1:50	Over night at 4°C	Cell Signaling Technology	Apoptotic cells
Polyclonal rabbit anti-Laminin	1:100	48 h at 4°C	Sigma-Aldrich	Capillary basement membrane
Polyclonal rat anti-BrdU	1:100	40-48 h at 4°C	Oxford Biotechnology	BrdU-labeled nuclei
Monoclonal mouse anti-BrdU	1:25	40 h at 4°C	Becton Dickinson	BrdU-labeled nuclei

Cresyl violet staining (papers I and IV)

Brain sections to be stained were rinsed 3 times in KPBS, mounted on glass slides and air dried overnight. The sections were briefly rinsed in H_2O two times and then subsequently dipped in 0.5% cresyl violet solution until sufficient staining was achieved followed by rinsing in H_2O, dehydration and coverslipping.

Silver staining (paper I)

The staining was performed according to the protocol by Nadler and Evenson (1983). Brain sections were washed in 0.1 M Tris buffer (pH 7.6) followed by

rinsing in H_2O. After pre-treatment in 4.5% NaOH and 8% NH_4NO_3, the sections were incubated in impregnation solution (5.4% NaOH/6.4% NH_4NO_3/0.2% $AgNO_3$ in H_2O) for 10 min and then washed in 31.6% ethanol/0.5% $NaCO_3$/0.12% NH_4NO_3. The staining was developed in 0.05% citric acid /0.55% formaldehyde/9.5% ethanol/0.12% NH_4NO_3 for 5 min. Sections were then rinsed in 0.1 M Tris buffer (pH 7.6), mounted, air-dried, dehydrated and coverslipped.

Fluoro-Jade staining (paper I)
The staining was performed according to the protocol originally developed by Schmued et al (1997). Brain sections were rinsed in KPBS, mounted on glass slides and air dried overnight. The mounted sections were then immersed in 100% ethanol for 3 min, 70% ethanol for 1 min and H_2O for 1 min. Pre-treatment in 0.06% potassium permanganate for 15 min was followed by rinsing in H_2O for 1 min and subsequent staining with Fluoro-Jade working solution (Histo-Chem, Jefferson, AR, U.S.A.) for 30 min. Following rinsing in H_2O the mounted sections were air dried, immersed in xylene and coverslipped.

Microscopical analysis
Brightfield microscopy (papers I, II, IV, V)
Cell proliferation, EBA-staining, possible cPARP-positive cells and pyknotic cells as indicated by cresyl violet staining was quantified in the GCL (including the SGZ), hilus and ML of the dentate gyrus (papers I, II, IV). Staining for c-fos was evaluated in the paraventricular nucleus (PVN), supraoptic nucleus (SON) and ventromedial hypothalamic nucleus (VMH) of the hypothalamus (paper V). An Olympus AX70 light microscope with a 40x objective was used. BrdU-positive cells were counted in the GCL (including the SGZ) and hilus (paper I). Cells lying within two cell diameters from the border between GCL and hilus were included in the GCL count. The PVN and the SON were defined through a UV-filter where these nuclei were easily recognizable, and according to Paxinos and Watson (1986). The VMH was defined according to Paxinos and Watson (1986). Cell counts were averaged and expressed as means per section.

Epifluorescence microscopy (papers II, III, IV, V)
Cell proliferation was assessed in the GCL (including the SGZ), hilus and ML of the dentate gyrus, using an Olympus AX70 fluorescence microscope with a 40x objective. BrdU-positive clustered cells (endothelial and neural) and BrdU-positive neurons were counted in the GCL (including the SGZ) (papers II, III, IV). Proliferated endothelial cells were counted in the GCL, hilus and ML of the dentate gyrus (papers II, III, IV) and in the PVN, SON and VMH of the hypothalamus (paper V). Cells lying within two cell diameters from the border between GCL and hilus were included in the GCL count. The total number of proliferated cells, defined as BrdU positive, and the number of proliferated endothelial cells, defined as BrdU and RECA-1 double positive, were counted

within the PVN, SON and the VMH. Cell counts were averaged and expressed as means per section.

Confocal microscopy (papers I, II, III)
Confirmation of double-labelling was performed on a Nikon confocal microscope using a 40x objective and BioRad software (BioRad, Burlington, MA, USA) (papers I and II) or a Leica TCS SL, Spectral Confocal Microscope (Leica Microsystems, Mannheim, Germany), with a 100x oil immersion lens objective and Leica Confocal Software, version 2.61 (Leica Microsystems, Mannheim, Germany) (paper III). For confirmation of BrdU/NeuN-double labelling, 25 BrdU-positive cells per animal were analysed for verification of colocalisation within the granule cell layer and hilus, respectively (papers I and II). The identity of BrdU labelled cells associated with RECA-1 vessels was determined by confocal analysis of a subset (n=100) of BrdU-positive nuclei in a triple staining for BrdU/RECA-1/Laminin (paper II). For determination of the percentage of BrdU-positive cluster cells in the subgranular zone, expressing RECA-1, 20 clusters per animal were analysed (papers II and III).

Stereology (paper IV)
The total number of endothelial cells in the ML of the DG was estimated using the optical fractionator method (West et al 1991). Every fourth horizontal section throughout the ML was sampled and analysis was performed employing the C.A.S.T-GRID system (Olympus, Denmark), consisting of an Olympus microscope with an x-y motor stage controlled by the C.A.S.T-GRID software and a microcator. Images acquired with a color digital camera were displayed live on a computer screen. Using a 1.25x objective with 2x zoom the ML was delineated on the screen in accordance with West et al (1978) and Paxinos and Watson (1986). Sections thickness was measured at three locations per section. Sampling was performed bilaterally within the delineated areas with a 100x objective with oil immersion lens. A three-dimensional probe consisting of a counting frame that was focused through a known depth of the section (an "optical disector") was used. The counting frame area was 260.9 μm^2 and the dissector height was 15 μm with a guard zone of 2 μm at the top of the section. The x-y steps giving the distance between sampling areas was 140 μm x 140 μm, generating counts of 150-300 sampled endothelial cells per animal.

The total vessel length in the ML, including potential arterioles, capillaries and small venules was estimated employing the global spatial sampling method utilizing isotropic virtual planes (Larsen et al 1998). Every fourth horizontal section throughout the ML was sampled and analysis was performed employing the C.A.S.T-GRID system. Structure delineation and section thickness measurement was performed as described above. Sampling was performed unilaterally within the delineated areas with a 40x objective with oil immersion lens. A three-dimensional sampling box consisting of a counting frame that was focused through a known

depth of the section was used. Within this 3D sampling box, isotropic virtual planes were generated with a fixed plane separation distance and the number of intersections between vessel spines and the virtual planes were counted according to counting rules described in Larsen et al (1998). The counting frame area was 4841 μm^2 and the dissector height was 15 μm with a guard zone of 2 μm at the top of the section. The x-y steps giving the distance between sampling areas was 300 μm x 300 μm, generating counts of approximately 400 vessel intersections per animal.

Statistical analysis

Values are presented as means ± standard error of the mean (SEM) throughout the text. Differences in proliferation following ECS- and/or CORT-treatment were in cases with only two groups analysed with Student's unpaired t-test (papers II and V) or, if three or more groups were compared, with one-way analysis of variance (one-way ANOVA) followed by either Bonferroni-Dunn post hoc test (papers I and II), Fisher's protected least significant difference (PLSD) post hoc test (paper III) or Scheffé's post hoc test (paper IV). Differences in total endothelial cell numbers and vessel length were analysed with Student's unpaired t-test (paper IV). In all cases differences were considered significant when $p \leq 0.05$. Regression analysis was performed to evaluate the correlation between total endothelial cell numbers and total vessel length (paper IV). All statistical analyses were performed with Statview software, version 5.0 (Abacus Concepts, Berkeley, CA, USA).

RESULTS AND COMMENTS

Corticosterone and ECS-treatment affect cell proliferation in the dentate gyrus (paper I)

At the time of the initiation of the experiments for paper I, a substantial amount of evidence had emerged that stress and high levels of glucocorticoids negatively affected cell proliferation and neurogenesis in the adult hippocampus (Alonso 2000; Cameron and Gould 1994; Cameron et al 1998; Gould et al 1997; Tanapat et al 1998). Furthermore, experiments in our own lab, spurred by the observations that epileptic seizures influence neurogenesis in the adult rat brain (Bengzon et al 1997; Parent et al 1997), had revealed that electroconvulsive seizures, a model for electroconvulsive therapy (ECT), causes a strong increase in hippocampal neurogenesis (Madsen et al 2000). The growing notion that decreased neurogenesis could be involved in the patophysiology of major depression (Jacobs et al 2000) and that mood stabilizing and antidepressant medication increased neurogenesis (Chen et al 2000; Malberg et al 2000) led us to combine the glucocorticoid corticosterone (CORT) and ECS-treatment in an attempt to elucidate to the potential role of neurogenesis modulation in the clinical effects of ECT. No previous studies had at the time investigated the neurogenic potential of an antidepressant treatment modality in a setting mimicking aspects of major depression, in this case hypercortisolemia. In order to do so we treated rats with CORT for three weeks in order to inhibit normal ongoing neurogenesis. At the end of this three week period we delivered either one or five ECS-treatments along with five days of BrdU-injections and then allowed the rats to survive an additional three weeks in order for newly formed neurons to mature.

In direct agreement with previous studies, CORT-treatment resulted in a substantial lowering of cell proliferation, with approximately 75% fewer BrdU-positive cells detected in the GCL and hilus compared to the number in control animals.

As previously shown, a single ECS-treatment profoundly increased BrdU-labelling in the GCL in vehicle-treated animals. A single ECS-treatment also restored the rate of proliferation in the CORT-treated animals back to baseline levels, with no differences in the percentage of double labelling for BrdU/NeuN between control animals and animals receiving CORT and a single ECS-treatment, thus showing that the rate of neurogenesis was restored. However, the situation in the hilus was completely different where the strong CORT-induced reduction in the number of BrdU-positive cells was still present despite ECS-treatment.

When CORT-treated rats received five ECS-treatments, the increase in BrdU-labelling in the GCL was the same as in vehicle animals receiving five ECS-treatments. Also, the percentage of BrdU/NeuN-double labelled cells was the same in the two groups. Thus, the rate of neurogenesis was now not only restored back to baseline levels but on par with that of the vehicle-treated rats receiving five ECS-treatments. Also in the hilus five ECS-treatments induced a significant increase in BrdU-labelling in CORT-treated rats compared to vehicle-treated

animals. However, the number of BrdU-labelled cells in the hilus was significantly lower in the CORT-treated animals receiving five ECS-treatments than in vehicle animals receiving five ECS-treatments. The majority of the BrdU-labelled cells in the hilus were not neurons and we thus concluded that ECS-treatment not only promotes neurogenesis but also the generation of non-neuronal cells. Furthermore the vast majority of the cells that still were inhibited by CORT were non-neuronal. Together, the results from paper I revealed a strong potential for ECS-treatment to counteract glucocorticoid-induced decrease in hippocampal neurogenesis, and to a lesser extent also counteract glucocorticoid-induced decrease in the proliferation of non-neuronal cells.

Endothelial cells and neural precursors proliferate in a coordinated fashion in response to ECS-treatment (paper II)

The observation that a large number of non-neuronal cells, mainly located outside the GCL, proliferate in response to ECS-treatment prompted us to investigate the phenotype of these cells and the dose dependence and temporal characteristics of their proliferative response. We noted that a large proportion of these cells, which were located in the ML, hilus and GCL of the DG, were associated with vessel-like structures. By triple staining for the capillary basement membrane component laminin, rat endothelial cell antigen-1 (RECA-1), a protein expressed on the luminal surface of endothelial cells and the proliferation marker BrdU, we were able to confirm that the proliferating vessel associated cells were confined between the vascular basement membrane and the luminal side of the vessel, thus indicating that these cells were endothelial cells.

A single ECS-treatment gave a very strong increase in endothelial cell proliferation in the GCL, hilus and ML, which was further enhanced by additional ECS-treatments. In agreement with paper I, ECS-treatment also induced proliferation of clustered cells in the SGZ. In order to determine how non-clustered endothelial cells primarily located in ML and hilus, and the clustered cells in SGZ responded to ECS-treatment we conducted a time course study. This revealed a sharp increase in proliferation of endothelial cells in the GCL, hilus and ML at day 2 following a single ECS-treatment. No significant increase in endothelial cell proliferation was seen at any other time point studied after a single ECS-treatment (day 0, 4, 6 and 8). The clustered cells in the SGZ displayed a proliferation maximum at day 4 with increased levels also at day 2 and 6, but at no other time point following the seizure. The increase in proliferation at their respective maximum was 14-fold for the endothelial cells and three-fold for the clustered cells.

Palmer and co-workers had previously described that a large fraction of the clustered cells in SGZ expressed endothelial cell markers (Palmer et al 2000). In order to determine if this was the case in our material we performed a confocal microscopy analysis of cell clusters in the SGZ of control animals and animals receiving five ECS-treatments. Twenty hours after the last of five days of BrdU-injections, we found that in control animals, almost 9% of the BrdU-labelled cells

also were positive for RECA-1, while in ECS-treated animals, this fraction had increased to 19%. Also the cluster size increased significantly after ECS-treatment.

We were interested in determining if the sharp ECS-induced increase in endothelial cell proliferation resulted in newly formed endothelial cells that were stable or short lived. Three weeks after a series of five ECS-treatments we still detected significantly elevated numbers of BrdU-labelled endothelial cells in all subfields of the DG as well as elevated numbers of BrdU-labelled cells in the SGZ/GCL, expressing NeuN, indicating a neuronal phenotype.

In summary, large numbers of endothelial cells in the DG proliferate in response to ECS-treatment. The proliferation of endothelial cells and neural precursors in response to ECS-treatment is dose dependent and occur in concert, with a close spatial relationship between these celltypes in the SGZ. Furthermore, both the BrdU-labelled neural and endothelial cells are still detected three weeks after ECS-treatment, with 80% of the cells in the SGZ expressing a marker for mature neurons at this time point.

Proliferating endothelial cells are not necessary for preserved ECS-induced neural precursor proliferation (paper III)

As previously mentioned, Palmer and co-workers (2000) had described a close spatial relationship between proliferating endothelial and neural cells in the SGZ, suggesting an "angiogenic niche" for hippocampal neurogenesis. Even if the authors suggested that angiogenesis and neurogenesis could depend on each other, their work was purely a description of a spatial relationship between vascular cells and neural precursors. However, other researchers had presented evidence that there might in fact be a causal link between angiogenesis and neurogenesis, with endothelium-derived BDNF promoting neuronal recruitment in the avian brain (Louissaint et al 2002). We had in two previous papers, respectively, described that (1) cells, now identified as endothelial cells, were unable to proliferate at a normal rate in response to ECS-treatment in the presence of corticosterone (paper I) and that (2) endothelial cells and neural precursors proliferated in concert in response to ECS-treatment (paper II). We thus found ourselves with a useful tool in our hands for dissecting the "angiogenic niche", namely, corticosterone. We asked if proliferating endothelial cells were necessary for the ECS-induced proliferation of neural cells in the SGZ. This question was addressed by administering BrdU while simultaneously subjecting the rats to five ECS-treatments and seven days of CORT injections.

The rats were killed 24 h after the last CORT-injection. CORT-treatment resulted in substantially decreased proliferation of clustered cells in the SGZ and an almost total loss of proliferating endothelial cells in the ML. The latter change was however non-significant when performing multiple comparison statistics but highly significant in a two-group comparison. After five ECS-treatments, the number of proliferating endothelial cells in the ML in CORT-treated animals was restored to baseline levels, but the normal ECS-induced proliferation response was blunted. In the SGZ, however, we detected similar amounts of BrdU-labelled clustered cells in

both vehicle-and CORT-treated animals subjected to five ECS-treatments. Given the neurogenic nature of the SGZ it was possible that we in this region would find a preserved endothelial cell proliferation despite CORT-treatment. However, after confocal microscopy analysis of the proliferative clusters in SGZ from vehicle and CORT-treated animals receiving five ECS-treatments, we found that in vehicle animals approximately 8% of the cluster cells were BrdU-labelled endothelial cells. This was in stark contrast to the situation in the CORT-treated animals where only a little more than 1% of the proliferating cluster cells were endothelial.

We conclude that it is unlikely that a preserved endothelial cell proliferation is a prerequisite for neural precursor proliferation to occur in response to ECS-treatment.

ECS-treatment increases the total number of endothelial cells and total vessel length in adult rat hippocampus (paper IV)

The results from paper III clearly show that endothelial cell proliferation is not necessary for ECS-induced neural precursor proliferation to occur. It also becomes clear that CORT strongly affects the basal proliferation of endothelial cells in the DG. It is tempting to speculate that such a strong inhibition of endothelial cell proliferation could render the hippocampal vascular system vulnerable and possibly lead to rarefaction of blood vessels over long time. ECS-treatment is however able to induce a profound endothelial cell proliferation in non-CORT-treated animals and also to a lesser extent in CORT-treated animals. We now wanted to find out if the ECS-induced endothelial cell proliferation could lead to an increase in vascularization of the DG.

Rats received 10 ECS-treatments over 10 days and were then allowed to survive 11 days after the last treatment. The total number of endothelial cells and the total vessel length in all vessels of the ML, the region where we had detected the strongest increase in proliferation, were estimated using unbiased stereological techniques. Possible apoptosis/necrosis of endothelial cells was assessed but no evidence of ECS-induced increases in those parameters could be found, suggesting that the endothelial cell proliferation could lead to a net increase in the total number of endothelial cells. Indeed, the ECS-treatment resulted in 30% more endothelial cells and 16% longer vessels being detected. The increase in total vessel length probably occurs by elongation of existing vessels since we could find no evidence for increased vascular sprouting in the ECS-treated animals.

We had thus for the first time showed that an antidepressant treatment was able to induce angiogenesis in the adult brain. What causes this angiogenic response? Patients receiving ECT in the clinic are routinely oxygenated in order to avoid them becoming hypoxic during the course of the seizure. Hypoxia is a well know inducer of angiogenesis and rats in the experimental setup are normally not oxygenated during the ECS-treatment. To investigate if the observed angiogenic response in the rats merely was a response to hypoxia, i.e. an experimental artefact, we oxygenated rats before, during and after the seizure and measured blood gas levels and endothelial cell proliferation. We also injected rats with the hypoxia

marker Hypoxyprobe™-1 in order to see if we could detect local tissue hypoxia. Our results showed that oxygenation did not affect the ECS-induced endothelial cell proliferation and we were not able to detect any tissue hypoxia, despite decreases in oxygenation measured in arterial blood samples after the ECS-treatment. We concluded that it was not likely that hypoxia solely was responsible for the angiogenic response, but rather a combination of brief hypoxia and an intense activation of the hippocampal neural network, associated with the ECS-treatment.

To summarize, the results from paper IV show an angiogenic response to ECS-treatment, resulting in an increase in vascularization of the adult rat DG. The results also lend support to the idea that these processes could take place in the human brain in response to electroconvulsive therapy.

ECS-treatment-induced endothelial cell proliferation is region specific and correlates with neuronal activation in the hypothalamus (paper V)

We had so far, in the hippocampus, been able to show that ECS-treatment counteracts CORT-induced inhibition of cell proliferation (paper I), induces concomitant endothelial cell proliferation and neurogenesis (paper II), causes an increase in neural precursor proliferation despite inhibited endothelial cell proliferation (III) and leads to an expansion of the vascular tree (paper IV). What was the situation in other areas of the limbic system? Did ECS-treatment have any effects on angiogenic processes, for example in the hypothalamus, also an area of great interest for depression?

Previous work had revealed an activation of neurons in specific hypothalamic nuclei in response to ECS-treatment, as indicated by staining for the immediate early gene c-fos (Samoriski et al 1997). We wanted to investigate the possible relationship between neuronal activation and endothelial cell proliferation in the hypothalamus of adult rats. Rats received one daily ECS-treatment for up to 10 days and were sacrificed two hours later in order to analyse the pattern of neuronal activation following ECS-treatment. To assess cell proliferation rats were given BrdU and received five ECS-treatments over five days. We detected a basal low grade proliferation of endothelial cells in the PVN, SON and VMH in control animals. This basal proliferation was increased two to three-fold in the PVN and SON and more than 20-fold in the VMH, following five ECS-treatments. The strongest increase in proliferation within the PVN was in the parvocellular area where we also detected the strongest c-fos induction. Of the three hypothalamic nuclei analysed, the strongest increase in endothelial cell proliferation was in the VMH where the induction of c-fos also was most pronounced. Endothelial cells made up approximately 75% of all proliferating cells in the PVN and SON in both control- and ECS-treated animals. In the VMH of control animals only 10% of the proliferating cells were endothelial but this fraction increased to almost 60% after five ECS-treatments. As evident from double staining for c-fos and RECA-1, c-fos expression was not found in endothelial cells in any of the hypothalamic nuclei,

indicating that the cells expressing this activation marker were neuronal and/or glial.

To summarise, we detected a convincing correlation between neuronal activation as indicated by c-fos expression and endothelial cell proliferation in the hypothalamus in response to ECS-treatment, both spatially and in regard to the magnitude of activation and proliferation.

GENERAL DISCUSSION

The main objective of the current thesis was to investigate ECS-induced cell proliferation in the limbic system of the adult rat and to identify which cells that proliferate and when they do so. In addition I wanted to try to understand the possible functional significance of the ECS-induced cell proliferation. This thesis provides evidence that ECS-treatment is able to override the inhibiting effect of corticosterone on neurogenesis, thus indicating that ECS-treatment can oppose stress-induced decreases in neurogenesis. We also shed some light on the role of endothelial cell proliferation in relation to ECS-induced neurogenesis in the context of the "angiogenic niche" for hippocampal neurogenesis. Regarding ECS-actions in other limbic structures, we detect proliferating endothelial cells, co-localizing with a marker for neuronal activation, in hypothalamic nuclei implicated in the patophysiology of depression. Furthermore we show that ECS-treatment is able to induce angiogenesis in the hippocampus, resulting in an increase of the total vessel length. This last observation could potentially be of importance for the development of new therapies for depression. There are two main questions that have arisen during this thesis work, which we to some extent have tried to answer in the papers, which I will elaborate further on in the following discussion.

Does ECS-treatment induce a permissive milieu for neuronal and vascular maturation?

The principle behind ECS-treatment constitutes of the induction of a controlled, electrically induced grand mal seizure. Thus one could expect finding similarities between cellular events taking place after epileptic seizures and ECS-tretament.

ECS-treatment induces neurogenesis (Madsen et al 2000; Malberg et al 2000; Scott et al 2000), gliogenesis (Wennstrom et al 2003; Wennstrom et al 2004), and, as has been shown in the current thesis, endothelial cell proliferation leading to angiogenesis (papers II-V). Increased neurogenesis is a well documented phenomenon after epileptic seizures (Bengzon et al 1997; Gray and Sundstrom 1998; Parent et al 1998; Parent et al 1997) and gliogenesis has also been described (Huttmann et al 2003; Niquet et al 1994). Eid et al (2004) describes microvascular proliferation as a "readily observed, but largely ignored phenomenon" in epilepsy, and cites a study from the late nineteenth century describing microvascular changes in the human hippocampus (Bratz 1899). Vascular sprouting in response to kainic acid-induced seizures is described in a study by Sperk et al (1983). However, with the exception of a conference abstract, describing transient changes in capillary density in adult rat hippocampus after status epilepticus (SE) (Nicoletti 2003), I have not been able to find any more recent studies concerning possible angiogenic responses to epileptic seizures.

What causes these neurogenic and angiogenic responses? There are similarities in the variety of genes and gene products that are induced in response

to epileptic seizures and ECS-treatment, and a few of these will be described in the following paragraph.

Expression of the immediate early gene product c-fos is generally taken as an indication of neuronal activation (Morgan and Curran 1991) and c-fos expression is induced in limbic structures by both epileptic seizures (Dragunow and Robertson 1987; Le Gal La Salle 1988; Morgan et al 1987) and ECS-treatment (Daval et al 1989; Nakajima et al 1989; Samoriski et al 1997). A novel immediate early gene, vascular early response gene (*Verge*), selectively expressed in endothelial cells has recently been identified (Regard et al 2004). *Verge* mRNA is highly expressed during developmental angiogenesis and induced by epileptic seizures and ECS-treatment. The authors suggest that *Verge* functions as a dynamic regulator of endothelial cell signaling and vascular function. Furthermore, ECS-treatment induces the expression of a number of genes and gene products such as growth factors and neuropeptides with neurogenic and angiogenic actions, for example fibroblast growth factor-2 (FGF-2), VEGF, BDNF and neuropeptide Y (NPY) (Altar et al 2004; Kondratyev et al 2001; Newton et al 2003; Nibuya et al 1995; Zachrisson et al 1995). FGF-2 and VEGF are well known endothelial cell mitogens that also have neurogenic actions (Ghosh and Greenberg 1995; Jin et al 2002). Furthermore, BDNF can both enhance endothelial cell survival and induce angiogenic sprouting (Kim et al 2004). Finally, NPY have been shown to stimulate angiogenesis (Zukowska-Grojec et al 1998) as well as proliferation of neuronal precursors (Howell et al 2005). All of the above described factors are also upregulated by epileptic seizures (Croll et al 2004; Hagihara et al 2005; Marksteiner et al 1989).

At a glance, there seem to be a striking similarity in the cellular responses after ECS-treatment and epileptic seizures. However there are a number of differences with regard to the survival of the newly generated cells as well as the stability of other cells in the brain.

Neuronal cell death, both apoptotic (Bengzon et al 1997; Sloviter et al 1996), generally restricted to the GCL, and necrotic (Fujikawa 1996; Sloviter et al 1996), mainly occurring in the hilus, is observed in various epileptic seizure models. Vascular pathological events, including degeneration of pericytes and thickening of capillary walls have been observed in brains from patients with complex partial seizures (Liwnicz et al 1990). Hypertrophy of endothelial cells has been noted after kainic acid-induced seizures in the developing rat brain (Nitecka et al 1984).

In contrast, neither excessive apoptotic cell death, unrelated to normal cellular turn-over, nor necrotic cell death and subsequent cell loss is normally detected after ECS-treatment. A proton magnetic resonance spectroscopic imaging study found no evidence of decreases in the hippocampal N-acetylaspartate signal after ECT, an indication that cell death or atrophy does not occur (Ende et al 2000). Dalby et al (1996) reported that ECS-treatment does not induce loss of somatostatin-positive hilar interneurons, sensitive to excitotoxic insults, an observation confirmed by Lukoyanov et al (2004), which however did find a decrease in the total number of hilar neurons two months after the last of six ECS-

treatments. However, the latter study utilised an ECS-paradigm with only two hours spacing between the fifth and the sixth ECS-treatment, in order to maximise the extracellular glutamate levels and increasing the likelihood of excitotoxic damage (Rowley et al 1997). Transient decrease in myelination of axons projecting to the DG, suggesting oligodendrocyte damage, has been described in a lesion model employing five repeated ECS-treatments during the same day (Meier et al 2004). In our own material (papers I and IV), different techniques utilised for detecting degenerating cells (Fluoro-Jade staining, silver staining, cresyl violet staining) or apoptotic cells (cPARP-staining) did, however, not reveal any evidence of dead or degenerating cells or increased apoptosis after ECS-treatment.

In fact, ECS-treatment has been described as having anti-apoptotic actions. Repeated, low intensity, so called minimal ECS-treatment (mECS), completely protect against adrenalectomy-induced apoptosis in the GCL (Masco et al 1999) and prevents neuronal apoptosis after SE (Kondratyev et al 2001).

A microglia-mediated inflammatory response, compromising the survival of newly born neurons is a recently described feature of SE (Ekdahl et al 2003). Microglia, activated and/or phagocytic to the same extent as after SE are however not detected after ECS-treatment (Wennström et al, unpublished observation). In contrast, increases in signaling of growth factors in the TGF-β family, implicated in anti-inflammatory responses in the CNS (for review see Bottner et al (2000) has been described after ECS-treatment (Dow et al 2003; Dow et al 2005), and there is preliminary evidence that ECS-treatment increase the expression of several anti-inflammatory genes without an accompanying increase in pro-inflammatory genes (LaBuz et al 2003). Clinically, a normalisation of elevated plasma levels of the pro-inflammatory cytokine TNF-α has been observed in depressed patients receiving ECT (Hestad et al 2003).

Recent and as yet unpublished results from our lab show that the majority of the neurons and endothelial cells formed after ECS-treatment survive for at least six months (Hellsten and Wennström, unpublished observation). This result stands in stark contrast to the situation after SE where merely 9% surviving BrdU-labelled neurons are detected six months after SE (Bonde et al 2005, unpublished result).

ECS-treatment has been compared to the phenomenon of ischemic preconditioning, in the sense that a repeated non-injurious low grade stimuli (mECS) can protect against the adverse effects of a high-intensity, long-term stimuli of the same type (SE) (Kondratyev et al 2001). Furthermore this treatment is shown to excert protective effects against an all together different type of insult, adrenalectomy (Masco et al 1999), suggesting that ECS-treatment can protect against insults completely unrelated to seizure activity, underscoring the possibly that ECS-treatment could have general trophic or neuroprotective effects in the adult brain.

To summarise, there is reason to believe that ECT-treatment, in addition to inducing cell proliferation, can create a permissive environment for newly formed neurons and endothelial cells by increasing the availability of growth factors,

inducing anti-apoptotic/neuroprotective programs and anti-inflammatory signaling, thus allowing them to mature and integrate in the brain.

New neurons, endothelial cells and blood vessels - what is the functional significance for the brain?

The function of adult neurogenesis is far from clear, but, as previously been described, it has been implicated in brain repair (Bengzon et al 1997; Dash et al 2001; Liu et al 1998; Parent et al 1997), memory function (Ambrogini et al 2000; Gould et al 1999; Shors et al 2001; Shors et al 2002) and also in the mechanisms of action for antidepressant treatment (Santarelli et al 2003). Not surprisingly, the functional significance of the ECS-induced neurogenesis is also open for speculation. If one ponders the different levels on which functionality can be discussed, the question of the role for ECS-induced neurogenesis becomes even more many facetted. Do the ECS-induced neurons behave like normal neurons? Do they integrate properly in the hippocampal network? On a behavioural functional level, what is the consequence of the ECS-induced increase in neurogenesis?

If we turn to the situation regarding new neurons formed during the normal physiological process of adult neurogenesis, it has been shown that they over time develop electrophysiological properties and finally behave like their more mature counterparts (van Praag et al 2002) as well as integrate in the hippocampal synaptic circuitry (Carlen et al 2002). Furthermore, newly formed neurons was after a number of weeks shown to partake in a hippocampus-dependent learning task as indicated by c-fos-expression after training in the Morris water maze, as well as responding to a seizure-induced synaptic stimulus (Jessberger and Kempermann 2003).

We know that neurons formed after ECS-treatment display normal granule cell morphology and dendritic processes with normal looking spines (Wennström et al, unpublished observation) and that they survive for at least six months (Hellsten and Wennström, unpublished observation). We have yet to address if the neurons formed after ECS-treatment behave functionally on both a cellular and circuitry level and if the behavioural outcome to ECS-treatment/ECT is due to the increase in neurogenesis.

In addition to mood improving effects, ECT is associated with anterograde and retrograde memory disturbances that most often are transient but sometimes persistent. Could any of these effects on either mood or cognition be due to the pronounced increase in neurogenesis?

As already mentioned and discussed, induction of neurogenesis has been proposed to be a prerequisite for the behavioural response to antidepressants in two depression/anxiety models in mice (Santarelli et al 2003). The effect of ECS-treatment in normalising the behaviour in these models has not been investigated. Furthermore, a similar blockade of ECS-induced hippocampal cell proliferation in order to reveal its possible role in any depression model has yet to be performed. The task of selectively inhibiting the proliferation of neuronal progenitors and

subsequent neurogenesis without affecting other cells proliferating in response to ECS-treatment is not easily accomplished (to say the least) and it remains to be seen if it is at all feasible.

Regarding the memory disturbances associated with ECS-treatment/ECT, it has been shown that ECS-treatment alters synaptic function, as indicated by attenuated long term potentiation (LTP) induction in the dentate gyrus, an effect that gradually resolves over several weeks, corresponding with the time for normalised memory function after ECT (Stewart et al 1994; Stewart and Reid 1993). The ECS-induced effect on synaptic function was abolished with pre-administration of a NMDA-blocker, suggesting that glutamatergic signaling could be involved in the amnesic effect of ECS-treatment (Stewart and Reid 1994).

However, ECS-treatment and fluoxetine (which also increase hippocampal neurogenesis (Malberg et al 2000)) was shown to have similar effects on synaptic function in the dentate gyrus, despite the fact that ECS-treatment causes memory disturbances and fluoxetine does not (Stewart and Reid 2000). The authors suggest that possible effects of ECS-treatment on memory function might be due to synaptic alterations in other hippocampal subfields or brain structures altogether and that the alterations in synaptic connectivity in the dentate gyrus rather have affective than cognitive effects.

Allowing ourselves to speculate, it is possible that the effect of ECS-treatment on synaptic function in the dentate gyrus is partly due to the increase in proliferation of neuronal progenitors, with newly proliferated neuronal progenitors altering dentate synaptic connectivity gradually as they are being integrated into the hippocampal network.

It remains to be shown if (1) neurogenesis is decreased in depressed patients and (2) if ECT increases neurogenesis in depressed patients. The improvement of mood in patients responding to ECT could be related to the possible proliferation of neuronal progenitors and subsequent maturation of these into fully integrated neurons. This is a process that, at least in the rodent brain takes weeks to be completed, a time span correlating with the time required for clinical treatment response.

It has been hypothesised that the function of hippocampal neurogenesis is to enable the brain to cope with novel situations and that neurogenesis works as a mechanism preparing the hippocampus for the processing of more complex input, possibly explaining why physical activity and learning is linked to proliferation and increased neurogenesis respectively (Kempermann 2002b). In this context, the ECS-induced proliferation of neuronal progenitors and subsequent neurogenesis could perhaps be likened to a rejuvenation of the hippocampal neuronal network. New neurons, if functioning properly, will integrate into the "stagnant" cellular network of the hippocampus of the depressed, enhancing neuronal plasticity and affecting synaptic plasticity, contributing to an increased ability to cope with complex input and new situations.

Having dealt with the possible functional significance of ECS-induced neurogenesis, we now turn to the role of the ECS-induced endothelial cell proliferation.

In the adult brain, endothelial cells divide rarely. However, endothelial cells proliferate at a fairly high rate in the SGZ, where proliferation of neural precursors and neurogenesis also occurs (Palmer et al 2000). This neural proliferation can continue in response to ECS-treatment despite a pronounced inhibition of endothelial cell proliferation by CORT (paper III).

It is overtly simplistic to assume that the proliferation of one cell type necessarily causes the proliferation of another. However, one could interpret a high level of endothelial cell proliferation as an indicator of a high degree of plasticity and the "angiogenic niche" for adult neurogenesis, with a variety of factors possibly affecting several celltypes, communicating and influencing each other in multiple ways, as a prime example of a plastic microenvironment.

In paper V of this thesis we describe that the PVN, SON and VMH of the hypothalamus display a relatively high basal level of endothelial cell proliferation, which is increased several times in response to ECS-treatment. We do not know the functional significance of either the basal or the ECS-induced hypothalamic endothelial cell proliferation.

If we again turn to the possible role of angiogenesis in adult neurogenesis, but now with emphasis on the hypothalamus, we find that the mammalian hypothalamus is generally not considered being a neurogenic region. However, postnatal neurogenesis of magnocellular neurons in the PVN and SON of the opossum has been reported (Iqbal et al 1995), as well as the generation of neurons in the vasopressin and oxytocin-containing nucleus (VON) of the pig hypothalamus (Rankin et al 2003). In addition it was recently reported that the ependymal layer of the third ventricle in the adult rat is a neurogenic region, possessing neural progenitors that can migrate into the hypothalamic parenchyma and express markers suggesting they have neuropeptide secreting functions (Xu et al 2005). We have yet to investigate if ECS-treatment induces hypothalamic neurogenesis.

It is however evident that there is an intense neuronal (and possibly glial) activation of hypothalamic nuclei, implicated in the patophysiology of depression, in response to ECS-treatment. The neuronal activation correlates with the ECS-induced endothelial cell proliferation both spatially and in magnitude, but the temporal relationship between neuronal activation and endothelial cell proliferation is not clear. Again, the increase in endothelial cell proliferation could be seen as an indicator of an active and possibly plastic brain region, in this case three hypothalamic nuclei with neurosecretory functions.

Endothelial cells have been shown to directly influence hormone release from neurosecretory axons in the median eminence by regulating to which extent specialized glial cells, tanycytes, wrap around the nerve endings, releasing hormone into the blood stream (De Seranno et al 2004). The endothelial cells excert their

effects via nitric oxide-signaling and the authors suggest that glial and endothelial cells might regulate neuronal function also in other areas of the brain.

The cerebral blood vessels are surrounded by a basal lamina, which main components are collagen IV, fibronectin and laminin. Mercier and co-workers have identified a network of basal laminae, termed fractones, in the neurogenic subependymal layer (SEL) of the lateral ventricle as well as in the area surrounding the third ventricle in the hypothalamus (Mercier et al 2002; Mercier et al 2003). These fractones contact blood vessels in both the SEL and in the hypothalamus, and in the latter structure forms a continuum with the basal lamina investing for example the PVN and the SON. The basal lamina connects various cell types such as macrophages, fibroblasts, pericytes, astrocytes, endothelial cells and precursor cells/neural stem cells, and the authors suggests that it serves as a route for communication between these different celltypes, a "dynamic frontline for functional interactions between neural and non-neural tissue".

Besides the possible roles for endothelial cells as important regulators of neuronal and glial function throughout the brain, endothelial cells also functions as building blocks of blood vessels.

As described in the introduction of this thesis, increased angiogenesis in the adult brain is normally associated with pathological events, but has also been described to occur in response to for example exercise, a situation where the balance between the demand and availability of oxygen is altered. The reverse situation, i.e. an impaired angiogenesis or a decrease in vascular function can lead to atrophy of the affected organ. In the brain, cerebrovascular dysfunction is a hallmark in vascular dementia but however, no evidence of an underlying impaired angiogenesis has been presented. Recently it was proposed that insufficient angiogenesis and vascular regression could represent a novel mechanism for the progression of Alzheimer's disease (Zlokovic 2005). Allowing ourselves to speculate, if chronic stress, as suggested by the findings of paper III, via high levels of glucocorticoids, can compromise the normal low-grade generation of endothelial cells, possibly resulting in vascular rarefaction over time, this could very well be at least part of the explanation for the reduced hippocampal volume seen in depressed patients. A link between cerebrovascular disease and late-life depression has been proposed (for reviews see Kales et al (2005); Thomas et al (2004)), but as yet no risk factors for vascular disease correlating with decreases in hippocampal volume has been identified (Hickie et al 2005). The latter study however revealed that patients with melancholia, in which hypercortisolemia is a common feature, exhibited the lowest hippocampal volumes.

Although conflicting results exist, several reports point to decreased blood flow in limbic and frontal brain regions in patients with major depression (Awata et al 2002; Bonne and Krausz 1997; Bonne et al 1996; Milo et al 2001). Interestingly, an increase in cerebral blood flow has been observed from one up to twelve weeks after clinically successful electroconvulsive therapy (Awata et al 2002; Mervaala et al 2001). Whether this increase is caused by an increased vascularization or a change in the regulation of the regional blood flow is at present not known.

However, given our finding that a series of ten ECS-treatments lead to increased vessel length in the rat hippocampus, it is not unreasonable to speculate that the long-term increase in blood flow observed after clinical ECT may in part be caused by an increase in vascularization.

In analogy with the interpretation of the role for ECS-induced neurogenesis as a rejuvenation of the neuronal network in the dentate gyrus, the angiogenic response after ECS-treatment can be taken as a means of boosting the angiogenic potential in this region. If chronic stress, as suggested by the findings of paper III, can compromise the normal low-grade generation of endothelial cells, possibly resulting in vascular rarefaction over time, ECS-treatment may be able to counteract such an effect.

CONCLUDING REMARKS

The results from this thesis points to the fact that ECS-treatment, an animal model of the antidepressant treatment ECT, seem to have general trophic effects in the adult brain. In response to ECS-treatment, new neurons and blood vessels are formed in the hippocampus, a region shown to be reduced in size in patients with depression, possibly due to long-term increases in levels of stress hormone. In addition this thesis show that ECS-treatment cause an angiogenic response in hypothalamic nuclei implicated in the patophysiology of depression, along with a strong neuronal activation. The functional significance of the increased neurogenesis and angiogenesis is not known, however, an increased vascularization could possibly lead to a better blood supply. If this angiogenic response is something that is exclusive to ECS-treatment, it could perhaps explain the efficacy to this treatment compared to antidepressant medication. Further studies of angiogenic mechanisms in conjunction with antidepressant treatment could possibly lead to the development of new and more effective therapies for depression.

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That's all folks!

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