A Glial Role in the Action of Electroconvulsive Therapy

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A Glial Role in the Action of Electroconvulsive Therapy

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ORIGINAL ARTICLES

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

I: Wennström M, Hellsten J, Ekdahl CT and Tingström A (2003): Electroconvulsive seizures induce proliferation of NG2-expressing glial cells in adult rat hippocampus. *Biol Psychiatry* 54 (10): 1015-102

II: Wennström M, Hellsten J and Tingström A (2004): Electroconvulsive seizures induce proliferation of NG2-expressing glial cells in adult rat amygdala. *Biol Psychiatry* 55 (5): 464-71

III: Wennström M, Hellsten J, Ekstrand J and Tingström A (2006): Corticosterone-induced inhibition of gliogenesis in rat hippocampus is counteracted by electroconvulsive seizures. *Biol Psychiatry* 59(2): 178-86.

IV: Wennström M, Ekdahl CT, Jansson L and Tingström A: Do electroconvulsive seizures induce reactive gliosis? (submitted)

ABBREVIATIONS

5-HT	serotonin
ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
B	basal nucleus of amygdala
BDNF	brain-derived neurotrophic factor
BrdU	bromodeoxyuridine
CA	cornu ammonis
Ce	central nucleus of amygdala
CNS	central nervous system
CRH	corticotropin releasing hormone
DAB	3,3"-diaminobenzidine
DSM-IV	dignostic and statistical manual of mental disorders, 4th edition
ECS	electroconvulsive seizures
ECT	electroconvulsive therapy
EEG	electroencephalogram
FGF-2	fibroblast growth factor 2
GCL	granule cell layer
GFAP	glial fibrillary acidic protein
GluT	glutamate transporter
HPA	hypothalamic-pituitary-adrenal
KPBS	potassium phosphate-buffered saline
L	lateral nucleus of amygdala
Me	medial nucleus of amygdala
ML	molecular layer
NA	noradrenaline
NG2	neuron-glia 2
PBS	phosphate buffered saline
PFC	prefrontal cortex
PLSD	protected least significant difference
OPC	oligodendrocyte progenitor
SE	status epilepticus
SGZ	subgranular zon
VEGF	vascular endothelial growth factor

SUMMARY

Major depression is a common and severe illness affecting a large number of individuals at some point during their lifetime. Researchers within both clinical and preclinical fields have searched for the biological key components underlying this disorder. Recent studies have shown that certain brain structures, such as prefrontal cortex (PFC), hippocampus and amygdala, are structurally and functionally altered in patients with major depression. Several brain-imaging studies describe reduced volume of these structures in depressed patients compared with healthy controls. The tissue alterations underlying the observed volume reduction is yet to be determined, but interestingly, *post mortem* studies of patients with depression have revealed a reduction in the number of glial cells in the amygdala and PFC. In recent studies this glial cell loss was attributed to a decrease in the number of oligodendrocytes in the amygdala and PFC.

Several clinical studies indicate that high levels of circulating cortisol might be involved in the structural changes seen in depressed patients. Similarly, preclinical studies have shown that elevated levels of corticosterone, the cortisol homologue in rat, are associated with volumetric and cellular changes in the rat hippocampus. Lately many studies have focused on the impact of corticosterone on cell proliferation and it has been shown that both neurons and glial cells are affected. Interestingly, it has been reported that the proliferation of a certain kind of glial cell, called chondroitin sulphate proteoglycan NG2 expressing glial cells (NG2+ glial cell), is dramatically downregulated. This glial cell type is believed to share a lineage relationship with oligodendrocytes.

Electroconvulsive therapy (ECT) is a widely used and effective treatment for severe depression. The underlying mechanisms are not fully understood, but it is known that ECT induces several molecular changes in the brain. In the present thesis we demonstrate that electroconvulsive seizures (ECS), an animal model for ECT, significantly increases the number of newly formed NG2+ glial cells, microglia and oligodendrocytes in the adult rat hippocampus and amygdala. We also show that ECS counteracts the inhibitory effect of corticosterone on proliferation of these three glial cell types and increase the hippocampal volume compared to control. Finally we demonstrate that ECS slightly increase glial cell activity, without inducing reactive gliosis, a process associated with cell damage and death in the brain.

Glial cell dysfunction may be of central importance in the pathophysiology of depression, and further knowledge on how antidepressant treatment affects proliferation, differentiation and survival in different glial cell populations may give clues to new therapeutic strategies.

SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING

Depression är en mycket vanlig och allvarlig sjukdom som drabbar ett stort antal människor någon gång i livet. På jakt efter effektiv antidepressiv behandling har forskare inom det medicinska fältet sökt efter biologiska processer kopplade till sjukdomen. På senare tid har ett flertal undersökningar visat att depression leder till förändringar inom vissa områden av hjärnan. Uppmärksammade studier har bland annat visat att deprimerade patienters prefrontala kortex (viktig bl a för målinriktat tänkande, ”säte för personligheten”), hippokampus (viktig för minnesinlagring) och amygdala (hjärnans ångestcentrum) uppvisar mindre volym än normalt. Vad denna volymminskning beror på är fortfarande okänt, men *post mortem*-studier på patienter som lidit av depression har avslöjat nerv- och gliacellsförändringar i dessa tre hjärnområden. Medan nervcellerna utgör själva stommen av det neuronala nätverket, är gliacellernas huvudsakliga uppgift att stödja och försörja nervcellerna. De nerv- och gliacellsförändringar som ses hos deprimerade patienter har delvis beskrivits som förändringar av cellernas morfologi, men man har även sett att antalet oligodendrocyter (gliaceller som är viktiga för nervcellskommunikation) minskar i både prefrontala kortex och amygdala

Ett flertal studier antyder att ”stresshormonet” kortisol kan ligga bakom de strukturella förändringar som ses vid depression. Ungefär hälften av de patienter som lider av depression har ofta en onormalt hög nivå av kortisol i blodet. Personer som behandlas med kortison (konstgjort kortisol) kan drabbas av depression, och patienter som lider av Cushings sjukdom (kortisolproducerande tumör) får ofta både depressioner och minskad volym av nämnda hjärnområden. Även i djurförsök har man kunnat visa att kortikosteron, råttans motsvarighet till kortisol, leder till volymminskningar och strukturella förändringar i hippokampus. Man har bland annat funnit att kortikosteron minskar nybildningen av neuron (nervceller) och gliaceller i hippokampus.

Elbehandling, eller på fackspråk elektrokonvulsiv terapi (ECT), är en av psykiatrins äldsta och mest effektiva behandlingsmetoder vid svår depression. Även om dess användning stöds av omfattande vetenskaplig dokumentation betraktas ECT fortfarande som en kontroversiell behandling av många, bland annat på grund av vanligt förekommande biverkningar i form av övergående minnestörningar. För att kunna ta fram bättre antidepressiva behandlingsformer har man sökt efter de exakta mekanismerna bakom ECT:s antidepressiva effekt. Idag vet man att ECT leder till en rad förändringar på molekylär nivå. Bland annat har man sett att ECT delvis påverkar samma processer i hjärnan som antidepressiva läkemedel, dvs modulerar signalsubstanser som t ex serotonin och noradrenalin. Intressant nog har undersökningar visat att ECT även påverkar uttrycket av andra substanser såsom neuropeptider och tillväxtfaktorer. Studier gjorda i vårt labb har visat att elektrokonvulsiv stimulering (ECS), en behandling på råtta som efterliknar ECT, flerfaldigt ökar nybildningen av neuron och blodkärlsceller i hippokampus. I samband med dessa undersökningar upptäcktes dessutom att ECS stimulerar nybildning av andra, ”icke-neuronala”, celltyper.

I de två första artiklarna som ingår i avhandlingen har vi karaktäriserat dessa ”icke-neuronala” celler. En stor procent av de celler som nybildades efter ECS i hippokampus och amygdala identifierades såsom kondroitinsulfatproteoglykan NG2-uttryckande gliaceller (NG2+ gliaceller). Denna gliacellstyp är relativ outforskad, men mycket talar för att de NG2-positiva gliacellerna är förstadier till oligodendrocyter. Vi fann även att ECS ökade antalet nybildade mogna oligodendrocyter och mikroglia, den senare en gliacelltyp som antas spela en viktig roll i hjärnans immunförsvar.

I den tredje artikeln använde vi oss av en depressionsmodell där vi, under en vecka, gav höga halter av kortikosteron till råttor (för att efterlikna de höga halter av kortisol som ses hos deprimerade patienter). I linje med tidigare studier, minskade nybildningen av mikroglia, NG2+ gliaceller och mogna oligodendrocyter i hippokampus hos de kortikosteronbehandlade råttorna. När dessa råttor fick ECS-behandling motverkades effekten av kortikosteron och nybildningen av gliacellerna återställdes till normal nivå. I studien fann vi även att ECS ökade volymen av vissa delområden i hippokampus.

Principen för ECT är att genom elektrisk stimulering av hjärnan framkalla ett kortvarigt epileptiskt krampanfall. Eftersom behandlingen på patienter kan ge minnesstörningar, och då man vet att krampanfall vid epilepsi leder till utbredda hjärnskador, ville vi i den fjärde och sista artikeln undersöka om ECS-behandling av råttor kan framkalla hjärnskada. När hjärnan skadas svarar mikroglia, NG2+ gliaceller och astrocyter (gliaceller som reglerar nervcellernas närliggande miljö) med att bli reaktiva. Detta fenomen kallas för reaktiv glios och kännetecknas bland annat av att gliaceller nybildas, ändrar sin morfologi dramatiskt och börjar uttrycka specifika ”reaktiva” markörer. Vår studie visade att gliaceller, som svar på ECS, genomgår en lätt morfologisk förändring, vilket ska ses som tecken på ökad gliacellsaktivitet. Trots den ökade gliacellsaktiviteten fann vi att ECS, i motsatts till epilepsi, varken ökar det ”reaktiva” marköruttrycket eller leder till de morfologiska gliacellsförändringar som man ser vid reaktiv glios. Detta resultat visar att ECS inte leder till någon omfattande cellskada, något som stämmer väl överens med tidigare studier där man inte kunnat påvisa någon celldöd efter ECS.

Artiklarna som ingår i denna avhandling antyder att ECT, en effektiv antidepressiv behandlingsmetod, kan motverka den gliacellspatologi man ser hos deprimerade patienter. Dessutom tyder våra resultat på att behandlingen inte leder till hjärnskada. Genom att studera effekten av stress och antidepressiv behandling kan vi närma oss en förståelse av mekanismerna bakom de förändringar i hjärnan som man funnit hos deprimerade patienter. På sikt kommer detta att kunna leda till säkrare och effektivare behandlingar för depression.

INTRODUCTION

Feelings of disappointment, sadness and grief are normal reactions to events in our daily lives. However, when such feelings linger for an extended time, disrupting social interests and impairing the ability to take care of everyday responsibilities, they may be symptoms of a mood disorder called depression. This disorder is very common and affects people all over the world, regardless of social status or sex. Depression is diagnosed twice as frequently in women than in men (Weissman et al 1993), and 14%–21% of the population (this varies between countries) is affected by this disorder at least once in their lives (Kessler et al 1994; Wittchen et al 1992). Depression is not an illness restricted to modern times: it has been described throughout the historical literature as far back as the fifth century BC (Hippocrates' writings). Today, depression is the fourth leading cause of disability worldwide, according to the World Health Organization (WHO), and by 2020 it is predicted that it will be the second leading cause of health problems after ischemic heart disease (WHO 2001).

Two of the more serious types of mood disorder are major depression and bipolar disorder. Major depression is characterized by a depressed mood lasting for more than two weeks. Important symptoms of depression are feelings of sadness, guilt or low self-worth, loss of interest or pleasure, hopelessness, disturbed sleep or appetite, low energy, and poor concentration (Hagop 2005 (DSM-IV)). Bipolar disorder leads to severe mood swings that oscillate between depression and mania, the latter being a state of extreme elation and unbounded energy. One particularly tragic outcome of mood disorder is suicide. Around 10-15% percent of patients formerly hospitalized with depression end their lives by committing suicide (Angst et al 1999)

Why do we become depressed?

Despite the fact that depression affects millions of people all over the world, it is still not understood what exactly causes the disorder. A number of theories have been presented over the years, and environmental influences and biological factors are considered to be important. Although no single gene has been linked to major depression, it is known that people whose relatives are affected by the disorder have a greater risk of becoming ill (Thase 2005). This indicates that vulnerability to depression can be inherited. However, because major depression also occurs in people with no family history of depression, the cause cannot be explained solely by genetics. Stressful events, such as dramatic and traumatic changes in our lives, or high expectations placed upon us by the surrounding environment, also seem to trigger the onset of major depression. In 1977, Zubin and Spring presented the stress/vulnerability model (Zubin and Spring 1977) as a way of understanding the interaction between environment and heredity in the development of depression. According to this model, an individual's vulnerability to stressful events depends on his or her biological, psychological and social prerequisites. This means that a person becomes ill when the stressor is far greater than his or her coping ability. It

also means that an individual with less vulnerability to stress can cope with a problem that would cause other individuals with higher vulnerability to become depressed.

Current methods to treat depression

Depression, like most psychiatric disorders, does not fall neatly into discrete categories. Each medical case differs from another, and the complete history of a patient's symptoms has to be evaluated to find the appropriate treatment. Aspects such as onset, duration, severity and reoccurrence of depression are taken into consideration, and the treatment of choice depends on the outcome of this evaluation. Below is a brief description of the three major types of antidepressant treatment used in psychiatry: psychotherapy, antidepressant drugs and electroconvulsive therapy (ECT).

Psychotherapy

Psychotherapy is primarily used to treat patients suffering from milder forms of depression. There are numerous different types of psychotherapy, but generally they all deal with different aspects of a patient's thoughts, feelings and personal interactions that are believed to cause or sustain an episode of depression (Svrakic and Cloninger 2005). Studies have shown that certain forms of psychotherapy can be as effective as antidepressant drugs when used to treat mild depression (Thase et al 1997).

Antidepressant drugs

Today, antidepressant drugs are used as first-line drugs of choice when treating mild or moderate depression. There are several classes of antidepressant drugs: tricyclic antidepressants, monoamine oxidase inhibitors, selective inhibitors of serotonin (5-HT), noradrenaline (NA) or both, and atypical antidepressant drugs (Kalat 2001). Although these drugs belong to different chemical groups and act via different molecular pathways, they all affect serotonergic and/or noradrenergic neurotransmission in the brain (Kalat 2001). The theory behind the therapeutic effect of antidepressants is based on the so-called monoamine hypothesis, which states that depression is caused by an impairment or dysregulation of monoaminergic activity in the brain (Schildkraut 1965). However, there are several indications that the monoamine hypothesis may be insufficient to explain the pathophysiology of depression. Firstly, there are drugs that can increase brain monoaminergic activity that are not clinically effective as antidepressants, such as cocaine and amphetamines. Secondly, not all depressed patients respond to antidepressant drugs, and thirdly, changes in monoamine levels at the synapse take place within hours, yet to obtain a therapeutic effect continuous administration of the antidepressant drugs for several weeks is often required (Rang et al 1998). This suggests that antidepressant drugs may exert their effects by promoting, via stimulation of neurotransmitter receptors, other molecular pathways involved in the pathophysiology of depression.

Electroconvulsive therapy

ECT, sometimes referred to as electroshock or shock treatment, is indicated when other antidepressant treatments have failed, when there is a psychotic component to the depression, or when there is a high risk of suicide (Fink 2005). The decision to use ECT must be evaluated for each individual, and informed consent by the patient must be given prior to the treatment (NIH & NIMH Consensus Conference (1985)).

The principle behind ECT is that a brief electrical current applied to the patient's head induces a generalized epileptic seizure (grand mal seizure). The patient is anesthetized during the procedure and given a muscle relaxant to minimize muscle contractions. Oxygen is administered to prevent hypoxia secondary to the convulsions. The antidepressant effect of ECT is related to the seizure activity rather than the electrical stimulation (Fink 2005).

The mechanisms behind the antidepressant effect of ECT are largely unknown; however, studies have shown that ECT affects several neurotransmitters, which correlates with the previously mentioned monoamine hypothesis. ECT seems to sensitize subtypes of the 5-HT receptor, thereby strengthening 5-HT-dependent neurotransmission. ECT also decreases the functioning of inhibitory NA and dopamine autoreceptors, resulting in an increased secretion of these neurotransmitters (for a review Ishihara and Sasa 1999). Although ECT seems to enhance neurotransmission, as antidepressant drugs do, the onset of action is more rapid (Fink 2005), suggesting that other molecular pathways are also affected. This will be further touched upon in the general discussion.

The most common side effects of ECT are confusion, memory loss for events that occurred close in time to the period of ECT treatment (retrograde and anterograde amnesia), and learning problems. Although most patients retain their memories and learning abilities, the severity and duration of the cognitive impairments varies between individuals (Fink 2005).

Today, ECT has an unfavorable reputation among the public. The incorrect use of ECT in the past (administration without anesthesia and neuromuscular blockade resulted in severe convulsions that sometimes caused injuries, including bone fractures), cognitive side effects and several unfavorable depictions in popular books and films, can explain the negative view the public has of ECT. Opponents of ECT claim that the cognitive side effects seen after treatment are signs of gross pathological brain changes. This has, however, not been supported by animal research or clinical studies (NIH & NIMH Consensus Conference (1985)).

Structural changes in the depressed brain

Clinical and preclinical studies have shown that certain brain structures are involved in the regulation of emotional behavior. The prefrontal cortex (PFC), anterior cingulate cortex, hippocampus and amygdala play crucial roles in the regulation of normal emotions (Thase 2005). It has recently been discovered that some of these brain areas, in particular the PFC, hippocampus and amygdala, are

structurally altered in depressed patients.

Prefrontal cortex

The PFC comprises the anterior part of the frontal lobes of the brain, lying in front of the motor and premotor areas. This region of the brain is thought to be involved in planning complex cognitive behaviors, and in the expression of personality and appropriate social behavior (Wood and Grafman 2003). Interestingly, it appears that the PFC of each hemisphere of the brain plays different roles in emotional reactions. Patients with damage to the left PFC are more likely to develop depressive symptoms compared with patients who have lesions in the right PFC (Gainotti 1972; Robinson et al 1985; Sackeim et al 1982). Indeed, most functional neuroimaging and MRI studies in patients with major depression have identified reductions in blood flow, glucose metabolism and tissue volume in the left PFC (Dolan et al 1992; Drevets et al 1998; Drevets et al 1997).

Hippocampus

The hippocampal formation lies deep within the temporal lobe. This structure, commonly called the hippocampus, is divided into several subregions: the subiculum, dentate gyrus, CA1, CA2 and CA3. The hippocampus plays a crucial role in the formation of new memories (Kandel et al 2000a). This implies that there ought to be high demands on remodeling of the neuronal network in the hippocampus. Indeed, convincing evidence has shown that neurogenesis (formation of new neurons) takes place in the dentate gyrus throughout adulthood (Altman and Das 1965; Eriksson et al 1998; Gould et al 1997). Neurogenesis occurs at the border between the dentate hilus and the granule cell layer (GCL), two subregions in the dentate gyrus. The newly formed neurons migrate into the GCL, where they differentiate into mature granule neurons, extend dendrites into the molecular layer, and project axons to pyramidal neurons in the CA3 region (Markakis and Gage 1999; Stanfield and Trice 1988).

The hippocampus is interconnected with brain structures involved in the regulation of emotional behavior, such as the hypothalamus, amygdala and PFC (Kandel et al 2000a). Therefore, the hippocampus may not just be important for memory encoding, but also for the regulation of mood. The fact that depressed patients often has memory disturbances (Hagop 2005 (DSM-IV)) provide further support for this idea. Older depressed patients are sometimes misdiagnosed as having dementia; however, this so-called *pseudodementia* resolves if the depressive episode is successfully treated (Whalley and Bradnock 1990). In recent years, a number of investigators have suggested that hippocampal neurogenesis might play a key role in depression. This hypothesis is based partly on the findings that several antidepressant drugs, as well as electroconvulsive seizures (ECS), (an animal model for ECT), increase neurogenesis (Chen et al 2000; Madsen et al 2000; Malberg et al 2000). Additionally, studies have shown that depletion of 5-HT and NA in the brain decreases neurogenesis (Brezun and Daszuta 1999; Kulkarni et al 2002). The importance of neurogenesis in depression has been investigated in a frequently

cited study by Santarelli, Saxe and colleagues (2003). This investigation demonstrates that when hippocampal neurogenesis is suppressed by irradiation, the anxiolytic/antidepressant effect of fluoxetine (selective serotonin reuptake inhibitor) is blocked. The authors conclude that hippocampal neurogenesis is necessary for the action of fluoxetine (Santarelli, Saxe et al 2003).

The most striking indication that the hippocampus is affected by depression comes from recent publications showing that depressed patients have a lower hippocampal volume. A number of brain-imaging and *post mortem* studies, show (with a few exceptions) that the hippocampal volume is significantly reduced in depressed patients (see meta-analysis Campbell et al 2004). A reduction in hippocampal volume has been correlated with severity of depression (Shah et al 1998; Vakili et al 2000) and with the length of time the depression was untreated (Sheline et al 2003). Furthermore, in a preclinical study in rodents, animals placed in an enriched environment had greater hippocampal volume compared with animals placed in a deprived environment (Kempermann et al 1997).

Amygdala

The amygdala is an almond-shaped structure located at the tip of the temporal lobe. This structure is involved in assigning emotional significance to cognitive events (Phelps 2006). Extensive research in rodents and primates has established the importance of the amygdala for emotional processing. Bilateral removal of the amygdala results in permanent disruption of emotional and social behavior (for review see Aggleton 1993), and damage to the amygdala can induce tameness in animals (Iversen et al 2000). In humans, electrical stimulation of the amygdala elicits feelings of fear and apprehension (Iversen et al 2000), and bilateral damage to amygdala impairs the processing of fearful facial expressions (Adolphs et al 1994).

The amygdala consists of a number of different subregions, of which the medial, central, lateral and basal nuclei are the most studied. The basal and lateral nuclei, often collectively referred to as the basolateral nucleus, are the main input nuclei. They receive information from other brain structures, such as the PFC, sensory cortex, thalamus and hippocampus (Iversen et al 2000), and are important in attaching emotional significance to complex stimuli (LeDoux 2000). The central nucleus is the output nucleus that relays information from the basolateral nucleus and makes extensive connections with the brain stem and hypothalamus (Iversen et al 2000), affecting autonomic functions that are an intrinsic part of the emotional response (increased heart rate, piloerection, stimulation of cutaneous blood flow, etc) (Purves et al 2001). The medial nucleus is believed to play an important role in sexual responses (Newman 1999).

Recent studies have demonstrated that the amygdala is functionally altered in depressed patients. Brain imaging studies of patients with major depression have revealed increased glucose metabolism and blood flow in the amygdala, and these findings were positively correlated with depression severity (Drevets 2003). Furthermore, depression-associated volumetric changes, as seen in the PFC and

hippocampus, also occur in the amygdala. However, reports on volumetric changes in the amygdala of patients with major depression have been inconsistent, showing both decreases (Mervaala et al 2000; Sheline et al 1998; von Gunten et al 2000) and increases in amygdala volume (Bremner et al 2000; Frodl et al 2002; Vilhardt 2005). A recent meta-analysis, based on a large number of investigations, concluded that increased amygdala volume is found in depressed children, whereas depressed adults show a decrease in amygdala volume (Haggerty et al 2004).

What causes depression-associated brain atrophy?

The volumetric changes of the PFC, hippocampus and amygdala in depressed patients mirror cellular changes in these areas. The precise mechanisms behind these changes are not well understood, however, several studies indicate that the 'stress hormone' cortisol might be involved. One of the many biological responses to stressful events is an increased secretion of the adrenal hormone cortisol. Stress stimulates the hypothalamus, via the nervous system, to secrete corticotropin-releasing hormone (CRH). CRH, in turn, causes the pituitary gland to release adrenocorticotropic hormone (ACTH) into the bloodstream, from which it enters the adrenal glands and stimulates cortisol secretion. This system is called the hypothalamo-pituitary-adrenal (HPA) axis. The HPA axis is controlled by a negative feedback loop in which excess cortisol, via receptors in the hippocampus and other levels, suppresses the production of CRH and ACTH (Vander et al 1994). Many patients with depression show elevated levels of circulating cortisol, suggesting that the negative feedback loop of the HPA axis is no longer present, resulting in an increased production of CRH, ACTH and cortisol (Catalan et al 1998; Galard et al 1991). High cortisol levels can be restored to normal levels with antidepressant treatment (Carroll et al 1976). Further evidence that cortisol might be involved in depression is that patients with Cushing's disease (an illness associated with hypercortisolemia) show high rates of depression (for review see Sonino and Fava 2002), and treatment with glucocorticoids (cortisol analogues) can precipitate a depressive episode (Brown et al 2004). Additionally, some antiglucocorticoid therapies have antidepressant properties (for review see Murphy 1997). In behavioral studies on animals, stress and chronic corticosterone-treatment induce depression-like behavior, memory deficits and impairments in behavioral flexibility (Cerqueira et al 2005; Gregus et al 2005; Kalynchuk et al 2004; Roozendaal 2002).

Several observations support the view that cortisol might play an important role in the volume alterations in the particular brain regions described above. For example, patients with Cushing's disease display decreased hippocampal volume (Starkman et al 1992). Increased cortisol levels in response to psychosocial stress in individuals with low self-esteem are correlated with a reduction in hippocampal volume (Pruessner et al 2005) and patients receiving chronic glucocorticoid therapy display smaller hippocampal volumes (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). Similarly, a link

between glucocorticoids and volume reduction in these regions of the brain has been demonstrated in animal studies. Rats exposed to corticosterone (the cortisol homologue in rat) showed a significantly reduced hippocampal volume (Sousa et al 1998), and psychosocial stress (which elevates cortisol levels) caused a nonsignificant trend towards reduced hippocampal volume in tree shrews. This volume reduction was reversed by the antidepressant tianeptine (Czeh et al 2001).

How cortisol causes these volumetric changes is not completely understood. A number of studies have focused on the impact of stress on neurons. In recent years, *post mortem* studies of patients with a history of depression have shown that the PFC, hippocampus and amygdala display a neuronal pathology that is described as changes in neuronal density and cell soma size (Bowley et al 2002; Rajkowska et al 1999; Stockmeier et al 2004). In preclinical studies exposure to stress and corticosterone-treatment induced a reversible atrophy of dendrites and caused changes in the structure of the mossy fibers (axons) in the rat hippocampus (Magarinos and McEwen 1995; Magarinos et al 1997). Similar findings, described as reorganization of dendrites in response to stress- and corticosterone, have also been found in the amygdala (Vyas et al 2002) and prefrontal cortex (Seib and Wellman 2003). Furthermore, it has been shown that prolonged exposure to corticosterone induces neuronal loss (Sapolsky et al 1985). Additionally, psychosocial stress and chronic corticosterone-treatment have been shown to have a suppressive effect on hippocampal neurogenesis (Cameron and Gould 1994; Czeh et al 2001; Hellsten et al 2002; Malberg et al 2000; McEwen 1999). Previous studies have also demonstrated that ECS, as well as antidepressant drugs, can reverse stress- and corticosterone-induced inhibition of hippocampal neurogenesis (Alonso et al 2004; Czeh et al 2001; Hellsten et al 2002; Malberg et al 2000). Although these findings imply that a suppression of neurogenesis may lie behind the depression-associated volumetric changes in the brain, there are a few important points to take into consideration. Firstly, no studies have detected changes in the size of the neuronal population in the brains of patients with a history of depression or high-dose steroid treatment (Lucassen et al 2001; Muller et al 2001). Secondly, volumetric studies on rats exposed to psychosocial stress have been unable to show any changes in the hippocampal GCL subregion, where new neurons are formed (Czeh et al 2001). Thirdly, volumetric changes occur in several brain regions (PFC, hippocampus and amygdala), however, only the hippocampus is known to produce new neurons. Thus, it is unlikely that the volume reductions in the brain can be explained solely by a corticosterone-induced downregulation of neurogenesis.

An important aspect of the previous mentioned study by Santarelli, Saxe and colleagues (2003), in which irradiation-induced downregulation of neurogenesis blocked the effect of fluoxetine, is that irradiation not only reduces the proliferation of neuronal progenitors located in the GCL but also reduces non-neuronal cells in the hilus of the dentate gyrus (Tada et al 2000). Could it be that these other cell types play a vital role in depression? Interestingly, studies in our own lab have shown that chronic corticosterone-treatment downregulates the

formation of new endothelial cells, i.e., angiogenesis, in the rat hippocampus (Ekstrand, unpublished data). Furthermore, it has been shown that ECS increases hippocampal angiogenesis, leading to an increased vascularization of the structure (Hellsten et al 2004; Hellsten et al 2005). ECS-induced angiogenesis also occurs in the PFC (Madsen et al 2005), amygdala (Jansson et al 2004) and hypothalamus (Jansson et al 2006), Taken together these results suggest that neurons might not be the sole actor in the pathophysiology of depression, and that other cell types might play a vital role.

A role for glial cells in depression?

Researchers have considered neurons to be the main actors in the brain for decades, neglecting the fact that they constitute a minor fraction of the cells present in the brain. The major cell population in the brain consists of cells collectively called glial cells. There are four major glial cell types in the brain: astrocytes, microglia, oligodendrocytes and neuron-glia 2-positive (NG2+) glial cells (a brief description of these glial cell types follows below). There are about 10 times more glial cells in the brain than neurons and it is naive to think that glial cells just "hang around" doing nothing. However, this was the general assumption about glial cells until quite recently. It was thought that glial cells filled up space in the brain, acting as glue and keeping neurons in the right place; hence, the name glia, which means glue in Greek. The first hint that this idea may be inaccurate came from a study on newborn rats in the 1960s (Diamond et al 1964). This study showed that rat pups living in an enriched environment displayed a higher glia-to-neuron ratio compared with pups living in a deprived environment, suggesting that increased neuronal activity demands increased glial support. Today it is well known that a continuous dialog between neurons and glial cells is crucial for the function of the neuronal network, and several studies have shown that a deficit in the glial structure disrupts this function.

Thus, might a glial deficit underlie the functional and structural changes seen in the brains of depressed patients? Indeed, *post mortem* studies indicate that this might be the case. These studies revealed, not only the previously described neuronal pathology, but also alterations in the glial cell populations in the PFC, hippocampus and amygdala of depressed patients. The glial pathology is described as changes in glial density and cell size, similar to the neuronal pathology observed in depressed patients (Bowley et al 2002; Rajkowska et al 1999; Stockmeier et al 2004). However, in contrast to the neuronal findings, the PFC and amygdala also show a reduction in glial cell populations (Bowley et al 2002; Ongur et al 1998; Rajkowska et al 1999; Stockmeier et al 2004). In recent studies, the reduction in glial cells in the PFC and amygdala of depressed patients has been shown to be due to a loss of oligodendrocytes (Hamidi et al 2004; Uranova et al 2004). Additionally, patients with depression display abnormalities in the transcription of genes involved in oligodendrocyte myelination and differentiation, as well as decreased levels of a transcription factor exclusively expressed by oligodendrocytes and their progenitors (Aston et al 2005). This suggests that a loss of oligodendrocytes and

their progenitors might be involved in the pathophysiology of depression. However, the involvement of other glial cell types cannot be excluded.

Microglia

Microglia are the smallest of the glial cells and make up 5-15% of all brain cells (Peters et al 1991). They are mostly known as resident immune cells of the CNS. Under pathological conditions, such as neurodegenerative disease, stroke, or tumor invasion, these cells become reactive, surround damaged and dead cells, and clear cellular debris from the area, much like the phagocytic macrophages of the immune system. In their reactive state, microglia synthesize a large number of factors, such as cytokines and growth factors (for review see van Rossum and Hanisch 2004). The function of resting (i.e., non reactive) microglia is poorly understood; however, recent studies show that these resting microglia are far from passive in the normal brain. By using their fine branches, which are highly mobile, they scan the surrounding tissue and provide extensive and continuous surveillance (Davalos et al 2005; Nimmerjahn et al 2005). Apart from being the 'watchdogs of the CNS', microglia are also thought to play a role in maintaining brain homeostasis (Lawson et al 1990) and to support neuronal functioning (for a review see (Vilhardt 2005).

Oligodendrocytes

The primary function of oligodendrocytes is to provide physical support to axons and to ensheath them with an insulating fatty protein called myelin. Each oligodendrocyte can supply myelin to several axons, and each axon can be insulated by myelin from several oligodendrocytes. Myelination is essential for the efficient conduction of action potentials down the axon of a neuron, and it increases the speed of nerve impulses (Kandel et al 2000b). Oligodendrocytes are highly vulnerable to changes in the environment; for example, increased levels of glutamate through overactivation of glutamate receptors (excitotoxicity) cause apoptosis and necrosis of these cells (for a review see Matute et al 2006). Because each oligodendrocyte can myelinate several neurons, damage to one oligodendrocyte can result in the loss of myelin from many axons. Myelin loss, known as demyelination, leads to poor neuronal transmission of the demyelinated segment (Kandel et al 2000b). A well-known demyelinating disorder is multiple sclerosis.

NG2+ glial cells

NG2+ glial cells constitute the major group of cells undergoing mitosis in the adult brain, and they represent about 5%–8% of all cells in the nervous system (Nishiyama et al 1999; Ong and Levine 1999). They are antigenically distinct from neurons, astrocytes, resting microglia and mature oligodendrocytes (for review see Nishiyama et al 1999) and are recognized by their stellate morphology and expression of the chondroitin sulphate proteoglycan termed Neuron-Glia 2 (NG2). The function of NG2+ glial cells is unknown; however, since these cells differentiate into mature oligodendrocytes *in vitro* and *in vivo* (Dawson et al 2000),

they are often regarded as oligodendrocyte progenitors (OPCs). This idea is further supported by the findings that many NG2+ glial cells express the platelet-derived growth factor alpha receptor and the O-antigen 4 (Nishiyama et al 1997; Reynolds and Hardy 1997), which are molecules expressed by OPCs. Interestingly, recent studies indicate that NG2+ glial cells might also act as neuronal progenitors. Convincing evidence has shown that NG2+ glial cells can differentiate into electrically excitable neurons (Aguirre et al 2004; Belachew et al 2003) and an exciting new study has shown that scattered NG2+ glial cells in the neocortex express markers that are also expressed by mature neurons (Dayer et al 2005).

The majority of NG2+ glial cells do not differentiate further and remain in their NG2-positive state for a significant time (Nishiyama 2001). This has led to the suggestion that NG2+ glial cells comprise a heterogeneous population, consisting of NG2-expressing progenitors and differentiated NG2+ glial cells called synantocytes (Butt et al 2002). The differentiated NG2+ glial cells seem to have a unique capacity for communication with nearby cells. They form multiple contacts with astrocytes, microglia, oligodendrocytes and neurons throughout the brain and are believed to make synaptic connections with neurons (Bergles et al 2000). The NG2+ glial cells might also play a role in the guidance of axonal outgrowth (for review see Butt et al 2005). Finally, NG2+ glial cells can become reactive and acquire an amoeba-like, phagocytic phenotype in response to CNS damage, as microglia do (Nishiyama et al 1997; Ong and Levine 1999). A recent study has shown that these reactive NG2+ glial cells are the source of the proinflammatory cytokine IL-1b and the neuroprotective agent IL-1ra (Fiedorowicz et al 2003).

Astrocytes

Astrocytes aid the surrounding neuronal network by regulating the microenvironment and providing nourishment to neurons. They are also involved in the formation and stabilization of synapses and are capable of modulating synaptic efficiency. Additionally, astrocytes synthesize and release growth factors and cytokines, promoting neuronal function, survival and growth (for a review see (Cotter et al 2001) indicating their importance in these processes. Astrocytes become reactive in response to changes in the CNS environment, such as increased levels of cytokines, neurotransmitters and growth factors (Barotte et al 1989; Caccamo et al 2005; Liberto et al 2004). Reactive astrocytes are characterized by changes in their morphology and upregulation of the intermediate filament glial fibrillary acidic protein (GFAP). Reactive astrocytes are involved in phagocytosis of cell debris, secretion of cytokines and various neurotrophic and growth factors (for review see Panickar and Norenberg 2005). Together with NG2+ glial cells and microglia, reactive astrocytes play a key role in the damaged-induced formation of 'glial scars', which serve to protect the underlying neural tissue.

AIMS OF THE THESIS

The main objective of this thesis was to investigate the potential role of glial cells in depression, more specifically the glial cell response to the ECS a model for the antidepressant treatment electroconvulsive therapy ECT. The specific aims were:

1. To investigate ECS-induced glial cell proliferation in adult rat hippocampus.

2. To investigate ECS-induced glial cell proliferation in adult rat amygdala.

3. To investigate the potential for ECS to counteract corticosterone-induced inhibition of glial cell proliferation.

4. To investigate whether ECS can induce reactive gliosis.

MATERIAL AND METHODS

Experimental animals

Adult male Wistar rats (Møllegaard breeding centre, Denmark), weighing 200 g at the beginning of the study, were used. Three rats were housed in each cage and kept on a 12 h light–dark cycle with access to food and water *ad libitum*. Experimental procedures were carried out according to the guidelines set by the Malmö-Lund ethical committee for the use and care of laboratory animals.

Animal treatments

Electroconvulsive seizure (ECS)-treatment (paper I-IV)

Bilateral ECS-treatment was delivered via silver electrode ear clips (Somedic Sales AB, Sweden) (50 mA, 0.5 s, 50 Hz unidirectional square wave pulses). The rats were monitored after the ECS-treatment to ensure that clonic movements of the face and forelimbs (indicative of limbic seizures) occurred for 20–30 s. Rats received a single ECS-treatment (paper I and IV) or 5 ECS-treatments over 5 days (paper I-IV). Control rats were sham-treated, that is, handled identically to the ECS-treated rats except that no current was passed.

Bromodeoxyuridine-treatment (paper I-III)

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 12h intervals over 5 days (papers I-III) or 4 injections of BrdU (100 mg/kg) in 2h intervals over 6h (time-course study, paper I)

Corticosterone-treatment (paper III)

A stock emulsion of corticosterone (C2505; Sigma-Aldrich, St Louis, MO, USA) (33.3 mg/ml) was prepared daily by vortexing in sesame oil (Sigma-Aldrich) for 10 min, followed by 60 min of sonication. Prior to every injection, the emulsion was vortexed briefly and administered as subcutaneous injections in the neck region (40 mg/kg) once daily for 7 days. Control rats received vehicle injections (sesame oil).

Status epilepticus (paper IV)

Rats were implanted with stainless steel stimulation/recording electrode (Plastics one, Roanoke, VA, USA) into the right ventral hippocampus (coordinates 4,8 mm caudal and 5,2 mm lateral to bregma, 6,3 mm ventral from dura, tooth-bar at 3,3 mm (Paxinos and Watson 1986)) under halothane anesthesia. Ten days following surgery, rats were subjected to electrically induced self-sustained status epilepticus (SE). The rats received suprathreshold stimulations consisting of 10 s trains of 1 ms biphasic square-wave pulses at a frequency of 50 Hz. The stimulations were interrupted every 10 min for 1 min to allow for electroencephalogram (EEG) recording and measurements of discharges (MacLab; AD Systems, Hastings; UK).

This stimulation and recording pattern was repeated for 60 min. After cessation of stimulations all rats exhibited self-sustained, continuous ictal EEG activity, which was associated with motor behavioral partial seizure. Behavioral convulsions and ictal EEG activity were arrested with pentobarbital (65 mg/kg i.p.) at 2 h after stimulation offset.

Histological procedures

Tissue processing

Rats were anesthetized with sodium pentobarbital (60 mg/ml) and in the absence of nociceptive reflexes, transcardially perfused with 0.9% saline, followed by 4% ice-cold paraformaldehyde. Following decapitation, the brain was removed from the skull and postfixed in 4% paraformaldehyde overnight at 4°C. The brains were left in 30% sucrose in PBS until they sank, and were then sectioned on a freezing microtome in 40 μm thick coronal sections (2.80 mm to 4.52 mm posterior to bregma (Paxinos and Watson 1986). The sections were stored in cryoprotectant solution at –20°C until stained (paper I-IV). Adrenal glands were dissected and weighed to assess the degree of atrophy as a measurement of the efficacy of the corticosterone-treatment (paper III).

Immunohistochemistry

Detailed protocols for the immunohistochemistry procedures can be found in papers I-V. Schematic protocols and a complete list of antibodies and serum used in the studies are shown in table 1 and 2.

Free-floating sections were washed in KPBS before staining procedures began. Sections to be stained for BrdU were pre-treated with 1M hydrochloric acid for 30 min at 65°C, in order to separate the DNA-chains and thereby expose the BrdU antigen (paper I-III). Sections to be developed with horseradish peroxidase catalysed 3,3"-diaminobenzidine (DAB) conversion were pre-treated with 3% H_2O_2 in 10% methanol in order to quench endogenous peroxidase activity (paper IV). Following these initial procedures, sections were pre-incubated in blocking solution containing 5% normal sera from animals in which the secondary antibodies were raised, and subsequently incubated with one or two primary antibodies in blocking solution. For DAB staining, 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 staining, fluorochrome conjugated secondary antibodies and/or biotinylated secondary antibodies followed by a streptavidin conjugated tertiary fluorochrome were used. Regularly used fluorochromes were Alexa 488 (green fluorescense) and Cy3 (red fluorescense). Sections to be double-stained for BrdU and cell-specific markers sensitive for HCl (indicated with an asterisks in table 1) were performed in sequence starting with the staining for the cell-specific marker following by fixation in 4 % PFA for 10 min before pre-treatment with HCl and subsequent BrdU staining.

Table 1. Antibodies			
Antigen	**Host**	**Source**	**Stains**
Chondrotin sulphate proteoglycan NG2 (NG2)	Rabbit	Gift from Dr Stallcup	NG2+ cells
Chondrotin sulphate proteoglycan NG2 (NG2)	Rabbit	Chemicon	NG2+ cells
CD11b (OX-42)	Mouse	Serotec	Microglia
Ionized calcium binding adaptor molecule 1 (Iba-1)	Rabbit	Wako	Microglia
2´,3´-cyclic nucleotide 3´-phophodiesterase (CNPase)	Mouse	Chemicon	Mature oligodendrocytes
Rip 1 (Rip)	Rabbit	HybridomaBank	Mature oligodendrocytes
Glial fibrillary acidic protein (GFAP)	Rabbit	Dakocytomation	Astrocytes
S-100β	Rabbit	SWANT	Astrocytes
Neuron-specific nuclear protein (NeuN)	Mouse	Chemicon	Post mitotic neurons
Doublecortin (DCX)	Rabbit	Santa Cruz	Neuronal progenitors
Nestin	Mouse	BD Bioscience	Reactive astrocytes
CD68 (ED1)	Mouse	Serotec	Reactive microglia, NG2+ cells
Ki67	Mouse	Novocastra	Proliferating cells
5-bromo-2`deoxyuridine (BrdU)	Rat	Oxford Biotechnology	BrdU-labeled nuclei

Cresyl violet staining (paper I and II)
Brain sections to be stained were rinsed 3 times in KPBS, mounted on glass slides and air dried overnight. The sections were then briefly rinsed in H_2O two times and subsequently immersed in 0.5% cresyl violet solution until sufficient staining was achieved. Sections were finally rinsed in H_2O, dehydrated and coverslipped.

Microscopical analysis
Epifluorescence microscopy (paper I-IV)
Cell proliferation was assessed in the molecular layer (ML), hilus and granular cell layer (GCL) of the dentate gyrus (paper I and III) and in the medial, basal, lateral and central nucleus of amygdala (paper II), using an Olympus AX70 fluorescence microscope with a 40x objective. The different brain subregions were defined with aid from an anatomical atlas of the adult rat brain (Paxinos and Watson 1986). Cells lying within two cell diameters of the granule cell layer border / hilar border were included in the GCL count. The total number of proliferating cells (BrdU+ or Ki67+) and the number of proliferating neuronal and glial cells (BrdU+ /NeuN+, NG2+, Iba-1+, OX-42+ or Rip+) were averaged and expressed as means per section.

Reactive glial cells, defined as Iba-1+/ED1+, NG2+/ED1+ or GFAP+/ Nestin+, were analyzed in representative coronal sections from the entire amygdala and hippocampus using an Olympus AX70 fluorescence microscope with a 40x objective (paper IV).

Table 2. Double immunofluorescense staining protocols

Antigen	HCl	Block	Primary antibody	Secondary antibody	Tertiary step	PFA	HCl	Block	Primary antibody	Secondary antibody
NG2/BrdU		NGS	rabbit α-NG2 1:500	biotin goat α-rabbit 1:200	avidin-Alexa 488 1:250	X	X	NDS	rat α-BrdU 1:200	Cy3 donkey α-rat 1:200
OX-42/BrdU		NGS	mouse α-Ox42 1:100	Cy3 goat α-mouse 1:200		X	X	NDS	rat α-BrdU 1:200	FITC donkey α-rat 1:200
OX-42/BrdU		NGS	mouse α-Ox42 1:100	Biotin horse α-mouse 1:200	avidin-Alexa 488 1:250	X	X	NDS	rat α-BrdU 1:200	Cy3 donkey α-rat 1:200
Iba-1/BrdU		NGS	Rabbit α-Iba-1 1:200	biotin goat α-rabbit 1:200	avidin-Alexa 488 1:250	X	X	NDS	rat α-BrdU 1:200	Cy3 donkey α-rat 1:200
S-100β/BrdU		NGS	rabbit α-S-100ß 1:1000	biotin goat α-rabbit 1:200	avidin-Alexa 488 1:250	X	X	NDS	rat α-BrdU 1:200	Cy3 donkey α-rat 1:200
CNPase/Brdu		NGS	mouse α-CNPase 1:100	Cy3 goat α-mouse 1:200		X	X	NDS		FITC donkey α-rat 1:200
Rip/Brdu		NGS	rabbit α-Rip 1:1000	biotin goat α-rabbit 1:200	avidin-Alexa 488 1:250	X	X	NDS	rat α-BrdU 1:200	Cy3 donkey α-rat 1:200
DCX/Brdu		NGS	rabbit α-DCX 1:1000	biotin goat α-rabbit 1:200	avidin-Alexa 488 1:250	X	X	NDS	rat α-BrdU 1:200	Cy3 donkey α-rat 1:200
NeuN /Brdu	X	NHS	mouse α-NeuN 1:100	biotin horse α-mouse 1:200	avidin-Alexa 488 1:250					
		NDS	rat α-BrdU 1:100	Cy3 donkey α-rat 1:200						
NG2/Ki67		NGS	rabbit α-NG2 1:500	biotin goat α-rabbit 1:200	avidin-Alexa 488 1:250					
			mouse α-Ki67 1:200	Cy3 goat α-mouse 1:200						
Iba-1/ED1		NGS	rabbit α-Iba-1 1:200	biotin goat α-rabbit 1:200	avidin-Alexa 488 1:250					
			mouse α-ED1 1:200	Cy3 goat α-mouse 1:200						
NG2/ED1		NGS	rabbit α-NG2 1:500	biotin goat α-rabbit 1:200	avidin-Alexa 488 1:250					
			mouse α-ED1 1:200	Cy3 goat α-mouse 1:200						
GFAP/nestin		NGS	rabbit α-GFAP 1:1000	biotin goat α-rabbit 1:200	avidin-Alexa 488 1:250					
			mouse α-nestin 1:1000	Cy3 goat α-mouse 1:200						

Secondary antibodies:
Cy-3 Donkey-anti-Rat (Jackson Immuno Research),
FITC Donkey anti-Rat (Jackson Immuno Research),
Biotin Horse anti-Mouse (Vector Laboratories Inc),
Biotin Goat anti-Rabbit (Vector Laboratories Inc)

Tertiary step:
Alexa 488 (Molecular Probes)

Serum:
Normal donkey serum (NDS) (Harlan Sera-Lab)
Normal Horse serum (NHS) (Sigma-Aldrich)
Normal Goat Serum (NGS) (Chemicon)

Confocal microscopy (paper I-III)
Confirmation of double-labelling was performed with a Nikon confocal microscope using a 40x objective and BioRad software (BioRad, Burlington, MA, USA) (paper 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+ cells, 50 BrdU+ cells per animal were analysed for verification of colocalisation within the granule cell layer and hilus, respectively (paper I). The identity of BrdU+ cells co-labelled with NG2, OX-42, Iba-1 and Rip antibodies was determined by confocal analysis of a subset of BrdU+ nuclei (paper I-III).

Brightfield microscopy (paper IV)
Morphological changes in glial cells were examined in representative coronal sections from the entire amygdala and hippocampus. An Olympus AX70 light microscope with a 40x objective was used.

Stereology
Volume of amygdala (paper II) and hippocampal (paper III) subregions was estimated using the Cavalieri principle. Every second section from each animal was de-lineated, using CAST-GRID software (Olympus, Albertslund, Denmark) and an Olympus BH-2 microscope with a 10× objective and a CCD-IRIS color video camera. Values of the cross-sectional areas of these regions were obtained. The total volume of the region of interest was calculated as the mean cross-sectional area multiplied by the rostro-caudal length of the region sectioned.

Statistical analysis
Differences in proliferation were statistically analyzed using Statview software, version 5.0 (Abacus Concepts, Berkeley, CA, USA). One-way analysis of variance (ANOVA) followed by Scheffé's post hoc test, Dunnett post hoc test or Fischer's PLSD tests were used when three or more groups were compared. In cases where only two groups were compared (paper III), Student's unpaired t-test was used. Values were presented as means ± standard error of the mean (SEM) throughout the text. Statistical significance were set to $p < 0.05$. All statistical analyses were performed with Statview software, version 5.0 (Abacus Concepts, Berkeley, CA, USA).

RESULTS AND COMMENTS

ECS-treatment upregulates proliferation of NG2 expressing glial cells in rat hippocampus (paper I)

While it is well known that ECT is an effective and fast-acting antidepressant treatment, side effects such as memory disturbance (Fink 2005) has been causing concern both in the general public and among professionals. In order to develop safer and better antidepressant treatments several studies have been performed to find the biological mechanisms underlying the antidepressant effect of ECT. Over the years many reports have shown that ECT induces several molecular changes in the brain.

As mentioned in the introduction, earlier studies in our own lab have shown that electroconvulsive seizures (ECS), can modulate the regenerating capacity of the adult rat dentate gyrus by increasing the formation of new neuron i.e. neurogenesis. From these studies it also became clear that apart from neurogenesis there was a prominent proliferation of other, non-neuronal, cells in the dentate gyrus in response to ECS. These cells were mainly found in the molecular layer (ML) and in the hilus of the dentate gyrus. While some of them were identified as endothelial cells (Hellsten et al 2004), a large percentage of the proliferating cells still remained unidentified. This prompted us to investigate the phenotypic characteristics of these other ECS-stimulated cells.

In order to do se, we collected hippocampal brain sections from BrdU-injected rats treated with ECS. By double staining with antibodies against BrdU together with various cell-type markers, we were able to identify a large proportion of the proliferating cells as NG2+ glial cells. To further investigate whether this ECS-induced NG2+ glial cell proliferation was dose-dependent, we conducted a dose-response study. When administering a single ECS-treatment we detected a strong increase in NG2+ glial cell proliferation in the ML, hilus and the granule cell layer (GCL). This increase was further enhanced by 5 ECS-treatments. Not only the number of proliferating NG2+ glial cells, but also the total number of NG2+ glial cells increased in all dentate gyrus subregions in response to 5 ECS-treatments, revealing a true increase in size of the NG2 glial cell population rather than an increased turnover of NG2+ glial cells.

In order to determine the NG2+ glial cell proliferation time-response to ECS we conducted a time course study, where we injected BrdU at different time-points (0, 2, 4 and 8 days) after a single ECS-treatment. This revealed a significant increase in proliferation of NG2+ glial cells in the ML, hilus and GCL on day 2 following a single ECS-treatment. No significant increase in NG2+ cell proliferation was seen at any other time point studied (day 0, 4 and 8). Additionally we found that a small fraction of the proliferating cells in ML at day 2 expressed the microglial/macrophage marker OX-42. Interestingy, no proliferating S100ß expressing glial cells (astrocytes) were detected at any time point after ECS. The proliferation in GCL (where the majority of the dividing cells are known to

become neurons) was significantely increased at day 2, peaked at day 4 and returned to baseline levels at day 8.

The fate of the proliferating cells were investigated by giving rats BrdU at day 2 or 4 after a single ECS-treatment and then letting them survive for an additional three weeks. The number of BrdU+ /NG2+ glial cells in ML and hilus were still significantly increased compared to control indicating that many of these cells retain their expression of NG2 over a long period of time. However, the fraction BrdU+/NG2+ glial cells out of all BrdU+ cells was decreased when compared to the study with short survival period. In agreement with previous reports a large percentage of the BrdU+ cells in the GCL expressed the neuronal marker NeuN. Given the fact that previous studies suggest NG2+ glial cells to be oligodendrocyte progenitors, we were surprised to find that none of the BrdU+ cells expressed the mature oligodendrocyte marker CNPase. At the time of this study we had no access to good antibodies against mature oligodendrocytes, and had therefore to settle with the antibody against CNPase. Today we know that the cellbodies of oligodendrocytes only weakly express CNPase, which might explain our failure to detect any mature oligodendrocytes in paper I. As we shall see in papers II and III a few BrdU+ cells indeed differentiate and begin to express the mature oligodendrocyte marker Rip.

In summary, paper I show that ECS-treatment increases the proliferation of NG2 + glial cells and microglia as well as the total number of NG2+ glial cells in the rat hippocampus. A sharp peak of NG2+ glial cell proliferation is seen two days after a single ECS-treatment, while ECS-induced proliferation of neuronal progenitors (in the subgranular zone) peaks at day 4.

ECS-treatment increases gliogenesis in the rat amygdala (paper II)

The observation that ECS increases NG2+ glial cells raises a crucial question: Are these cells important for the antidepressant effect of ECT? As mentioned in the introduction previous *post mortem* studies have revealed that depressed patients show a reduced number of oligodendrocytes in the amygdala. These intriguing findings together with the fact that NG2+ glial cells often are referred to as oligodendrocyte progenitors (see introduction), urged us to investigate whether ECS also increase NG2+ glial cell proliferation in the rat amygdala.

We analyzed the cell proliferation after 5 ECS-treatments in the central, lateral, basal and medial nuclei of amygdala. Interestingly, we found that proliferation of NG2+ glial cells in response to ECS varied between the different nuclei. In the central, lateral and basal nuclei NG2+ cell proliferation was greatly increased by ECS, but this was not the case in the medial nucleus. Moreover, we found that in response to ECS more than 90% of all cells proliferating in the central, lateral and basal nuclei expressed NG2, whereas only about 40 % of the cells in the medial nucleus were NG2+ glial cells. Staining against the microglial antigen OX-42 also revealed a specific proliferation pattern. The number of proliferating microglia increased in response to ECS in all nuclei, but the percentage of microglia were very low in the central, basal and lateral nuclei (about

1 %), whereas more than 10 % of the proliferating cells in the medial nucleus expressed OX-42.

In order to investigate whether some of the NG2+ glial cells differentiated into mature oligodendrocytes, we conducted a survival study where rats treated with 5 ECS were let to survive for three weeks. This study revealed that, even though a large percentage of the BrdU+ cells still expressed NG2, a small fraction of the BrdU+ cells expressed the mature oligodendrocyte antigen Rip. Furthermore, the number of BrdU+/Rip+ cells (i.e. newly formed oligodendrocytes) was significantly increased by ECS.

In summary, paper II shows that ECS-treatment increases the number of newly formed NG2+ glial cells, microglia and mature oligodendrocytes in the rat amygdala, and that the proliferation response differs between the different amygdala nuclei.

ECS-treatment counteracts the inhibitory effect on gliogenesis in corticosterone-treated rats (paper III)

The results in paper I and II suggest that the antidepressant treatment ECT might be able to counteract the oligodendrocyte loss seen in depressed patients, by increasing the number of newly formed oligodendrocytes. In order to further investigate this we needed a rat model that mimic depression in humans. The observation that depression is associated with elevated cortisol levels (Dinan 2001) suggests that animals chronically treated with corticosterone can mimic some aspects of human depression. Since corticosterone treatment is known to induce depression-like behavior, impair declarative memory and reduce hippocampal volume (Gregus et al 2005; Roozendaal et al 2002; Sousa et al 1998) (similar to what is seen in depressed patients), it has been considered as a satisfactory rodent model for depression. Interestingly, a previous study has also shown that corticosterone not only suppresses neurogenesis (see introduction) but also proliferation of NG2+ glial cells (Alonso 2000). Given the previous studies showing that ECS-treatment reverse the suppressive effect of corticosterone on neurogenesis (Hellsten et al 2002), we wanted to study whether ECS also could counteract corticosterone-induced inhibition of NG2+ glial cell proliferation.

In order to investigate this, rats were given five days of BrdU-injections along with ECS-treatment (once daily for five days) and corticosterone-treatment (once daily for seven days). In agreement with previous reports (Alonso 2000), the corticosterone-treatment resulted in a substantial inhibition of microglial and NG2+ glial cell proliferation in the ML and hilus of the dentate gyrus. We also found that ECS-treatment indeed was able to counteract corticosterone-induced inhibition of gliogenesis and restore the proliferation of NG2+ glial cells and microglia to baseline levels. Analogously, this study revealed that corticosterone was able to decrease the total number of NG2+ glial cells in ML and hilus, and that this effect also was counteracted by ECS. The total number of microglia did not decreased after corticosterone-treatment, but increased in response to ECS in the ML and GCL.

Since previous reports have shown a reduction in the hippocampal volume in rats chronically treated with corticosterone, we measured the volume of the three different dentate gyrus subregions in ECS- and corticosterone-treated rats using stereology (employing the Cavalieri principle). While no significant reductions in the overall hippocampal volume of corticosterone-treated rats was detected (a plausible explanation for this may be that the corticosterone-treatment period was too short to give detectable changes), we found that the hilar volume increased in response to ECS.

In order to investigate the fate of glial cells proliferating in response to ECS- and corticosterone-treatment we conducted a survival study where we allowed BrdU-injected ECS- and/or corticosterone-treated rats to survive for an additional three weeks. As described in our previous studies many of the BrdU+ cells remained in their NG2+ state, and the effect of ECS and corticosterone on microglia and NG2+ glial cell proliferation looked by large the same as the in ECS /corticosterone study with short survival. Interestingly, we found that corticosterone also inhibited the number of newly formed mature oligodendrocytes in the ML and hilus. This decrease in the number of oligodendrocytes was, just like the corticosterone-induced decrease in the number of other glial celltypes, reversed by ECS, and the number of BrdU+ oligodendrocytes was returned to baseline levels.

In summary we show that in an animal depression model, where levels of corticosterone are elevated, the number of newly formed microglia, NG2+ glial cells and oligodendrocytes in the rat hippocampus is reduced and that this reduction can be counteracted by ECS.

ECS-treatment does not induce reactive gliosis (paper IV)

Increased gliogenesis is a prominant feature in reactive gliosis, a process where microglia, NG2+ glial cells and astrocytes rapidly become strongly activated (reactive) in response to cellular damage in the the brain (see introduction). The reactive glial cells interact with each other at the lesion sites, where they phagocyte cellular debris, secrete inflammatory mediators and growth factors as well as play a key role in orchestrating the formation of a glial scar that protects the underlying neural network. Generally, reactive gliosis is characterized by glial cell proliferation, dramatic changes in glial cell morphology (swollen amoebic cells with retracted processes - indicating a phagocytic phenotype) and upregulation of glial specific marker (see introduction).

Prolonged seizure activity, as in status epilepticus (SE) is associated with extensive neuronal damage or death as well as reactive gliosis (Beach et al 1995; Lynch et al 1996; Mathern et al 1996). Our previous studies showed that ECS, with seizure activity lasting only about 30 seconds increase proliferation of both microglia and NG2+ glial cells in the rat hippocampus and amygdala. In these studies we were not able to detect any astrocyte proliferation, however it is known that ECS can induce a measurable activation of astrocytes, seen as an upregulation of glial fibrillary acidic protein (GFAP) (Kragh et al 1993). Since ECS-induced

gliogenesis might be interpreted as an indicator of reactive gliosis, we found it interesting to investigate whether microglia, NG2+ glial cells and astrocytes of ECS-treated adult rats also display morphological and biochemical features typical for reactive gliosis.

To address this question, rats were given either a single ECS-treatment, and were then allowed to survive for different time-periods (2 hours, 1 day, 2 days and 7 days), or a single ECS-treatment once daily for five days followed by a 2 days survival period. Rats exposed to SE- or sham-treatment were used as positive and negative controls, respectively. In order to detect changes in glial cell morphology, representative brain sections from the amygdala and hippocampus were stained with antibodies against NG2 (NG2+ glial cells), Iba-1 (microglia) and GFAP (astrocytes).

This study revealed that microglia and NG2+ glial cells display discrete morphological changes 2 hours after ECS. Slight retractions of glial cell processes were seen, and a somewhat more intense staining, maybe caused by thicker cell processes or increased expression of the glia cell marker. Moreover, astrocytes showed small morphological changes, characterized by slightly shortened, less smooth cellular processes and a darker appearance (possibly reflecting an increased expression of GFAP (Kragh et al 1993)) 2 and 7 days after ECS-treatment. The subtle alterations in glial morphology was in sharp contrast to the dramatic cellular remodelling seen after status epilepticus (SE) where the glial cells lost most of their cellular processes and assumed an amoebic cell shape.

Even though no amoebic glial cells were seen in rats exposed to ECS-treatment, it cannot be excluded that the slight alterations in glial morphology seen after ECS could be an indication of reactive gliosis. We therefore stained for ED1, a marker for phagocytic activity in microglia and NG2+ glial cells (Damoiseaux et al 1994; Dzwonek 2005) and nestin, an intermediate filament known to be upregulated in reactive astrocytes (Clarke et al 1994). We found that glial cells after ECS-treatment, in contrast to after SE, did not upregulate these two markers.

In summary, paper IV shows that even though ECS-treatment increases glial activity it does not induce reactive gliosis in rat hippocampus and amygdala.

GENERAL DISCUSSION

Since the introduction of the monoamine hypothesis that proposed that depression is caused by a deficit in the monoaminergic neurotransmission, depression has been regarded as a neurochemical disorder. Consequently, much research has focused on the role of neurotransmitters such as serotonin and norepinephrine in the pathophysiology of depression. In recent years, however, several studies have revealed that certain brain structures such as the prefrontal cortex (PFC), amygdala and hippocampus, all implicated in the regulation of emotional behavior, show volume reductions in depressed patients. Further investigations demonstrated that the volume reductions were at least partly explained by changes in the densities and sizes of neurons and glial cells. In addition, some studies showed a reduced number of glial cells in the PFC and amygdala. These data suggest that not only neurons but also glial cells may play an important role in the pathophysiology of depression. In light of these intriguing findings we became interested in investigating the glial cell response to electroconvulsive seizures (ECS), a rat model of the antidepressant treatment electroconvulsive therapy (ECT).

ECS stimulate proliferation of glial cells

Our first and most striking finding was that ECS-treatment increases the proliferation of glial cells. In the studies of this thesis we found that this gliogenic response occurred in the dentate gyrus of the hippocampus and in the main core nuclei of the amygdala, but we now know that ECS-induced gliogenesis is also found in other areas, such as the prefrontal cortex (Pohlman et al 2005), entorhinal cortex, piriform cortex and hypothalamus (Wennström and Jansson, unpublished observations). NG2+ glial cells showed the greatest response to ECS and constituted the majority of the dividing cells in these areas, but proliferation of microglia was also significantly enhanced. Furthermore, we found that the number of newly formed oligodendrocytes was significantly increased three weeks after ECS-treatment. As the mechanisms by which ECS may induce glial proliferation are poorly understood, however, a consideration of the rationale of ECS might be helpful.

By inducing a so-called grand mal seizure, ECS-treatment gives rise to a wave of glutamatergic excitation (activation of neurons through stimulation of glutamate receptors) spreading through the limbic system and other brain areas implicated in emotional behavior (Kandel et al 2000c). Interestingly, the expression of glutamate receptors is not restricted to neurons. Several studies have shown that glial cells also express these receptors (for review see Matute et al 2006). One implication of this interesting finding is that glial cells can receive information and respond to signals coming from nearby neurons. Generally, it is believed that neuro-glial glutamate transmission is a so-called spillover transmission where glutamate molecules that escape the interneuronal synaptic cleft activate receptors on the glial cell (Overstreet 2005). There is conclusive evidence, however, that one of the glial

cell types, the NG2+ glial cell, has the unique capability of forming true synaptic connections with neurons (Bergles et al 2000; Lin and Bergles 2004). This neuro-glial synapse is a clear exception to the traditional view of glial cell functioning and offers a new perspective on the role of glial cells in the brain. Apparently such cells can be involved in cell–cell communication in a manner much more direct than previously thought. It has recently been suggested that neurons can regulate proliferation of NG2+ glial cells via glutamate receptors expressed on NG2+ glial cells (Butt et al 2005). Proliferation of microglia can also be mediated by glutamate receptors, at least *in vitro* (Serio et al 2005). In summary, it is thus possible that increased glutamatergic activation, as occurs during ECS-treatment, can directly induce gliogenesis.

Our studies show that the proliferative response to ECS does not occur until two days after seizure induction. It is most likely that a chain of events -maybe initiated by glutamate receptor activation, followed by an upregulation of specific genes which in turn leads to the synthesis of glial mitogens, explain the observed delay in proliferative response. Indeed, it has been shown that factors involved in cell proliferation, survival and differentiation, such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor-2 (FGF-2) are upregulated in response to ECS (Mallei et al 2002; Newton et al 2003; Nibuya et al 1995). These factors, collectively referred to as growth factors or neurotrophic factors, are produced by neurons (Chang et al 2003; Samii et al 1999; Woodward et al 1992), endothelial cells (Gualandris et al 1996; Louissaint et al 2002; Seghezzi et al 1998) and activated glial cells (Dougherty et al 2000; Gehrmann et al 1996; Knott et al 2002; Panickar and Norenberg 2005; Sköld et al 2005). BDNF is foremost known to promote neurogenesis and survival (Pencea et al 2001; Zigova et al 1998). However, recent studies have shown that both NG2+ glial cells and astrocytes express the BDNF receptor TrkB (Condorelli et al 1995; Skup et al 2002), and preliminary results have shown that BDNF can mediate proliferation of NG2+ glial cells (Hallbergson et al 2005). Although microglia express very low levels of TrkB receptors, it has been shown that BDNF can induce proliferation of these cells *in vitro* (Zhang et al 2003). VEGF is mostly known as a key mediator of angiogenesis, but this growth factor has also been shown to induce neurogenesis *in vitro* and *in vivo* (Jin et al 2002) as well as microglial proliferation *in vitro* (Forstreuter et al 2002). FGF-2, like VEGF, is a very potent angiogenic growth factor (Presta et al 2005), but previous studies have shown that this growth factor also induce NG2+ glial cell proliferation (Butt and Dinsdale 2005; Goddard et al 1999) and neurogenesis (Ghosh and Greenberg 1995).

As mentioned in the introduction, the glial pathology found in depressed patients has been attributed to a loss of oligodendrocytes in some brain areas. Furthermore, it has been shown that depressed patients display abnormalities in genes expressed exclusively by oligodendrocytes and their progenitors. These interesting findings suggest that oligodendrocytes may be directly involved in the pathophysiology of depression. It is thus particularly interesting that ECS-treatment increases the number of newly formed oligodendrocytes. This increase

could possibly be explained by the finding that ECS upregulates growth factors (such as BDNF) implicated in the maturation of oligodendrocytes (Du et al 2003). However, given that NG2+ glial cells can differentiate into mature oligodendrocytes, it is also possible that the increased number of oligodendrocytes seen three weeks after ECS is a reflection of an increased pool of oligodendrocyte precursor cells (OPCs). Support for this theory comes from our finding that the proportion of newly formed NG2+ glial cells decreases three weeks after ECS. In addition, unpublished results show that the proportion of newly formed oligodendrocytes is even larger six months after ECS-treatment, while the fraction of NG2+ glial cells is decreased (Wennström and Hellsten unpublished results). Given these findings, and the fact that the number of oligodendrocytes is reduced in depressed patients, it is tempting to speculate that the antidepressant effect of ECT might involve, at least in part, an effect on oligodendrocyte renewal. Interestingly a recent study shows that frontal white matter reductions in depressed patients are reversed by ECT-treatment (Nobuhara et al 2004).

ECS counteract corticosterone-induced glial loss

Our next important finding was that the number of newly formed NG2+ glial cells, microglia and oligodendrocytes in the rat hippocampus decreased significantly in response to corticosterone-treatment. These findings are particularly interesting as they indicate that the human counterpart to corticosterone, the stress hormone cortisol, might be involved in the oligodendrocyte loss seen in depressed patients. As mentioned in the introduction, several investigators consider cortisol-induced atrophy as a possible explanation for brain volumetric changes seen in depressed patients. This assumption should be seen in the light of the well-established observation that cortisol levels are often increased in depressed patients. Furthermore, it is well known that high levels of glucocorticoids, either in patients with Cushing's disease or caused by steroid treatment of (for example) inflammatory disorders, can lead to depression as well as hippocampal volume reduction. Finally, in preclinical studies it has been shown that corticosterone can induce both hippocampal atrophy and depression-like behaviors.

Our finding that corticosterone suppresses the formation of new NG2+ glial cells and microglia is in line with studies by other researchers showing glucocorticoid-induced downregulation of these cells (Alonso 2000; Ganter et al 1992). Since both immature oligodendrocytes and microglia contain glucocorticoid and mineralocorticoid receptors (Tanaka et al 1997a; Vielkind et al 1990) it is plausible that the suppression of proliferation is induced by a direct effect of corticosterone on these cells. Furthermore, several studies have shown that stress and corticosterone downregulate growth factors involved in glial proliferation (Heine et al 2005; Molteni et al 2001; Smith et al 1995).

The decreased number of newly formed oligodendrocytes may be a consequence of corticosterone-induced downregulation of NG2+ glial cell proliferation (a decrease in the OPC pool). This is analogous to the idea that ECS may increase the number of mature oligodendrocytes by increasing the number of

precursors (see above). However, as corticosterone downregulates BDNF and as BDNF has been shown to be involved in the differentiation of oligodendrocytes, it is also possible that corticosterone-treatment leads to a suppression of oligodendrocyte maturation.

Interestingly, we found that the inhibitory effect of corticosterone on glial cell renewal was counteracted by ECS. How ECS-treatment opposes this inhibitory effect remains to be elucidated. However, given that ECS create a permissive environment for cell proliferation by increasing the availability of growth factors (see above), it is possible that ECS can interfere directly with the suppressive effect of corticosterone by upregulating factors that are downregulated by corticosterone. Indeed, it has been shown that ECS counteract stress-induced downregulation of BDNF (Nibuya et al 1996). It could also be that the two treatments affect cell proliferation in independent manners. The findings that ECS can oppose the effect of corticosterone on gliogenesis suggests that depression-associated oligodendrocyte loss might (at least in part) be caused by elevated levels of cortisol often found in depressed patients, and that ECT may function to oppose such glial loss.

No signs of cellular damage were noted after ECS

As previously discussed, ECS-induced gliogenesis may, directly or indirectly, be mediated via increased glutamate transmission. It is well established, however, that too much glutamate is toxic and can lead to cell death (Rothstein 1996). This so-called excitotoxicity is caused by sustained glutamate receptors stimulation and since neurons and glial cells express glutamate receptors they are both susceptible to glutamate-induced excitotoxicity (Matute et al 2006). Excitotoxic cell damage is typically seen after prolonged seizures, such as status epilepticus (SE) (Bengzon et al 1997; Sloviter et al 1996) As ECS-treatment is associated with a surge of glutamate release, it is reasonable to assume that ECS could also induce excitotoxic cell damage.

In the fourth article of this thesis we show, however, that ECS does not lead to reactive gliosis. Since reactive gliosis is the inflammatory response to cell damage wherein glial cells remove dead cells and cellular debris and replace lost tissue with a glial scar (see introduction), it can be concluded that no major cell death occurs in response to ECS. This result is in line with several studies that have failed to show cellular death after ECS (Dalby et al 1996; Ende et al 2000; Hellsten et al 2002). The absence of cellular damage may be explained by the fact that the seizure duration is much shorter (lasting about 30 seconds) in ECS compared to status epilepticus (SE). Indeed, previous studies have shown that the extent of brain damage seen after chemically induced SE is dependent on the duration of the seizure (Hsieh 1999; Lemos and Cavalheiro 1995).

Glia-mediated cell-protection: mechanisms behind the antidepressant effect of ECS?

As mentioned above, stress or glucocorticoids have been hypothesized to underlie the volumetric changes seen in depressed patients. A large number of animal studies, showing that stress and corticosterone can inflict cellular damage (such as dendritic atrophy, reduced neurogenesis and neuronal loss) as well as reductions in hippocampal volume, support this hypothesis. The hypothesis that depression may be a result of atrophy or loss of brain cells, as has been observed in *post mortem* material from depressed patients, has led to the idea that the antidepressant effect of ECT may involve an ability to reverse or prevent such cellular alterations (Newton et al 2003). Could it be that the glial response seen after ECS are important in such protective role?

Glial cells produce neuroprotective agents

Although our studies showed that glial cells do not become reactive in response to ECS we found discrete changes in glial cell morphology indicating a certain degree of activation shortly after ECS-treatment. It is well known that fully reactive glial cells produce both inflammatory mediators and neuroprotective agents (see introduction), but what are the functional characteristics of a glial cell that is activated only to a small degree?

It is reasonable to assume that glial cell activity should depend on the extent of changes in the surrounding environment. As an example, it has been suggested that, after a minor disturbance in the environment, microglia tend to produce neuroprotective agents and growth factors in an attempt to support and rescue endangered neurons. Not until all hope is gone and the neuronal damage is irreversible will the microglia become reactive, begin to engulf cellular debris and turn into what might be regarded as "merciful killers" (van Rossum and Hanisch 2004). Further support for this idea comes from a recent study on rats living in an enriched environment, where microglia increased their activity slightly, but not to the extent that they acquired the typical reactive phenotype. These activated microglia were shown to play an important role in the maintenance of hippocampal neurogenesis and spatial learning (Ziv et al 2006). It is thus tempting to speculate that slightly activated glial cells, similar to the ones we observe after ECS, secrete molecules with a more "protective" profile.

As previously mentioned, growth factors known to derive partly from activated glial cells are upregulated in response to ECS. VEGF and FGF-2 have been reported to reduce excitotoxic damage to cultured hippocampal neurons (Bonthius et al 2003; Matsuzaki et al 2001; Svensson et al 2002) and BDNF is known to be important in the survival and maintenance of neurons (Hashimoto et al 2004; Lowenstein and Arsenault 1996). Interestingly, several studies suggest that BDNF can both be involved in the pathophysiology of depression and act as a mediator of the effect of antidepressants. Clinical studies have suggested that low BDNF levels are associated with depression (Karege et al 2002; Lang et al 2004) and it has been reported that the levels of BDNF were increased in *post mortem*

tissue from patients receiving antidepressant treatment at the time of death (Chen et al 2001). In preclinical studies, it has been shown that BDNF and its receptor TrkB are upregulated by several antidepressant treatment modalities (Nibuya et al 1995) and that BDNF itself has antidepressant properties (Shirayama et al 2002; Siuciak et al 1996).

Other glia-derived "protective" molecules have also been found to be upregulated in response to ECS. In a preliminary report, it was shown that a single ECS-treatment increased the expression of several anti-inflammatory mediators in the hippocampus and prefrontal cortex (Labuz et al 2003) while the expression of proinflammatory molecules was not increased. In addition, increased levels of growth factors belonging to the TGF-β family, implicated in anti-inflammatory responses in the CNS (for review see Bottner et al 2000) have been noted after ECS-treatment (Dow et al 2005).

All above mentioned findings suggest that ECS, via activation of glial cells, may enhance the expression of protective molecules that could be important for reversing stress-induced cellular changes. The ability of ECS to reverse the cortiocosterone-induced downregualtion of gliogenesis (as mentioned above), neurogenesis and angiogenesis (as mentioned in the introduction) may serve as examples.

Glial cells are involved in neurite outgrowth

The above mentioned changes in neuronal density and size in the prefrontal cortex, the hippocampus and the amygdala of depressed patients, as well as the observations that stress and corticosterone treatment induce dendritic atrophy and reduced spine densities in the corresponding areas in rat brain (see introduction), suggest that shortening or loss of neurites (axons and dendrites) may be involved in the pathophysiology of depression. Outgrowth and stabilization of neurites and formation of dendritic synapses are strictly regulated processes, depending both on secreted stimulatory or repulsive factors, and adhesion / guidance molecules laid down in the extracellular matrix or expressed on the surface of nearby cells (Kandel et al 2000d). Interestingly, it has been observed that processes from NG2+ glial cells often enclose dendrites and dendritic synapses (Ong and Levine 1999) and that NG2+ glial cells produce a number of extracellular matrix molecules that compromise the so-called perineuronal net (Celio et al 1998; Sandvig et al 2004). This net is important for the stabilization of dendritic synapses. Moreover, it has been shown that the chondroitin sulphate proteoglycan NG2 can inhibit axonal outgrowth both *in vitro* and *in vivo* (for review see Tan et al 2005). Conversely, preliminary results suggest that NG2+ glial cells can also promote regeneration of retinal axons and outgrowth of cultured hippocampal axons (Butt et al 2005; Yang et al 2004). This indicates that NG2+ glial cells may both inhibit and promote axonal outgrowth. Interestingly, it has been demonstrated that growth factors such as BDNF can also promote sprouting of granule cell neurites in the hippocampus (Hashimoto et al 2004; Lowenstein and Arsenault 1996).

A reduction in NG2+ glial cells, as occurs after corticosterone-treatment,

could therefore lead to a rarefaction of the supportive perineural network, a loss of guidance cues both in the extracellular matrix and on processes from NG2+cells, and most likely also to a shortage of glia-derived trophic factors. This could in turn result in poor axonal outgrowth, shrinking of dendritic trees and eventually neuronal death. In addition, other glial cells would be affected.

Previous studies have shown that ECS increases hippocampal neurogenesis (see introduction) and induces sprouting of granular mossy fibers in the hippocampus (Chen et al 2001; Vaidya et al 1999). Both of these processes involve outgrowth of axons. Our studies show that ECS-induced proliferation of NG2+ glial cells in the hippocampus is most pronounced in the hilus of the dentate gyrus. This is also the area into which the new axons are reaching, searching for their targets in the hippocampal subregion CA3 (Markakis and Gage 1999) In line with the above reasoning, ECS-induced production of growth factors and newborn NG2+ glial cells may be important for stimulation and guidance of axon outgrowth.

Glial cells regulate glutamate homeostasis

The role of glutamate in the patophysiology of depression has recently attracted much attention. A number of studies have revealed that glutamate receptor modulators show antidepressant-like effects in animal models of depression (for review see Palucha and Pilc 2005). In one study the glutamate receptor antagonist ketamine was shown to produce an antidepressant effect with a surprisingly rapid onset (Berman et al 2000). Furthermore, depressed patients display abnormalities in the glutamatergic system, and both increases (Altamura et al 1995; Binesh et al 2004; Mauri et al 1998; Sanacora et al 2004) (Hashimoto et al 2005) and decreases (Auer et al 2000; Pfleiderer et al 2003; Rosenberg et al 2005) in glutamate levels have been reported. The cause of these alterations is unknown, but, interestingly, a recent *post mortem* study of depressed patients revealed a down-regulation of glial cell specific glutamate transporters (GluT's) (Choudary et al 2005).

These GluT's are predominately expressed by astrocytes and are essential for the maintenance of glutamate homeostasis (for review see Matute et al 2006). By regulating synaptic glutamate levels the transporter molecules prevent extracellular glutamate concentrations from rising to toxic levels (Rothstein 1996). The protective role of these glutamate transporters was demonstrated in a study where transgenic mice, with a deletion in a one of the glutamate transporter genes, showed signs of excitotoxic brain damage (Tanaka et al 1997b).

The expression of glutamate transporters is dependent on the extracellular milieu. In response to increased levels of glutamate and growth factors astrocytes increase their activity as well as the expression of glutamate transporters (Duan et al 1999; Suzuki et al 2001; Zelenaia et al 2000). Interestingly, recent findings have shown that also activated microglia upregulate glutamate transporters (van Landeghem et al 2001). It may thus be that activated microglia help astrocytes in removing excessive glutamate in order to prevent neuronal and glial cell death. The knowledge of NG2+ glial cells still is rather limited, and it is not known whether

these cells, in an activated state, also upregulate GluT's. However, given that processes from NG2+ glial cells are interposed with glutamatergic synapses in the hippocampus (Ong and Levine 1999), it cannot be excluded that NG2+ glial cells also take part in the regulation of glutamate signaling.

Since ECS can activate glial cells, it is possible that this treatment also upregulates GluT's. It is tempting to speculate that ECS, by activating glial cells, may oppose the downregulation of GluT's seen in depressed patients and thereby normalize glutamate levels.

CONCLUDING REMARKS

The results of this thesis demonstrate that ECS-treatment, an animal model of the antidepressant treatment ECT, increase glial activity and induce formation of new glial cells in the rat hippocampus and amygdala. This glial response is particularly interesting given the intriguing findings that depressed patients display a glial pathology in these regions. Since glial cells respond to their environment, communicate via synapses as do neurons, and have an important role in neuroprotection, it is likely that a glial deficit leads to serious disturbances in the neuronal network. It could thus be argued that ECS could prevent or protect against cellular damage possibly by reversing glial loss. Glial cell dysfunction may be of central importance in the pathophysiology of depression and further knowledge on how antidepressant treatment affects proliferation, differentiation and survival of different glial cell populations may lead to new therapeutic strategies.

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