Shaping the brain through experience: effects of stressful life events on hippocampal neurogenesis, morphology and function

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Chapter 1

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Chapter 1. General Introduction

1. Stress
You can feel stressed (out), be under a lot of stress, find something stressful, or have stress. Although the word stress is commonly used in every day life, the exact definition of stress is not a simple one. In the context of this thesis, stress refers to the encounter of a potentially threatening, generally uncontrollable situation (or so-called stressor) and the subsequent physiological response of the body to this threat. Stressors can be psychological or physical in nature and may last for considerable periods of time -like an unhappy family life- or can be as acute as a spider dropping from the ceiling.

1.1. The stress response
In a stressful situation, certain physiological systems are activated that enable an appropriate behavioural response (De Kloet et al., 2005). First of all, via the autonomic nervous system sympathetic activity is enhanced, resulting in higher levels of noradrenaline; parasympathetic activity is suppressed. Collectively, this decreases digestive activity, increases heart rate and blood pressure and heightens attention, which is sometimes referred to as the ‘fight or flight (or fright) response’ (Cannon, 1929). Next to this, the other major stress system is activated: the hypothalamo-pituitary adrenal (HPA) axis (figure 1). The end product of the HPA-axis is the stress hormone cortisol (or corticosterone in rodents), which facilitates a.o. release of glucose from storage sites and enhances cognitive processing. Through this, the stress response ensures effective coping with threats and is for this reason highly adaptive and strongly preserved throughout evolution (Korte et al., 2005). Yet, when stress is persistent and fighting or fleeing does not result in the relief of the stressor, prolonged activation of the stress system may threaten bodily homeostasis (McEwen and Wingfield, 2003; de Kloet et al., 2005).

These different phases of stress were noticed by Hans Selye as early as in 1936. Selye described an initial alarm response, a subsequent phase of resistance and a final stage of exhaustion (Selye, 1998). Indeed, a vast amount of literature has shown that long term glucocorticoid exposure can increase the chance to develop e.g. metabolic disorders and various mental illnesses such as post traumatic stress disorder and depression.

1.2. Corticosteroid action and negative feedback
After release of corticosterone from the adrenal cortex into the circulation, the hormone reaches all parts of the body and passes the blood brain barrier to enter the brain. Via the brain and pituitary gland it terminates its own production through negative feedback regulation, reinstating homeostasis. Corticosterone binds to two receptors, the mineralocorticoid (MR), (Reul and de Kloet, 1985) and glucocorticoid receptor (GR), (McEwen and Wallach, 1973). Negative feedback primarily occurs via the paraventricular nucleus (PVN) of the hypothalamus and pituitary, where corticosterone binds GR and
Figure 1. The hypothalamo-pituitary adrenal (HPA) axis. Activation of the HPA-axis upon stress (de Kloet et al, 2005), leads to the production of corticotropin releasing hormone (CRH) in the paraventricular nucleus (PVN) of the hypothalamus, which subsequently stimulates the pituitary gland to produce adrenocorticotropin hormone (ACTH). ACTH reaches the adrenal cortex, which produces corticosterone that is released into the circulation. Corticosterone crosses the blood brain barrier and terminates its own production in the brain via a negative feedback regulation. Corticosterone inhibits CRH and ACTH production in the hypothalamus and pituitary respectively, but also reaches higher brain areas such as the hippocampus. Besides mediating part of the functional response to stress at a cognitive level, the hippocampus contributes to the negative feedback regulation in an indirect manner. Photograph by E.H. Velazquez

inhibits corticotropin-releasing hormone (CRH) production and subsequent adrenocorticotropin hormone (ACTH) release. Keller-Wood and Dallman, 1984. Both MR and GR are intracellular receptors that reside in the cytoplasm. Upon ligand binding, these receptors translocate to the nucleus where they act as transcription factors and regulate gene transcription (Joels and de Kloet, 1989). The two receptor types are differentially distributed throughout the brain; the MR is mainly expressed in the limbic system, with a high expression level in the hippocampus (Ahima and Harlan, 1991; de Kloet et al., 2005).
whereas the GR has a more uniform distribution (Fuxe et al., 1985). Corticosterone has a 10-fold higher binding affinity to the MR compared to the GR (Reul and De Kloet, 1985). Therefore MR is mostly occupied under baseline conditions, whereas the GR is only activated when glucocorticoid levels show a substantial rise. The latter occurs only during the stress response and the peak of the diurnal rhythm (i.e. before the onset of the active phase).

Although the almost constant occupation of the MR may raise questions as to its functional role, various studies have shown that tonic MR activation is necessary for neuronal development and viability (Wossink et al., 2001). Adrenalectomy (i.e. removing all glucocorticoids) for example, increases granule cell death by apoptosis (Gould et al., 1991). MR activation maintains excitatory tone in hippocampal neurons, which indirectly facilitates the inhibitory GABAergic input on CRH producing neurons in the PVN (Miklos and Kovacs, 2002; Herman et al., 2003). Furthermore, recent studies show that corticosterone can change the excitability of CA1, amygdala and dentate gyrus neurons via a lower-affinity membrane MR receptor in a rapid, non-genomic fashion (Karst et al., 2005; Pasricha et al, in preparation). This greatly enhances the speed and capacity of MR-mediated physiological responses, and thereby their range of action (Joels et al., 2008).

Whereas the MR is responsible for the appraisal and immediate response to stress, GR controls the stress response, ensures subsequent consolidation of relevant information (Oitzl and de Kloet, 1992; Oitzl et al., 2001) and promotes recovery from stress (Sapolsky et al., 2000; de Kloet et al., 2005). The hippocampus plays an important role in both MR- and GR-dependent processes, via changes in gene expression of approximately 70-100 genes involved in a.o. plasticity and structure (Datson et al., 2001). In summary, due to the differential receptor distribution, binding affinity and time course of action in response to corticosterone, stress can initiate a complex but tightly orchestrated response (Joels and Baram, 2009), with the hippocampus as one of its major targets and regulators.

### 1.3. Stress as a risk factor for depression

A large number of studies have shown convincing evidence for the involvement of stress in (the vulnerability to) depression. First, stressful experiences, particularly early in life, increase the risk to develop depression (Heim and Nemeroff, 2001; McEwen, 2003). Second, about 50% of all patients suffering from depression display a dysregulation of components of the HPA axis. For instance, in a subset of patients (often suffering from psychotic depression), hypercortisolemia is often observed (Gibbons and McHugh, 1962; Gold et al., 1986; Belanoff et al., 2001a). Moreover, depressed patients usually demonstrate an impaired negative feedback as measured by the CRH/dexamethasone suppression test (Heuser et al., 1994) and increased levels of CRH in the PVN (Raadsheer et al., 1995). Third, in patients with Cushing's disease that suffer from high cortisol levels due to a benign tumor in the pituitary gland, depression-like symptoms are common (Kelly et al., 1983). Finally,
normalization of HPA axis parameters has been associated with, and seems to be a prerequisite for the successful treatment of depression (Holboer et al., 1982). Consistent with this, treatment with the GR-antagonist mifepristone was recently reported to ameliorate symptoms of psychotic depression (Belenoff et al., 2001b; Belanoff et al., 2002; Flores et al., 2006). Interestingly, mifepristone was already effective after 4 days of treatment.

Major depressive disorder is a severe mood disorder affecting many people worldwide and in the U.S., about 6.7% of the population over 18 years old is affected by depression (Kessler et al., 2005). It is the leading cause of disability for ages 15-44 (World Health Organization, 2004). Depression is a heterogeneous disorder that is diagnosed according to the criteria formulated in the diagnostic statistical manual of mental disorders (DSM-IV-TR), which states that a depressive episode is characterized by (at least five of) the following symptoms: depressed or irritable mood, anhedonia, changes in bodyweight (loss or gain), insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or excessive guilt, decreased ability to think or concentrate, and thoughts of death or suicide. Although it is still not exactly known what causes low mood in depression, the original observation that monoamine reuptake inhibitors increase mood (Kuhn, 1957) has led to the formulation of the monoamine hypothesis (Coppen, 1967; Hirschfeld, 2000), attributing symptoms to a neurochemical imbalance of neurotransmitters like serotonin, noradrenaline or dopamine. Nowadays, this still forms the basis for most treatment strategies.

Based on twin studies, depression has an estimated heritability of 40% and indeed, multiple genes appear to be involved in the vulnerability to develop depression (Sullivan et al., 2000). Other factors that can render an individual vulnerable are a.o. gender; women develop depression more often than men (Kessler et al., 1993; Bland, 1997; Ustun, 2000). In addition, there is a strong environmental component and adverse life events are known to render an individual more vulnerable. At the moment, the traditional neurochemical explanation no longer dominates depression research and it seems more and more likely that changes in the stress system, for example resulting in an altered structural plasticity of a.o. the hippocampus (see section 2.3), may play a role as well. At the moment, the search for novel antidepressants concentrates increasingly on the stress system, and compounds that modulate HPA-axis activity have so far shown moderately promising results (Berton and Nestler, 2006).
2. The Hippocampus
The hippocampus is one of the major target areas of corticosterone. Below I will give an overview of the main anatomical and functional properties of the hippocampus.

2.1. Anatomy
In the human brain, the hippocampus can be found bilaterally in the temporal lobe and bears the remarkable resemblance of a seahorse, after which it is named. In rodents, this analogy may be less obvious, but the hippocampus is still remarkably recognizable due to its laminar organisation. The hippocampus is an old cortical structure (archicortex) and contains only three layers, instead of the usual six in the other cortical regions. The major subfields that can be distinguished in the hippocampal formation are the dentate gyrus (see box 1), the CA3 and CA1 region which together form the so-called trisynaptic circuitry (figure 2). This circuitry is a (mainly) unidirectional processing unit, where

![Diagram of the hippocampus and its connections](image-url)

**Figure 2.** The hippocampus. A. The left hemisphere of the rat brain (around bregma -4.2), with the hippocampus located roughly in the middle B. A schematic drawing of the trisynaptic circuitry of the hippocampus. The dentate gyrus (DG) receives input from the entorhinal cortex, after which it projects to the CA3 and finally the CA1 region. After that, information leaves the hippocampus via the subiculum (S). Photograph by E.H. Velzing
information enters the hippocampus at the DG through the fibres of the perforant path that originate in the entorhinal cortex. The principal neurons of the DG process this input and relay it to the pyramidal neurons of the CA region (from Cornu Ammonis; Ammon’s horn). Via the mossy fibres the signal arrives from the dentate in the CA3 after which it is further projected to the CA1 via the Schaffer collaterals. The CA1 neurons in turn project to the subiculum (part of the hippocampal formation) and the deep layers of the entorhinal cortex (this is, however, a highly simplified description; for an extensive review see Van Strien et al., 2009). In rodents, the hippocampus runs along the so-called septotemporal axis, starting in the dorsal part of the brain, close to the septum, to the ventral or temporal part, which is in close proximity to the amygdala (an almond-shaped nucleus, involved in the processing of emotions). Both the hippocampus and the amygdala are part of the limbic system, an evolutionary old system that is implied in the regulation of emotions and the detection of novelty (Broca, 1878; Papez, 1995).

2.2. Function
The hippocampus plays a major role in learning and memory processes. Although it is to a large extent, not the site for long term memory storage, it is sometimes referred to as the ‘gateway to memory’. A well known patient who is often discussed in this respect is Henry Molaison (H.M.), who suffered from severe epilepsy. As a final treatment option, his left and right medial temporal lobe containing both hippocampi (and amygdala) were surgically removed. This resulted in a disability to form new memories (anterograde amnesia) and in addition to this, he lost a substantial part of his old memories, at least the more recent ones (retrograde amnesia) (Scoville and Milner, 2000). Reports from other patients have confirmed that the hippocampus is important in declarative memory, i.e. the memory of events and facts, but not procedural memory or working memory (Zola-Morgan et al., 1986). The idea that the hippocampus is a temporary storage site for information, that is later consolidated in the neocortex comes from the observation that H.M.’s long term memory was still intact is referred to as the ‘standard’ theory of memory consolidation (Squire, 1986). A recent study using MRI further supports this idea of ‘temporary storage’; Takashima and colleagues demonstrated that the need for hippocampus activation while recalling previously stored information, decreases over time (Takashima et al., 2006).

**BOX 1. Dentate gyrus structure, function and development**

All experiments described in this thesis that assess morphological and electrophysiological properties of the hippocampus, were performed in the hippocampal dentate (tooth-shaped) gyrus of the rat. The dentate gyrus (DG) (Scharfman, 2007) is the first hippocampal subfield in the trisynaptic circuitry and consists of a principal cell layer (the granule cell layer) that contains
mainly granule neurons, a molecular layer where the apical dendrites of granule cells reside and a polymorphic layer or hilus, which is enclosed by the granule cell layer (GCL). Granule neurons have a cone-shaped apical dendritic tree and an axon running through the hilus projecting to the CA3. The (approximately 3 cells wide) border between the hilus and the granule cell layer is called the subgranular zone, a germinative matrix where adult neurogenesis occurs (see paragraph 3).

Besides granule neurons, two other main cell types can be distinguished in the dentate gyrus, the mossy cell and the dentate pyramidal basket cell. The mossy cells are located in the hilus and receive extensive projections from the granule neurons, mainly glutamatergic, and project back upon the granule cells in another part along the septotemporal axis. The pyramidal basket cells are GABAergic interneurons that can be found in the subgranular zone. These cells form a strong inhibitory input on the granule cell bodies (Sik et al., 1997). The dentate gyrus receives its major input from layer II of the entorhinal cortex through the perforant path (Steward and Scoville, 1976). The perforant path can be subdivided in the medial and lateral perforant path; the lateral path projects mainly to the outer part of the molecular layer and the medial perforant path to the inner part. Functionally, the dentate gyrus has been proposed as a ‘filter’ for the hippocampus (Hsu, 2007). The granule cell population of the dentate (of approximately 1 million neurons in the rat, Boss et al., 1985), outnumbers the CA3 pyramidal cell population. Multiple granule cells project upon a single CA3 neuron. In parallel, it is unlikely that different CA3 pyramidal cells receive input from the same subset of dentate neurons (Amaral et al., 2007).

Regarding the development of the dentate gyrus (Altman and Bayer, 1990b, 1990a), it develops mostly postnatally and retains a unique form of structural plasticity into adulthood i.e. the birth of new neurons. In short, the central nervous system starts as a hollow structure, the neural tube, from which the hollow inside develops into the ventricular system. The brain develops in an inside-out fashion and neurons are generated from precursor cells aligning the inside of the tube, the so-called subventricular zone, which is the primary germinative matrix. From this site, neurons migrate towards the outside and create the cortical regions. All hippocampal neurons are also generated in the ventricular zone and in rats, and cells from this primary matrix migrate into place around embryonic day 18 (CA-region). A secondary germinative matrix builds up at the subventricular zone and from this, around the time of gestation (embryonic day 21), the outer one-third of the dentate migrates into place (figure 3). However, most of the dentate gyrus is developed postnatally during which the suprapyramidal layer is formed first, followed by the infrapyramidal layer. Between postnatal day (PND) 3 and 14 the migration a secondary and tertiary germinative matrix occurs a process characterized by high levels of cell proliferation. At the border of the granule cell layer and the hilus, cell division from the tertiary germinative
matrix continues into adulthood (although at a lower level) giving rise to the process of adult neurogenesis.

Figure 3. Dentate gyrus development in the rat. All cells originate in the primary matrix of the subventricular zone (SVZ) along the wall of the lateral ventricle. On the day of gestation E21/22, the outer one-third of the granule cell layer migrates into place (granule cell layer: dark grey; CA-region: light grey). Around PND 3 the migration of the inner two-third of the granule cell layer occurs i.e. the secondary germinative matrix, which, after further division gives rise to the tertiary germinative matrix that is eventually confined to the subgranular zone (PND 8). This tertiary matrix represents the site of future adult neurogenesis. Adapted from Kempermann, 2006, based on Rickmann et al, 1987; Altman and Bayer, 1990a, 1990b.

In rodents, spatial memory can be regarded as a form of declarative memory and is easily testable. The hippocampus is necessary for spatial learning and memory, as can be shown in a frequently used spatial navigation task for rodents: the Morris water maze task (Morris, 1984). In the Morris water maze animals learn to escape a circular swimming pool via a platform that is hidden under the water surface. The hippocampus-dependent version of the task
involve the integration of spatial information from distal cues outside the pool. With this information animals are believed to form a spatial map that they use to navigate towards their goal i.e. the platform. An intact hippocampus is crucial for the performance in this task (Schenk and Morris, 1985). Animals with a hippocampus damage however, are still able to find a platform that has been marked in some way (regarded as non-spatial or cued learning), (Schenk and Morris, 1985). The idea that the hippocampus can form a spatial map of the surrounding environment was discovered by O’Keefe and Dostrovsky in 1971 who found that hippocampal neurons only fire in a specific spatial location; a phenomenon that is referred to as ‘place cells’ (O’Keefe and Dostrovsky, 1971). Both granule neurons and pyramidal neurons of the CA region function as place cells.

In which form memories are stored and what can be considered as the actual ‘storage site’ of information, has intrigued scientists for centuries. At the cellular level, a synaptic contact between two neurons can be strengthened when repeated firing of the presynaptic cell is followed by activation of the postsynaptic cell. This principle is best described by ‘neurons that fire together, wire together’ and is called Hebbian learning (Hebb, 1949). This form of synaptic plasticity can result in strengthening of certain neuronal connections for a substantial period of time, and was shown at the network level through the process of long-term potentiation (LTP), (Bliss and Lomo, 1973). Long-term potentiation (or, when connections weaken: long-term depression) is considered to be the cellular substrate for learning (Pastalkova et al., 2006; Whitlock et al., 2006).

As mentioned in section 1.2, the hippocampus is highly affected by stress and stress hormones. Corticosterone has profound effects on a.o. neuronal firing rates and synaptic plasticity and, through that, on learning and memory (Joels et al., 2006). Depending on the timing and intensity, stress and/or corticosterone can enhance (Korz and Frey, 2003; Wiegert et al., 2006) or impair (Foy et al., 1987; Diamond et al., 1992; Alfèrez et al., 2002) the degree of LTP or even induce LTD (Kim and Diamond, 2002). In summary, the hippocampus is a very interesting target when studying the effects of stress on the brain, not only because it is involved in the stress response and highly affected by stress hormones, but also because this brain region is a key component of the everyday cognitive abilities of animals and humans alike.

2.3. The hippocampus in depression

As mentioned, the hippocampus is one of the major targets and modulators of the HPA-axis. Depression and other stress-related disorders are believed to affect the hippocampus is several ways. First, a reduction in hippocampal volume has been observed in depressive patients (Axelson et al., 1993; Bremin et al., 2000; Sheline, 2003; Campbell et al., 2004; Jansen et al., 2004; Videbech and Ravnkilde, 2004) that seems to correlate with disease severity (MacQueen et al., 2003). A second observation supporting the involvement of the hippocampus in depression is that patients can suffer from changes in cognitive
functioning such as declarative memory (Wolkowitz et al., 1995; Belanoff et al., 2001a) in parallel with hippocampal volume loss (Sheline et al., 1999).

The underlying mechanisms of hippocampal volume loss are still under debate. The question emerges whether a smaller hippocampus in depression is a cause or consequence of this disorder. A twin study in patients suffering from Post Traumatic Stress Disorder (PTSD), which is also characterized by hippocampal volume loss, demonstrated that combat veterans with PTSD and their healthy, non-exposed twin brothers both have a smaller hippocampus than control subjects (Gilbertson et al., 2002). In depression however, the causative relation is less clear, although a recent study showed that both depressed patients and people at high (genetic) risk for depression have a smaller hippocampus and that early life adversity also causes volume reduction (Rao et al., 2010). Interestingly, in women volume reduction was reported only in patients that also experienced childhood abuse (Vythilingam et al., 2002), indicating that, at least in women, this environmental factor may play an important role in the development of depression.

The source of the volume reduction in depressed patients is not resolved yet. It could be caused by lower cell numbers or by a reduction in the neuropil, glia, myelination or water content, for example. If cell numbers are responsible, the question can be raised whether this is due to increased apoptosis or decreased (developmental or adult) neurogenesis (see section 3). Until now, no differences have been reported in the amount of apoptosis in post mortem tissue of depressed patients (Lucassen et al., 2001; Muller et al., 2001). Whether adult neurogenesis could play a role in the etiology of depression has been subject to debate. Data from animal studies show that monoamine levels may be necessary for the maintenance of neurogenesis (Brezun and Daszuta, 1999; Jacobs et al., 2000) and in addition to this, adult neurogenesis is upregulated by many antidepressant treatments (Duman et al., 2001; Malberg and Schechter, 2003; Banasr and Duman, 2007) and downregulated by stress (see section 3.5). These findings led to the formulation of the ‘neurogenic hypothesis of depression’ (Jacobs et al., 2000; Duman, 2004), stating that decreased levels of neurogenesis may be involved in the etiology of depression and that normalizing neurogenesis as a result of antidepressant treatment will contribute to the recovery from depression. This hypothesis was later supported by the finding that the behavioural effect of both tricyclic antidepressants (TCAs) and selective serotonin re-uptake inhibitors (SSRIs) require adult hippocampal neurogenesis (Santarelli et al., 2003). This hypothesis is questioned however, considering the low occurrence of neurogenesis in adult humans (Reif et al., 2006; Boldrini et al., 2009; Lucassen et al., 2010a). Moreover, recent studies have found that antidepressants can have both neurogenesis-dependent and neurogenesis-independent mechanisms of action (Surget et al., 2008; David et al., 2009). It seems that neurogenesis, although an important component of hippocampal structural plasticity, is a representative substrate of depression- or antidepressant-induced changes, rather then a pivotal causal player in this process (Sahay and Hen, 2007; Lucassen et al., 2010b).
3. Adult hippocampal neurogenesis

Neurogenesis in adulthood is restricted to a very limited set of brain regions. This process is extremely sensitive to life experiences, as will be explained below.

3.1. Historical perspective

For long, the brain was thought to be post-mitotic and incapable of generating new neurons during adult life. However, studies in the 1960's by Joseph Altman using tritiated [3H]-thymidine, which incorporates into the DNA of dividing cells, demonstrated the presence of newborn neurons in the adult rat brain (Altman, 1962). He found that neurogenesis was mostly restricted to the subgranular zone (the border of the granule cell layer with the hilus) in the dentate gyrus (Altman, 1963; Altman and Das, 1965) and the subventricular zone (SVZ), from which cells migrate to the olfactory bulb (Altman, 1969; Kaplan and Hinds, 1977). The initial findings were received with scepticism and it was not until the early 1990's that adult neurogenesis became an accepted phenomenon. This was mainly attributable to pioneering studies on neurogenesis in songbirds (Goldman and Nottebohm, 1983), showing functional integration of newborn neurons into the neuronal network (Paton and Nottebohm, 1984) and an experience-dependent regulation of neurogenesis (Barnea and Nottebohm, 1994; Kirm et al., 1994; Barnea and Nottebohm, 1996). Adult hippocampal neurogenesis in rodents was confirmed by Gould and colleagues, revealing the identity of the newborn cells as neurons by double-labelling with the neuronal marker “neuron-specific enolase” (Cameron et al., 1993). Around the same time, the synthetic thymidine analogue BrdU (5-bromo-3'-deoxyuridine, see also box 2) was introduced (Kuhn et al., 1996), greatly facilitating neurogenesis research. BrdU, like [3H] thymidine, incorporates into the DNA of dividing cells but allows immunohistochemical processing thereby enabling stereological quantification. A few years later, the existence of adult hippocampal neurogenesis in the human brain was demonstrated (Eriksson et al., 1998). Together, these findings triggered an exponential increase in scientific interest and publications, reflecting a shift in neuroscience dogma from structural rigidity to an adaptive structural plasticity.

3.2. From stem cell to neuron

Adult neurogenesis is regarded as a remnant of brain development that continues to occur in certain restricted areas (Seri et al., 2001). The stem cell population in the hippocampal dentate gyrus originates from the SVZ (Rickmann et al., 1987), whereas the SVZ itself remains the other site of adult neurogenesis. These two neurogenic regions of the adult brain (of which I will focus on the dentate gyrus from now on) provide a permissive microenvironment for the generation of new neurons from neural stem cells (NSCs); a multiple phase process that, as a whole, is referred to as neurogenesis (see box 2). Stem cells have been isolated from adult hippocampal tissue (Ray et al., 1993; Palmer et al., 1995) and the subgranular zone seems particularly
suited as a neurogenic niche due to its intense vascularization (Palmer et al., 2000) and regional specific astrocyte populations (Song et al., 2002).

Still, it has been disputed whether the hippocampus actually contains stem cells with unlimited self-renewal properties (Seaberg and van der Kooy, 2002). So far, no exclusive ‘neural stem cell marker’ exists, making it difficult to exactly identify the stem cell pool. Nevertheless, it has been shown that putative NSCs in the subgranular zone have properties of astrocytes and show a morphological resemblance to radial glia cells (Seri et al., 2001). NSCs generate so-called transiently amplifying progenitor cells by asymmetrical division. These transiently amplifying progenitor cells are not considered genuine stem cells, but can undergo symmetrical cell division, expanding the progenitor pool, or can undergo asymmetrical division, generating cells of the neural lineage that differentiate into neurons. During the latter process, cells leave the precursor cell stage and enter a post-mitotic phase during which differentiation and maturation takes place (Kempermann et al., 2004).

The different developmental stages from stem cell to mature neuron can be identified by combining information regarding the proliferative ability (Kempermann et al., 2004), protein expression pattern, differentiation status (Seki, 2002; Brandt et al., 2003; Kronenberg et al., 2003; Mignone et al., 2004; Seri et al., 2004; Steiner et al., 2004), and specific morphological (Filippov et al., 2003; Plumpe et al., 2006) and electrophysiological properties (Fukuda et al., 2003; Ambrogini et al., 2004b). Different ways of categorization have been proposed (Seri et al., 2001), but here I will describe the nomenclature according to Kempermann et al, 2004. The NSCs of the subgranular zone which show morphological characteristics of radial glia are named type-1 cells and possess a long process protruding the GCL (Seri et al., 2001) (Seri et al, 2001; Filippov et al, 2003). These astrocyte-like cells express the intermediate filaments GFAP (glial fibrillary acidic protein, a marker for astrocytes) and nestin (used as a marker for precursor cell activity). The transiently amplifying progenitor cells are categorized as type 2 and type 3 cells. Type 2 cells express nestin, have short processes that run parallel to the granule cell layer and are highly proliferative. Some of these cells express the microtubule-associated protein doublecortin (DCX, see box 2), a marker for immature neurons (Francis et al., 1999) defining them as type 2b (leaving cells without DCX with the label type 2a). Type 3 cells are DCX positive but do no longer express nestin. Although some of the type 3 cells can still divide, most of these young neurons undergo morphological maturation, radial migration and extend dendrites into the granule cell layer and subsequent molecular layer. For more details on the morphological maturation, see box 2.

**BOX 2: Visualizing neurogenesis**

Newborn neurons can be identified immunohistochemically in brain sections, based on the expression of certain proteins and/or the use of birth-date markers like BrdU. Neurogenesis is a multi-stage process, and there are
unique proteins expressed by the cell during each stage. Here, I will describe three commonly used ways to identify neurogenesis ranging from cell proliferation, cell survival to neuronal maturation.

**Cell proliferation** A common way to identify proliferating cells is by immunohistochemical staining for the cell cycle marker Ki-67. The protein Ki-67 is expressed during all active phases of cell division (G1, S-phase, G2 and mitosis) but is absent in resting cells (G0). Its exact function in cell cycle related activities is unknown (Scholzen and Gerdes, 2000). Since the cell cycle of adult hippocampal neurogenesis lasts around 24 hours, quantification of Ki-67 positive cells reflects the number of proliferating cells as present during the last day.

**Newborn cell survival** As mentioned in the text, the use of 5-bromo-3′-deoxyuridine (BrdU) greatly facilitated research in adult neurogenesis. This synthetic thymidine analog incorporates into the DNA of dividing cells by binding to adenosine in double-stranded DNA. If labelled cells divide again, both daughter cells will also be labelled with BrdU, although the chance of dilution increases after multiple divisions. BrdU can be detected in the nuclei of cells using immunohistochemistry and provides information about the proliferation or survival rate of cells, depending on the time between injection and sacrifice of the animal. BrdU is thought to incorporate into DNA within 2 hours and can be detected for years. This provides a useful tool for timing studies and at the moment also other halogenated thymidine analogs exist (like IdU and CtdU), offering possibilities of studying survival of multiple cell population born at different time points in greater detail (Vega and Peterson, 2005).

**Neuronal maturation/ neurogenesis** The protein doublecortin or DCX is a microtubule associated protein (MAP), which is a family of proteins involved in the stabilization of microtubules and critical to the migration and maturation of developing neurons (Francis et al., 1999; Meyer et al., 2002) and is frequently used in adult neurogenesis research (Rao and Shetty, 2004). In humans, a mutation in the X-linked DCX gene, results in the neuronal migration disorder called “doublecortex syndrome” in women, or lissencephaly in men (des Portes et al., 1998). DCX expression is thought to be restricted to cells of neuronal lineage (Meyer et al., 2002) and is expressed (in rats) typically in cells up to 14 days old, but it can still be present in 3 week old cells as well (Brown et al., 2003). Although a subpopulation of DCX-positive cells is still dividing (Brown et al., 2003; Kronenberg et al., 2003), the majority of DCX-positive cells are in the phase of neuronal maturation. DCX facilitates neurite outgrowth and cell migration by stabilizing microtubuli and it is expressed in the cytoplasm, neurites and growth cones of young neurons (Froicourt et al., 2003; Schaar et al., 2004). It is possible to distinguish different phases of maturation through morphological characterization of DCX-positive cells as was categorized by Plompe et al, 2006, figure 4, and applied in chapters 3 and 4.
3.3. Numbers and significance
In the young adult rat, around 9000 new neurons are generated per day (Cameron and McKay, 2001), reflecting approximately 1% of the total granule cell population. The number of BrdU-positive cells after a single injection is doubled in the first 24 hours, reaching a maximum number after 3 days in mice (Kronenberg et al., 2003) or 7-10 days in rats (Hastings and Gould, 1999; Palmer et al., 2000). Double-labelling with Ki-67 indicated that cells exit the cell cycle after 4 days and during the next three weeks around 50% of these newborn cells die (Dayer et al., 2003), most likely due to apoptosis (Biebl et al., 2000). After this period, granule cells that survive remain stable in numbers for a long time (Dayer et al., 2003; Kempermann et al., 2003). Newborn neurons are targeted by axons originating in the entorhinal cortex (Toni et al., 2007), and in addition develop functional synapses on their target cells in the CA3 region (Hastings and Gould, 1999; Toni et al., 2008).

Although the rate of adult neurogenesis rapidly declines during aging in rodents (Kuhn et al., 1996; Heine et al., 2004a), low levels persist in very old animals (Kuhn et al., 1996; Kempermann et al., 1998) which results in a low but steady addition of new neurons to the granule cell layer. Adult born neurons most likely replace part of the granule cell population that was formed early in ontogeny (Dayer et al., 2003) initially resulting in a net growth of the GCL in

![Figure 4. Morphological categorization of doublecortin positive cells in subtypes A through F. On the left side, a description of the morphological characteristics of DCX-positive cells, ranging from small soma and without neurites (A) to a more mature neuronal phenotype with one large primary dendrite that extends into the molecular layer (F). On the right side, an representative example of doublecortin-stained brain material from 7 weeks old animals. Here, several of these morphological cell types can be distinguished. (Adapted from Plompe et al., 2006).](image)
rodents, most likely during the first year of life (Bayer et al., 1982; Boss et al., 1985). Later in life, a constant cell number in the GCL has been observed, indicating that there is a balance between the levels of neurogenesis and apoptosis (Boss et al., 1985; Rapp and Gallagher, 1996; Merrill et al., 2003).

Not only is neurogenesis one of the most remarkable forms of structural plasticity found in the adult brain, it alters the dentate gyrus throughout life and is highly influenced by environmental factors (see 3.4). What exactly makes the dentate such a unique, permissive environment for neurogenesis, facilitating the development from stem cell to neuron, is as yet unknown, but would be very useful regarding its potential for a.o. regenerative medicine. Why neurogenesis exists and what its exact function is, is also still up for debate, but it appears to be conserved among mammals (Gueneau et al., 1982; Gould et al., 1998) including humans (Eriksson et al., 1998). Although the number of adult born neurons may seem low, due to the filter function of the dentate gyrus and its non-synchronous sparse firing patterns, the addition of newborn, highly excitable (Schmidt-Hieber et al., 2004) neurons to an existing network can have a relative large impact on hippocampal information processing (Kempermann, 2006, Chapter 6) and may therefore make a unique contribution to hippocampal function.

3.4. A role for neurogenesis in learning?
Given the well established role of the hippocampus in learning and memory (section 2.2), the idea that neurogenesis may be involved in this function has attracted considerable attention. Several studies observed parallels that support this notion. First, neurogenesis is enhanced after learning. Second, learning is enhanced after increased levels of neurogenesis and third, reduced levels of neurogenesis impair learning. Here, some of these findings will be summarized, although a more detailed discussion can be found in the addendum.

The observation that learning increases neurogenesis in the hippocampus, was initially made by Elizabeth Gould and colleagues in 1999, who found that both Morris water maze learning and trace eye-blink conditioning increased the survival of one week old cells in the adult hippocampus of rats (Gould et al., 1999). A large number of studies have followed up on this finding and report ambiguous results in other learning tasks (Olariu et al., 2005; Van der Borght et al., 2005a; Moshapel et al., 2006). Moreover, even studies using spatial water maze learning do not all find effects of learning on cell survival (van Praag et al., 1999a; Van der Borght et al., 2005b) and in some studies a decrease was reported (Ambrogini et al., 2004a). Detailed studies from the group of Nora Abrous indicate that the phase of learning may have a different impact on cell survival, explaining some of the discrepancies found (Dobrossy et al., 2003). In addition to this, especially regarding the use of the Morris water maze, other components like stress and exercise (both factors that were shown to respectively increase and decrease rates of neurogenesis, section 3.5) can play a role in the contradictory outcomes.

Chapter 1. General Introduction
The second and third observations regarding the potential role of neurogenesis in learning essentially state that the level of neurogenesis can predict learning performance and studies correlating the levels of neurogenesis with learning performance, show that more neurogenesis is associated with better learning (Kempermann and Gage, 2002; Drapacu et al., 2003). Also, higher levels of neurogenesis induced by environmental enrichment (Nilsson et al., 1999; Bruel-Jungerman et al., 2005) and exercise (van Praag et al., 1999b; Stranahan et al., 2006; Van der Borght et al., 2007) result in an increased learning performance. However, other changes in (structural) plasticity can be expected after these environmental manipulations such as an increased angiogenesis (Van der Borght et al., 2009) that can promote hippocampal function. Indeed, some studies indicate that the behavioural changes after enrichment may be neurogenesis independent (Meshi et al., 2006).

More direct approaches focusing on the ablation or reduction of neurogenesis have been performed by treating animals with cytostatic agents like methylazoxymethanol (MAM), (Shors et al., 2002), focal irradiation (Madzen et al., 2003; Rola et al., 2004; Snyder et al., 2005; Saxe et al., 2006; Saxe et al., 2007; Clelland et al., 2009) or genetic manipulation (Saxe et al., 2007; Scobie et al., 2009). Overall, these studies support a role for adult neurogenesis in some but not all forms of hippocampus dependent learning.

3.5. Other regulators of neurogenesis
Several elements of the environment of an organism can modulate neurogenesis. This has been demonstrated for example by studies employing an enriched housing environment in rodents (Kempermann et al., 1997) resulting in an increase in cell survival. In addition to this, physical exercise in a running wheel was shown to induce a major increase in the proliferation of cells (van Praag et al., 1999a). Next to enrichment and exercise, a large number of factors have been identified to affect a particular phase of neurogenesis, ranging from internal factors like aging (Kuhn et al., 1996; Heine et al., 2004a), hormones (Cameron and Gould, 1994; Tanapat et al., 1999; Galea, 2008; Garza et al., 2008), seizures (Bengzon et al., 1997; Parent et al., 1997) and ischemia (Liu et al., 1998) to external factors like alcohol intake (Nixon and Crews, 2002) toxic substances (Jooen et al., 2009), and antidepressants (section 2.3). Although the underlying regulatory mechanisms have not all been elucidated yet, it is likely that at least some of the factors regulating neurogenesis converge onto common pathways that influence the microenvironment of the newborn cells.

An important internal regulating system is the hormonal status of the animal. For example, the sex hormones testosterone and estrogen can differentially regulate the levels of newborn neurons (Galea, 2008), although the net effects of these hormones on neurogenesis does not cause gross differences between males and females (Tanapat et al., 1999; Tanapat et al., 2005). In female rodents, neurogenesis fluctuates throughout the oestrous cycle, peaking in pro-oestrous, when estrogen levels are high (Tanapat et al., 1999) suggesting a stimulating effect of this hormone on neurogenesis. However, exogenous
administration of estrogen has differential effects on cell proliferation by subsequently increasing and suppressing it (Ormerod and Galea, 2001; Ormerod et al., 2003). Testosterone has been reported to stimulate neurogenesis, specifically at the level of cell survival (Spritzer and Galea, 2007).

The stress hormone corticosterone is considered the main mediator of the inhibitory effects of stress on adult hippocampal neurogenesis. In 1994 Cameron and Gould observed a decreased number of new neurons after corticosterone administration but an increase after adrenalectomy. Other studies found that adrenalectomy prevented age-induced (Cameron and McKay, 1999) and stress induced (Tanapat et al., 2001) decreases in neurogenesis and promotes neuronal differentiation and survival (Wong and Herbert, 2004).

It is still unknown whether stress and glucocorticoids exert their effect on neurogenesis in a direct or indirect manner (or both) and although the MR and GR are co-localized in adult granule cells, during the process of adult neurogenesis MR and GR expression is variable (Garcia et al., 2004). The MR is not expressed in proliferating precursor cells, but only in mature neurons. MR activation is necessary, though, for cell survival and conditional MR knockout mice show less neurogenesis and a lower total granule cell number (Gass et al., 2000). The GR is found in 30-50% of the progenitor cell population i.e. type 1 and 2a cells and indeed, in vitro GR activation can suppress cell division in hippocampal progenitor cells (Yu et al., 2004). GR expression in differentiating cells is transient since it is not expressed in type 2b cells (Garcia et al., 2004). Four weeks after cell division 90% of the adult born neurons express both MR and GR, so that receptor expression may coincide with the final stage of maturation (Garcia et al, 2004). The effects of corticosteroids on adult hippocampal neurogenesis are complex and, for example, may depend on NMDA-receptor activity (Gould et al., 1997). Also, serum corticosterone concentrations do not always predict changes in neurogenesis as shown by some stress studies (Thomas et al., 2006; Day et al., 2009). This also applies for e.g. physical exercise, that leads to an increase in corticosterone levels and neurogenesis. The exact impact of stress on newborn neurons therefore depends on the type of stressor and the exact phase of neurogenesis, and its (long term) consequences are still unknown.
4. Modelling stress-related pathology in animals

In vulnerable individuals, stress can increase the chances to develop depression, possibly through a combination of a progressive increase in allostatic load (Sapolsky et al., 1986; McEwen and Wingfield, 2003), changes in MR/GR balance (De Kloet et al., 1998) and an altered structural make-up of a.o. the hippocampus (Lupien et al., 2009). Ultimately, this prolonged state of stress may turn into a state of pathology and precipitate depression. In order to study the underlying mechanisms of this transition from stress to stress pathology, several animal models have been developed that generally use high-impact stressors like stress during development, or long term unpredictable stress during adult life.

4.1. Early life stress

Animal models of stress during development usually involve prenatal or early postnatal stress. Prenatal stress is often applied by restraining the pregnant dam. It was found that elevated levels of corticosterone reach the offspring and can program several aspects of the adult HPA-axis and neural development (Weinstock, 2001; Maccari and Morley-Fletcher, 2007). The effects of prenatal stress can be profound, but in this thesis I will focus on stress during the postnatal period.

In rodents, exposure to postnatal stress is usually implemented by disturbing the relationship between the mother (dam) and infant during the first two weeks of life. Rats and mice are relatively immature at the time of birth and the survival of pups is completely dependent on the dam who provides food, warmth and tactile stimulation (which facilitates defecation). During the first two weeks, the stress system is still in development and mild to moderate stressors do not evoke a corticosterone response in pups. This period is called the stress hyporesponsive period (SHRP) and lasts between postnatal day 1-12 (mice) or 3 - 14 (rats), and is ensured by the presence of the dam (Sapolsky and Meaney, 1986; Levine, 1994; Schmidt et al., 2003). The SHRP is thought to exert a protective function to prevent high glucocorticoid levels during early postnatal development to reach the brain. It is believed to result from a decreased sensitivity of the pituitary to CRH and of the adrenal cortex to ACTH (Stanton et al., 1988; Rosenfeld et al., 1991; Dent et al., 2000).

4.1.1. Role of maternal care

An important initial observation regarding the complex role of the early environment on the development of adult stress responsiveness was made by Seymour Levine in the 1950’s who studied effects of shock exposure in rat pups. For his experiment two control groups were used; briefly handled pups and completely undisturbed litters (Levine et al., 1956). Surprisingly, the non-disturbed animals showed a hyper-emotional response as compared to the handled group. Later, it was found that early handling results in lower stress-induced corticosterone levels due to an enhanced negative feedback (Meaney et al., 1993; Plotsky and Meaney, 1993). This beneficial effect of early handling is
attributed to an increased level of maternal care in response to nest disturbance (Francis and Meaney, 1999). Subsequent studies by the lab of Michael Meaney revealed that natural variation in the amount of licking and grooming (LG) and active “arch-back” nursing (ABN) tightly regulate future aspects of HPA-axis development and behaviour. Animals from low-LG/ABN mothers display a more anxious behavioural phenotype and have an impaired negative feedback compared to the high-LG/ABN offspring, which is likely related to a decreased expression of hippocampal GR (Liu et al., 1997) and MR (Champagne et al., 2008). It was found that the long term downregulation of GR in low LG/ABN offspring is caused by lasting changes in the epigenome (Weaver et al., 2004). Low LG/ABN offspring show higher levels of DNA methylation at the promoter region of the GR gene, which lowers transcription activity, explaining the lower GR expression levels compared to high LG/ABN animals. This demonstrates that persistent changes due to epigenetic programming are under the regulatory control of maternal care, emphasizing the programming potential of the early life environment. Studies comparing high LG/ABN offspring with low LG/ABN offspring have revealed other remarkable findings. In terms of structural changes, a reduction in the level of adult hippocampal neurogenesis was found (Bredy et al., 2003) a reduced complexity of individual hippocampus neurons (Champagne et al., 2008; Bagot et al., 2009), although an increase in the complexity of neocortical neurons was reported (Smit-Rigter et al., 2009). At the cognitive level, low-LG/ABN offspring are a.o impaired in hippocampus dependent spatial learning (Liu et al., 2000), but show an enhanced memory formation of a fearful context, pointing to a possible adaptive programming of early life adversity (Champagne et al., 2008; Champagne et al., 2009).

4.1.2. Separating mother and offspring
In rodents, brief separations of mother and offspring during the first two weeks of life, as described above, are referred to as early handling and are not regarded as stressful. However, when separation lasts for more than three hours, a rise in both basal and stress induced ACTH and corticosterone levels can be observed, thereby disturbing the SHRP (Stanton et al., 1988; Kulin et al., 1990; Rosenfeld et al., 1992; Schmidt et al., 2004). The physiological response to lasting maternal absence is caused by a number of factors. As mentioned before, the presence of the dam is essential for the pups’ survival at the level of temperature regulation, nutrition and tactile stimulation, that facilitates defecation. All these factors were shown to play a role in the maintenance of homeostasis; first of all, it should be mentioned that rodent pups cannot yet maintain their body temperature, and long term separation studies are therefore performed in a heated cage. Second, since starvation is known to cause a stress response in adult animals (Dallman et al., 1999) it is likely to contribute to the activation of the HPA-axis during separation. Indeed, blocking the metabolic changes in separated rodent pups, by applying a high dose of glucose a.o., was shown to postpone HPA-axis activation (Schmidt et al., 2006). Third, the lack of anogenital stimulation by the mother prevents normal defecation. Indeed,
simulating licking and grooming during separation by stroking pups with a brush, was shown to prevent the rise in ACTH and normalized CRH and MR mRNA levels in the PVN (Suchecki et al., 1993). Combined stroking and feeding completely prevented HPA-axis activation and the subsequent rise in corticosterone (van Oers et al., 1998; van Oers et al., 1999).

Various separation protocols are used and a clear distinction can be made between repeated maternal separation (MS) of 3 hours or more on a daily basis, versus single but prolonged separation (referred to as maternal deprivation: MD) which usually lasts for 24 hours. Besides this, there is considerable variation in the timing of MS or MD, i.e. at which postnatal day(s) the separation is employed. Also, the exact separation protocol varies between studies; litters can be separated as a whole, or split in half, where the siblings that remain in the nest serve as control. In some cases, pups are isolated from each other as well. The final important consideration is the control group. There is a general consensus that a completely undisturbed early environment is not beneficial and often a form of ‘animal facility rearing’ (AFR) is used, which indicates that the nest is disturbed for cage cleaning. The disadvantage of this control group is that it is likely to differ between labs and experiments and even this minor variation may induce inconsistencies between studies (Pryce and Feldon, 2003).

4.1.3. Effects of separation

Separation protocols have been shown to slow normal development as measured by neurological reflexes and somatic development like eye opening or locomotor activity (Ellenbroek et al., 2005; Mesquita et al., 2007; Farkas et al., 2009). In addition, elevated corticosterone levels due to MS/MD coincide with the postnatal development of some of the major anatomical components of the HPA-axis, such as the dentate gyrus of the hippocampus (Altman and Bayer, 1990b, 1990a). Indeed, it was shown that separation can induce an immediate increase in apoptosis in the CA1, CA3 and dentate gyrus (Zhang et al., 2002; Llorente et al., 2009) as well as an increase in the number of astrocytes (Llorente et al., 2008).

Reports on the long-term effects of separation on HPA axis activity, behaviour and neural correlates later in life are not always consistent, which is probably due to the variation in protocols (for a summary of relevant data, see table 1). In short, long-term effects of separation on the stress system usually point to a hyperactive HPA axis, as revealed by elevated basal corticosterone levels in some studies (Penke et al., 2001; Workel et al., 2001), and/ or an increased stress responsivity in others (Plotsky and Meaney, 1993; Biagini et al., 1998; Kulminichev et al., 2002; Lehmann et al., 2002; Aisa et al., 2007, 2008). However, these effects are dependent on the age of the animal and also a decreased stress responsivity has been reported (Workel et al., 2001). Behaviourally, animals often show higher levels of anxiety and are impaired in hippocampus dependent learning tasks like the Morris water maze (Oitzl et al., 2000; Uysal et al., 2005; Choy et al., 2008). At a neuro-anatomical level a
reduction in mossy fibre density after MS was found (Huot et al., 2002) and in another study, hippocampal cell number after 24th MD (PND 9) was reported (Fabricius et al., 2008). It should be mentioned that the latter study pooled male and female offspring in an odd ratio that may be a confounding factor. At the level of structural plasticity, neural cell adhesion molecule (NCAM) expression (Aisa et al., 2009) and synaptic development (Andersen and Teicher, 2004) were reduced, in addition to a downregulation of brain-derived neurotrophic factor (BDNF), (Roceri et al., 2002; Choy et al., 2008; Aisa et al., 2009; Kikusui et al., 2009) and NMDAR subunit levels (Roceri et al., 2002).

The effects of MS or MD on adult neurogenesis were mostly reported in male offspring and are primarily found at the level of cell proliferation, resulting in a lower level of cell genesis in the dentate gyrus (Mirescu et al, 2004; Aisa et al, 2009) but not cell survival (Mirescu et al, 2004; Greisen et al 2005). One study used also female offspring and reported an increased survival independent of sex (Petersen et al, 2008). In mice, no effects of MS on proliferation in adulthood were found (Navailles et al., 2008), although early weaning did reduce cell survival (Kikusui et al, 2009).

### Questions:
- What are the short term effects of stress during development on different phases of hippocampal neurogenesis post-weaning? (chapter 2)
- Are the effects of early life stress on neurogenesis, structure and function different in males and females? (chapter 2, 3 and 4)
- What are the long-term structural and functional consequences of exposure to early life stress? (chapter 3 and 4)

### 4.2. Chronic stress

Although acute, short-term stress during adult life normally does not threaten health, chronic or repeated exposure to uncontrollable stressful events can have a strong impact on bodily allostasis (McEwen and Wingfield, 2003). In rodents, chronic stress can be modelled in several ways. Some studies use chronic restraint stress i.e. immobilizing the animal for a certain period of time (a.o. Magarinos and McEwen, 1995), exposure to repeated footshock stress (a.o. Dagyte et al, 2009) or to chronic unpredictable stress where multiple physical and psychosocial stressors are alternated (Herman et al., 1995). Although animals can habituate to certain paradigms (Dallman, 1993), chronic unpredictable and uncontrollable stress can lastingly increase baseline corticosterone levels or induce an abnormal circadian rhythm, (Herman et al, 1995) and change HPA-axis set points (Cullinan and Wolf, 2000; Joels et al., 2004; van Gemert and Joels, 2006). In terms of behaviour, increases in anxiety are commonly reported (Conrad et al., 1999), which is persistent even after recovery and most likely dependent on glucocorticoid exposure (Vyas et al., 2004). At the level of the hippocampus, chronic stress reduces dendritic length, complexity and the amount of synaptic contacts in CA3 neurons (Watanabe et
al., 1992; Magarinos and McEwen, 1995; Magarinos et al., 1996; Magarinos et al., 1997), and at the same time, this is paralleled by a hypertrophy of amygdalar neurons (Vyas et al., 2002). Dendritic atrophy in CA3 cells occurs in association with learning impairments (Luine et al., 1994; McEwen and Magarinos, 1997). Furthermore, chronic stress induces a reduction in the number of hippocampal astrocytes (Czech et al., 2006).

As described in section 3.5, stress and the stress hormone corticosterone can affect adult hippocampal neurogenesis. The effects of stress on adult neurogenesis have been shown in several species, with various stress paradigms. Both psychosocial and physical stressors can inhibit one or more phases of the neurogenic process (Gould et al., 1997; Czech et al., 2002; Pham et al., 2003; Heine et al., 2004b; Westenbroek et al., 2004; Dagle et al., 2009; Veena et al., 2009). A downregulation of proliferation was observed in some (Heine et al., 2004, Czech et al., 2001, Veena et al., 2009), but not all (Dagle et al., 2009; Snyder et al., 2009; Torner et al., 2009) chronic stress paradigms. With respect to adult born cell survival, chronic stress reduces the number of young neurons (Dagle et al., 2009, Pham et al, 2003, Torner et al, 2009).

Question: How does chronic unpredictable stress affect different phases of neurogenesis, and is it possible to normalize this process by short-term blockade of the glucocorticoid receptor? (chapter 5)
<table>
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<td>MS 3h: (PND 2–14), male rats, (Ploot and Meaney, 1993)</td>
<td>↑ CORT stress compared to early handling</td>
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<td>MS 3h: (PND 2–14), rats, Kalinichenko et al. (2002)</td>
<td>↑ ACTH and CORT in response to stress</td>
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<td>MS 3h: (PND 3–10), rats, Wigler and Neumann, 1999</td>
<td>↑ baseline CORT ↓ CORT stress</td>
<td>↑ anxiety</td>
<td>↑ depressive like behaviour</td>
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<td>MS 3h: (PND 2–21) male, female rats, (Aisa et al., 2007; Aisa et al., 2009)</td>
<td>↑ CORT stress response in females ↓ GR hippocampus</td>
<td>↑ depressive behaviour ↓ hippocampus memory</td>
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<td>MS 3h: (PND 3–12), rats, Sletten et al. (2006)</td>
<td>↑ basal CORT females, ↓ CORT stress</td>
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<td>MS 3h: (PND 2–14), female rats (Petersen et al., 2008)</td>
<td>↑ adult hippocampal neurogenesis in female offspring</td>
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<td>MS 3h: (PND 1–14), rats (Mirescu et al., 2004)</td>
<td>↓ adult neurogenesis (proliferation and 1-week cell survival)</td>
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<td>MS 3h: (PND 2–14), rats, Rocrean et al. (2004)</td>
<td>↓ BDNF levels in the PFC (stress induced)</td>
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<td>MS 3h: (PND 2–14), male rats, (Gorrie et al., 2005)</td>
<td>↑ BDNF levels ↑ adult hippocampal neurogenesis</td>
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<td>MS 4h: (PND 2–20), male and female rats, (Andersen and Tricher, 2004)</td>
<td>↓ synaptic development in the hippocampus, but not prefrontal cortex, amygdala</td>
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<td>MS 5: (PND 2–4) male rats (Rajput et al. 2006)</td>
<td>↑ CORT in response to stress</td>
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<td>MS 6h: (PND 2–12) male and female rats (Spivey et al., 2009)</td>
<td>↓ thickness of the prefrontal cortex exp. males</td>
<td>↑ impulsivity exp. males</td>
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<td>MS 12h: (PND 9 and 11) rats, (Garner et al., 2007)</td>
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<td>↓ water maze performance</td>
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<td>MD 24h: (PND 5), male and female rats (Nautanto et al., 1996)</td>
<td>↓ GK and MK in males, not females</td>
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<tr>
<td>MD 24h: (PND 4, 9 or 18) male, female rats (Lehmann et al., 1999, 2002)</td>
<td>↑ baseline CORT ↑ CORT in response to stress</td>
<td>↑ water maze acquisition and active avoidance ↓ anxiety</td>
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<td>MD 24h: (PND 5) male rats, (Wenk et al., 2001; Oztal et al., 2010)</td>
<td>↑ basal CORT adult ↓ basal CORT, impaired feedback adults ↑ basal CORT old age</td>
<td>↓ water maze acquisition young, adult ↔ water maze in old age: but E-distribution</td>
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<td>MD 24h: (PND 9), rats, Rocrean et al. (2002)</td>
<td>↓ BDNF, NMDAR subunits</td>
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<td>MD 24h: (PND 9), Chen et al. (2008)</td>
<td>↓ BDNF</td>
<td>↓ water maze performance</td>
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<td>MD 24h: (PND 9), Fabiuciu et al. (2008); male and female mice</td>
<td>↓ hippocampal neurons</td>
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<td>Weaning from PND 14, male &amp; female mice (Kasai et al., 2009)</td>
<td>↓ BDNF and neurogenesis in males</td>
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5. Outline of this thesis

The overall aim of this thesis is to evaluate the effects of stressful life events on the structural and functional development and plasticity of the hippocampal dentate gyrus in the rat. Specifically, we studied how different phases of neurogenesis are affected by early life stress and what the structural and functional consequences are in adulthood. Since women are more vulnerable for the development of depression, and early life stress is a prerequisite for the volume reduction in depressed women, we questioned whether sex differences are present in the extent to which the hippocampus is affected structurally and functionally. In addition to this, we used a model of chronic, unpredictable stress in adult animals and studied whether effects of chronic stress on neurogenesis could be normalized by blocking glucocorticoid action.

• In chapter 2, short-term effects of 24h maternal deprivation (PND 3) on dentate gyrus structure are studied. In this model, maternal deprivation coincides with postnatal dentate gyrus development. We assessed how cell proliferation, survival, neuronal maturation, gliogenesis and glia cell numbers are affected in 3 week old animals, with a specific focus on the possible differences between male and female offspring.

• In chapter 3 and 4, the effects of 24h MD (PND 3) are studied in adult females and males respectively. At a structural level, we evaluated the effects of MD on different phases of neurogenesis as well as total granule cell number, granule cell morphology and DG volume. In addition, functional consequences at the network and behavioural level were examined. Because evidence exists that moderate early life adversity (i.e. low-LG-ABN offspring) can result in an adaptive behavioural phenotype later in life -i.e. under stressful conditions- we studied the degree of long-term potentiation both under baseline conditions and in the presence of the stress hormone corticosterone. At the behavioural level, we tested basal anxiety levels and hippocampus functioning using (1) a hippocampus-dependent spatial learning test and (2) hippocampus-dependent contextual and amygdala-dependent cued fear conditioning.

• In chapter 5, we exposed adult rats to 21-day chronic, unpredictable stress. In this experiment, animals were subjected twice daily to alternating physical and emotional stressors, a protocol that was previously shown to induce elevations in basal corticosterone levels (Herman et al, 1995) and affect adult neurogenesis (Heine et al, 2004). To examine whether these effects of chronic stress are mediated by GRs we applied the GR-antagonist mifepristone (RU38486). In humans, short term blockage of the GR using mifepristone was already shown to relieve psychotic depressed patients of their symptoms. Here, we examined (1) how chronic stress impacts different
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phases of hippocampal adult neurogenesis and (2) whether short term, high dose mifepristone treatment can normalize this.

- In chapter 6, the results are summarized and the implications are discussed.

- In the addendum we evaluated how the various components of Morris water maze learning -spatial learning, stress and physical exercise- contribute to changes in adult hippocampal neurogenesis. We critically discuss the related literature on the role of neurogenesis in hippocampal learning.
6. References


Chapter 1. General Introduction


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