Acute and long-term effects of chronic social stress on cognition: The role of synaptic cell adhesion molecules.

Dissertation

der Fakultät für Biologie

der Ludwig-Maximilians-Universität München

vorgelegt von

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geboren am 24.08.1981

in München

München, Oktober 2011

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Tag der mündlichen Prüfung: 25.04.2012

Ich habe die Erfahrung gemacht,
dass Leute ohne Laster auch sehr wenige Tugenden haben.

- Abraham Lincoln -

(1809 - 1865)

Für meine Eltern

Abstract

Cell adhesion molecules play a pivotal role in synaptic plasticity and memory as they influence development, maintenance and remodelling of synaptic contacts. Novel synaptic CAMs, including neuroplastin, nectin, SynCAM, and neurexin/neuroligin, may be promising candidates for studies on cognition as they are synapse-specific and able to form and modulate new synapses. Furthermore, it is widely accepted that chronic stress and aging impair cognitive functions. The aims of this thesis are (I) to assess the impact of aging and chronic stress on cognitive performance, (II) to study the contribution of novel synaptic CAMs in these processes and (III) the assessment of the therapeutic value of synaptic CAMs to treat cognitive diseases at aging or stress-related cognitive dysfunctions.

First, male CD1 mice were subjected to chronic social stress and the cognitive performance in young (3 months) and aged animals (15 months) was tested in a variety of cognitive paradigms. Brains were removed under basal conditions and 2 hours after learning for analysis of hippocampal CAM expression levels. In the second part, the effects of Nptn-derived mimetic peptides, Enplastin and Narpin, on cognitive performance were investigated either under basal conditions using acute intra-hippocampal injections or following stress and/or aging using chronic subcutaneous injections.

It was shown that aging impairs spatial learning and that chronic social stress has both acute and long-term adverse effects on cognitive performance, which correlate with structural and functional parameters. In addition, these cognitive differences could be correlated with altered CAM dynamics on mRNA level. The modulation of cognitive performance via Nptn-derived mimetic peptides was possible.

In conclusion, these findings confirmed that stress and aging induce cognitive deficits and it is hypothesised that alterations in CAM dynamics play an important role in the underlying processes. There might even be a causal link between stress-induced cognitive disorders or age-related cognitive deficits and CAM dynamics. Future studies will provide further insights into the molecular substrates of stress/aging-induced cognitive impairment.

Zusammenfassung

Zelladhäsionsmoleküle (CAMs) spielen eine wichtige Rolle bei synaptischer Plastizität sowie Lernen und Gedächtnis, da sie die Entwicklung, die Aufrechterhaltung und den Umbau von synaptischen Kontakten beeinflussen. Kürzlich entdeckte, sogenannte neuartige synaptische CAMs wie Nptn, Nectin, SynCAM, und Neurexin/Neuroligin, sind daher vielversprechende Kandidaten für die Kognitionsforschung. Sie sind synapsen-spezifisch, fähig neue Kontakte zu bilden und diese zu modulieren. Es ist weithin bekannt, dass chronischer Stress und Altern kognitive Fähigkeiten einschränken können. Die Ziele dieser Doktorarbeit sind (I) den Einfluss von chronischem Stress und Altern auf die kognitive Leistung zu erfassen, (II) den Beitrag von neuartigen synaptischen CAMs innerhalb dieser Prozesse zu bewerten und (III) den therapeutischen Wert dieser CAMs für die Behandlung von altersbedingten oder stress-induzierten kognitiven Störungen zu analysieren.

Dafür wurden männliche CD1 Mäuse verschiedenen Alters chronischem sozialem Stress ausgesetzt und anschließend die kognitive Leistung in einer Reihe von Verhaltenstests untersucht. Um die Expression der CAMs im Hippocampus zu messen, wurden die Hirne entweder unter basalen Bedingungen herauspräpariert oder 2 Stunden nach Lernen. Außerdem wurden die Effekte von Nptn-abgeleiteten mimetischen Peptiden, Enplastin und Narpin, auf die kognitive Leistung untersucht, entweder unter basalen Bedingungen (mit Hilfe von akuten intra-hippocampalen Injektionen) oder nach Stress und/oder im Alter (mit Hilfe von chronischen subkutanen Injektionen).

Es wurde bestätigt, dass Altern die Lernfähigkeit vermindert und dass chronischer Stress sowohl negative akute als auch negative Langzeiteffekte auf die Kognition hat. Diese konnten mit strukturellen und funktionalen Parametern in Verbindung gebracht werden. Veränderungen der Kognition gingen einher mit veränderten CAM-Expressions-Mustern (auf mRNA Ebene). Die Modulation von kognitiver Leistungsfähigkeit durch mimetische Peptide war unter bestimmten Bedingungen möglich.

Diese Ergebnisse lassen die Schlussfolgerung zu, dass die veränderte Expression von CAMs eine wichtige Rolle spielt für Prozesse des Alterns sowie bei Stress-

und Lernvorgängen. Es könnte sogar sein, dass eine kausale Beziehung zwischen CAMs und altersbedingten oder stress-induzierten kognitiven Störungen vorliegt. Weitere Studien sind nötig, um diese Ergebnisse zu spezifizieren.

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1 Introduction

It is not stress that kills us; it is our reaction to it.

Hans Selye (1907 – 1982)

1.1 Stress

Stress is an aspect of our daily lives; hence, the term stress is more and more used in the colloquial language. Stress is elicited by specific external or internal stimuli and includes physically and mentally challenging processes, which have the aim to enhance the organisms' ability to adapt to novel demands. In the biological context, the term stress was first coined by Walter Cannon (1932) with his hypothesis of the fight-or-flight reaction. He showed in an animal model that a hazardous situation induces the sympathetic nervous system (SNS) to release catecholamines, increase respiratory, cardiovascular activity and tonicity (Ulrich-Lai and Herman, 2009), while digestive and reproductive mechanisms are inhibited (Sapolsky et al., 2000). Thereby, the animal is prepared for either fighting harder or escaping faster. This reaction was viewed as a stress response and part of a homeostatic process to ensure survival (Holmes et al., 2006).

In 1936, Hans Selye, who is deemed the founder of scientific stress research, introduced his thesis of the general adaptation syndrome (Selye, 1936; Selye, 1950; Selye and Fortier, 1950) in which he described a generalised physiological stress response comprised of three phases: initially, there is the alarm reaction during which the inner homeostasis is disrupted and the SNS is activated. Second is the stage of resistance with adaptive mechanisms reaching an optimal level, but in case of persisting stress, adverse effects occur due to the high levels of catecholamine and cortisol. At last, the stage of exhaustion comes into effect. It is characterised by the loss of adaptive capacity and symptoms such as an insufficient energy mobilisation, a weakened immune defence and an impaired ability to reproduce and might even result in death (Neylan, 1998).

Selyes' theories have been refined, but the concept of a threatened homeostasis remains. As all living organisms strive towards a dynamic equilibrium, versatile mechanisms are directed towards achieving and reinstating stability (Stott, 1981; McEwen, 1998; de Kloet et al., 2005). During these processes of allostasis (Sterling and Eyer, 1988; McEwen, 2001; McEwen, 2005), it does not matter if the threat is real or imaginary (McEwen, 2000a).

Often neglected by the general public, stress can act in beneficial ways if the challenge is mild and controllable and the adaptive changes are activated for a short period of time (Luine et al., 1996). In such cases of avoidance of a chronic overstimulation, the term eustress is used (Selye, 1975b; Milsum, 1985). On the contrary, if the adaptive system fails to be shut off efficiently or if the stress response is inadequate, the organism is unable to cope with challenges in an effective manner and the inner homeostasis may remain disrupted. In this case of allostatic overload, the stressor is uncontrollable and unpredictable (Koolhaas et al., 2011) and the stressful situation can be described as experience of distress (Engelmann et al., 2004). It was hypothesised that the costs of allostasis chronically accumulate, thereby converting the organism into a state of increased vulnerability, which facilitates the development of certain pathologies (McEwen and Wingfield, 2003).

1.1.1 Chronic stress as a risk factor

Stress is known to be related to a variety of complex diseases, but the same stressor can elicit different reactions in different individuals (Selye, 1975a). After a life-threatening event for example, only 20 - 25 % of all individuals develop post-traumatic stress disorder (PTSD), whereas others recover completely without any apparent psychological long-term damages (Green et al., 1998; Breslau, 2001). These inter-individual differences in vulnerability to disease are thought to be produced by genetic predispositions and environmental factors such as priming life events, particularly in early life (de Kloet et al., 2005). However, it is important to note that stress per se is in most cases not sufficient to induce an illness, but rather increases the vulnerability to develop a certain disease (de Kloet et al., 2005; Schmidt et al., 2008).

Chronic stress at work, for instance, has become a major risk factor for metabolic syndrome, especially in western societies. Metabolic syndrome includes a cluster of symptoms (amongst others central obesity and hypertension) that in turn increase the risk for cardiovascular diseases (Strike and Steptoe, 2004; Rozanski et al., 2005; Chandola et al., 2006) and also raise cardiovascular mortality, for example in middle-aged men (Ohlin et al., 2004). Accumulating evidence supports these findings and reveals chronic stress as a key player in the onset and progression of coronary heart disease, which has become the leading cause of premature death in the western world (Brydon et al., 2006; Hassan et al., 2008; Sterlemann et al., 2010). Besides physical illnesses, a lot of psychiatric diseases are associated with elevated stress levels. For example, psychosocial distress has been linked to the risk to develop Alzheimer's disease in old age (Wilson et al., 2003; Wilson et al., 2006; Wilson et al., 2007). Excessive stress experience also imposes a risk factor for PTSD (de Kloet et al., 2005). Moreover, numerous studies have demonstrated that major depression, one of the most common mental disorders worldwide, can be facilitated by chronic stress exposure (Coplan et al., 1996; Holsboer, 2000; Kendler et al., 2000; van Praag, 2005; Weinstein et al., 2010). The concept of a causal relationship between chronic stress and affective disorders like major depression is supported by the finding of an altered or disturbed hypothalamic-pituitary-adrenal axis (HPA axis) function and elevated corticotropin-releasing hormone (CRH) levels in depressed patients (Nemeroff et al., 1984; Holsboer, 2000; de Kloet et al., 2005; Tichomirowa et al., 2005; Ising et al., 2007; Marques et al., 2009). In order to understand the mechanisms underlying affective disorders, the system regulating the responses to stress has to be considered more closely.

1.1.2 Hypothalamic-pituitary-adrenal axis regulation

Apart from the fast reaction of the SNS, which is the driving force for the fight-or-flight reaction inducing the release of catecholamines (adrenalin and noradrenalin), there is another slower and in its actions more persistent major control module, the HPA axis. Any threat to homeostasis, real like pain perception or predicted like the recognition of a predator, triggers HPA stress responses

(Herman et al., 2003; de Kloet and Derijk, 2004). HPA axis activation begins with parvocellular neurons in the paraventricular nucleus (PVN) of the hypothalamus. These cells release CRH and arginine vasopressin (AVP) from the median eminence (Frank and Landgraf, 2008). The neuropeptides, CRH and AVP, reach the anterior pituitary gland via the portal blood system, where they bind to their respective receptors. The occupation of the corticotropin-releasing hormone receptor type 1 (CRHR 1) by CRH stimulates the synthesis of pro-opiomelanocortin (POMC), which is a precursor of the adrenocorticotropic hormone (ACTH), while AVP, which binds to the arginine vasopressin receptor 1B (V1b), works as a co-expressed peptide to amplify the ACTH response. By these means, CRH and AVP stimulate the release of ACTH in a synergistic manner (Gillies et al., 1982; DeBold et al., 1984; Herman et al., 2003). Circulating ACTH reaches the secretory cells in the *zona fasciculata* of the adrenal cortex and binds to specific receptors leading to on-site secretion of glucocorticoids (GCs) into the blood stream. As the main hormonal products of the HPA axis, GCs (cortisol in humans and corticosterone in rodents) act at multiple levels and control a great variety of behavioural and physiological adaptations. In the periphery, GCs mainly contribute to the mobilisation of energy for example by increasing cardiovascular activity and glucose metabolism, while at the same time GCs are responsible for the dampening of inflammatory mechanisms as well as the suppression of reproductive and digestive functions (Munck et al., 1984; Sapolsky et al., 2000; Sorrells and Sapolsky, 2007). In the brain, they modulate fear and anxiety-related behaviour (Tronche et al., 1999) as well as learning and memory processes (Oitzl and De Kloet, 1992; Sandi and Rose, 1994; Sandi, 1998; Shors, 2001; Oitzl et al., 2001).

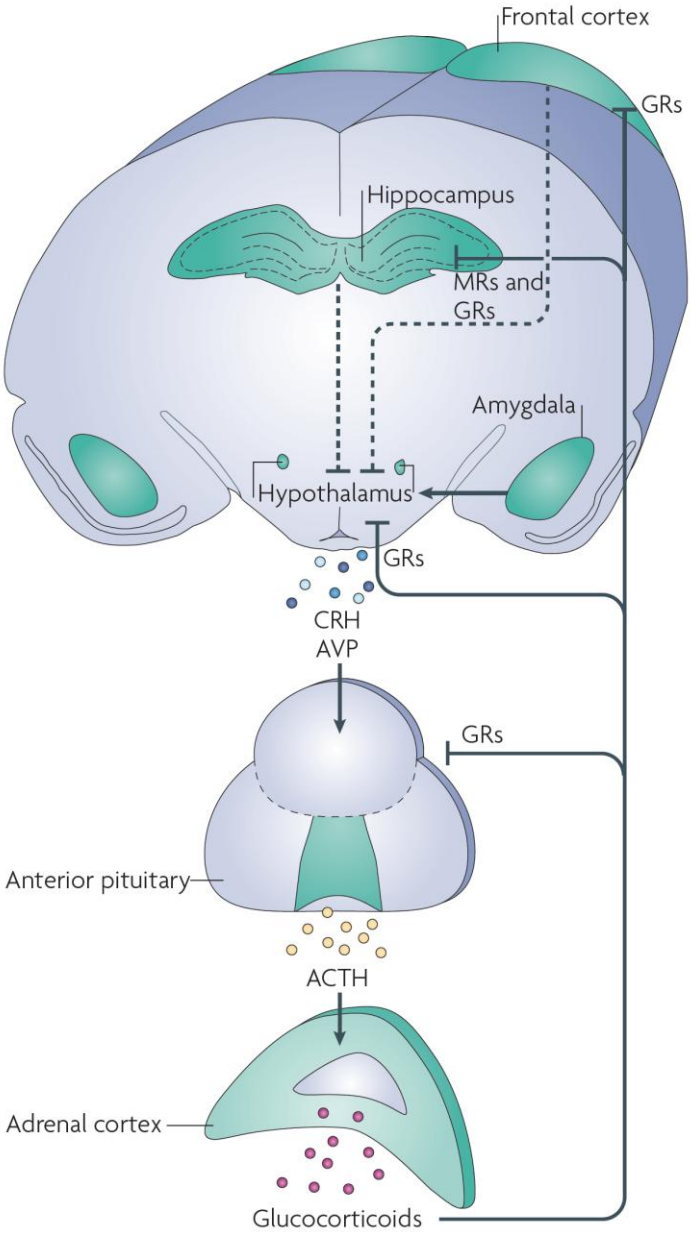

Figure 1: **Overview of the HPA axis.** The PVN of the hypothalamus integrates stress-relevant information and receives input from the amygdala (excitatory) as well as from the hippocampus (inhibitory). Activation of the HPA axis initiates the secretion of CRH and AVP into the hypophysial portal system. At the anterior pituitary, CRH and AVP promote ACTH secretion into the blood stream. ACTH in turn binds to its specific receptors in the adrenal cortex, thereby stimulating the release of GCs from the *zona fasciculata*. GCs regulate the release of CRH, AVP and ACTH via binding to 2 types of glucocorticoid receptors, the GR and the MR, and thereby inhibit their own synthesis; adapted from Lupien (2009).

The nature of a stressor, which activates the HPA axis, can vary in numerous ways. Tangible stressors are recognised by somatic, visceral or circumventricular sensory pathways (for example a bodily injury) and trigger a reactive response. Mentally evoked stressors are centrally generated (for instance a social challenge) and lead to an anticipatory response (Herman et al., 2003; Dedovic et al., 2009). It is important to note that there are also appetitive stimuli, for example sexual behaviour or victory during a social defeat, which activate the HPA axis in the same manner like aversive ones (Koolhaas et al., 2011). However, whether a certain situation is actually perceived as stressful by an individual depends on

several factors, such as the speed of recovery of the HPA axis, the controllability and predictability of the stimulus. Psychological stressors result in an appraisal of the threat, for example when recognising a predator. They are mainly channelled through limbic structures such as the amygdala, whereas physiological stressors (for example hypoxia) pose an immediate threat to the body and are transmitted to the PVN via the brainstem nuclei (Herman and Cullinan, 1997).

Usually after an adequate stress response, the HPA axis activation is efficiently shut off. A prolonged state of hyperactivation and chronically elevated GC levels lead to somatic dysregulation and stress-related affective diseases. As a means of restoring the HPA axis to its normal function and evading a pathological overshooting, GCs terminate the stress response as part of a direct negative feedback loop at different levels of the HPA axis.

1.1.3 Negative feedback mechanisms

To prevent a dysregulation of the HPA axis elicited by persistent stress, a negative feedback loop is activated via glucocorticoid (GR) and mineralocorticoid receptors (MR) (De Kloet et al., 1998; Pariante et al., 2004). Receptor occupation with GCs normalises the activity of the stress system on several levels: the synthesis of CRH in the hypothalamus, the ACTH synthesis in the anterior pituitary and the synthesis of GCs in the adrenals are inhibited. MRs exhibit a particularly dense expression in limbic regions such as the hippocampus, the medial amygdala and the septum, while GRs have been found ubiquitously in the brain (Joels et al., 2006), but are most abundant in the PVN and the hippocampus. MRs display a tenfold higher affinity for corticosterone than GRs and are thus continuously occupied by GCs. Due to this receptor diversity, de Kloet and colleagues (1987) formed the hypothesis that “tonic influences of corticosterone are exerted via hippocampal MRs, while the additional occupancy of GRs with higher levels of corticosterone mediates feedback actions aimed to restore disturbances in homeostasis” (Reul et al., 1990). This means, that in contrast to MRs, which are involved in the basal regulation of the HPA axis, GRs are only largely saturated at high levels of circulating GCs, which is the case during stress responses or during the circadian corticosterone peak (Joels et al., 2006). High GC levels in the

morning and low levels in the evening are typical for humans and other diurnal mammals; for rodents, which are nocturnal mammals, it is vice versa (Gass et al., 2001).

Many studies suggest a crucial role of the hippocampus in the feedback inhibition of the HPA axis (Jacobson and Sapolsky, 1991; van Haarst et al., 1997; Furay et al., 2008). Nestler and colleagues (2002) have shown that hippocampal stimulation leads to a reduction in GC secretion, whereas hippocampal lesions increase the GC release (Dunn and Orr, 1984; Herman et al., 2005). Moreover, the hippocampus promotes the feedback inhibition of CRH in the PVN via indirect GABAergic innervation (Herman and Cullinan, 1997).

MRs are responsible for behavioural reactivity in novel situations (Oitzl et al., 1994) and maintenance of neural circuits related to stress (de Kloet et al., 2005), while GRs facilitate recovery and storage of information in preparation for future challenges (de Kloet, 2003). The relationship between GCs and cognition will be further elucidated in the next chapter.

1.1.4 Chronic stress, cognition and aging

In the literature, the effects of stress on cognition are described differently and sometimes even confusingly: on the one hand, it is generally known that stress can be linked to cognitive impairment. On the other hand, stressful life events are very well remembered up to the point that people are unable to forget their adverse experience for example in PTSD. “The direction of changes in memory performance –improvement or impairment – depends on whether the stress is experienced closely linked in time to and within the context of the information to be learned” (Joels et al., 2006). In contrast to acute stress, which can be memory facilitating (Dalm et al., 2009; Sandi, 2011), chronic stress promotes disease, causes neuronal degeneration (Nestler et al., 2002; Conrad, 2008) and attenuates learning and memory (Sandi and Pinelo-Nava, 2007). Learning describes the process of acquiring new information, while memory refers to the ability of retrieving information, which was acquired in the past and was then stored for the future. A variety of studies in healthy human subjects have demonstrated that stress hormones have direct but reversible effects on memory and cognition

(Bremner, 1999; Heffelfinger and Newcomer, 2001): administration of GCs (Keenan et al., 1995; Kirschbaum et al., 1996) or dexamethasone (dex), which is a synthetic GC (Newcomer et al., 1994), hampers cognition concerning verbal declarative memory. Seeman and colleagues (1997) were able to verify that reduced cortisol levels result in an improved memory function, while stress-induced elevation of cortisol levels leads to memory deficits (Lupien et al., 1997). Younger subjects are less susceptible to stress exposure and its resulting detrimental effects compared to older subjects (Keenan et al., 1995). Hence, the effects of chronic stress on cognition appear not only in dependence of the duration of the exposure (acute vs. chronic), but also in dependence of the timing "with the highest impact on structures that are developing at the time of the stress exposure (stress in prenatal periods) and those undergoing age-related changes (in aged individuals)" (Lupien et al., 2009).

Current data indicate the structural basis for stress-related cognitive deficits: during prolonged and severe stress, hippocampal neurons, particularly CA3 (cornu ammonis 3) pyramidal neurons, are affected by atrophic processes such as the reduction in dendritic branching and loss of dendritic spines (McEwen, 2000a). As a consequence of these processes, even cell death may occur (Uno et al., 1989; Sapolsky et al., 1990). The hippocampus is a structure, which has an essential role in learning and memory (Zola-Morgan and Squire, 1990), hence, hippocampal atrophy and cell loss have unfavourable consequences for cognitive function, for example spatial cognition (Conrad et al., 1996; Wright and Conrad, 2005; Conrad, 2006; McLaughlin et al., 2007). Although dendritic retraction is a reversible process (Conrad et al., 1999), the susceptibility for damage is increased, which enables a normally innocuous event, for instance a metabolic challenge, to cause permanent harm in a stress-compromised hippocampus (Conrad, 2008). Hippocampal atrophy can also be found in several stress-related diseases like Cushing's syndrome (a rare disease characterised by hyper-secretion of GCs) or depression. As these diseases are associated with severe memory deficits, it was suggested that hippocampal atrophy may lead to such cognitive deficits (Sapolsky, 2000). The notion of the hippocampus as a target during stress exposure followed by cognitive impairment is further supported by the finding that hippocampus-independent memory processes seem to be more resistant to chronic stress (Ohl

and Fuchs, 1999). In addition to atrophy, chronic stress also leads to a declined capacity to generate new granule cell neurons in the adult hippocampal dentate gyrus (DG) (Fuchs and Gould, 2000). Although controversial, there is evidence that such hippocampal neurogenesis contributes to memory formation (Burke and Barnes, 2006; Tan et al., 2010; Deng et al., 2010; Nihonmatsu-Kikuchi et al., 2011). Electrophysiological studies have shown that chronic stress also reduces long-term potentiation (LTP) in the CA1, CA3 and the DG of the hippocampus (Pavlides et al., 2002; Alfarez et al., 2003; Gerges et al., 2004). LTP is reflected in a persistent increase in the firing rate of synapses after high-frequency stimulation and is an underlying mechanism for synaptic plasticity. It is often considered to be a crucial phenomenon at the cellular basis of learning and memory processes (Sandi, 2004).

Disadvantageous effects, similar to those seen after stress experience (see above), can be revealed in geriatrics. Aging is reflected by a decreased ability to adapt to stress and recover from it (Lupien et al., 1997). Furthermore, it is associated with an increase in basal corticosterone levels (Sapolsky et al., 1983; Sonntag et al., 1987) and an enhanced HPA activity accounts at least partly for differences in the occurrence of age-related hippocampal pathology and cognitive deficits (Issa et al., 1990). Moreover, it has been shown that adrenalectomy in middle-aged rats can have protective effects for cognitive function later in life (Landfield et al., 1981; Montaron et al., 2006). It is generally known that normal aging is associated with impairments on multiple levels, for instance sensory or motor impairments, leading to reduced muscle strength or increased reaction time (Kumar and Foster, 2007). More important, aging (as well as stress exposure) impairs memory function as it is accompanied by functional alterations in neurons such as a considerable reorganisation in the hippocampal circuitry resulting in an altered synaptic efficacy in the senescent brain (Rapp et al., 1999; Smith et al., 2000) and changes in activation patterns concomitant with a global loss of integrative function (Kramer et al., 2004; Bishop et al., 2010). In addition to these functional alterations, structural changes, for example the loss of neurons in the hippocampus or the prefrontal cortex (PFC) and a reduced dendritic and axonal arborisation (Shankar, 2010), take place. However, the loss of neurons with aging is now recognised to be less decisive than initially estimated. Besides apoptotic

mechanisms, the birth of new hippocampal neurons is inhibited, which might be a vital process during cognitive aging (Bizon and Gallagher, 2005) and could be regarded as an indicator of the cognitive state of aged animals (Montaron et al., 2006).

In general, these age-related modifications in brain structure and function are characterised by large individual differences. Modifications are not uniform across the whole brain (Glisky, 2007) and impact each individual differently (Buckner, 2004; Reinvang et al., 2010). The evident variability in cognitive functioning in young subjects increases in senescent humans (Laursen, 1997; Unverzagt et al., 2001) as well as in aging animals (Deupree et al., 1991; Gallagher et al., 2003) meaning that some individuals suffer from substantial cognitive decline as they age, while others exhibit only little or no impairment. Convincing data indicate that this variability might originate from challenging life events such as infection (Wofford et al., 1996) or psychological stress (VonDras et al., 2005), which increase the cognitive vulnerability in old age. This hypothesis is supported by the finding that the normal loss of hippocampal neurons during aging is even accelerated by stress experience (Landfield et al., 1981; Sapolsky, 1985; Uno et al., 1989; Bishop et al., 2010). Lupien and colleagues (1997) hypothesised that the altered responsiveness to GCs might be an essential factor explaining the genesis of memory deficits in aged populations.

In conclusion, chronic stress and aging both lead to neuroanatomical and neurochemical changes. Both have detrimental effects on cognition, but each individual is differently affected in dependence of its own vulnerability. Furthermore, chronic stress facilitates adverse effects during aging and, vice versa, aging promotes detrimental stress effects. Today, with an increased life expectancy, the percentage of senior citizens in the population is constantly growing and along with it the demand for elucidation of mechanisms behind successful aging (Buiza et al., 2008) and human health at old age (Vijg and Campisi, 2008). With almost 50 % of adults over the age of 85 troubled by Alzheimer's disease (in the United States), cognitive deterioration has become one of the most crucial health threats in old age (Bishop et al., 2010). The determination of the factors (either genetic or environmental factors or a combination of both) accounting for the enormous variability in the vulnerability to

aging, has become an increasingly pressing issue, as this may provide insights into the processes underlying age-related cognitive decline (Chapman et al., 2010). Therefore, it is useful to incorporate data from studies using animal models.

1.2 The mouse as model organism in scientific research

Although animal models do not reproduce human psychopathology in every detail, their need in scientific stress research is evident (McKinney, 1984). Due to ethical reasons, it is impossible to manipulate living conditions in human subjects, for instance to conduct studies concerning adverse life events. Here, investigators have to fall back on patients, who had to endure specific events such as child abuse, domestic violence or a natural disaster. Besides ethical issues, it is hardly possible to control genetic factors or environmental prehistory of patients, and subjects cannot be randomised to treatment groups (Shively, 1998).

Over the last decade, the mouse has emerged as an ideal model organism for various reasons and has become the premier animal to study the basis of human pathological conditions (Peters et al., 2007). Mice are small mammals, therefore easy to handle; they require little space and can be kept cost-effectively; they have a short gestation period (19 – 21 days) and produce fertile offspring at a high rate, approximately every 10 weeks. Despite the obvious differences between the human and the mouse brain, there are neuronal structures working in specific circuits that have been evolutionarily conserved, for example the limbic regulation of emotion by the hippocampus and the amygdala (Cryan and Holmes, 2005). Additionally, mice and humans share several hereditary diseases such as diabetes, cancer or heart disease. The fact that 75 % of mouse genes are in an orthologous relationship with human genes is also significant; from the estimated 20210 mouse and 19042 human protein-coding genes, 15000 are functionally related and originated from a common ancestor (Church et al., 2009). Most important, there is a wealth of information resources available due to the broad range of employments of mice in scientific research over the past 100 years. Taken as a whole, mice offer all the necessary resources for the investigation of

genetic, molecular and environmental mechanisms of human pathology, for example pathologies related to chronic stress experience.

1.2.1 Animal models for chronic stress

According to Willner (1990; Willner and Mitchell, 2002), there are three criteria that need to be addressed, when creating a suitable animal model: face validity, predictive validity and construct validity. Face validity implies a similarity of symptoms between the model and the clinical condition modelled. However, this criterion can only be fulfilled in a restricted manner as it is impossible to model complex psychological concepts, for instance suicidal tendencies of depressed patients in an animal (Cryan and Holmes, 2005). Predictive validity refers to the pharmacologic correlation, thus, to the extent to which clinically effective drugs, which influence the pathological state, have an equal impact in the model. Problems arise, if there are no effective drugs available for the disease. To achieve high construct validity, the model's and the disorder's theoretical rationales have to be investigated and the underlying neurobiological mechanisms should be homologous for the two (Fuchs, 2005). Nonetheless, the etiology for a lot of diseases is far from clear. In this case, specific risk factors that have been identified for a particular pathology should be implemented. Although it can be challenging to fulfil all 3 of these criteria, there are numerous validated animal models available, for example for chronic stress and depression. Sometimes, chronic stress models are utilised to study depression. In this case, these models often employ a form of social instability due to the fact that in humans the majority of stress-related diseases can be traced back to social stressors (Brown and Prudo, 1981) and that social factors are the most potent key stimuli to trigger disease, not only in humans, but also in social animals like mice (Bartolomucci et al., 2005). Thereby, the fundamental point is not the social status itself, but the stability of the social situation (Sachser et al., 1998).

A promising model for chronic psychosocial stress has been developed by Fuchs and colleagues (2005) and is conducted in male tree shrews. It is based on their highly territorial behaviour towards intruders and forces two males to coexist, who in turn exhibit dominant/subordinate behaviour. Other commonly used models for

chronic social stress (CSS), particularly in the mouse or rat, are chronic social defeat (CSD) paradigms and the visible burrow system (VBS). During the CSD, one animal is regularly confronted with a larger and more aggressive conspecific (Koolhaas et al., 1997). In the VBS, rats live in mixed-sex groups and display different types of behaviours (offensive or defensive), which can be associated with the development of social hierarchies (Blanchard et al., 1995). Although these animal models hold a high degree of face and predictive validity, they are labour-intensive, need a lot of space and thus restrict the amount of animals that can be studied. These limitations avert the use of studies, which need a large scale of animals, for instance drug screenings. To avoid such restrictions, Schmidt and colleagues (2007; 2008) developed a novel CSS paradigm, which considers not only the underlying biological mechanisms of stress-related human pathologies, symptoms and treatment options, but is also easy to apply and applicable for large-scale studies.

1.2.2 The chronic social stress paradigm

The CSS paradigm, which was used for this study, is based on the disruption of the social hierarchy between group-housed, adolescent, male mice (see chapter 2.2). It was developed to fulfil all three criteria of validity with a high degree (Schmidt et al., 2008) and evade the restrictions still borne by other animal models. Many pre-existing models are problematic with regard to the applied stressor (continuity and adaption) and applicability (Schmidt et al., 2007).

A variety of studies investigates effects during chronic stress exposure or directly afterwards, but studies reporting on the long-term effects of chronic stress remain rare. However, it is also important to tap these long-term stress effects, as human malignancies often develop with a latency of several years or even decades, for instance after childhood trauma. Effects occurring acutely after chronic stress exposure are likely to reflect mostly the non-pathological adjustments following stress (Schmidt et al., 2007) and can be considered rather a physiological support of behaviour than an actual stress response (Koolhaas et al., 2011). As it is known that some stimuli are perceived as stressful only in the beginning (Martinez et al., 1998) and furthermore to maximise construct validity, the employed social stress

stimulus has to be truly chronic with persisting effects, even when the stressor is discontinued. Our CSS model meets these requirements as it offers an unavoidable, stressful social situation, where the stressor is constantly present over a prolonged period of time without the animals being able to adapt to it. Applying this CSS paradigm, Schmidt and colleagues (2007) showed persistent stress effects after 1 week of recovery. These stress effects reflect alterations in HPA axis functioning, which can be found in humans after chronic stress exposure as well. Hence, face validity is given. These alterations in HPA axis function regarding adrenal sensitivity, corticosteroid receptor expression and anxiety-behaviour and could be prevented via antidepressant treatment, thereby fulfilling the predictive validity criterion. Furthermore, this model leads to neuroendocrine and behavioural alterations retrievable 12 months after the cessation of the stressor (Sterlemann et al., 2008; Sterlemann et al., 2010), thereby exhibiting actual long-term stress effects.

Another characteristic, which adds further evidence for the validity of this CSS model, is its timing. It is conducted during adolescence, which represents a time frame of high vulnerability. Adolescence in rodents is defined slightly different from author to author: according to Tirelli (2003), it encompasses the time between PD 21 and 59. Spear (2000) considered PD 28 to 46 as adolescent. As in the model CSS was applied for 7 weeks long from PD 28 to PD 77, the paradigm covered this phase and early adulthood in any case. Adolescence is a shaping and highly adaptive period with ongoing neuroendocrine and behavioural changes (Tsoory and Richter-Levin, 2006). Due to these developmental processes and the fact that the basis for social interactions is built in the adolescent phase, adolescent animals are highly vulnerable for imprinting factors, especially for social stressors.

All in all, the recently developed CSS paradigm is easy to apply, cost-effective, as there is no need for special equipment, it enables a high throughput with relatively little effort and it closely mimics the human situation of social stress with regard to the three validity criteria. Thus, it is an ideal tool to investigate complex stress-related questions, such as the involvement of novel cell adhesion molecules (CAMs) in chronic stress and aging.

1.3 Cell adhesion and cell adhesion molecules

In the last chapters, the entwined concepts of chronic stress, cognition and aging have been discussed. Another subject that is important in this context is cell adhesion (CA). The remodelling in neuronal structure, which appears during aging, learning processes and also after chronic stress exposure, needs "destabilisation of membrane proteins that are involved in the organisation and maintenance of neural circuits, the simultaneous interaction with the cytoskeleton and the translation of neurotrophic signals" (Sandi, 2004). Consequently, CA and its mediation by CAMs play an important role in chronic stress, aging and cognition.
On the one hand, CA describes a targeted cell assembly and on the other hand the establishment of boundaries between distinct cell types. CA emerges either by binding of one cell to another or to the extracellular matrix (ECM) (Papusheva and Heisenberg, 2010). CAMs, which navigate CA, are transmembrane glycoproteins that typically consist of three units: an extracellular domain, one segment passing through the membrane and an intracellular anchor region. They emerge in the remodelling of the cytoskeleton (Gumbiner, 1993) and are crucial during both early development, like embryonic organ development, and life-long maintenance of three-dimensional structures. CAMs are evident in tissues undergoing development in adult organisms (Gumbiner, 1996) and regulate normal tissue function (Aplin et al., 1998) and tissue regeneration (Chen et al., 2004; Rao and Winter, 2009). They are involved in numerous processes such as wound healing (Hynes and Lander, 1992), proliferation as well as cell survival (Hynes, 1999) and signal transduction leading to the regulation of cell growth and differentiation (Rosales et al., 1995). CAMs are not merely static structures that keep cells together by adhesion, but dynamic regulators of synaptic function able to receive and integrate signals from the extracellular environment (Dalva et al., 2007). Thereby, it is important to note that even if CA is reduced or abrogated, CAMs still retain their signalling abilities (Cavallaro and Dejana, 2011). CAMs, which are located at synaptic membranes and bridge the synaptic cleft (see *Figure 2*), are vital in synapse formation and stabilisation (Sandi, 2004). Synapses are asymmetric cell junctions with highly specialised and protein-dense membrane regions such as the active zone on the presynaptic side and the postsynaptic

density (PSD) on the opposed side. To enable neurons to communicate through synapses, specific proteins, for instance neuronal CAMs (nCAMs), are accumulated at both sides of a neuronal cell contact, thereby forming trans-synaptic protein complexes (Li and Sheng, 2003). In this study, the focus lies on these nCAMs, which are regarded as the “backbone of synapses” (Giagtzoglou et al., 2009) and build the foundation for memory and learning. They are essential during synaptogenesis, but also in mature synapses. In the developing nervous system, they mediate neurite outgrowth including growth cone motility and migration of precursor cells (Gumbiner, 1993) and control processes such as target recognition for new synapses, synapse differentiation, integrity and stability of the synapse. In the mature synapse, they are responsible for the regulation of synaptic structure, function and plasticity (Dalva et al., 2007).

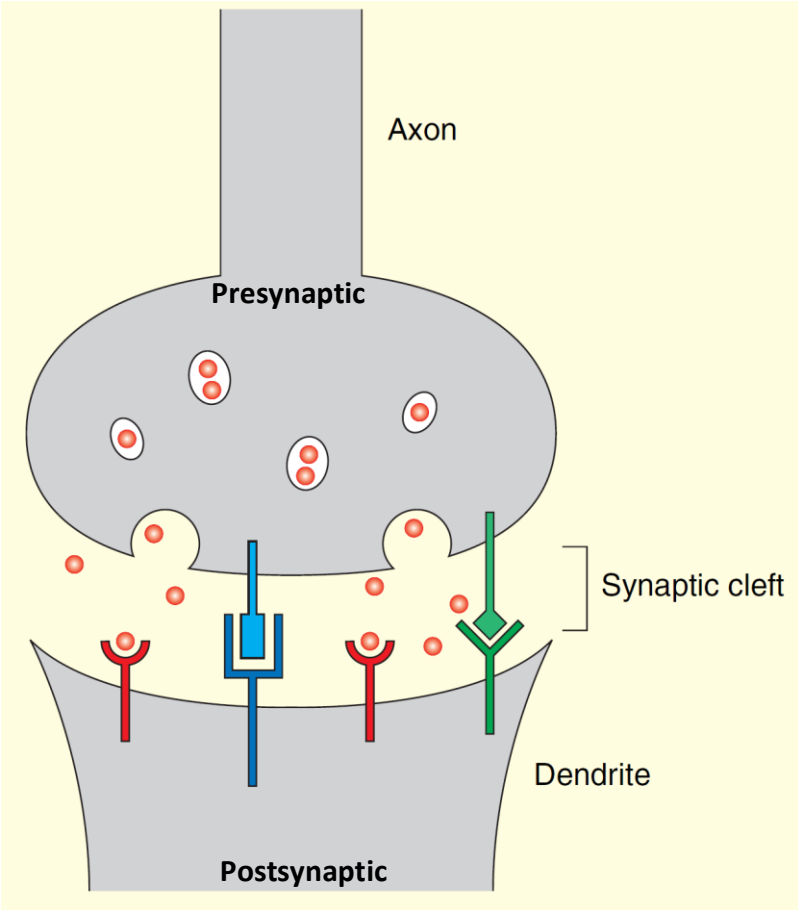

Figure 2: **Scheme of a mature synapse.**
On the presynaptic side, neurotransmitter molecules (red circles) are transported in vesicles. Upon the arrival of an action potential, they undergo exocytosis by fusion with the plasma membrane and are released into the synaptic cleft. They travel to the opposite side and then bind to postsynaptic receptors (red). The precise alignment of cell contacts is established by ligand-receptor pairs (blue and green) such as nCAMs; adapted from Abbas (2003).

There are four main CAM families: integrins, cadherins, selectins and the immunoglobulin superfamily (Ig-SF) (Hynes, 1999; Aricescu and Jones, 2007) with the latter being particularly significant for this study. Polypeptides belonging to the Ig-SF are characterised by the presence of a specific protein domain, the Ig-fold, which has been conserved over time and may be called evolutionary ancient. This Ig-related domain can appear in varying numbers within a single polypeptide (Brümmendorf and Rathjen, 1995). It is often accompanied by a second type of protein module, the fibronectin type III (Fn3) (Vaughn and Bjorkman, 1996), which was first detected in the adhesive ECM protein fibronectin (Hynes, 1999). The Ig-fold is composed of 70-110 amino acid residues. It is embedded in a beta-sheet structure of two antiparallel beta-sheets, which are connected by hydrophobic interactions (Brümmendorf and Rathjen, 1995). These two opposing beta-sheets are stabilised by disulfide bonds formed by pairs of cysteine residues (Vaughn and Bjorkman, 1996). The Fn3 repeats are folded into this beta-sheet (Hynes and Lander, 1992). Although Ig-SF members have the same core structure, the peripheral regions can differ in their composition. Hence, Ig-SF members are quite diverse (Chothia and Jones, 1997). Sequencing of the human genome disclosed the Ig-SF as the largest CAM family with the distinctive protein domain widely represented in vertebrates (Lander et al., 2001), but also in invertebrates such as *Drosophila melanogaster* or *Caenorhabditis elegans* (Vogel et al., 2003).

Almost three decades ago, NCAM (neural cell adhesion molecule) was discovered as one of the first membrane proteins participating in CA between neurons (Thiery et al., 1982). It is a prototype member of the Ig-SF and indicated early the importance of CAMs from this family in synaptic plasticity (Scholey et al., 1993; Lüthl et al., 1994). NCAM is comprised of two Fn3 repeats and five Ig-domains (Yamagata et al., 2003) and forms homophilic complexes (via self-self interactions) (see *Figure 3*). A multitude of subsequent studies on NCAM further highlighted its role as they revealed NCAM to be highly expressed in the CNS and to have a fundamental role during memory formation (Cremer et al., 1994; Ronn et al., 1995; Fox et al., 1995; Muller et al., 1996; Becker et al., 1996; O'Connell et al., 1997). Due to stress-related alterations in its gene expression (Sandi et al., 2001; Grootendorst et al., 2001; Touyarot and Sandi, 2002; Venero et al., 2002), NCAM

might be involved in processes by which corticosterone affects memory formation (Sandi and Loscertales, 1999).

All novel synaptic CAMs investigated in this study belong to the Ig-SF except for Neuroligin (Nlgn) and Neurexin (Nrxn), which pose an independent group for themselves (Yamagata et al., 2003).

1.3.1 Novel synaptic cell adhesion molecules

Besides already well characterised CAMs like NCAM, there are a couple of novel CAMs, which just have been recently identified: Nectin, SynCAM or Necl (nectin-like protein), Nrxn/Nlgn (see *Figure 3*) and Neuroplastin (Nptn).

All Nectins have three extracellular Ig-folds and are associated with the actin cytoskeleton through afadin, a Nectin- and actin-filament-binding protein (Kakunaga et al., 2005). Nectin 1 (Nec 1) and Nectin 3 (Nec 3) are implicated both in homophilic and heterophilic binding (Narita et al., 2011), whereas the heterophilic interaction between Nec 1 and Nec 3 is regarded as the strongest one in the various possible combinations between all Nectin members (Sakisaka and Takai, 2004). Nec 1 and Nec 3 are located in an asymmetric manner at pre- and postsynaptic sites of membranes for example in the adult mouse CA3 (Honda et al., 2006). This “asymmetric localisation of Nec 1 at the terminus of an axon and Nec 3 at dendrites plays a major role in defining selective interactions between an axon and dendrites”, as until now it remained elusive “why an axon initiates contact with a dendrite, but not with other axons and why a dendrite seeks contact with axons, but not with other dendrites” (Ogita et al., 2010). Inhibiting the formation of the Nec 1-Nec 3-dimer leads to reduced synapse size (Mizoguchi et al., 2002). Overall, Nectins are required as the structural and functional basis for synapses (Irie et al., 2004), sometimes in cooperation with cadherins (Takai and Nakanishi, 2003). Nonetheless, the question remains as to how synapses are regulated in detail via Nectins.

Besides Nectins, there are SynCAMs, also called Necls, which show a Nectin-like structure with three Ig-folds in the extracellular space, a single transmembrane region and a carboxy-terminal intracellular tail (Biederer, 2006). There are interactions between SynCAM 1 / Necl 2 and Nec 3 as well as between

SynCAM 3 / Necl 1 and Nec 1 (Irie et al., 2004; Biederer, 2006). However, their functional roles still have to be determined. SynCAMs appear in pre- and postsynaptic cell compartments and are known to prefer heterophilic over homophilic CA with SynCAM 1 / Necl 2 and SynCAM 2 / Necl 3 being the strongest heterophilic pair (Fogel et al., 2007). SynCAM 1 / Necl 2 and SynCAM 2 / Necl 3 are both highly expressed in the hippocampus and seem to be able to organise synapses and contribute to their function (Fogel et al., 2007). Biederer and colleagues (2002) demonstrated that SynCAM over-expression leads to an increase in spontaneous synaptic activity and that SynCAM in non-neuronal cells drives neurons in near proximity to form synapses onto the non-neuronal cells. Besides their involvement in late stages of neural circuit formation for instance during synaptogenesis, a recent study confirmed that SynCAMs are also involved during early developmental steps in the spinal cord of chickens: SynCAM 2 / Necl 3 could be identified in the floorplate, thus being a key player in axon guidance (Niederkofler et al., 2010).

Nlgns, which do not belong to the Ig-SF, share some similarities with SynCAMs: they are postsynaptic membrane proteins and both have an intracellular anchor organised around a PDZ-based scaffold (scaffolding proteins containing multiple protein-binding motifs) (Sheng and Sala, 2001). They operate via the same presynaptic signalling pathways (Washbourne et al., 2004) and furthermore, SynCAMs and Nlgns are the only known CAMs sufficient to promote presynaptic differentiation and maturation during artificial synapse induction (Li and Sheng, 2003; Sara et al., 2005; Wittenmayer et al., 2009). Additionally, SynCAM 1 and Nlgn 1 both promote excitatory synaptic transmission (Washbourne et al., 2004).

The binding partners for postsynaptic Nlgns are Nrxns, a family of presynaptic cell surface receptors (Missler et al., 1998). Rodents and other mammals express 6 main Nrxns (1 to 3 and α or β) (Tabuchi and Südhof, 2002) and four main Nlgns (1 to 4) (Bolliger et al., 2008), whereby Nrxns undergo extensive splicing, which leads to hundreds of variants (Ullrich et al., 1995). Nrxns and Nlgns form a tight trans-synaptic complex with a rank order of affinities depending on the involved isoforms and splice variants (Comoletti et al., 2006). Nlgn 1 and Nlgn 2 are differentially located, meaning presynaptic Nrxn interacts with postsynaptic Nlgn 1 at excitatory (glutamatergic) synapses only, while at inhibitory synapses

(GABAergic), Nrxn binds to Nlgn 2 (Song et al., 1999; Graf et al., 2004; Chubykin et al., 2007). When interfering with the Nlgn-Nrxn interaction in culture, synapse formation is inhibited (Li and Sheng, 2003). Moreover, it has been shown that mutations in genes that encode Nlgns or Nrxns are linked to cases of autism (Jamain et al., 2003), mental retardation (Talebizadeh et al., 2006) and learning disability (Südhof, 2008).

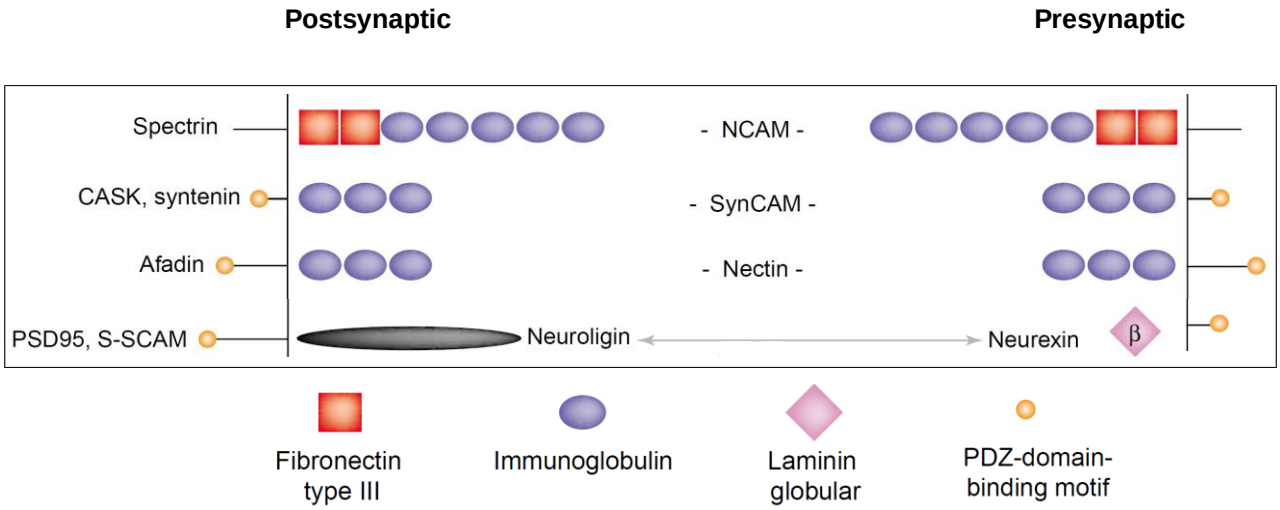

Figure 3: **Synaptic adhesion molecules with known ligands.** The extracellular domain of NCAM is designed out of five Ig-domains and two Fn3 repeats. Except for NCAM, the depicted adhesion molecules possess a binding motif (small yellow circle) that binds to PDZ proteins. SynCAM and Nectin exhibit similar extracellular structures, both being comprised of three Ig-domains. Nlgn and Nrxn do not belong to the Ig-SF and thus do not contain an Ig-domain. On the left, there are adhesion molecules, which are present postsynaptically (Nlgn). The right side illustrates CAMs believed to be present in presynaptic membranes (Nrxn). CAMs presented in the middle are capable of homophilic binding, hence they appear both pre- and postsynaptically (NCAM, SynCAM, Nectin); adapted from Yamagata (2003).

Nptn, another member of the Ig-SF, appears in two isoforms, Np 55 and Np 65 (formerly known as gp 65 and gp 55), generated by alternative splicing from a single gene and named according to their molecular weight (Owczarek et al., 2010). These two isoforms can be discriminated by the presence of the Ig 1-domain: Np 65 is comprised of three extracellular Ig-domains including the Ig 1-domain, while Np 55 has two Ig-folds with the Ig 1-domain being absent (Langnaese et al., 1998). Unlike Np 65, which is neuron-specific, Np 55 is widely expressed in rodent tissues including all brain regions (Langnaese et al., 1998). Until now, Np 55 has not been detectable in humans, thus, Np 65 is the main human isoform. In the rodent forebrain, Np 65 is evident in the cortex,

hippocampus, putamen, amygdala and the thalamus (Smalla et al., 2000). Only Np 65 is involved in homophilic binding (Buckby et al., 2004) and exhibits a central role in synapse stabilisation and memory formation. LTP increases the Np 65 level, while antibodies against Np 65 prevent LTP (Smalla et al., 2000). The role of Np 55 is only poorly understood, but Owczarek and colleagues (2010) were able to demonstrate that Np 55 binds in a heterophilic manner to fibroblast growth factor receptor 1 (FGFR 1), which is important during development, maintenance and regeneration of the CNS. This activation of FGFR 1 by Np 55 induces neurite outgrowth.

All nCAMs specified above are promising candidates for studies on cognition as they are synapse-specific, able to form new synapses and able to recruit the synaptic machinery. Nonetheless, Nptn proved to be of particular value for studies involving mimetic peptides.

1.3.2 Mimetic peptides

Mimetic peptides are synthesised polymers, which might mimic biological effects of the original peptide from which they are derived. They are not complete copies, but normally encompass a motif of several amino acids, which poses an active and rather small section. In the past, scientists were able to reveal the impact of mimetic peptides on cognition. FGL, a mimetic peptide for NCAM, was verified to improve hippocampus-dependent memory formation in the long term (Cambon et al., 2004; Secher et al., 2006), to prevent impairment of spatial memory as well as the reduction of hippocampal neurogenesis related to chronic stress (Borcel et al., 2008). Moreover, FGL was shown to facilitate synaptogenesis (Cambon et al., 2004), axonal outgrowth (Kiselyov et al., 2003) and neuronal survival in hippocampal cultures (Neiiendam et al., 2004). These findings indicate mimetic peptides of nCAMs as a new tool with therapeutic relevance for the treatment of stress-related disorders and impairments.

As mentioned above, Np 55 is comprised of two Ig-domains (Ig 2 and Ig 3) (Empson et al., 2006), functions as a FGFR ligand and is not involved in homophilic binding. This finding was further supported by microsphere binding experiments showing that only constructs including the Ig 1-domain are capable of

mediating homophilic binding, while the Ig 2 and/or Ig 3-module are not sufficient (Smalla et al., 2000). X-ray crystallography revealed the crystal structure of the ectodomain of Np 55: Ig 2 and Ig 3 are "positioned in an extended conformation, with Ig 3 oriented at an angle of ~ 45° to the Ig 2 module axis" (Owczarek et al., 2010) (see *Figure 4*). The two modules are linked via a short region comprised of only three amino acids. The Ig 2-domain consists of two beta-sheets (connected by a cysteine bridge) with overall eight beta strands, thus forms a classical beta-sheet structure (see chapter 1.3). The Ig 3-domain has 12 additional residues compared to Ig 2 and has nine beta strands instead of eight (Owczarek et al., 2010). The heterophilic interaction between Np 55 and FGFR is mediated via a motif in the Ig 2-domain. From this motif, the mimetic peptide for Np 55, which is called Narpin, is derived (see *Figure 4*). The motif is remarkably homologous to a motif in the first Ig-domain of FGFR 1 (similarities: 77 %) (Owczarek et al., 2010).

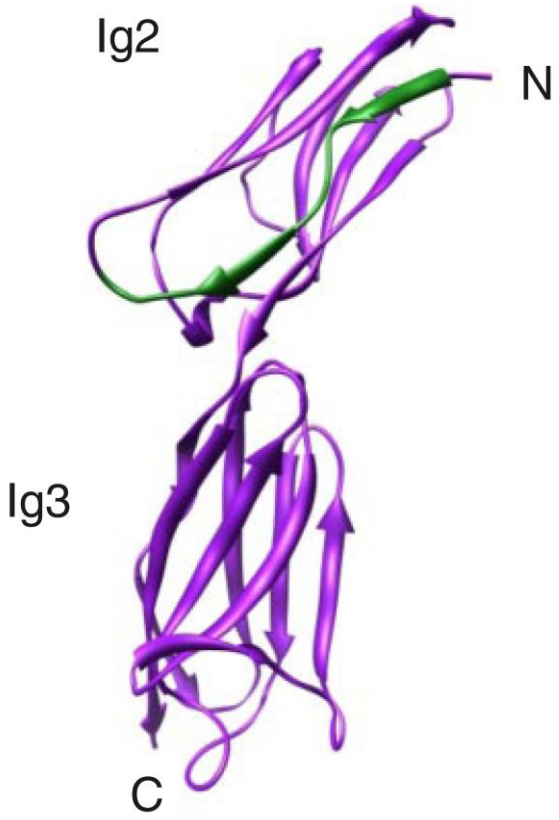

Figure 4: **Three-dimensional structure of the ectodomain of Np 55.**
The Ig 2-domain with the N-terminus and the Ig 3-domain with the C-terminus are depicted. Both modules consist of several beta strands (lilac). The Narpin motif is derived from the A and B strands and an interconnecting loop (green) in the Ig 2-domain; adapted from Owczarek (2010).

Owczarek and colleagues (2010) revealed that Narpin, like Np 55, binds to FGFR 1, that both induce neurite outgrowth in cell culture of hippocampal neurons and that Narpin has antidepressive-like effects in rats during the forced swim test (FST). It is hypothesised that Np 55-induced signalling may be implicated in synaptic plasticity *in vivo.*

In contrast to Np 55, Np 65 is characterised by the presence of a third Ig-domain (Ig 1), which allows homophilic binding (Owczarek et al., 2011). The putative binding site is located in the F-G loop of the Ig 1-domain (see *Figure 5*). This loop is completely exposed to its environment, oriented perpendicularly to the surface of its Ig 1-module and able to establish a contact with the F-G loop of an opposing Nptn molecule. The Ig 2-module is oriented at an angle of ~ 45° to the Ig 1-module axis. The mimetic peptide for Np 65, Enplastin, is derived from the homophilic binding site. It was shown that both, Np 65 and Enplastin activate intracellular signalling pathways via FGFR and p38 mitogen-activated protein kinase (p38-MAPK) and that they induce neurite outgrowth with Enplastin being a stronger agent during these processes. Disruption of Np 65 homophilic binding via Enplastin inhibits neurite outgrowth *in vitro* and impairs the initial phase of spatial learning in rats in the Morris water maze (MWM) (Owczarek et al., 2011).

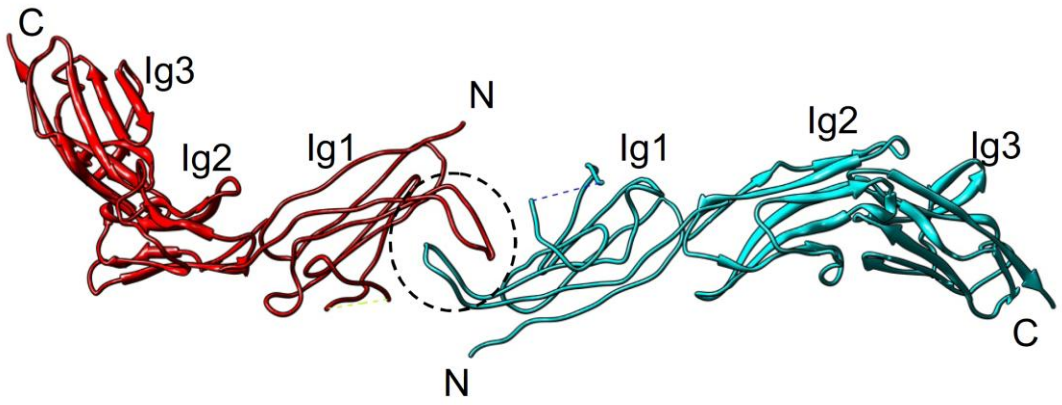

Figure 5: **Three-dimensional structure of the ectodomain of Np 65.** The homophilic interaction between two extracellular Np 65 domains (red and turquoise) is depicted. The binding site is located in the F-G loop of the Ig 1 domain (encircled). For both molecules, the Ig 1 domain with the N-terminus, the Ig 2 domain and the Ig 3 domain with the C-terminus are indicated; adapted from Owczarek (2011).

1.4 Scope of the thesis

Chronic stress and aging attenuate cognitive functions and are often accompanied by diseases such as major depression or Alzheimer's disease. The aims of this thesis are (I) to assess the impact of CSS and aging on cognitive performance, (II) to determine the contribution of novel nCAMs in these processes and (III) the assessment of the therapeutic value of nCAMs to treat cognitive diseases at aging or stress-related cognitive dysfunctions. To pursue these objectives, several experiments were conducted, which addressed the following issues.

- Effects of learning experience and/or stress on expression levels (mRNA and protein level) of recently identified nCAMs.
- Effects of Nptn-derived mimetic peptides (Enplastin and Narpin) on cognition under basal conditions (intra-hippocampal injections).
- Effects of Enplastin and Narpin on cognition following stress and/or aging (subcutaneous injections). Are peptides able to compensate for already existing cognitive deficits?

The detailed design and background of each experiment will be introduced separately in chapter 2.3.

2 Material and Methods

2.1 Experimental animals

All experiments were carried out with male CD1 mice delivered from the Charles River Laboratories (France or Sulzefeld, Germany). CD1 is a genetically variable outbred mouse strain (Rice and O'Brien, 1980; Chia et al., 2005). Female mice were excluded from all experiments to avoid confounding factors, for example estrogens, which are known to affect cognitive performance in female rodents (Grootendorst et al., 2004) as well as the different activation of cortical areas by stress in dependence of the stage of oestrus (Figueiredo et al., 2002). Furthermore, there are large gender differences in the susceptibility to individual stressors and reliable stress models are mostly validated for male animals (Palanza et al., 2001; Palanza, 2001).

As all animals were ordered from a supplier, they were allowed to habituate to the animal facilities of the Max Planck Institute of Psychiatry in Munich for at least one week before starting any experiments. The animals were 4 to 12 weeks of age on the day of arrival, depending on the experiment. All animals were housed in groups of four until postnatal day 77 (PD 77), then they were singly housed in standard Plexiglas cages (22 cm x 16.5 cm x 14 cm (l x w x h)) (see *Figure 6)*. Housing and testing of the mice took place under standard conditions with a 12 h/12 h dark/light cycle with lights on at 7:00 am, constant temperature of 22 ± 2° C and humidity of 55 ± 5 %. Food (Altromin 1324, Altromin GmbH, Germany) and drinking water were provided *ad libitum*.

All experiments were performed according to current regulations of the European Communities Council Directive 2010/63/EU. All efforts were made to minimise animal suffering during the experiments. The protocols were approved by the committee for the Care and Use of Laboratory Animals of the Government of Upper Bavaria, Germany.

Figure 6: **Standard Plexiglas cage with bedding and additional nesting material.**

2.2 Chronic social stress procedure

To assess the consequences of CSS, animals underwent the CSS procedure (see *Figure 7)* described previously by Schmidt and colleagues (2007), where the social stress is constantly present and covers an important life stage. The CSS paradigm was conducted during the animals' adolescent phase from age 4 weeks to 12 weeks. By changing the cage composition of the animals twice per week, each mouse had to face regularly three unknown cage mates in a new, clean cage. The rotation schedule was randomised in order to minimise the possibility of a repeated encounter of the same mice. An exemplary rotation schedule for 64 mice is depicted in *Table 1.* As the animals kept on fighting for a distinct social hierarchy, the change of cage mates created an unstable social environment, which the animals could not adapt to and could not avoid. Control animals remained in the same group of four throughout the whole 7 weeks, therefore being able to build a stable hierarchy. Animals with major wounds or overly aggressive animals were excluded. In all cases, these were less than 3 %. After 7 weeks of group-housing, all animals (control and stress animals) were separated and singly housed. In contrast to other species, single-housing itself does not pose a stressor

in male mice. It has no impact on behaviour or stress response (Arndt et al., 2009) and it does not affect immuno-endocrine parameters (Bartolomucci et al., 2003). Body weight was monitored once per week throughout the CSS procedure.

To investigate normal aging in CD1 mice, acute and long-term effects of CSS on nCAMs and the effects of CSS during adolescence on aging-induced memory decline, animals were single-housed for 12 months upon completion of the CSS paradigm.

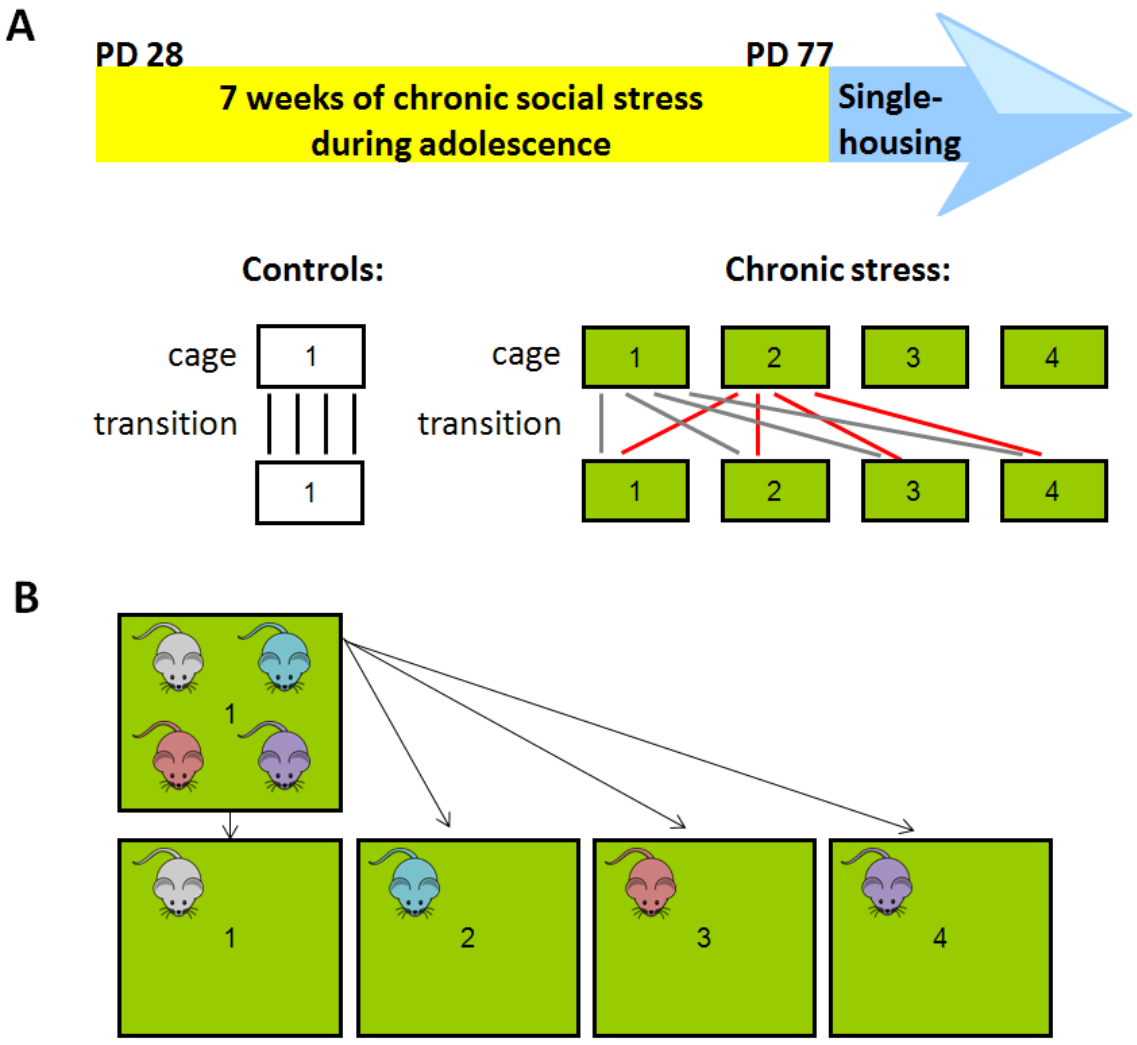

Figure 7: **CSS paradigm. (A)** Control mice (depicted as black lines) were housed in groups of four, remaining with the same cage mates. CSS mice (depicted as grey and red lines) were group-housed for 7 weeks as well, changing the group composition randomly twice per week. White boxes represent the control group cages; green boxes represent the CSS group cages. **(B)** Transition of four CSS mice to the new and clean cages.

Table 1: **CSS rotation schedule.** The cage composition of 64 mice (16 cages with four animals each) is disrupted twice per week for 7 weeks. Digits represent the animals 1 to 64, digits in bold represent the cages 1 to 16.

cage	**1**				**2**				**3**				**4**				**5**				**6**				**7**				**8**			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
week 1	1	5	9	13	2	6	10	14	3	7	11	15	4	8	12	16	17	21	25	29	18	22	26	30	19	23	27	31	20	24	28	32
week 1	1	6	11	16	2	7	12	13	3	8	9	14	4	5	10	15	17	22	27	32	18	23	28	29	19	24	25	30	20	21	26	31
week 2	1	7	9	15	6	12	14	4	11	13	3	5	16	2	8	10	17	23	25	31	22	28	30	20	27	29	19	21	32	18	24	26
week 2	1	12	3	10	7	14	5	16	9	4	11	2	15	6	13	8	17	28	19	26	23	30	21	32	25	20	27	18	31	22	29	24
week 3	1	17	33	49	12	28	44	60	3	19	35	51	10	26	42	58	7	23	39	55	14	30	46	62	5	21	37	53	16	32	48	64
week 3	1	28	35	58	17	44	51	10	33	60	3	26	49	12	19	42	7	30	37	64	23	46	53	16	39	62	5	32	55	14	21	48
week 4	1	44	3	42	28	51	26	49	35	10	33	12	58	17	60	19	7	46	5	48	30	53	32	55	37	16	39	14	64	23	62	21
week 4	1	51	33	19	44	26	12	58	3	49	35	17	42	28	10	60	7	53	39	21	46	32	14	64	5	55	37	23	48	30	16	62
week 5	1	26	35	60	51	12	17	42	33	58	3	28	19	44	49	10	7	32	37	62	53	14	23	48	39	64	5	30	21	46	55	16
week 5	1	12	3	10	7	14	5	16	9	4	11	2	15	6	13	8	17	28	19	26	23	30	21	32	25	20	27	18	31	22	29	24
week 6	1	7	9	15	6	12	14	4	11	13	3	5	16	2	8	10	17	23	25	31	22	28	30	20	27	29	19	21	32	18	24	26
week 6	1	6	11	16	2	7	12	13	3	8	9	14	4	5	10	15	17	22	27	32	18	23	28	29	19	24	25	30	20	21	26	31
week 7	1	5	9	13	2	6	10	14	3	7	11	15	4	8	12	16	17	21	25	29	18	22	26	30	19	23	27	31	20	24	28	32
week 7	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32

cage	**9**				**10**				**11**				**12**				**13**				**14**				**15**				**16**			
	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
week 1	33	37	41	45	34	38	42	46	35	39	43	47	36	40	44	48	49	53	57	61	50	54	58	62	51	55	59	63	52	56	60	64
week 1	33	38	43	48	34	39	44	45	35	40	41	46	36	37	42	47	49	54	59	64	50	55	60	61	51	56	57	62	52	53	58	63
week 2	33	39	41	47	38	44	46	36	43	45	35	37	48	34	40	42	49	55	57	63	54	60	62	52	59	61	51	53	64	50	56	58
week 2	33	44	35	42	39	46	37	48	41	36	43	34	47	38	45	40	49	60	51	58	55	62	53	64	57	52	59	50	63	54	61	56
week 3	9	25	41	57	4	20	36	52	11	27	43	59	2	18	34	50	15	31	47	63	6	22	38	54	13	29	45	61	8	24	40	56
week 3	9	20	43	50	25	36	59	2	41	52	11	18	57	4	27	34	15	22	45	56	31	38	61	8	47	54	13	24	63	6	29	40
week 4	9	36	11	34	20	59	18	57	43	2	41	4	50	25	52	27	15	38	13	40	22	61	24	63	45	8	47	6	56	31	54	29
week 4	9	59	41	27	36	18	4	50	11	57	43	25	34	20	2	52	15	61	47	29	38	24	6	56	13	63	45	31	40	22	8	54
week 5	9	18	43	52	59	4	25	34	41	50	11	20	27	36	57	2	15	24	45	54	61	6	31	40	47	56	13	22	29	38	63	8
week 5	33	44	35	42	39	46	37	48	41	36	43	34	47	38	45	40	49	60	51	58	55	62	53	64	57	52	59	50	63	54	61	56
week 6	33	39	41	47	38	44	46	36	43	45	35	37	48	34	40	42	49	55	57	63	54	60	62	52	59	61	51	53	64	50	56	58
week 6	33	38	43	48	34	39	44	45	35	40	41	46	36	37	42	47	49	54	59	64	50	55	60	61	51	56	57	62	52	53	58	63
week 7	33	37	41	45	34	38	42	46	35	39	43	47	36	40	44	48	49	53	57	61	50	54	58	62	51	55	59	63	52	56	60	64
week 7	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64

2.3 Experimental design and background

2.3.1 Experiment 1: Effects of CSS in young animals and the acute impact on novel synaptic CAMs

It is known from a great variety of studies (see chapter 1.1.4) that chronic stress induces cognitive impairment. To assess the impact of memory loss related to stress on novel synaptic CAMs, we investigated region-specific changes of nCAM expression on mRNA and protein level in young, male CD1 mice. At the date of testing, animals were between 11 to 13 weeks old. Additionally, physiological, neuroendocrine and behavioural data were collected.

The first batch of animals included 32 animals, which were subjected to the CSS paradigm, and 32 controls. Animals were divided into subgroups, each consisting of control and stress animals in equal parts. The first group of 32 mice were tested in the following order in object recognition (OR), Y-maze and MWM (see chapter

2.7.1, 2.7.3 and 2.7.4). Behavioural testing started during the last week of the CSS procedure, when the animals were 11 weeks old. They were sacrificed in the first week of single-housing, 2 hours after the last MWM trial. The second group included 32 basal animals without learning experience. These naïve mice were sacrificed directly after the CSS procedure, 4 days after the last cage rotation. For this first batch of animals, experimenters collected brain tissue for in situ hybridisation (ISH: chapter 2.6.1), adrenal glands and thymus for determining organ weights (chapter 2.5.3) as well as blood samples for the analysis of plasma corticosterone levels (chapter 2.6.3).

To investigate the effects of CSS on nCAM protein levels, the experiment was repeated with a second batch of animals, which was used for Western blot (WB) sampling (chapter 2.6.4). After cessation of the CSS procedure, animals were sacrificed at two time points, either directly after the CSS paradigm under basal conditions (n = 20 naïve animals) or 4 hours after the last MWM trial (n = 18 animals with learning experience). The time course for experiment 1 is depicted in *Figure 8*.

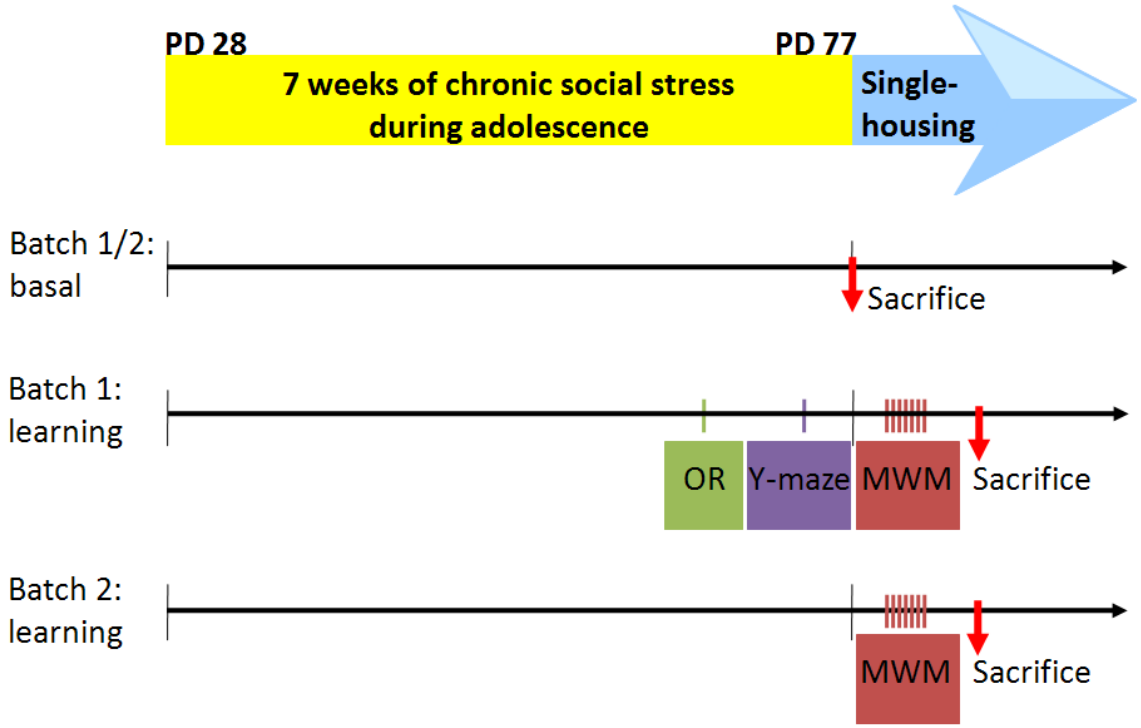

Figure 8: **Schedule for experiment 1.** CSS started at PD 28. In both batches, one group of animals was killed under basal conditions, directly after the CSS procedure. A second group of batch 1 was tested during the last week of CSS and sacrificed during the first week of single-housing, 2 hours after the last MWM trial. A second group of batch 2 was tested during single-housing and sacrificed 4 hours after the last MWM trial. Coloured tick marks represent one day of testing.

2.3.2 Experiment 2: Effects of CSS in aged animals and the long-term impact on novel synaptic CAMs

Similar to experiment 1, this study was designed to evaluate the impact of memory loss related to stress, but also to aging on novel synaptic CAMs. It is well known that aging is accompanied by a certain degree of cognitive decline (see chapter 1.1.4). Hence, the study was conducted in aged (15 months of age), male CD1 mice. Expression patterns of different nCAMs on mRNA and protein level, as well as physiological, neuroendocrine and behavioural parameters were analysed.

Animals went through the CSS procedure as already described. Afterwards, they were single-housed for 12 months. Mice with evident diseases or other age-related impairments were excluded from the experiment; some animals also died of old age before testing. Upon reaching the age of 15 months, the number of healthy, residual animals amounted to a total of 31 individuals (n = 15 controls and n = 16 stress animals) in batch 1. All mice were first tested in OR and then in the

MWM. Analogous to experiment 1, there were two different time points of sacrifice after testing: basal animals were sacrificed 2 weeks after the MWM (n = 8 controls, and n = 8 stress animals) to allow recovery to baseline, while the second animal group was sacrificed directly after learning, namely 2 hours after the last MWM trial (n = 7 controls and n = 8 stress animals). Brains and adrenal glands were removed and blood samples collected.

To examine the effects of CSS on protein level, the experiment was repeated with a second batch of animals, which was used for WB analysis (chapter 2.6.4), blood and adrenal sampling. One year after CSS cessation upon an age of 15 months, animals were sacrificed under basal conditions (n = 10 control and n = 10 stress animals). The schedule for the whole study is shown below in *Figure 9*.

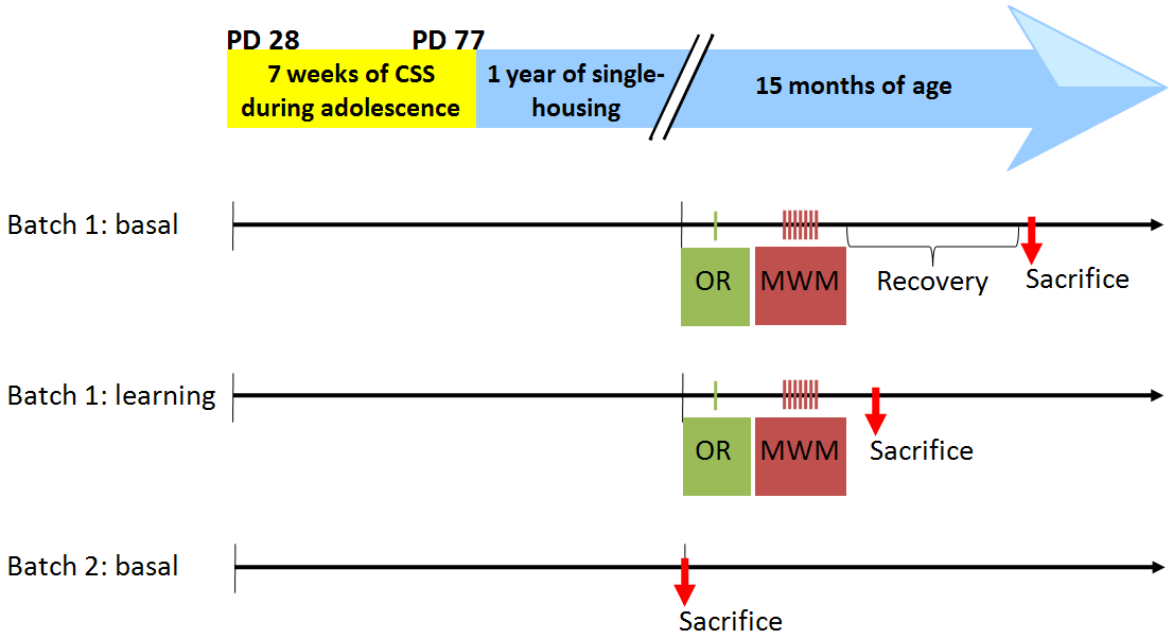

Figure 9: **Schedule for experiment 2.** After 7 weeks of CSS during adolescence, animals were singly housed for 12 months. All animals of batch 1 were behaviourally tested. There were two time points of sacrifice: basal animals were sacrificed after a recovery period of 2 weeks after the last test. Another group was sacrificed 2 h after learning. Animals of batch 2 were not tested behaviourally, but sacrificed under basal conditions, 1 year after CSS cessation. Coloured tick marks represent one testing day.

2.3.3 Experiment 3: Regulation of novel synaptic CAMs by dexamethasone

In order to test whether nCAMs can be regulated directly via the GR, animals received subcutaneous (s.c.) injections (see chapter 2.4.2) of dex and were sacrificed at five different time points after treatment. Dex is a very potent synthetic glucocorticoid. It has a 30 times higher affinity compared to its naturally produced analogue and has been widely used in scientific research to assess HPA axis negative feedback sensitivity to glucocorticoids (Oxenkrug et al., 1984; Ribeiro et al., 1993; Cole et al., 2000).

In this study, 12 weeks old, male and single-housed CD1 mice were injected with a high dose of dex of 10 mg/kg body weight. A total of 100 animals were injected: 50 animals received dex, while the other 50 served as control group and received Ringer solution. Animals were sacrificed at five different time points: 1 h, 2 h, 4 h, 8 h and 24 hours after the single injection. For each time point, 20 animals (n = 10 controls and n = 10 dex animals) were injected. Brain samples for ISH were collected as well as blood samples for a corticosterone radioimmunoassay (RIA). The time course for experiment 3 is illustrated below in *Figure 10*.

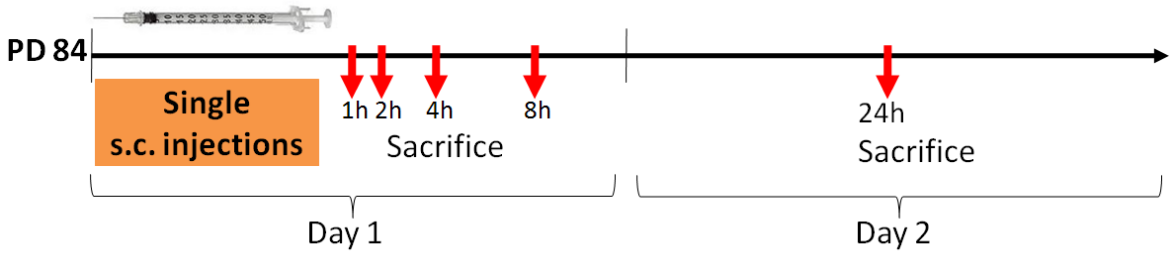

Figure 10: **Schedule for experiment 3.** Animals were singly housed and upon reaching the age of 12 weeks injected with either dex or Ringer (n = 50 animals each). There were five different time points of sacrifice: 1 h, 2 h, 4 h, 8 h after the injection (day 1) or 24 h after the injection (day 2).

2.3.4 Experiment 4: Acute treatment of young animals with mimetic peptides and the impact on cognition under basal conditions

The aim of this study was to modulate cognition in young animals by a single intra-hippocampal injection (see chapter 2.4.3) of a certain mimetic peptide, Enplastin or Narpin (see chapter 1.3.2 and chapter 2.4.1).

Animals were 9 weeks old on the date of arrival and singly housed. After the acclimatization phase, animals were subjected to stereotactic surgery (see chapter 2.4.3) during which two cannulas were bilaterally implanted into the hippocampus. The mice were allowed to recover from the procedure for 1 to 2 weeks. At the age of 12 weeks, they were tested in OR and 1 week later in the Y-maze. All animals were sacrificed on the day of their last test. Brains were extracted for localisation of cannula placement (see chapter 2.4.3).

Animals were treated with one of the peptides or belonged to the control group receiving artificial cerebrospinal fluid (vehicle animals). The substances were injected either before or during training (*Figure 11*): the first and the second batch received intra-hippocampal injections 30 minutes before the acquisition trial (batch 1: n = 12 vehicle- and n = 12 Narpin-treated animals; batch 2: n = 20 vehicle- and n = 20 Enplastin-treated animals), while the animals of the third and fourth batch received the substances during the training, directly after the acquisition trial (batch 3: n = 12 vehicle- and n = 12 Narpin-treated animals; batch 4: n = 12 vehicle- and n = 12 Enplastin-treated animals).

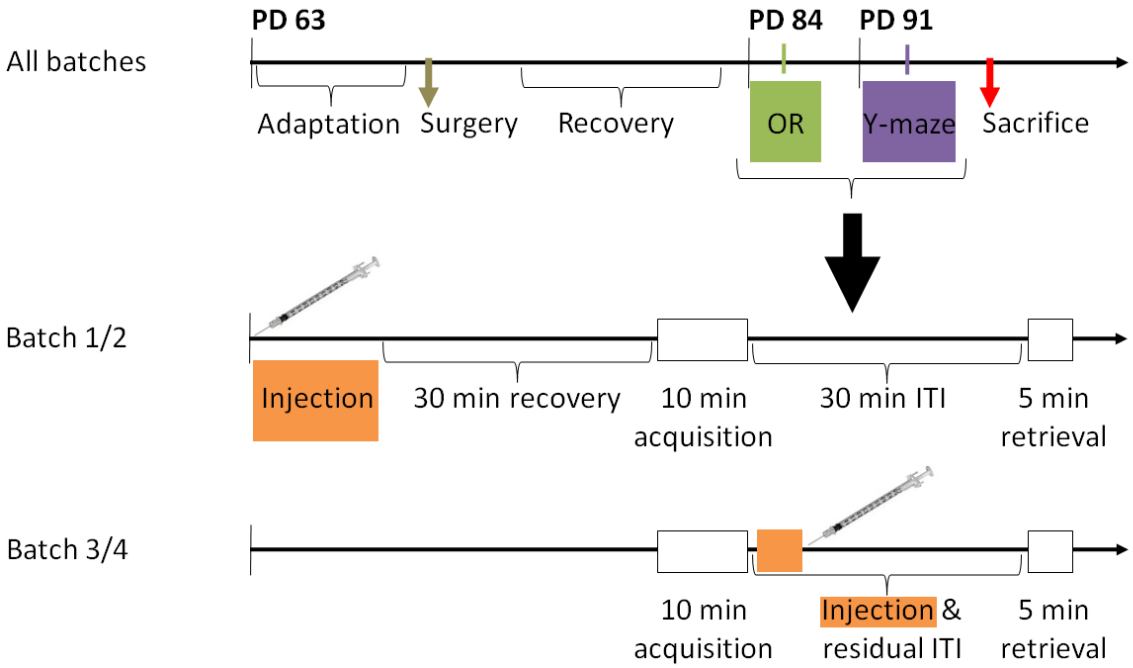

Figure 11: **Schedule for experiment 4.** All animals were subjected to stereotactic surgery. They recovered from the procedure and then were injected intra-hippocampally. Injections of batch 1 (Narpin) and batch 2 (Enplastin) were conducted before the training (OR and 1 week later Y-maze), 30 minutes prior to the acquisition. Injections of batch 3 (Narpin) and batch 4 (Enplastin) were given during the training, directly after the acquisition. Coloured tick marks represent one day of testing.

2.3.5 Experiment 5: Chronic treatment of young animals with mimetic peptides and the impact on cognition after CSS

The consequences of a chronic treatment with mimetic peptides in young animals on cognition were assessed in experiment 5. The main question was if it would be possible to reverse or at least improve cognitive deficits elicited by CSS during adolescence.

A total of 64 animals underwent the CSS procedure as described earlier, another 64 animals served as controls. During the last week of the CSS procedure, the animals received s.c. injections of either Narpin (n = 32), Enplastin (n = 32) or Ringer (n = 32) on 6 consecutive days. From day 1 to day 4, the injections started at 9:00 am and afterwards animals immediately went back to their home cages. In contrast, on day 5 and 6, mice were tested in the Y-maze (on day 5) and in OR (on day 6) with injections 2 hours prior to testing. Hence, injections started at 7:00 am on day 5 and 6. Animals were sacrificed the next day, 24 hours after the last training. Experimenters collected brains and blood samples. The time course is demonstrated in *Figure 12*.

A fourth batch of 32 animals was used to generate additional WB samples for experiment 1 and animals were neither injected nor tested behaviourally, but sacrificed after the cessation of the CSS paradigm (not depicted).

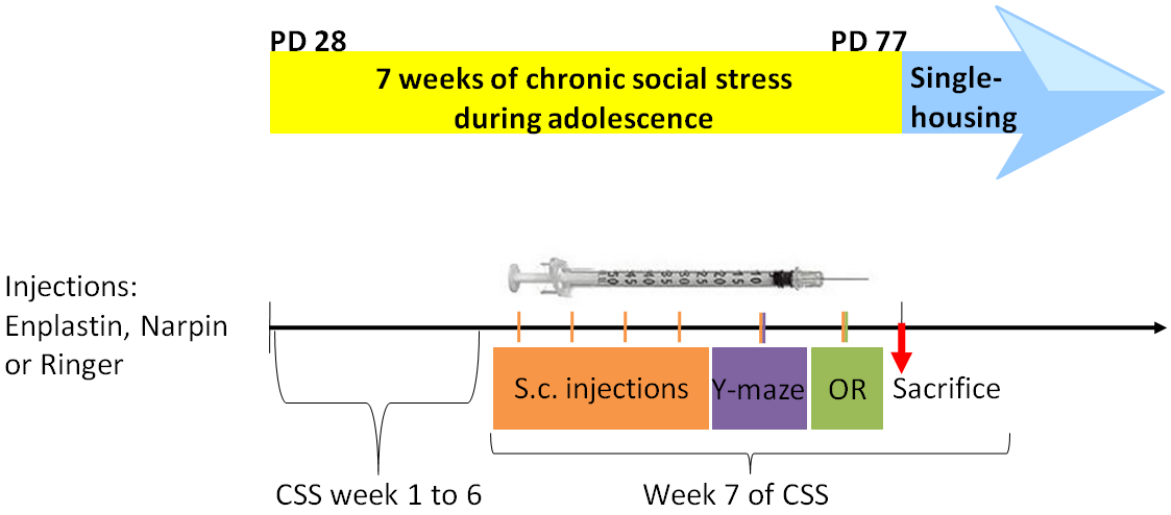

Figure 12: **Schedule for experiment 5.** Animals were subjected to the CSS procedure and treated by s.c. injections with Enplastin, Narpin or Ringer on 6 consecutive days during week 7, the last week of the CSS procedure. On testing days (day 5 + day 6), injections were conducted 2 hours before testing. The following day (day 7), animals were sacrificed. Coloured tick marks represent one day.

2.3.6 Experiment 6: Chronic treatment of aged animals with mimetic peptides and the impact on cognition after CSS

The objectives of experiment 6 were similar to those of experiment 5. The consequences of a chronic mimetic peptide treatment on cognition were investigated, but in this case in aged animals. Another main point of interest was if it would be possible to attenuate cognitive deficits elicited by CSS and/or aging as it is generally acknowledged that aging can even amplify stress-related cognitive deficits.

A total of 128 animals were used. Mice underwent the CSS procedure as described earlier and were single-housed for one year. Some animals died of old age, while others suffered from apparent diseases such as tumours and had to be excluded. The number of healthy, residual animals without any evident age-related impairments amounted to 51 control and 42 stress animals. Like in experiment 5, animals received s.c. injections of either Narpin (n = 24), Enplastin (n = 24) or

Ringer (n = 23) on 6 successive days. The injections were timed that upon starting behavioural testing, all animals had reached the age of 15 months. On the first 4 days, injections started at 9:00 am, while on day 5 and day 6 injections were given from 7:00 am, 2 hours prior to testing (Y-maze and OR). The injected animals were sacrificed 24 hours after behavioural testing. Brain halves and trunk blood samples were collected. *Figure 13* shows an overview of the whole study. One additional batch of animals was used to generate additional WB samples for experiment 2. They were neither injected nor tested behaviourally (not depicted).

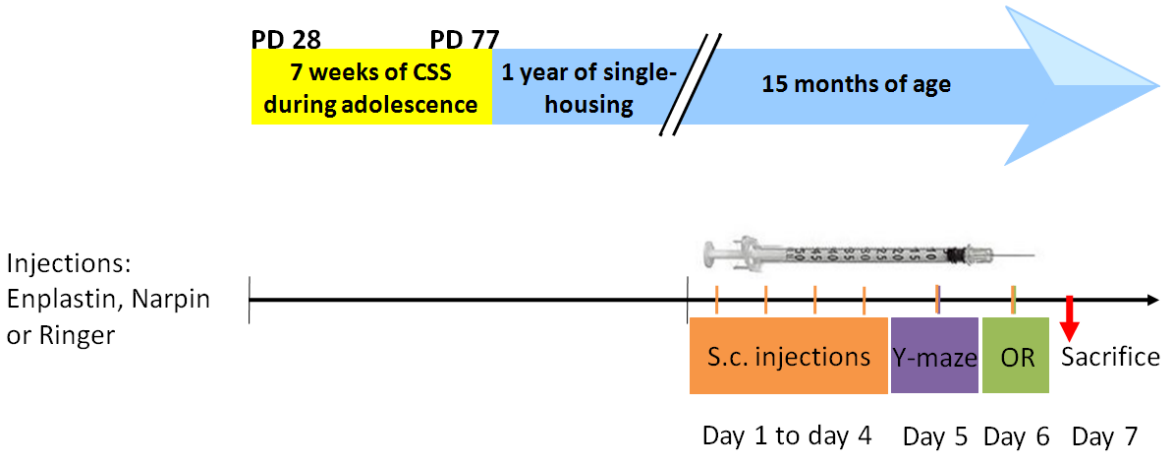

Figure 13: **Schedule for experiment 6.** Animals underwent the CSS procedure and were then single-housed for one year. At the age of 15 months, animals were given s.c. injections of Enplastin, Narpin or Ringer on 6 consecutive days. On testing days (day 5 + day 6), injections were conducted 2 hours before testing. The next day (day 7), animals were sacrificed.

2.4 Application of substances

2.4.1 Mimetic peptides

Two different mimetic peptides, Enplastin and Narpin, were applied. They were both derived from the recently identified nCAM Nptn and were synthesised as tetrameric peptides with two branching points by Schafer-N (Copenhagen, Denmark) with a purity of at least 85 %. The peptides' C-termini were blocked by an amide group (CONH2), while the N-termini (NH2) remained free. The peptides' amino acid sequences are depicted in *Table 2.* In *Table 3*, the one-letter abbreviation system for amino acids can be seen (Coligan, 2001).

According to the method of administration, s.c. injection or intra-hippocampal injection (see chapters 2.4.2 and 2.4.3.), the mimetic peptides, which were delivered in powder form, had to be diluted. In case of repeated injections, for example on several consecutive days, the stock solution was freshly prepared on a daily basis.

Table 2: **Amino acid sequences for Enplastin and Narpin.** Amino acids are abbreviated.

Mimetic peptide	Peptide sequence
Enplastin	DPKRNDLRQNPSITWIR
Narpin	RIVTSEEVIIRDS

Table 3: **One-letter code system for the 20 amino acids.**

Amino acid	1-letter code	Amino acid	1-letter code
Alanine	A	Leucine	L
Arginine	R	Lysine	K
Asparagine	N	Methionine	M
Aspartate	D	Phenylalanine	F
Cysteine	C	Proline	P
Glutamate	E	Serine	S
Glutamine	Q	Threonine	T
Glycine	G	Tryptophan	W
Histidine	H	Tyrosine	Y
Isoleucine	I	Valine	V

2.4.2 Subcutaneous injections

In order to produce an adequate stock solution for s.c. injections, the powdery mimetic peptides were first dissolved in Ringer solution and then diluted to a final dosage of 10 mg/kg body weight. To perform the s.c. injections in mice in a correct manner, the loose skin above the animals' neck and shoulders was lifted, thereby

already restraining the animal (Fox et al., 2002). The needle was inserted in parallel to the animals' back into the skin fold above the scruff.

For the detection of mimetic peptides in the brain and to demonstrate that they crossed the blood brain barrier via s.c. injections (see chapter 2.6.2), mimetic peptides were biotylised at the N-terminus by Schafer-N during the process of synthesis. As biotin is a relatively small ring system (see *Figure 14*), it can be easily attached and is very unlikely to interfere with the peptides' efficacy.

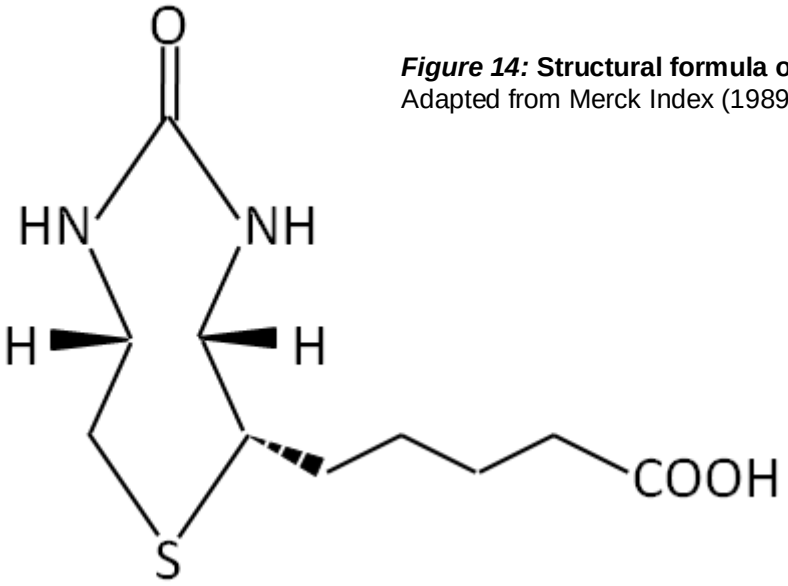

Figure 14: **Structural formula of biotin.**
Adapted from Merck Index (1989).

Beside the administration of mimetic peptides via s.c. injections, this method was also used to conduct a dex treatment in mice (see chapter 2.3.3) and investigate the regulation of nCAMs by this potent synthetic glucocorticoid. To this end, the dex stock solution was diluted with NaCl and each animal was injected with approximately 100 µl of dex solution (concentration = 10 mg/kg body weight).

2.4.3 Stereotactic surgery and intra-hippocampal injections

Experimental animals were implanted with bilateral guidance cannulas with the CA3 as target area. This enabled us to apply mimetic peptides intra-hippocampally at different time points, for example before or during testing.

Mice were sedated with pentobarbital sodium diluted 1:20 in 0.9 % NaCl (Invitrogen GmbH, Karlsruhe, Germany; 0.1 ml/10 g body weight; intra-peritoneal). To provide an analgesic effect as well, animals received s.c. injections of Metacam

(Meloxicam; Boehringer Ingelheim Pharma GmbH & Co. KG, Germany, 0.5 mg/kg body weight). After ensuring a sufficient anaesthetic depth, the animals were placed in a stereotactic apparatus (Type 430005-M/P, TSE Systems GmbH, Germany) and fixed by insertion of metal pins into the auditory canals as well as lowering of the front teeth into a metal trough. To protect the animals' eyes against dehydration and potential consequential damages like blindness, eyes were dabbed with eye ointment (Bepanthen Augen- u. Nasensalbe, Bayer AG, Leverkusen, Germany). Under additional, local anaesthesia with lydocaine (Xylocain, AstraZeneca GmbH, Wedel, Germany), the skull was exposed and stainless steel cannulas (10 mm, 26 gauge (G)) were bilaterally implanted, just above the CA3 region. In accordance to a stereotactic mouse brain atlas (Paxinos and Franklin, 2001), cannulas targeted the following coordinates relative to bregma: posterior: + 2.1 mm, lateral: ± 2.2 mm and ventral + 1.6 mm. For each cannula, a hole was drilled. The cannulas were fixed in the skull by locally applied instant adhesive (UHU GmbH & Co. KG, Bühl, Germany) and dental cement (Paladur, Heraeus Kulzer GmbH, Germany), 2 screws (length: 1.2 mm, diameter: 2 mm, custom-built from stainless steel, Paul Korth GmbH, Germany) and a final layer of dental cement covering the exposed skull. After a successful surgery, animals had a recovery period of 1 to 2 weeks. To minimise animal suffering, Metacam was constantly administered via the drinking water until the decapitation of the animals.

For the intra-hippocampal injections, mimetic peptides were diluted in artificial cerebrospinal fluid to a concentration of 1 µg/µl. A total volume of 1 µl was infused in the left and right CA3 of the hippocampus. Injections were administered over 1 minute by means of injection cannulas (30 G, Type 5 x SS304, Hamilton Bonaduz AG, Switzerland) that extended the tip of the guidance cannulas by 1 mm. After a successful injection, the injection cannula was left in place for another minute to allow diffusion of the injectate. During this procedure, animals were not anaesthetised and had to be hand-held and fixed carefully by an experienced colleague, while a second person conducted the injection. Correct cannula placement was determined by post mortem histological verification (see *Figure 15*). Only mice with bilaterally correct placement were included in the final analysis.

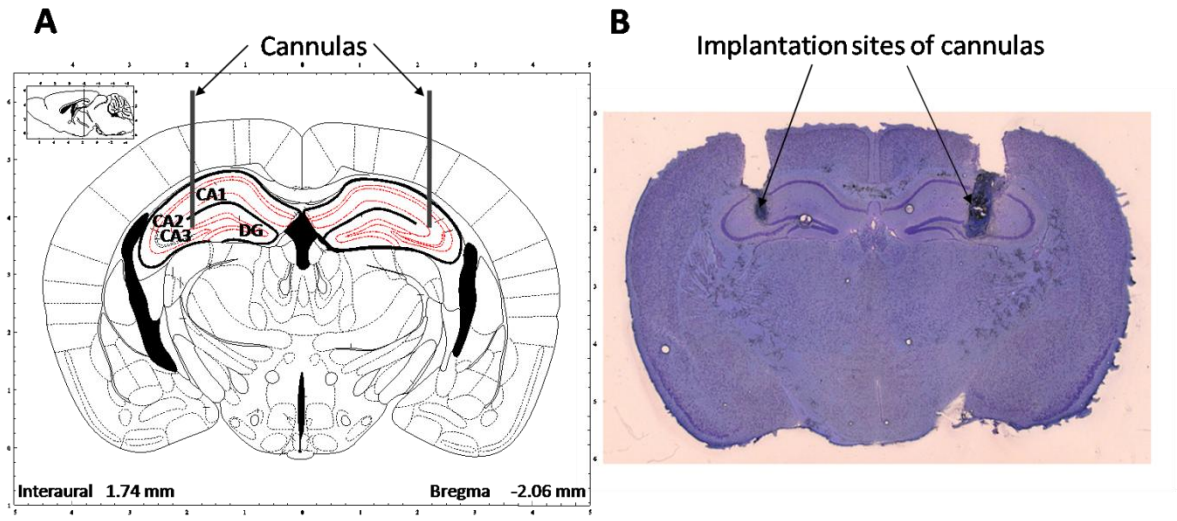

Figure 15: **Verification of cannula placement. (A)** Coronal brain section illustrating the locus of correct implantation (CA3) (Paxinos and Franklin, 2001). **(B)** Post mortem localisation of cannula placement in the hippocampus.

2.5 Sampling procedures

2.5.1 Blood preparation

Figure 16: **CD1 mouse shortly before tail cut.** The black arrow indicates the tail vein.

Blood was collected individually in labelled 1.5 ml EDTA-coated microcentrifuge tubes (Kabe Labortechnik, Germany). To retrieve trunk blood, animals were decapitated after anaesthesia with isoflurane (Abbott GmbH & Co. KG, Wiesbaden, Germany). For tail blood collection (20 µl), a small incision with a razor blade was inflicted in the dorsal tail vein (see *Figure 16*) (Fluttert et al., 2000). This method needs no anaesthesia and is considered relatively stress-free.

To exclude interference with the basal corticosterone levels, the time between the first handling of the animals and sampling was less than 1 minute. This was an

important step, as glucocorticoids can increase within 3 minutes in the plasma after a stressful stimulus (Dallman, 2005). Blood samples were constantly kept on ice. The contained plasma and cell components were then separated by centrifugation for 15 minutes at 8000 rpm (revolutions per minute) at 4° C. Plasma was transferred to new, labelled 1.5 ml microcentrifuge tubes and stored at - 20° C. Corticosterone was measured using a standard radio immuno assay (RIA) (see chapter 2.6.3).

2.5.2 Brain tissue preparation

After decapitation, brains were dissected from the skull (see *Figure 17 A*), shock-frozen in 2-methylbutane (Carl Roth GmbH, Karlsruhe, Germany) and were stored at - 80° C for ISH (see chapter 2.6.1).

For immunohistochemistry (IHC, see chapter 2.6.2), animals were deeply anaesthetised by intraperitoneal (i.p.) injection of ketamine/xylazine (Sigma-Aldrich, Germany). After ensuring a sufficient anaesthetic depth by examining the hind limb pedal withdrawal reflex (Buitrago et al., 2008), animals were slowly perfused intracardially. Perfusion started with 0.9 % saline and was followed by 4 % paraformaldehyde (PFA). PFA leads to fixation of tissues by interlinking proteins. Upon completion of the perfusion, brains were extracted and additionally post-fixed overnight in 4 % PFA at 4° C (Chen et al., 2001). This was followed by three washing steps in 1 x PBS and an overnight incubation in 20 % sucrose solution at again 4° C. The sucrose solution served as cryoprotectant, partially dehydrating the tissue and thereby preventing the development of ice crystal, which could cause formation of artefacts. Until further use, perfused brains (see *Figure 17 B*) were stored at - 80° C.

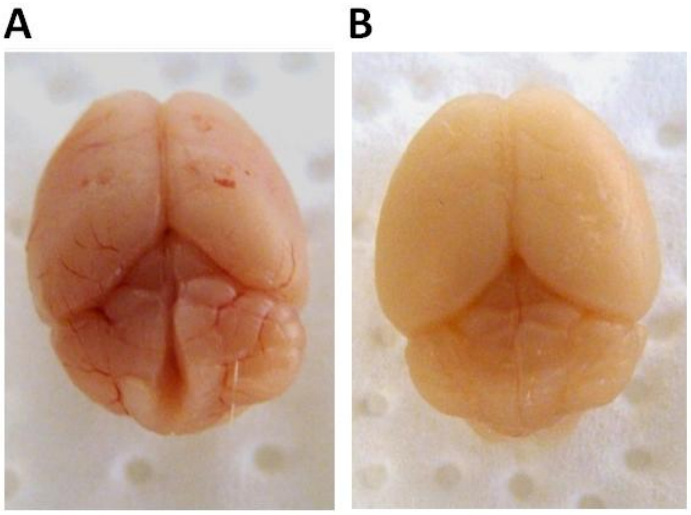

Figure 17: **Two differently treated mouse brains. (A)** Freshly dissected mouse brain (no perfusion) containing blood. **(B)** Bloodless mouse brain after perfusion.

For ISH, frozen brains were mounted on polyfreeze tissue medium (Tissue Tek O.C.T. Compound, Sakura Finetek, Staufen, Germany). Brains were sectioned at -20° C in a cryostat (Leica CM 3050, Bensheim Germany) in the coronal plane (four to five brain slices per slide). Slices were sectioned at 18 µm cutting thickness and thaw mounted on Super Frost Plus slides (Menzel GmbH, Braunschweig, Germany) coated with polylysine, which improves cell adherence. Conversely for IHC (free floating sections), Super Frost slides without coating (Carl Roth GmbH, Karlsruhe, Germany) were used (8 slices per slide). To ensure stability of the slices as well as a large yield, slice strength was 25 µm. For both cases, ISH and IHC, brain sections were dried on a hot plate at approximately 30° C and stored at -80° C. All efforts were made to prevent RNA degradation elicited by RNAse contamination.

For WB analysis, animals were decapitated and the brain was removed. Hippocampus extraction was performed on ice: the cerebral cortex covering the hippocampus had to be pulled up. The hippocampus was then separated from the surrounding tissue and carefully taken out. It was stored on ice in a 1.5 ml tube until homogenisation (see chapter 2.6.4).

2.5.3 Organ preparation

Adrenal and thymus glands were removed, stored on ice in tubes with physiological saline solution (0.9 % NaCl) and dissected from fat (Pearse, 2006b). Before weighing the organs, they were dried for a few seconds on a dust-free paper tissue. In aged animals, the extraction of the thymus was foregone, as it is known that with increasing age, the thymus gland undergoes a progressing involution (Birmingham and Grad, 1954; Delima and Walford, 1975).

2.6 Analytics

2.6.1 In situ hybridisation

Specific gene expression in brain slices was localised and quantified by means of labelled complementary ribonucleotide probes, which bind to the mRNA sequence of interest. Investigated brain regions were different areas of the dorsal hippocampal formation (CA1, CA3, DG) and the PFC. For our protocol, ^{35}S-UTP labelled ribonucleotide probes (NCAM, Nec 1, Nec 3, Nlgn 1, Nlgn 2, Nptn, Nrxn 1 and SynCAM) were used. Note that the Nptn ribonucleotide probe was designed to recognise both prevalent isoforms, Np 55 and Np 65. The Nec 3 ribonucleotide probe was designed to recognise all three prevalent splice variants, alpha, beta and gamma. These three isoforms differ in their size from alpha being the biggest splice variant to gamma being the smallest (Satoh-Horikawa et al., 2000). The respective primer sequences for in situ probe design are depicted in *Table 4*. ISH was performed as described previously (Schmidt et al., 2002; Schmidt et al., 2007). Briefly, sections were fixed in 4 % PFA and acetylated in 0.25 % acetic anhydride in 0.1 M triethanolamine/HCl. Subsequently, brain sections were dehydrated in increasing concentrations of ethanol. The antisense mRNA probes were transcribed from the respective linearised plasmid. Sections were saturated with 90 µl of hybridisation buffer containing approximately 1.5×10^6 cpm (counts per minute) ^{35}S-UTP labelled riboprobe. Afterwards, brain sections were coverslipped and incubated overnight at 55° C. The following day, sections were rinsed in 4 x SSC (standard saline citrate), treated with RNAse A (20 mg/l) and washed in increasing concentrations of SSC solutions at room temperature.

Finally, sections were washed in 0.1 x SSC for 1 h at 65° C and dehydrated through increasing concentrations of alcohol. The slides were exposed to Kodak Biomax MR films (Sigma-Aldrich, Germany) and developed. Exposure durations varied for the different nCAMs between a minimum of 10 hours for Nptn and a maximum of 2 weeks for Nec 1. Autoradiographs were digitised and relative expression levels were determined by optical densitometry utilising the freely available computer program Scion Image (Beta 4.0.3, Scion Corporation, Frederic, USA). The mean of four measurements of two different brain slices was calculated for each animal. The data were analysed blindly, always subtracting the background signal of a nearby structure not expressing the gene of interest from the measurements.

Table 4: **Primer sequences for in situ probe design.**

name	forward primer (5' to 3')	reverse primer (5' to 3')	insert size (bp)
NCAM	GATCAGGGGCATCAAGAAAA	GGAGGCTTCACAGGTCAGAG	475
Nec 1	GGCCATCTACAACCCGACTA	AAACGGTAACGGCTGATGAC	405
Nec 3	AGCCGTTACATTCCCACTTG	ATTGTCCATCCAACCTGCTC	485
Nlgn 1	GGGGATGAGGTTCCCTATGT	GGATCATCCTGTTTGGCAGT	458
Nlgn 2	TGTGTGGTTCACCGACAACT	CTCCAAAGTGGGCAATGTTT	401
Nrxn 1	AGTTGTACCTGGGTGGCTTG	TCACACGTCCTGCATCTAGC	495
Nptn	GAGGATTCAGGCGAATACCA	TTTCAGCCAGAATTCCCAAG	419
SynCAM	GAAGGACAGCAGGTTTCAGC	CTAGATAGCGCTGGGTCTGC	431

2.6.2 Immunohistochemistry

IHC was utilised to confirm that mimetic peptides crossed the blood brain barrier via s.c. injections. It is a technique based on the principle of antibodies binding specifically to antigens in biological tissues (Ramos-Vara, 2005).

Here, immunofluorescence for free-floating brain sections was conducted via an indirect approach (see *Figure 18*): one antibody (unconjugated goat-anti-biotin) was employed against the antigen being investigated; a second labelled antibody

(donkey-anti-goat) was utilised against the first one. Briefly, after transferring the brain slices from the slides into the net wells, a short pre-treatment with 0.2 % Triton X-100 was applied. This step macerates the cells, thereby preparing the samples for the incubation with the first antibody and enabling a broad and more even coverage. To clean the samples from this detergent, several washes in 1 x PBS were interposed. Background staining was reduced by treatment with blocking serum for 1 hour (with serum from the host species of the second antibody), in this case donkey serum. Afterwards, the samples were incubated with the first antibody overnight at 4° C. The following day, incubation with the second (fluorescence) antibody was conducted for 1 hour at room temperature. From here it was important to protect the slides from direct light incidence, thus each step was performed in a semi-dark room. To wash out the second antibody, samples were repeatedly rinsed in 1 x PBS and finally in distilled water. Afterwards, sections were mounted on Super Frost slides, dried in a horizontal position, dehydrated in ascending concentrations of ethanol and dried again. At last, the sections were covered with Vectashield Mounting Medium and stored light-proof at 4° C until microscoping.

As the injected peptides were biotylised during their synthesis, the employed first antibody (unconjugated goat-anti-biotin) specifically recognised the biotin-labelled mimetics, which were meant to spread from the injection site via the bloodstream through the body, finally across the blood brain barrier and into the brain. The second antibody (donkey-anti-goat), specifically bound to epitopes of the host species from which the first antibody was derived. It was directly linked to a fluorochrome (Alexa Flour 488) to enable immunofluorescence (Coons and Kaplan, 1950).

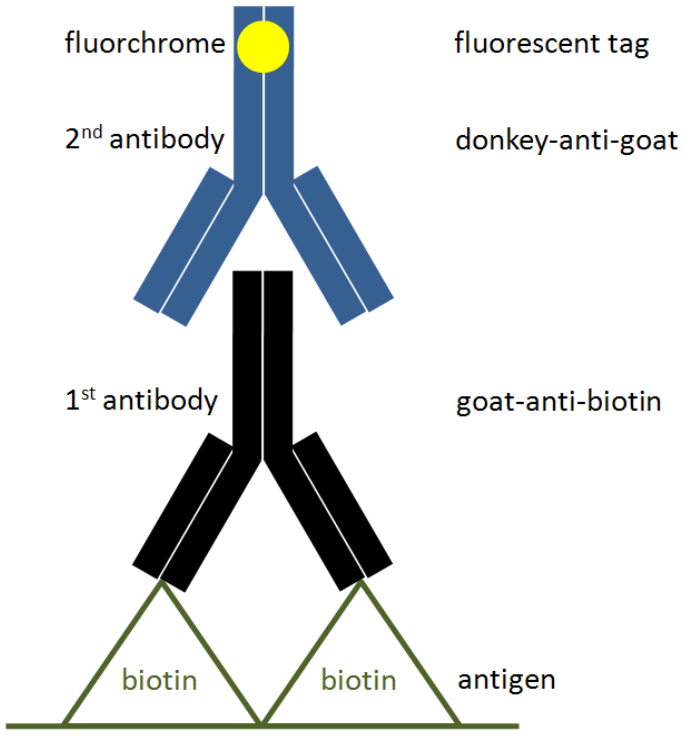

Figure 18: **IHC mechanisms.** The primary antibody (black) binds to the antigen (green), while the secondary antibody (blue), which is associated with a fluorchrome (yellow), binds to the first antibody. Via this indirect technique, the presence of biotylised mimetic peptides in the brain can be confirmed.

2.6.3 Corticosterone radioimmunoassay

For the quantitative analysis of plasma corticosterone (see chapter 1.1.2), a commercially available radioimmunoassay (RIA) kit (ImmunoChem™ Double Antibody Corticosterone ^{125}I RIA Kit, MP Biomedicals, LLC, Orangeburg, NY, USA; sensitivity of 6.25 ng/ml) was utilised. The RIA represents a competitive binding assay where endogenous corticosterone with unknown concentration (from the plasma samples) and a solution of radioactively (^{125}I) labelled corticosterone with a known concentration compete for a limited amount of binding sites on an anti-corticosterone antibody. This antibody binds to both labelled and unlabelled corticosterone as it does not differentiate between the two. The magnitude of labelled antibody-corticosterone-complex and the amount of endogenous and unlabelled corticosterone are in inverse proportion: the higher the corticosterone concentrations in the investigated sample, the lower the amount of binding of labelled corticosterone to the antibody and the lower the radioactivity that can be measured after centrifugation. After the specific binding of the second antibody, which only binds to antibody-corticosterone-complexes, the samples were

centrifuged (15 minutes at 3000 rpm at 4° C). This separates antibody-bound corticosterone from unbound corticosterone. The supernatant containing free corticosterone was discarded, while the radioactivity of the pellet containing precipitated antibody-antibody-corticosterone complexes was measured with a gamma counter (Packard Cobra II Auto Gamma; Perkin-Elmer, Waltham, MA, USA). For the quantification of the actual corticosterone concentrations from the samples, a standard curve built from known corticosterone concentrations had to be generated. Gamma counter values were compared to the standard curve, thereby revealing the absolute concentrations of corticosterone from the samples (Shimizu et al., 1983).

Processing of all blood samples was performed according to the manufacturers' manual. To obtain results in the optimum range of the standard curve, 10 µl of each plasma sample was diluted either 1:100 or 1:200, depending on the expected value of the sample. For instance, for basal samples with expected low corticosterone values, a higher concentration (1:100) was used and correspondingly, for CSS samples with expected higher corticosterone levels, the used concentration was lower (1:200). High and low controls were included in the kit and provided proof of a successful radioimmunoassay.

2.6.4 Western blot

The WB is a protein immunoblot, which enables the detection of specific proteins in a given tissue sample. By means of gel electrophoresis, denaturised proteins are separated by their molecular weight and afterwards transferred to a membrane. On the membrane, the investigated protein can be detected by use of an antibody specific for the target protein.

All hippocampi were homogenised in lysis buffer composed of Tris, sucrose and EDTA, as well as a ready-to-use mixture of different proteinase inhibitors (Sigma-Aldrich Chemie GmbH, Germany). Brain tissue was manually shredded by repeated pipetting, punching with the pipette tip and in the end via use of syringes of decreasing size. Homogenised hippocampi were shortly centrifuged to eliminate cell debris. The supernatant containing soluble proteins, plasma membranes and internal membranes, was used as protein sample. Protein concentration was

determined according to Lowry (1951) with the BioRad DC protein kit (BioRad, München, Germany). 40 µg protein per lane were loaded on 10 % SDS-Page. The proteins were separated by their molecular weight by means of gel electrophoresis. In the process, voltage conditions (120 V) were kept constant for 1.5 to 2 hours. To transfer the separated proteins to a nitrocellulose membrane (Protran BA85, 45 µm, Schleicher und Schüll, Dassel, Germany), the gel was blotted at constant current conditions (200 mA) for 40 to 50 minutes. The membrane was blocked for 1 hour in 5 % blocking solution, thereby reducing nonspecific binding. The last step on day 1 was the overnight incubation at 4° C with the respective primary antibody. All antibodies were diluted in TBS-T (dilutions: 1:500 to 1:5000). The following primary antibodies were used for WB analysis: Nptn (Abcam ab83063), Nlgn 1 (Synaptic Systems 129003), Nec 3 (Abcam ab633931) and Nec 1 (Abcam ab66985 and Santa Cruz sc-28639). Incubation with the second antibody (Goat-Anti-Rabbit IgG/HRP, DAKO P044801-2) lasted 2 hours and was conducted at room temperature. Antibody detection was performed with Amersham ECL analysis systems according to the manufacturers' instructions. The resulting ECL signal was exposed to Super-RX-films (Fujifilm Medical X-Ray film, Amersham Buchler, Braunschweig, Germany) with varying exposing times from several seconds (for example 10 seconds for samples of aged animals for Nec 1) to several minutes (for example 3 minutes for Nlgn 1). To ensure the efficacy of the protein transfer and to check if the membrane contained same amounts of protein for the different samples, the membrane was stripped with mild stripping buffer (Abcam, Cambridge, UK) and then incubated overnight at 4° C with a primary Actin antibody (Santa Cruz sc-1616). Actin is ideal to pose as control protein: it is part of the cytoskeleton, therefore apparent in all eukaryotes and can be found to a similar extent in different individuals of the same species. Hence, for samples containing the same amount of protein, a similar Actin signal was expected.

On day 3, incubation with the second antibody (Polyclonal rabbit-anti-goat IgG/HRP, Abcam) lasted 2 hours at room temperature. Antibody detection was performed as described before with Amersham ECL analysis systems. Blot autoradiographs were digitised and quantified by densitometry using Quantity one 4.6.2 analysis system (Bio-Rad, München, Germany). All data were expressed as

relative grey values. The measured adjusted volume of a specific nCAM was divided by the measured adjusted volume of the respective Actin. As a result, the values were normalised to the amount of the respective protein content. By setting the control group (for example basal animals without any testing experience) to 100 %, the relative percentages of other groups (for example animals after learning) were calculated.

2.7 Behavioural testing

Behavioural tests were performed in the same room, where the animals were housed. All tests were carried out between 8:00 am and 12:00 pm to minimise effects of hormonal variations elicited by the circadian pulsatility of glucocorticoids (Lightman et al., 2008). After each trial, behavioural apparatuses were freed from faeces and sensory traces with water to limit olfactory cues for following animals. If animals were tested in several paradigms one after the other, testing was ordered from least to most stressful to minimise the likelihood that behavioural performance would be influenced by previous testing experience (McIlwain et al., 2001). Tracking of the animals was accomplished by means of an automated video tracking software (Anymaze 4.20; Stoelting Co., Wood Dale, IL). To exclude an apparatus bias, animals with different treatment and condition were randomly distributed. The illumination was conducted in an equal manner for all setups. All specific apparatuses are described below.

2.7.1 Object recognition test

Recognition memory is the ability to distinguish the familiarity of things that were previously encountered. Our OR protocol is based on the innate propensity of rodents for novelty (Ennaceur and Delacour, 1988) and their capability to remember earlier encountered objects. The OR test consisted of an acquisition trial of 10 minutes, an intertrial interval (ITI) of 30 to 35 minutes and the retrieval trial, which lasted 5 minutes. The apparatus is depicted in *Figure 19 A & B*. During the acquisition trial, a single object (glass saltshaker) was positioned in the centre of an open field box (OF box: 50 cm x 50 cm x 50 cm (l x w x h)) and the animal

was free to explore its environment and the object. During the ITI, animals recovered in their respective home cage. For the retrieval trial, the saltshaker used during the acquisition (referred to as known object) was exchanged with a clean and olfactory neutral replica. The replica and a novel object (metal double nipple) were positioned in the two opposite corners of the OF. By these means, the animal could neither use any olfactory nor any spatial cues related to the objects' positions. The animal was allowed to explore the OF and the two objects. For the analysis, the focus lied on the animal's interaction with the objects, which is mainly represented by sniffing the objects. To detect the animals' interaction as object exploration, the nose-point of the mouse had to be within the object zone surrounding the object. Behaviours like using the object as platform to attempt escape from the OF box were not rated as object exploration.

As CD1 mice have a high level of novelty-seeking behaviour, it was expected that an animal able to remember the known object, will spend more time exploring the novel object. The percentage of the duration exploring the unknown object compared to the duration in percent exploring the novel object was calculated. A higher preference for the novel object was rated as intact recognition memory. For our protocol and experimental procedure, this was a mostly hippocampus-independent process. Other studies (Ennaceur et al., 1997; Gaskin et al., 2003; Winters et al., 2004) have confirmed that to judge the familiarity of complex objects, the hippocampus itself and an array of interconnected limbic structures is not fully required.

Both objects were easy to distinguish, but at the same time similar in height and width (see *Figure 19 C & D)*. It has been shown that OR tests are useful models to assess recognition memory in rodents (Dodart et al., 1997). During testing, automated scoring of OR behaviour was not sufficient. Instead, an unbiased person blind to the experimental groups, rescored the recorded tests afterwards.

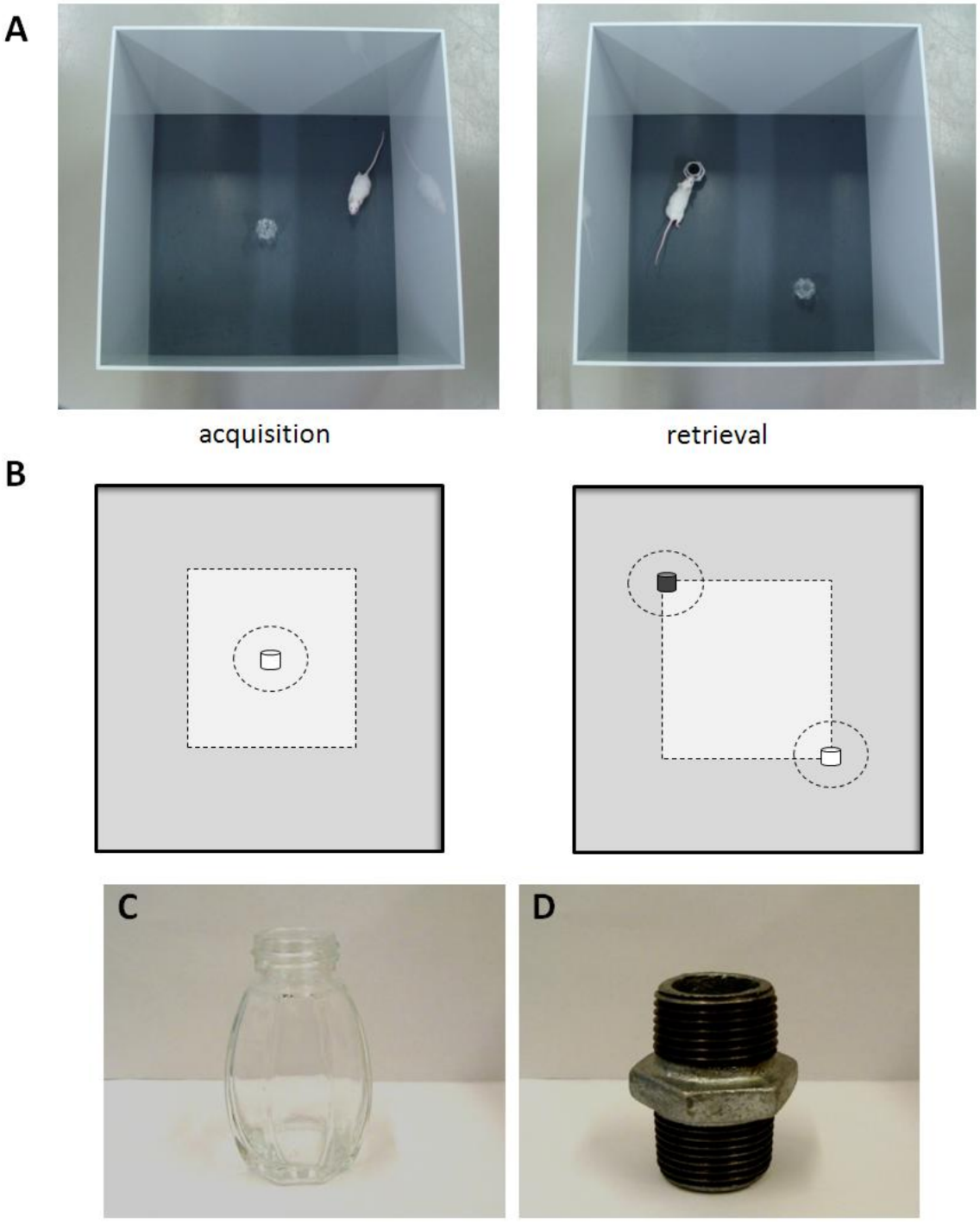

Figure 19: **Object recognition test. (A)** Experimental setup. **(B)** Schematic overview. The OF box is virtually divided into an inner zone (dashed square with 25 cm x 25 cm) and an outer zone. The object zone surrounding the objects is depicted as dashed circle.**(C)** Saltshaker from trial 1, referred to as known object during trial 2. **(D)** Double nipple, referred to as novel object (only apparent in trial 2).

2.7.2 Y-maze design 1: spontaneous alternation behaviour

The grey polyvinyl Y-maze apparatus (see *Figure 20*) consisted of three arms (30 cm x 10 cm x 15 cm (l x w x h)), which were identical except for the inner labelling. Arms were positioned at equal angles of 120°. One arm was marked with triangles, the second arm with bars and the third arm with plus signs, all made from white adhesive tape. These bright visual cues were easy to distinguish from the dark Y-maze walls and enabled the animals to differentiate between the three arms. Additionally, there was one visual cue (sun, half-moon or star) on each of the three walls surrounding the apparatus. Via personal communication by M. Wolferstätter (research group Landgraf), it could be excluded that mice have a preference for specific marks.

The centre of the Y-maze was the starting point. Each animal had 5 minutes to explore the three accessible arms. To be able to calculate the spontaneous alternation of the animals, the tracking system recorded the animals' full rotations, among other things. A full rotation is carried out if the mouse visits all three arms one after the other without reverting to a previously visited arm. An arm entry was counted, when all four limbs of the mouse were within an arm. Alternation behaviour is thought to reflect working memory capacity (Sarter et al., 1988). A high percentage of full rotations were detected as intact working memory. The percentage of spontaneous alternation for each animal was calculated as follows:

$$\frac{\left[\left(\sum \text{full rotations}\right) \times 3 - 2\right] \times 100}{\sum \text{arm entries}}$$

Animals with a high spontaneous alternation exhibited a high number of full rotations, whereas animals with a low spontaneous alternation tended to return to the arm they just had visited and hence displayed a small number of full rotations.

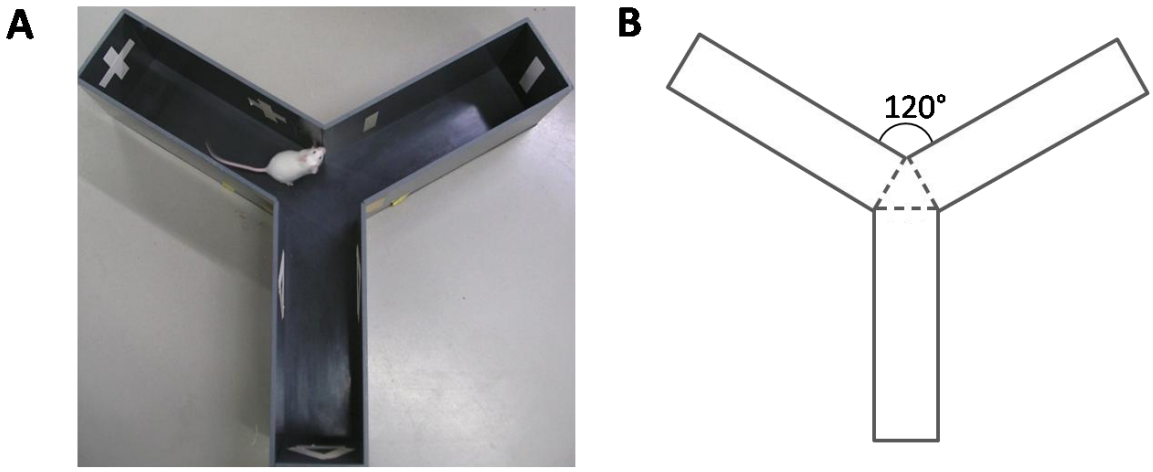

Figure 20: **Spontaneous alternation Y-maze test. (A)** Experimental setup. **(B)** Schematic overview. All three arms were accessible for exploration.

2.7.3 Y-maze design 2: spatial memory

For this task, the same apparatus (see *Figure 21*) as described above was used. In contrast to design 1, this test comprised of two trials, the acquisition (10 minutes) and the retrieval trial (5 minutes), separated by an ITI of 30 minutes, which the animals spent in their respective home cage. During the acquisition, a removable PVC board blocked one of the three arms. The inaccessible arm was referred to as the novel arm during the retrieval. At the beginning of the acquisition, the mouse was placed into the central zone facing one of the two accessible arms. By exploring the environment, the animals had the possibility to memorise the spatial orientation of these arms based on the inner- and extra-maze cues. It is known that mice do not rely on nonspatial cues, but rather on visuospatial orientation to solve a task like the Y-maze (Dellu et al., 2000). After 30 minutes ITI, the animals were reintroduced to the apparatus with the centre zone as their starting point and now all three arms accessible for exploration. Previous studies have shown that mice in general seek to explore novel environments and that CD1 mice have a spatial memory span of 1 hour (Dellu et al., 2000). Hence, it is expected that animals, which remember the spatial constellation from the acquisition, prefer to reside in the novel arm. The Y-maze test has been confirmed as a valid method to test hippocampus-dependent spatial memory (Dellu et al., 1992; Olton and Markowska, 1994; Conrad et al., 1996). To assess spatial memory performance, the percentages of time spent in the novel

arm compared to the percentages of the time spent in the known arms were calculated. The number of entries into the novel arm was measured as well.

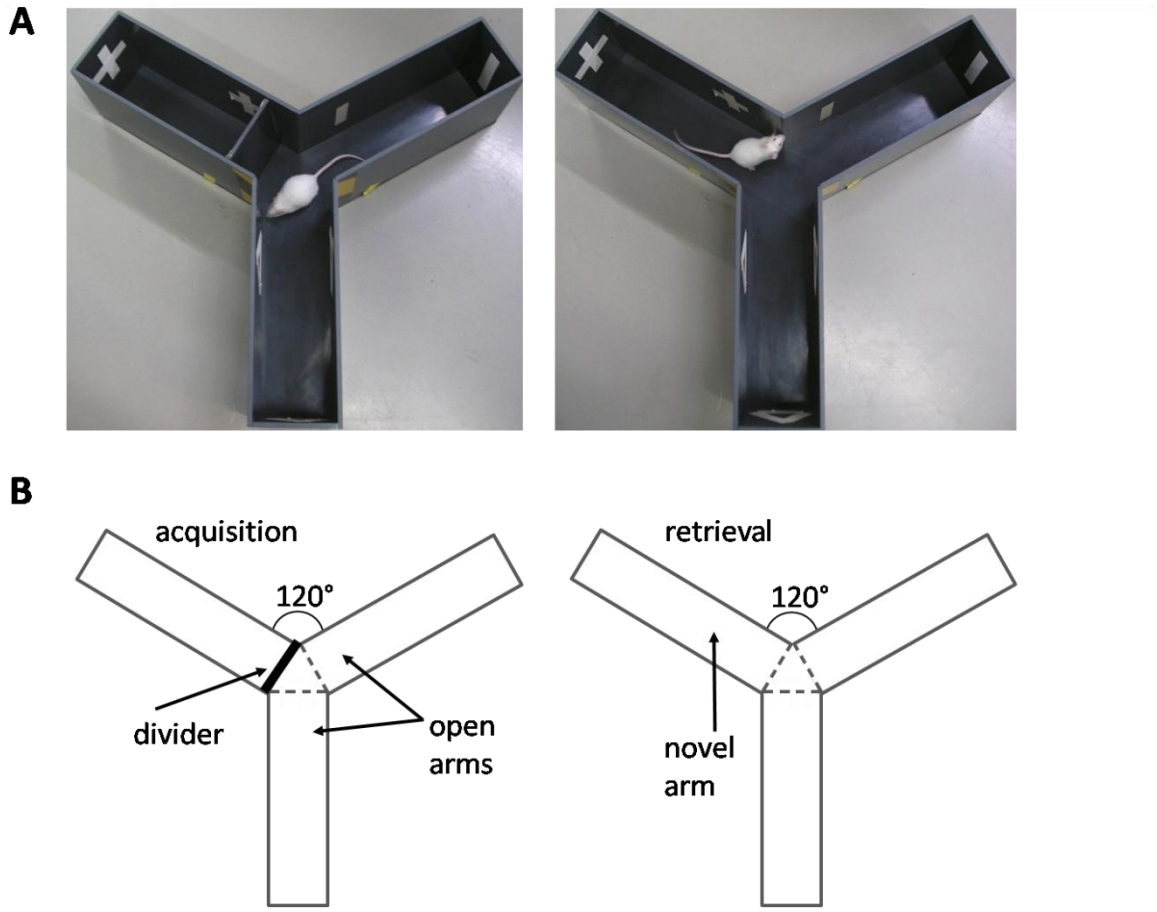

Figure 21: **Spatial Y-maze test. (A)** Experimental setup. **(B)** Schematic overview: during trial 1, only two arms were accessible, while during trial 2, all three arms were free for exploration.

2.7.4 Morris water maze

The MWM, originally described by Richard Morris (1984), has become one of the most frequently used scientific tools in behavioural neuroscience. Today, it is a standard test to assess hippocampus-dependent memory performance and spatial learning in rats and mice (D'Hooge and De Deyn, 2001).

The test was carried out as described previously (Sterlemann et al., 2010) with some adaptations. One day before the start of the MWM, the animals' backs were coloured from head to tail with a blue band (Porcimark marking spray, Kruuse, Denmark) to facilitate automatic tracking by the video system. Animals were tested in a water filled circular pool (110 cm in diameter) at room temperature (21 ± 1° C),

where the water reached a height of approximately 20 cm (see *Figure 22 A*). The water was rendered opaque by mixing it with chalk. This ensured that the submerged, white-painted platform (10 cm in diameter) blended with the background and hence stayed invisible. Several extra-maze visual cues were attached to the walls in a distance of 50 to 100 cm, clearly visible for the animals. Animals were supposed to navigate a direct path to the hidden platform and escape the water, which posed an aversive environment, by using the spatial information delivered by the distal cues around the pool. The area of the basin was virtually divided into four quadrants called back left, back right, front left and front right (see *Figure 22 B*). For each trial, which lasted 60 seconds, the mouse was carefully released into the water. In case of repeated trials, there was an ITI of 10 minutes. After each swim, animals were gently dried with a towel and then could recover in their home cage, which was kept under a red heat lamp to avoid hypothermia.

On the first day of the MWM, animals completed a single free swimming trial without a platform. During this pre-training, animals grew accustomed to the water, to the swimming and the whole apparatus. Besides that, potential preferences for a certain quadrant could be excluded. The second day served as visual training with a visible platform elevated 1 cm above the surface. Animals completed four subsequent trials, while the location of the platform varied for each trial. The starting position of the mice was always located in the opposing quadrant to the target. If animals succeeded in navigating to the target and climbed onto it, they were immediately removed from the basin. If they failed, animals were guided to the target by hand and placed onto it for a few seconds before rescue. By these means, animals got accustomed to the procedure that climbing onto the platform ends the test, thus terminates the exposure to the aversive environment. After a recovery period of 2 days, animals received a spatial training for 3 days, where the platform was submerged 1 cm beneath the surface. On each day, animals performed in four consecutive trials with varying starting positions, while the location of the platform remained the same (quadrant back-left). During this phase, escape latencies as well as distance travelled were measured. The next day, the platform was removed to conduct a probe trial and measure the animals' preference for the quadrant, where the target had been one day ago. The

objective of this single trial was to determine if the animal remembered the target's position learned during spatial training. Therefore, the time spent in the different quadrants was measured. After 3 days of recovery, animals had to perform a reversal learning task. The submerged and invisible platform received a new location (quadrant front-right) in the opposite quadrant compared to the spatial training. During four subsequent trials, the animals' cognitive flexibility to learn a new platform position was assessed. Cognitive flexibility implies the ability to inhibit a previously learned strategy in order to develop a novel strategy more fitting and appropriate to the change in demands (Clapcote and Roder, 2004). Parameters of interest were the escape latency and distance travelled to reach the target. The timetable for the whole procedure is depicted in *Figure 22 C*.

Spatial learning as well as cognitive flexibility were estimated across repeated trials. Short escape latencies were rated as intact spatial learning. Reference memory was assessed by capturing the preference for the platform area, when the platform was absent (Vorhees and Williams, 2006).

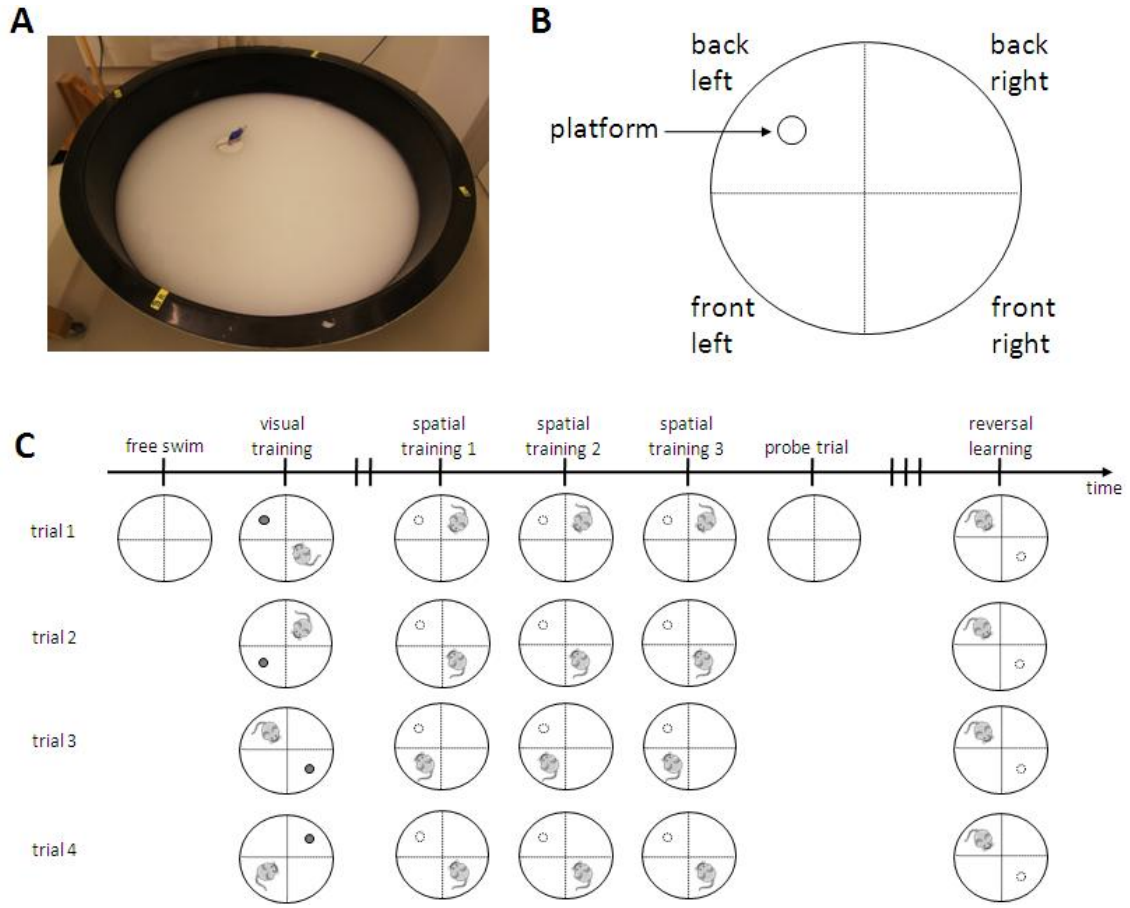

Figure 22: **MWM test. (A)** Experimental setup. **(B)** Schematic overview: the apparatus was divided into four quadrants, **(C)** Overview of the timetable with varying platform positions and varying starting positions of the animal. Free swim and probe trial did not include a platform and consisted of a single trial. Visual training, spatial training and reversal learning consisted of four trials per day. During visual training the platform was visible with varying positions, while during spatial training and reversal learning the platform was submerged beneath the water surface (dashed circle) in a fixed position. Tick marks on the time axis represent one day.

2.8 Statistics

For statistical comparisons, the commercially available software package SPSS 16.0 was used. Simple comparisons of two independent groups were made by two-tailed, unpaired t-tests for parametric measurements. All data comparisons concerning more than two groups were performed by the appropriate analysis of variance (ANOVA), which, in case of significance, was followed by post-hoc unpaired t-tests. For an interaction effect, significance was accepted at a level of $p < 0.1$, followed by post-hoc testing. For main effects such as group or condition effects, the significance level was set at $p < 0.05$. To compare multiple time points,

repeated-measures ANOVA was used. More than two groups without additional variables were compared via a one-way ANOVA. In the event of more than two groups with additional variables, a two-way ANOVA was applied to determine the target variable. For all cases (except interaction effects, see above), significance was accepted at a level of $p < 0.05$. One asterisk or one number sign indicate a significance level of $p < 0.05$; two asterisks or two number signs indicate a significance level of $p < 0.01$. To enable the estimation of the animals' OR performance based on the mRNA expression, a Pearson correlation was used. The Pearson correlation is specified with a number between - 1 and + 1 measuring the degree of association between two variables with correlations of - 1 and + 1 being a perfect correlation. The respective graphs were created with SigmaPlot 11.0 (Systat Software Inc., Chicago, IL, USA). All results are shown as means ± standard error of the mean (SEM).

3 Results

3.1 Experiment 1: Effects of CSS in young animals and the acute impact on novel synaptic CAMs

Region-specific changes of nCAM expression in young animals (11 to 13 weeks of age) after CSS exposure were analysed. Chronic stress influences physiological, neuroendocrine and behavioural factors. Thus, these parameters were included in the analysis as well. After the CSS exposure, animals were either tested in several learning paradigms (after learning) or remained naïve (basal) without testing experience.

3.1.1 Physiological data

In order to assess the impact of CSS exposure on physiological parameters, body and organ weights of basal animals were measured. Those data were acquired directly after the CSS exposure, on the day of sacrifice. Independent t-tests revealed no significant differences in body weight between the two groups (control and stress; $T_{26} = 0.294$; $p = 0.771$) as well as in thymus weight ($T_{26} = 1.305$; $p = 0.203$; see *Figure 23 A*). Nonetheless, for adrenal gland weights, which pose a sensible marker for stress effects, an independent t-test showed a significant increase in stressed animals ($T_{26} = 6.356$; $p < 0.01$; see *Figure 23 B*).

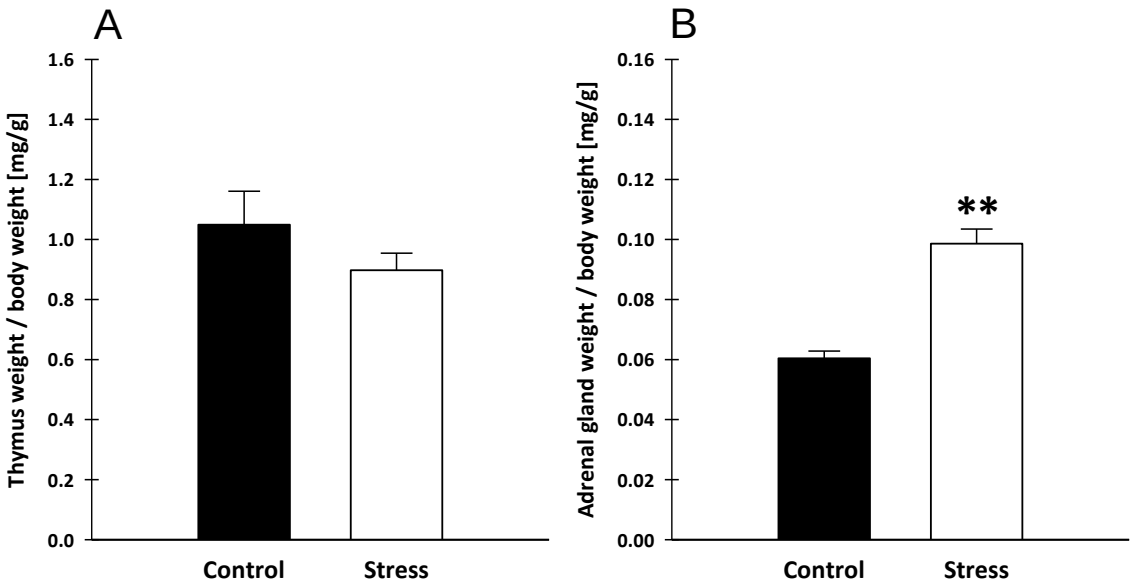

Figure 23: **Organ weights related to body weight. (A)** Thymus: directly after CSS exposure, there were no significant differences in thymus weights between the groups. **(B)** Adrenal glands: at the end of the CSS procedure, adrenal gland weights were significantly increased in stressed animals (** significantly different from the control group, $p < 0.01$).

3.1.2 Neuroendocrine data

For the analysis of neuroendocrine factors, blood samples were collected. Plasma corticosterone levels were determined in basal animals in the morning during the circadian nadir, in the last week of the CSS procedure. ANOVA revealed differences in corticosterone levels, based upon the condition of mice (basal or after learning; $F_{1, 55} = 48.157$; $p < 0.01$; see *Figure 24*). Post-hoc independent t-tests exposed significantly elevated levels in the basal stress group compared to controls ($T_{25} = 2.450$; $p < 0.05$; see *Figure 24*). Blood samples from animals with learning experience were acquired 2 hours after the last trial of behavioural testing (morning to midday), 2 weeks after the cessation of the CSS procedure. Within the batch of animals after learning, corticosterone levels did not differ significantly. However, post-hoc t-tests confirmed that the corticosterone levels of animals after learning were increased compared to basal animals within the same condition (control: $T_{24} = 6.557$; $p < 0.01$; stress: $T_{28} = 3.917$; $p \leq 0.01$; see *Figure 24*).

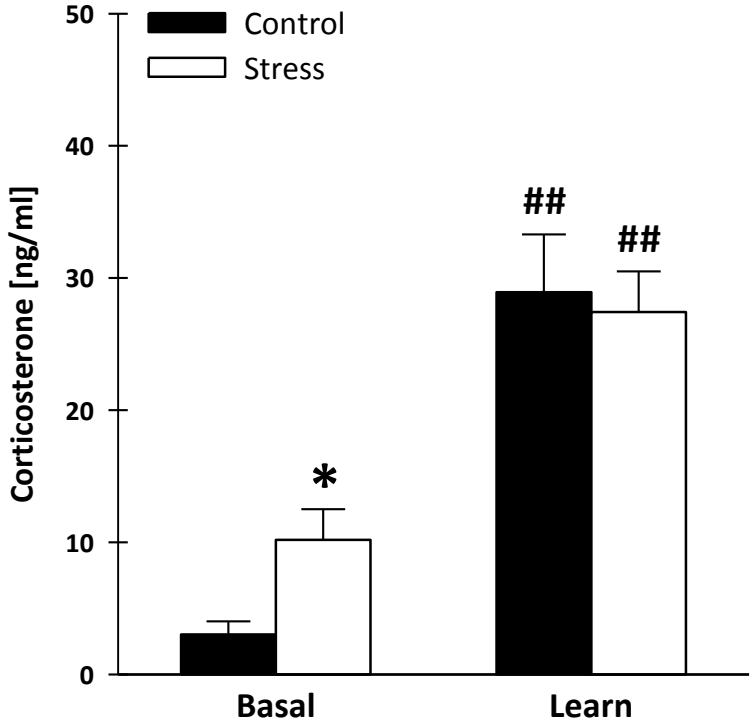

Figure 24: **Plasma corticosterone levels.** Basal animals revealed elevated corticosterone levels during the CSS procedure compared to control animals. Animals after learning showed increased corticosterone levels compared to basal animals (* significantly different from the control group, $p < 0.05$; ## significantly different from respective basal animals, $p \leq 0.01$).

3.1.3 Behavioural data

Several behavioural tests were conducted to examine the animals' general locomotion, cognitive performance and stress-coping behaviour. First, to observe hippocampus-independent short-term memory, the OR test was carried out. Significant differences were found within the control group: animals spent significantly more time with the novel object than with the known object ($T_{60} = 11.683$; $p < 0.01$; see *Figure 25*). In contrast, stress animals spent equal amounts of time with both objects. Additionally, control animals spent significantly more time with the novel object than stress animals ($T_{60} = 3.596$; $p \leq 0.01$; see *Figure 25*).

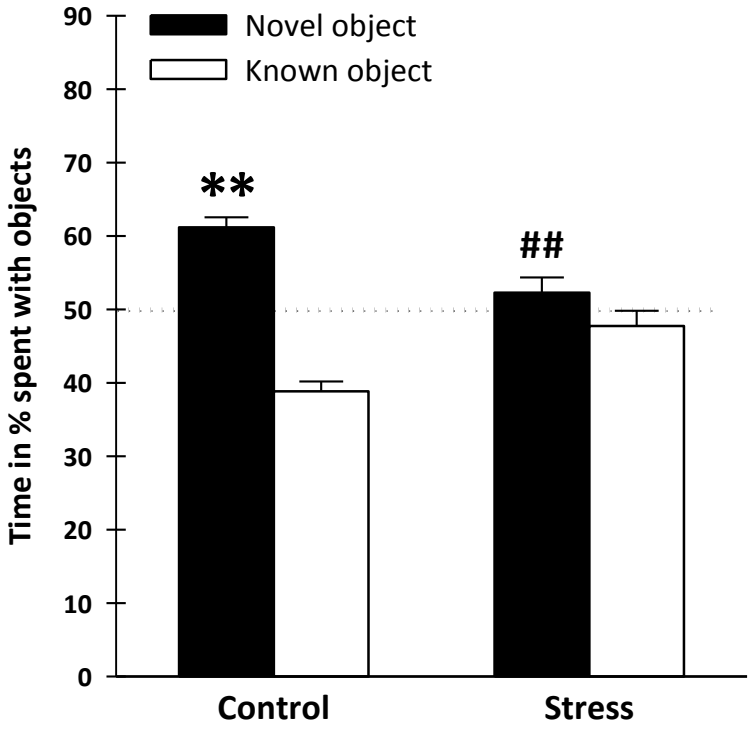

Figure 25: **Time spent with objects.** Control animals explored the novel object to a significantly greater extent than the known object. Also, controls took significantly more time to explore the novel object than the stress animals. The dotted line represents chance level at 50 % (** significantly different from known object time, $p < 0.01$; ## significantly different from control animals, $p \leq 0.01$).

Second, to test hippocampus-dependent short-term memory as well as general locomotion, animals had to perform in the spatial Y-maze. Both groups, control and stress animals, spent significantly more time in the novel arm than in the known arms (control: $T_{60} = 7.845$; $p < 0.01$; stress: $T_{53} = 4.902$; $p < 0.01$; see *Figure 26*). The results for novel arm time did not differ significantly between both groups ($T_{56} = 1.300$; $p = 0.199$). This finding was further supported by the analysis of the number of head entries into the arms: both groups entered the novel arm more often than the known arms (control: $T_{60} = 6.675$; $p < 0.01$; stress: $T_{54} = 5.885$; $p < 0.01$), but the total number of novel head entries did not differ between stress and control animals ($T_{56} = 0.814$; $p = 0.419$). The analysis of the parameters time immobile and total distance travelled disclosed that both groups were similarly active ($T_{58} = 1.061$; $p = 0.293$) and travelled an equivalent distance in total ($T_{59} = 0.730$; $p = 0.468$).

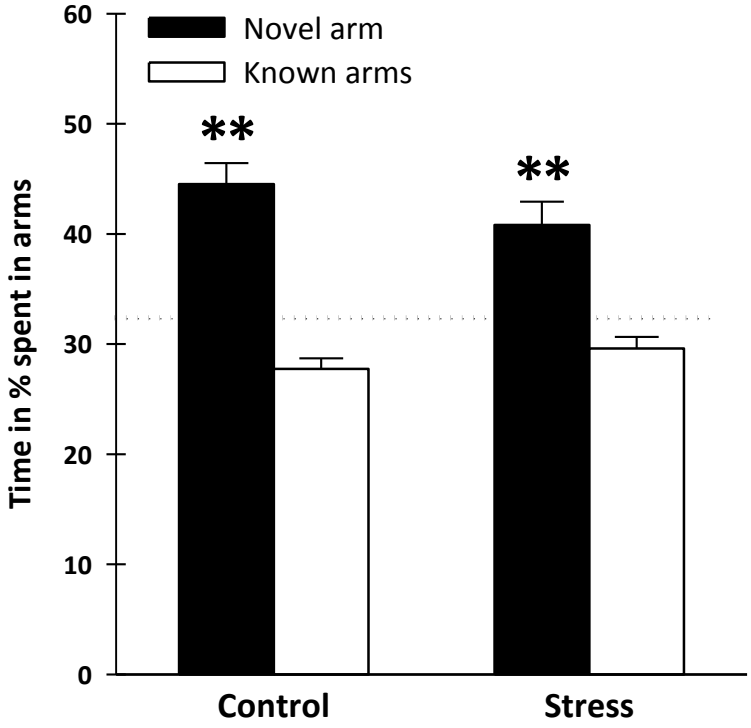

Figure 26: **Time spent in the arms of the Y-maze.** Control animals, as well as stress animals, spent significantly more time in the novel arm than in the known arms. No difference between the two groups (control and stress) was detected. The dotted line represents chance level at 33 % (** significantly different from known arm time, $p < 0.01$).

Third, animals had to perform in the MWM, which represents a test for hippocampus-dependent long-term memory. Over the course of the experiment, all animals appeared to learn as they approached the platform faster and faster: during the visual training, animals exhibited escape latencies between 35 to 55 seconds, while during the last trial of the spatial learning, escape latencies ranged between 10 to 25 seconds. Nevertheless, the results revealed no significant differences between control and stress animals, except for the spatial day 2. Here, a repeated measures ANOVA indicated a group effect (control or stress; $F_{1, 25} = 4.390$, $p < 0.05$; see *Figure 27*). Post-hoc t-tests supported the finding that at least for some trials (trial 2 and trial 3) on spatial day 2, the controls learned more effectively and reached the platform faster than the stress animals (trial 2: $T_{27} = 2.873$; $p < 0.01$; trial 3: $T_{28} = 1.785$; $p < 0.1$; see *Figure 27*). However, for trial 3, this finding was merely a trend. Similar results were produced by the data set containing the total distances travelled: there were no significant group effects (control and stress) except for spatial day 2, when the controls travelled

less until they found the platform (trial 2: $T_{26} = 2.500$; $p < 0.05$; trial 3: $T_{27} = 2.311$; $p < 0.05$).

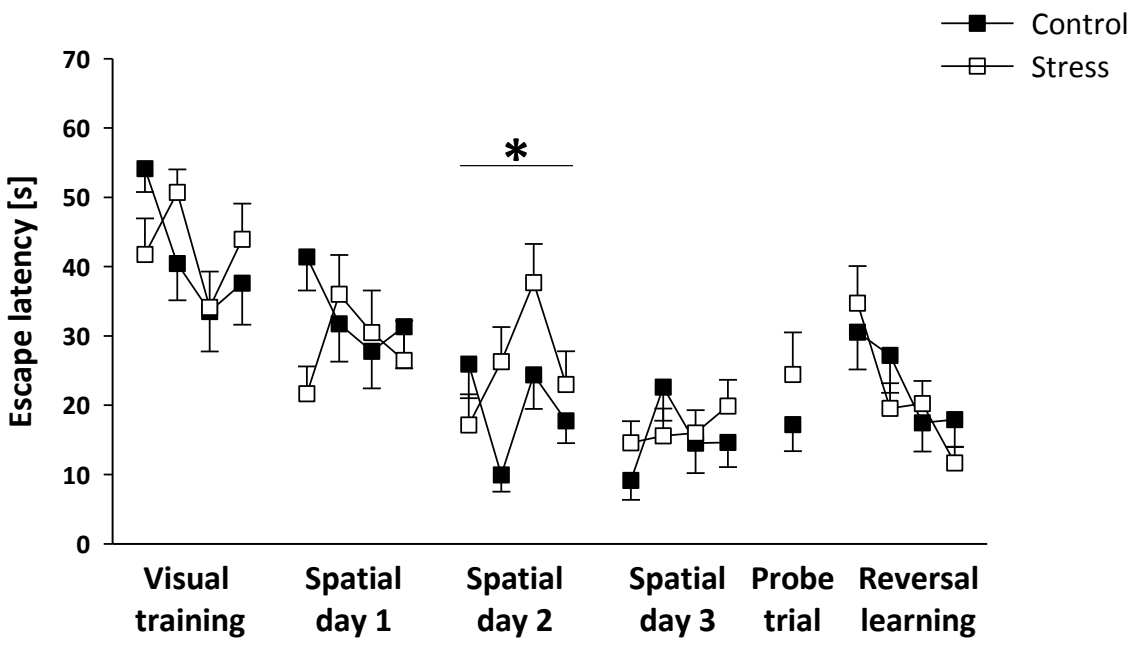

Figure 27: **Escape latencies in the MWM.** Overall, all animals exhibited reduced escape latencies over time. Only on spatial day 2, a significant difference between the two groups (control and stress) was detected (* significantly different from controls, $p < 0.05$).

To measure the animals' preference for the former target quadrant (the back-left quadrant, where the platform was localised during all trials of the preceding spatial training), a probe trial was carried out. Control animals, in comparison to stress animals, spent significantly less time in the former target quadrant and at the same time significantly more time in the other sectors of the basin (target quadrant: $T_{27} = 3.173$; $p < 0.01$; non-target quadrants: $T_{27} = 3.152$; $p < 0.01$; see *Figure 28*). Stress animals significantly preferred the former target quadrant compared to the non-target quadrants and spent most of the time there ($T_{28} = 7.358$; $p < 0.01$; see *Figure 28*). Those results could be reproduced by the data set for distance travelled per quadrant: stress animals travelled a longer distance in the former target quadrant than controls ($T_{27} = 2.315$; $p < 0.01$) and controls travelled a longer distance in the non-target quadrants than stress animals ($T_{26} = 2.492$; $p < 0.05$).

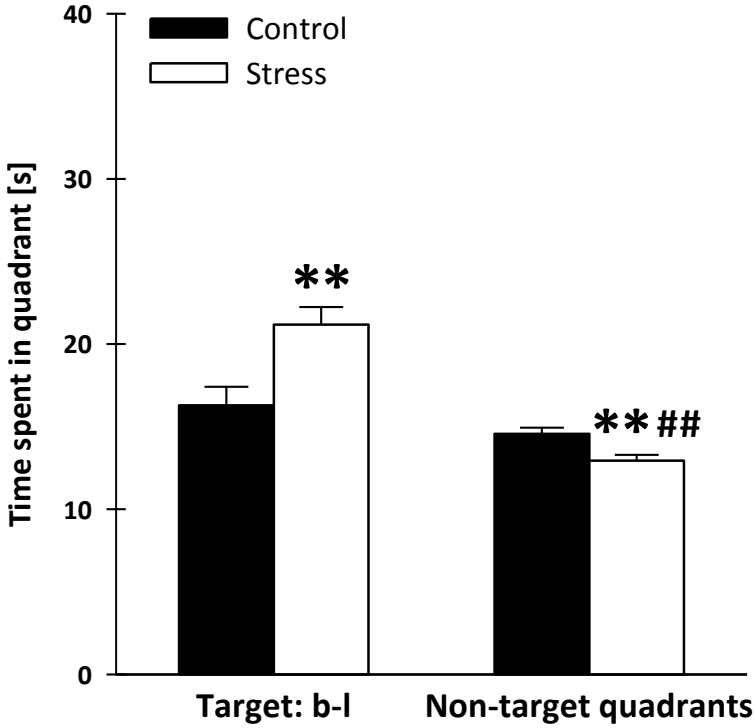

Figure 28: **Time spent in the quadrants of the MWM.** Stress animals were found to search for the platform mostly in the former target quadrant (back-left); compared to controls, they explored this quadrant significantly longer. They spent enhanced periods of time in the back-left quadrant compared to the non-target quadrants. By contrast, in comparison to stress animals, the controls spent more time in the non-target quadrants (** significantly different from controls, $p < 0.01$; ## significantly different from target quadrant, $p < 0.01$).

3.1.4 Expression levels of nCAM-mRNA

Expression patterns on mRNA-level were assessed both in the hippocampus and the PFC and for several nCAMs. While there were a variety of regulation patterns in the hippocampus for the different nCAMs (NCAM, Nec 1, Nec 3, SynCAM, Nlgn 1, Nlgn 2, Nrxn, Nptn), no effects at all could be demonstrated in the PFC. Thus, in the following, only the results for hippocampal expression levels are presented.

ANOVA indicated a difference based on the condition (before and after learning; $F_{1, 43} = 10.121$, $p < 0.01$; *see Figure 29*) for NCAM, which is a well-studied synaptic CAM that has been discovered almost three decades ago. Post-hoc t-tests showed a significant group effect in the DG between control and stress animals under basal conditions ($T_{19} = 2.090$; $p \leq 0.05$; see *Figure 29*): NCAM was up-regulated after stress experience. Within the “after learning” batch, no effect was present. Post-hoc t-test confirmed another finding: in stress animals, NCAM was

down-regulated after learning (T_{20} = 3.308; p ≤ 0.01; see *Figure 29*). No further significant effects could be demonstrated in the other regions of the hippocampus, CA1 or CA3. Until stated otherwise, a group effect indicates a significant difference between control and stress animals, while a condition effect relates to a significant difference between animals without learning (basal) and animals with learning experience (learn).

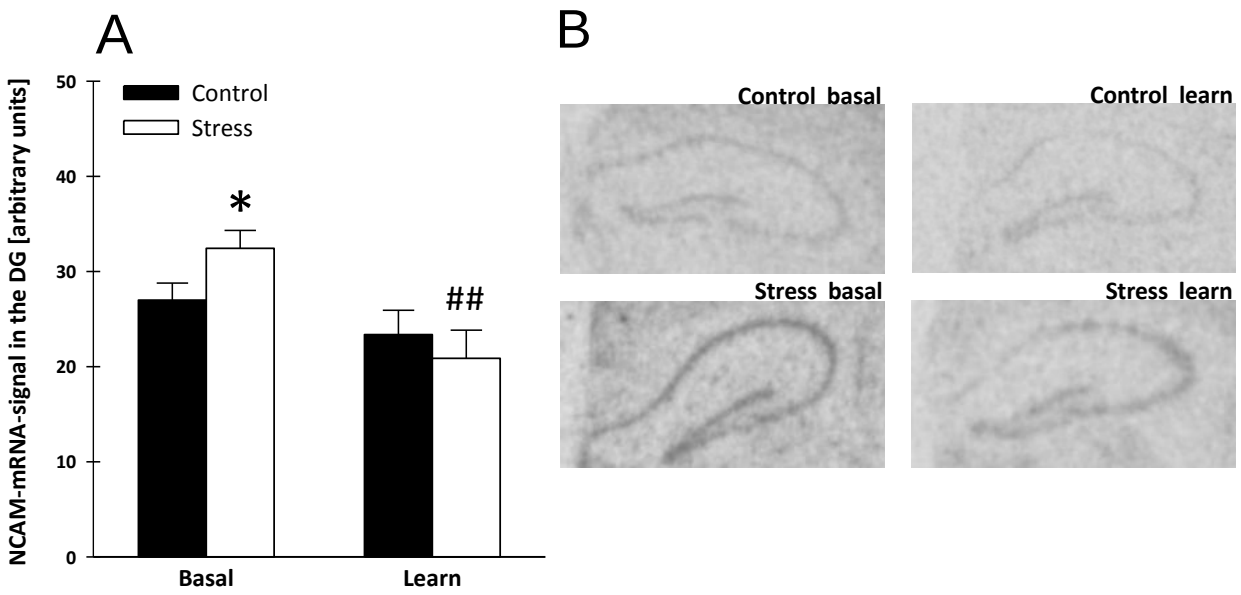

Figure 29: **NCAM-mRNA-signal in the DG. (A)** In stress animals under basal conditions, an up-regulation of NCAM-mRNA was detected compared to controls of the same condition. Additionally, there was an effect across conditions: after learning, NCAM-mRNA was found to be down-regulated in stress animals compared to the basal condition (* within the basal batch, significantly different from controls, p ≤ 0.05; ## significantly different from basal stress animals, p < 0.01). **(B)** Depicted are representative NCAM-mRNA autoradiograms of controls (at the top) and stress animals (at the bottom), either under basal conditions (on the left) or after learning (on the right). If not stated otherwise, this layout will be the same for all following autoradiograms.

Another group of important novel, synaptic CAMs consists of Nectins. They work as basic modules in synapse structure and function. The results for Nec 1 exposed differential regulations in the CA1 and the DG. In the CA1, ANOVA pointed toward both a group effect ($F_{1, 44}$ = 19.200, p < 0.01; see *Figure 30*) and an interaction effect of group*condition ($F_{1, 44}$ = 9.811, p < 0.01; see *Figure 30*). Further analysis via post-hoc t-tests revealed a down-regulation of Nec 1-mRNA in control animals after learning compared to the basal controls (T_{20} = 2.432; p < 0.05; see *Figure 30*) as well as an up-regulation of Nec 1-mRNA in stress animals after learning compared to the basal stress animals (T_{21} = 2.166; p < 0.05; see *Figure 30*).

Additionally, Nec 1-mRNA was up-regulated in stress animals after learning compared to the controls of the same condition (T_{21} = 4.940; $p < 0.01$; see *Figure 30*). There was no significant effect within the batch of basal animals.

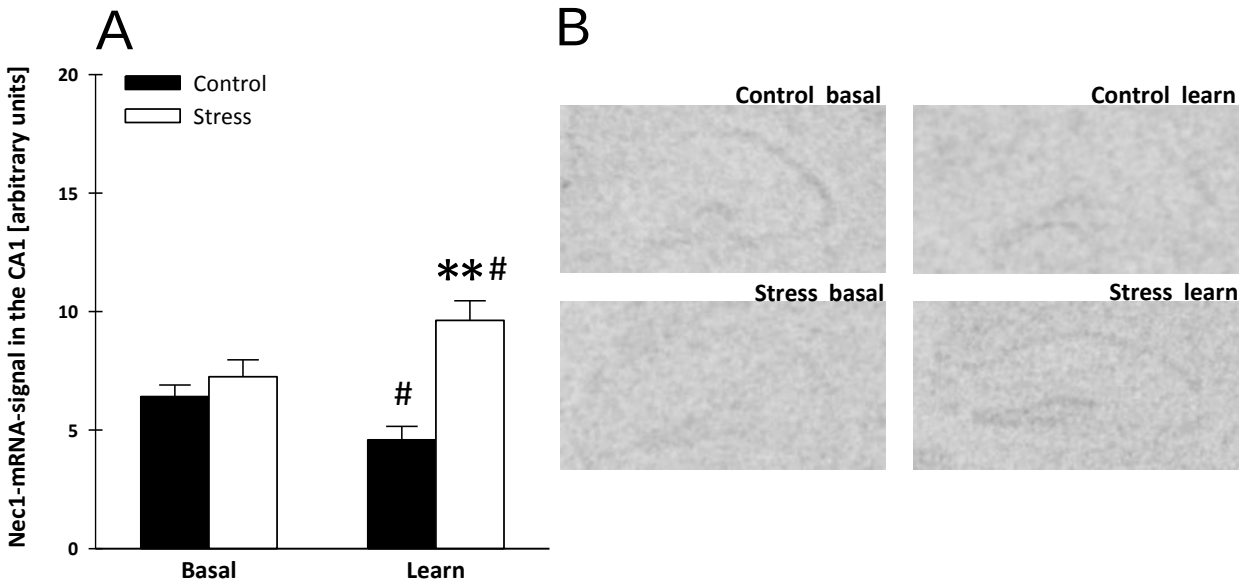

Figure 30: **Nec 1-mRNA-signal in the CA1. (A)** In controls after learning, a down-regulation of Nec 1-mRNA was identified compared to controls without learning experience. Within the batch of animals after learning, there was an up-regulation of Nec 1-mRNA in stress animals compared to controls. Along with this, Nec 1-mRNA was found to be up-regulated in stress animals after learning compared to the basal condition (** within the learn batch, significantly different from controls, $p < 0.01$; # significantly different from the respective basal animals, $p < 0.05$). **(B)** Representative Nec 1-mRNA autoradiograms are displayed.

In the DG, ANOVA disclosed a condition effect ($F_{1, 45}$ = 9.501, $p < 0.01$; see *Figure 31*) for Nec 1. In contrast to the results for Nec 1-mRNA expression in the CA1, there was a down-regulation of Nec 1-mRNA in stress animals after learning compared to the respective basal animals (T_{22} = 3.204; $p < 0.01$; see *Figure 31*). No further significant effects could be demonstrated in the DG.

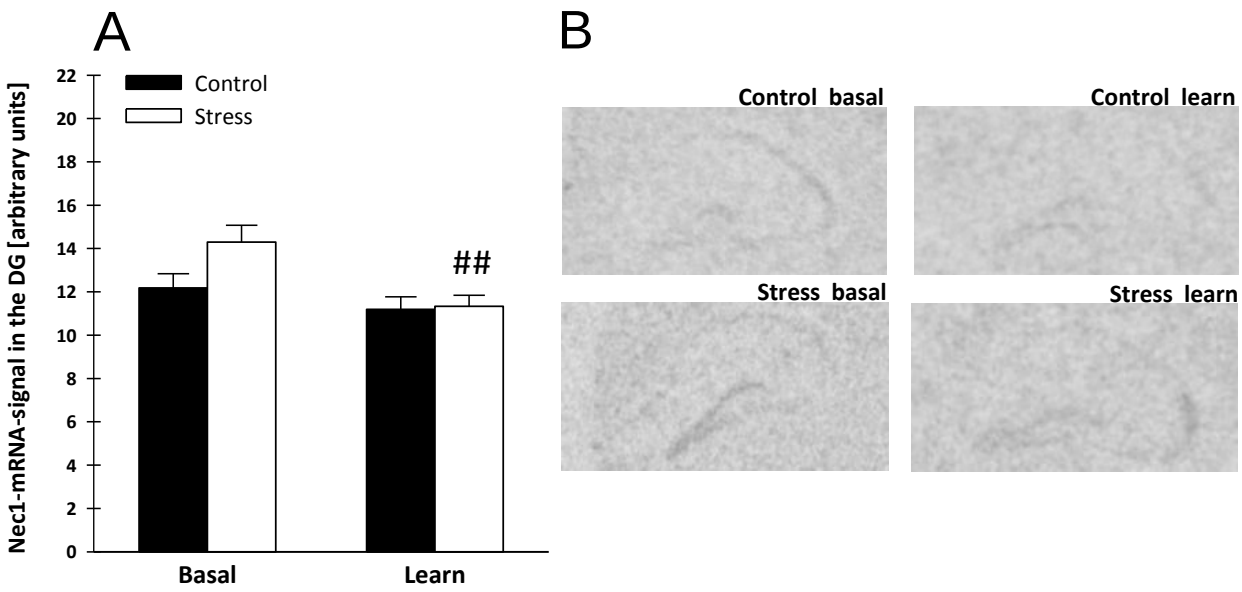

Figure 31: **Nec 1-mRNA-signal in the DG. (A)** In stress animals, there was an effect across conditions: after learning, Nec 1-mRNA was found to be down-regulated compared to the basal condition in stress animals (## significantly different from basal stress animals, $p < 0.01$). **(B)** Representative Nec 1-mRNA autoradiograms are shown.

Nec 1 is engaged in heterophilic interactions and forms adhesion complexes, for example with Nec 3, which is its strongest binding partner. The Nec 3-mRNA results disclosed both similarities and differences in the expression patterns compared to Nec 1. For Nec 3, significant results were found in the CA3 and the DG (in contrast to Nec 1 with significant differences in the CA1 and the DG): ANOVA revealed a condition effect ($F_{1, 45} = 9.116$, $p < 0.01$; see *Figure 32*) in the CA3; in stress animals, this effect withstood post-hoc t-tests ($T_{22} = 2.498$; $p < 0.05$; see *Figure 32*) and exposed a down-regulation of Nec 3-mRNA in stress animals after learning that was also shown for Nec 1 in the DG. A subsequent analysis of Nec 3-mRNA in the DG via ANOVA identified a group effect ($F_{1, 45} = 12.139$, $p < 0.01$; see *Figure 33*) as well as an interaction effect of group*condition ($F_{1, 45} = 7.334$, $p \leq 0.01$). Post-hoc t-tests were able to demonstrate a down-regulation in stress animals after learning for Nec 3 in the DG ($T_{22} = 3.145$; $p < 0.01$; see *Figure 33*), which was alike to the regulation patterns seen before both for Nec 1 in the DG and Nec 3 in the CA3. Furthermore, in animals after learning, Nec 3-mRNA was down-regulated in the DG of stress animals compared

to the controls (T_{20} = 4.893; p < 0.01; see *Figure 33*). This group effect remained elusive for Nec 1 and was limited to the DG for Nec 3.

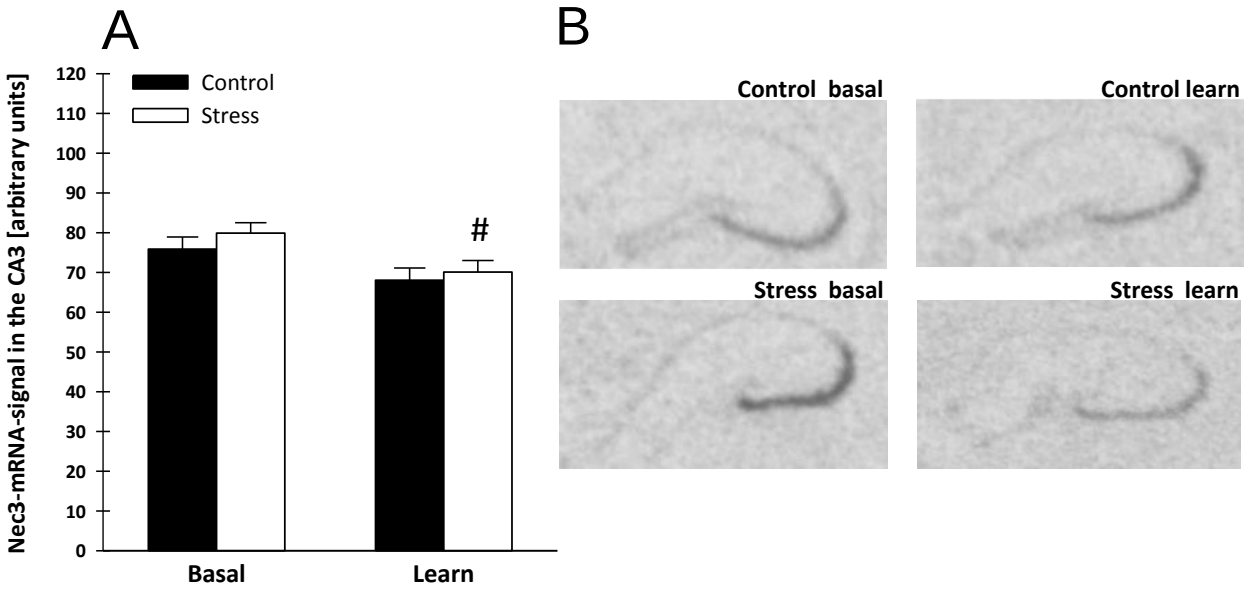

Figure 32: **Nec 3-mRNA-signal in the CA3. (A)** In stress animals with learning experience, a down-regulation of Nec 3-mRNA was detected (# significantly different from basal stress animals, p < 0.05). **(B)** Representative Nec 3-mRNA autoradiograms are displayed.

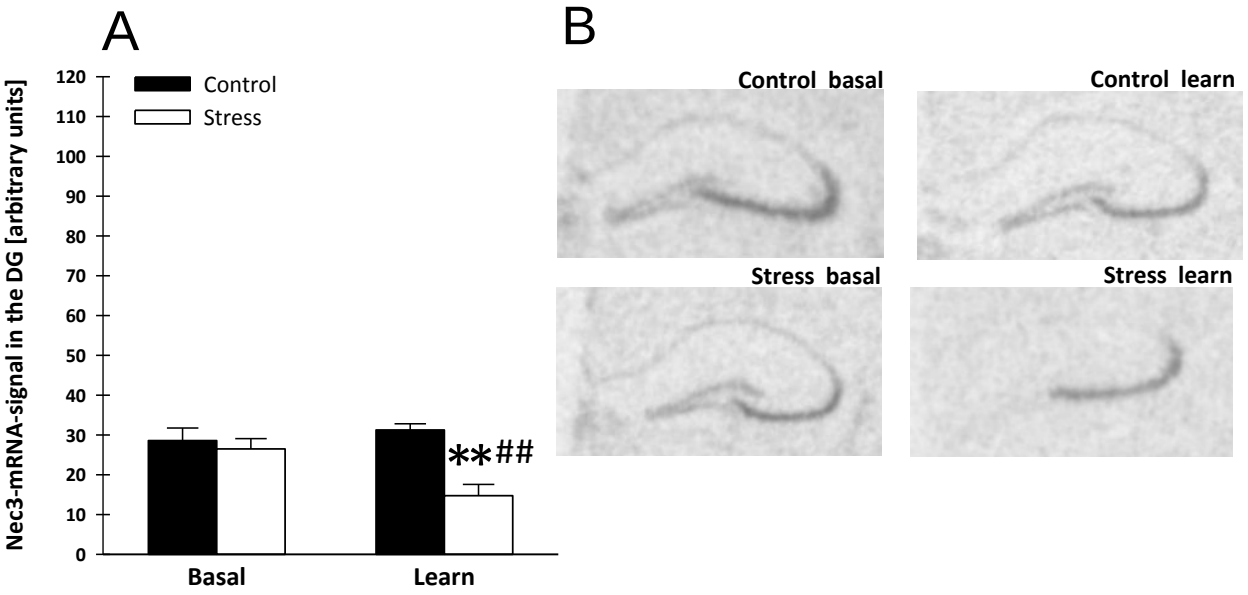

Figure 33: **Nec 3-mRNA-signal in the DG. (A)** In stress animals after learning, a down-regulation of Nec 3-mRNA was detected. In addition, there was a down-regulation in stress animals after learning compared to controls of the same condition (** within the learn batch, significantly different from control animals, p < 0.01; ## significantly different from basal stress animals, p < 0.01). **(B)** Representative Nec 3-mRNA autoradiograms are shown.

Nectins are structurally related to another group of novel CAMs, SynCAMs. Besides the structural resemblance, SynCAMs and Nectins interact in heterophilic adhesion processes. The results for SynCAM displayed significant effects with similar regulation patterns in the whole hippocampus (CA1, CA3, DG). In the CA1, ANOVA indicated an interaction effect of group*condition ($F_{1, 46} = 4.810$, $p < 0.05$; see *Figure 34*). Post-hoc t-tests were able to confirm a significant effect within control animals: SynCAM-mRNA was up-regulated after learning compared to controls under basal conditions ($T_{21} = 2.178$; $p < 0.05$; see *Figure 34*). Moreover, post-hoc testing revealed a significant effect within animals with learning experience: SynCAM-mRNA was down-regulated after stress exposure compared to controls ($T_{22} = 2.511$; $p < 0.05$; see *Figure 34*). In the CA3, ANOVA indicated a group effect ($F_{1, 46} = 6.117$, $p < 0.05$; see *Figure 35*): akin to the situation in the CA1, SynCAM-mRNA was down-regulated within the learn batch after stress exposure ($T_{22} = 2.735$; $p < 0.05$; see *Figure 35*). In the DG, ANOVA indicated a group effect as well ($F_{1, 45} = 7.447$, $p < 0.01$; see *Figure 36*): again, SynCAM-mRNA was down-regulated after CSS and learning compared to the respective controls ($T_{21} = 3.330$; $p < 0.01$; see *Figure 36).* In the DG, ANOVA also pointed toward an interaction of group*condition ($F_{1, 45} = 7.412$, $p < 0.01$; see *Figure 36*): within stress animals, SynCAM-mRNA was down-regulated after learning ($T_{21} = 2.178$; $p < 0.05$; see *Figure 36*).

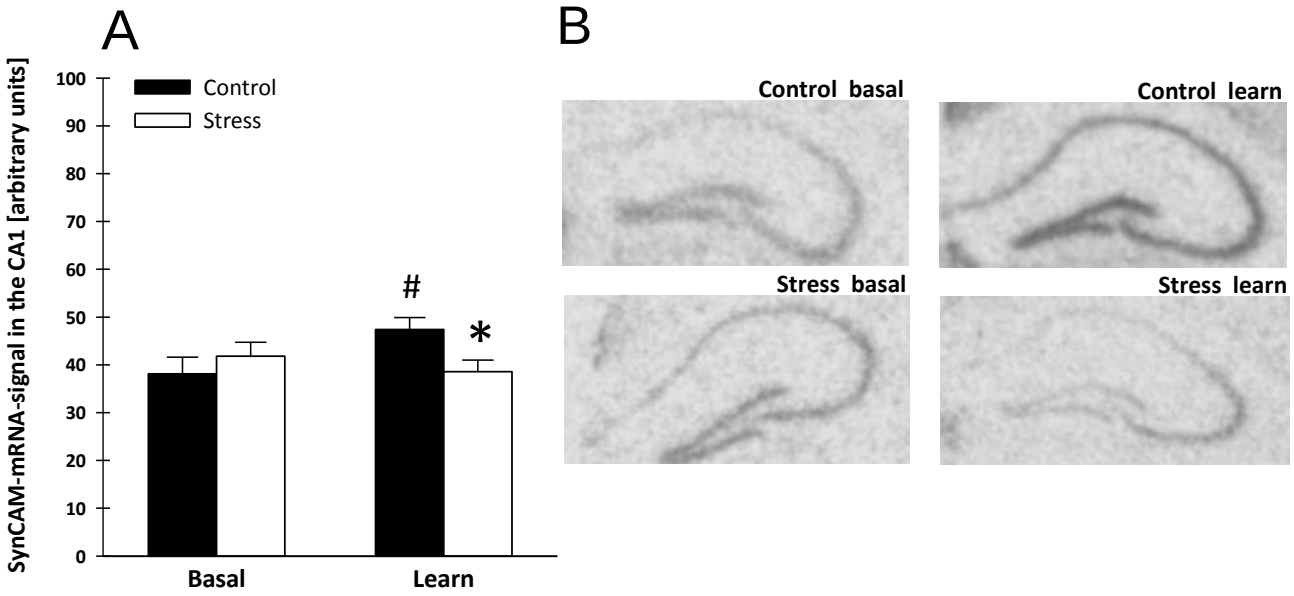

Figure 34: **SynCAM-mRNA-signal in the CA1. (A)** There was a down-regulation within animals with learning experience after stress exposure. Moreover, in control animals after learning, an up-regulation of SynCAM-mRNA was discovered compared to the basal controls (* within the learn batch, significantly different from controls with $p < 0.05$; # significantly different from basal control animals, $p < 0.05$). **(B)** Representative SynCAM-mRNA autoradiograms are shown.

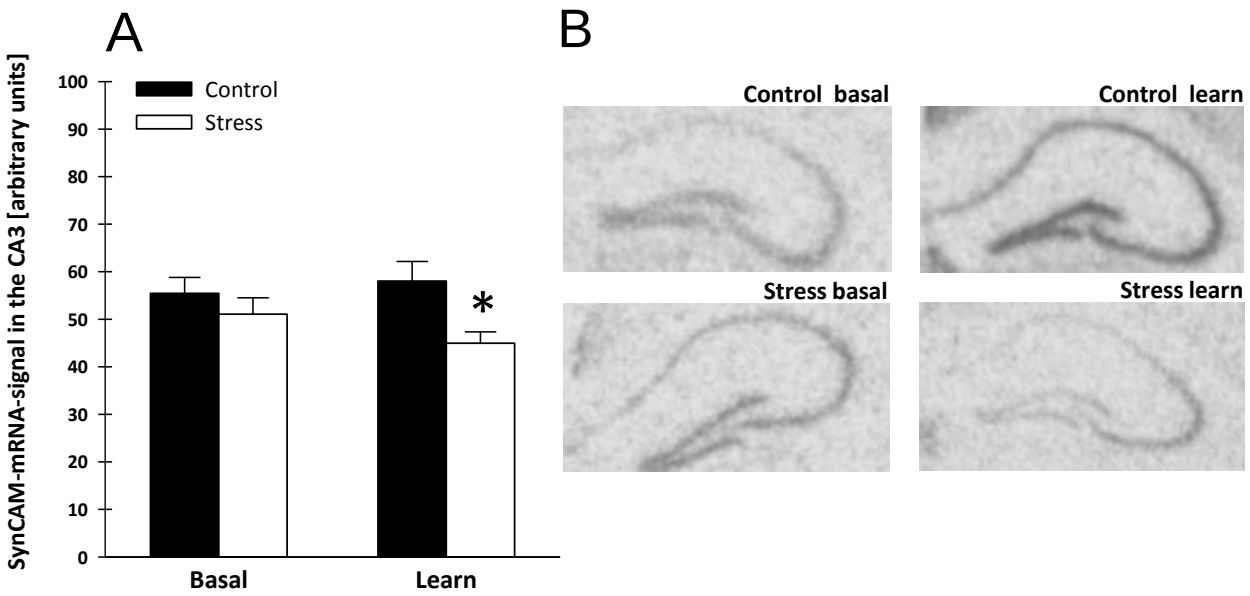

Figure 35: **SynCAM-mRNA-signal in the CA3. (A)** Similar to the situation in the CA1, there was a down-regulation of SynCAM-mRNA within animals with learning experience after stress compared to the controls (* within the learn batch, significantly different from controls, $p < 0.05$). **(B)** Representative SynCAM-mRNA autoradiograms are displayed.

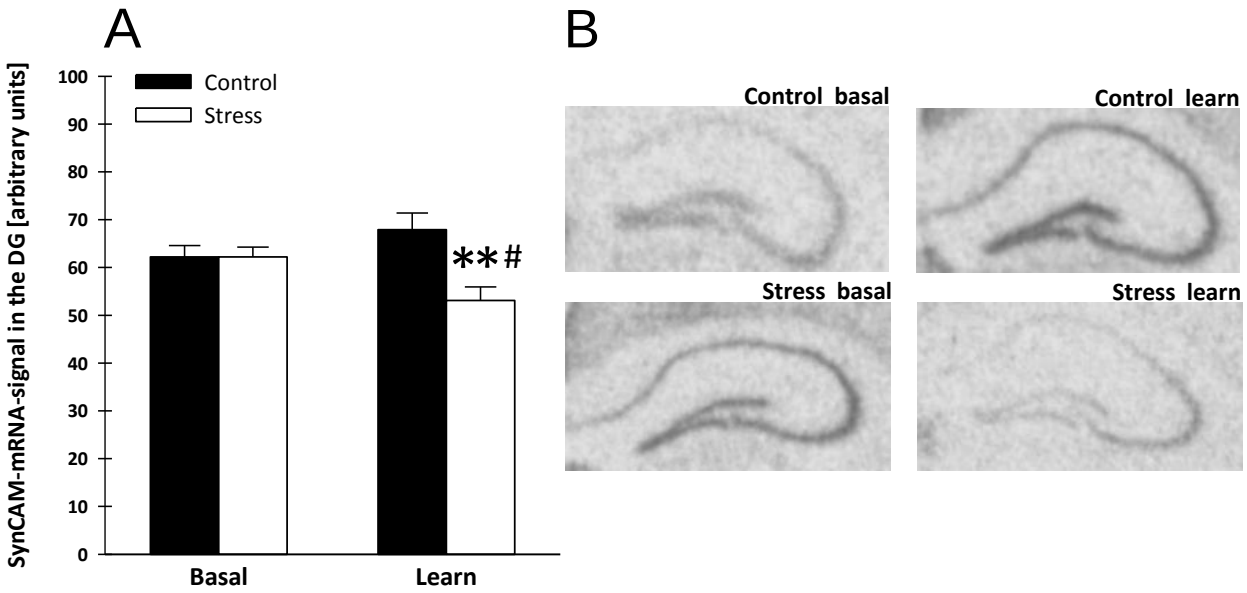

Figure 36: **SynCAM-mRNA-signal in the DG. (A)** In stress animals after learning, a down-regulation of SynCAM-mRNA was detected compared to controls of the same condition. Additionally, there was a down-regulation in stress animals after learning compared to basal stress animals (** within the learn batch, significantly different from controls, $p < 0.01$; # significantly different from basal stress animals, $p < 0.05$). **(B)** Representative SynCAM-mRNA autoradiograms are depicted.

SynCAMs share structural and functional components with Nlgns, for example during artificial synapse induction. Nonetheless, Nlgns and their presynaptic binding partners, Nrxns, can be summarised in a class of their own. The data for Nlgn 1 showed significant effects limited to the DG. ANOVA pointed toward a condition effect ($F_{1, 43} = 7.578$, $p < 0.01$; see *Figure 37*). This finding withstood post-hoc t-tests, which revealed a down-regulation of Nlgn 1-mRNA in stress animals after learning ($T_{21} = 2.562$; $p < 0.05$; see *Figure 37*). No further significant effects could be demonstrated in the other regions of the hippocampus, CA1 or CA3.

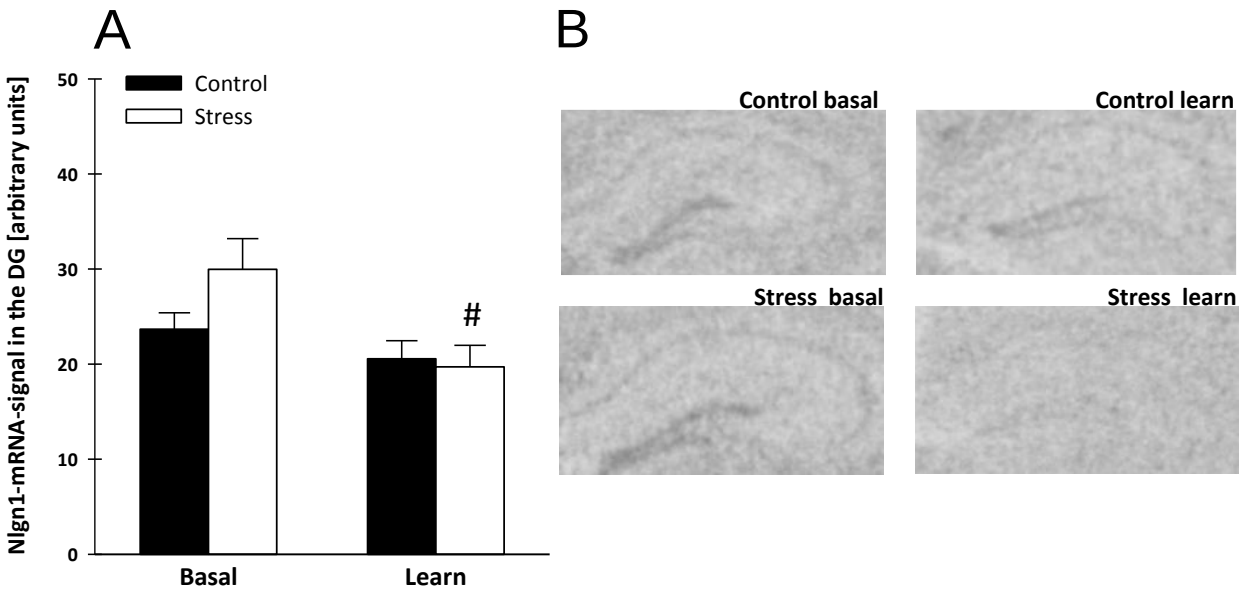

Figure 37: **Nlgn 1-mRNA-signal in the DG. (A)** In stress animals after learning, a down-regulation of Nlgn 1-mRNA was detected compared to basal stress animals (# significantly different from basal stress animals, $p < 0.05$). **(B)** Representative Nlgn 1-mRNA autoradiograms are displayed.

For Nlgn 2, there were no significant effects. Overall, the total mRNA-signal was weak, probably due to technical problems. Additionally, the standard variation was rather high. Overall, these findings established Nlgn 2 as an nCAM, which was not regulated by CSS or learning experience.

The situation for Nrxn, which is the presynaptic binding partner of Nlgn, resembled the one for Nlgn 2. In the CA1 and CA3, the total mRNA signal was very weak. In the DG, the signal strength was indeed higher. Nonetheless, no significant effects could be identified; Nrxn remained unaffected by CSS and learning.

The results for Nptn, another important nCAM, were more promising. ANOVA pointed to an interaction of group*condition in the CA1 ($F_{1, 46} = 3.415$; $p < 0.1$; see *Figure 38*) and allowed post-hoc t-tests. In the CA1, independent t-tests disclosed a group effect in the learn animals: Nptn-mRNA was down-regulated after stress exposure compared to controls ($T_{21} = 2.894$; $p < 0.01$; see *Figure 38*). ANOVA indicated an interaction of group*condition also in the DG ($F_{1, 46} = 8.333$; $p < 0.01$; see *Figure 39*). Post-hoc t-tests verified the down-regulation of Nptn-mRNA within the learn animals after stress exposure ($T_{21} = 3.199$; $p < 0.01$; see *Figure 39*) and thus, the appearance of this effect in the CA1 as well as in the DG. In addition, post-hoc t-tests confirmed the up-regulation of Nptn-mRNA in control animals after

learning compared to basal controls ($T_{22} = 2.882$; $p < 0.01$; see *Figure 39*). No effect was present in the CA3.

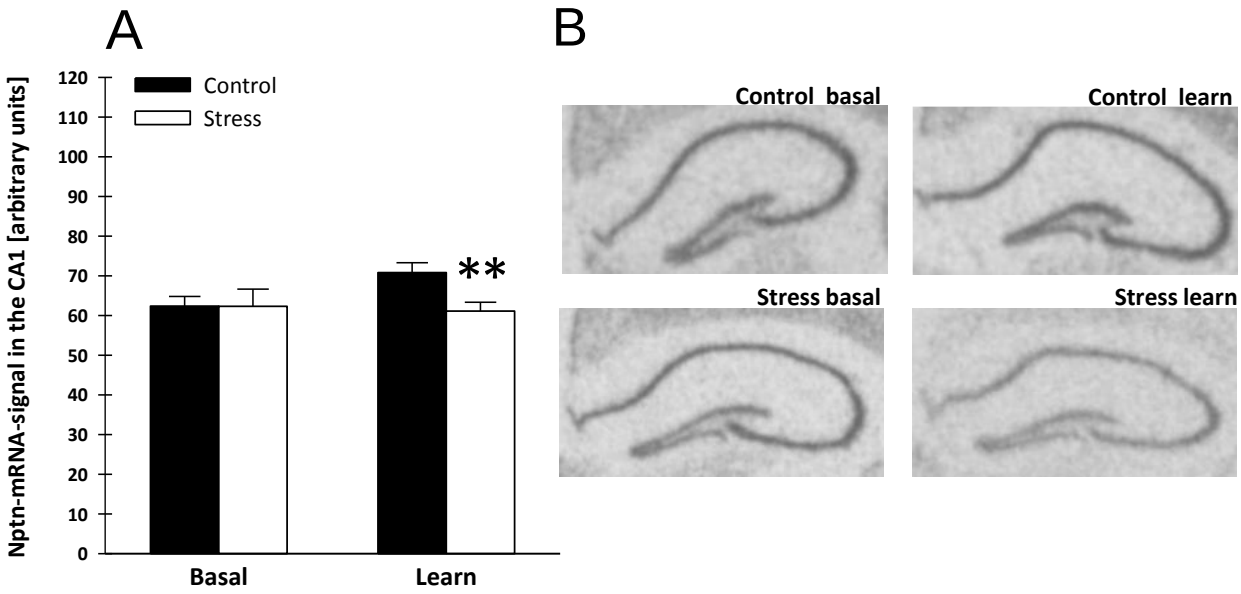

Figure 38: **Nptn-mRNA-signal in the CA1. (A)** In stress animals after learning, a down-regulation of Nptn-mRNA was identified compared to controls of the same condition (** significantly different from controls within the learn batch, $p < 0.01$). **(B)** Representative Nptn-mRNA autoradiograms are displayed.

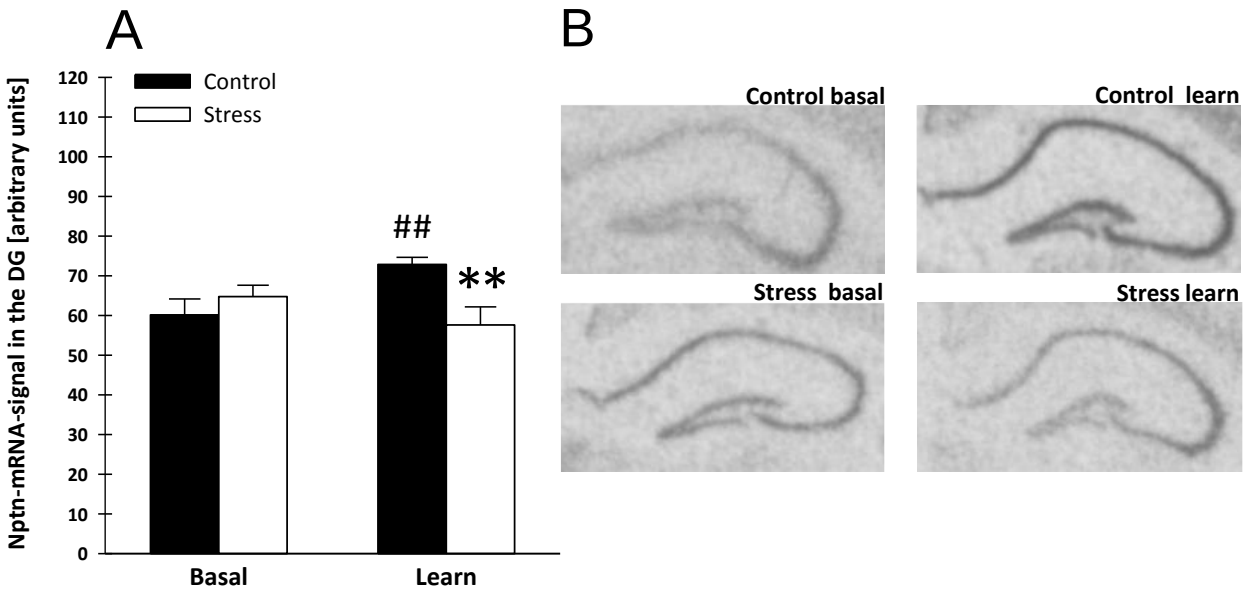

Figure 39: **Nptn-mRNA-signal in the DG. (A).** The down-regulation of Nptn-mRNA in stress animals after learning compared to learn controls that was seen in the CA1 could be reproduced in the DG. Furthermore, there was an up-regulation in controls after learning compared to basal controls (** significantly different from controls within the learn batch, $p < 0.01$; ## significantly different from basal controls, $p < 0.01$). **(B)** Representative Nptn-mRNA autoradiograms are shown.

Table 5 gives a clearer overview of all the previously presented nCAM-mRNA results. A striking effect was found for NCAM (DG), Nec 1 (DG), Nec 3 (CA3, DG), SynCAM (DG) and Nlgn 1 (DG). For all these nCAMs, a condition effect within the stress animals was verified, namely a down-regulation of nCAM-mRNA in animals after learning compared to the basal stress animals. Moreover, there was a robust effect that appeared for SynCAM (CA1, CA3, DG) and Nptn (CA1, DG): in animals with learning experience, nCAM-mRNA was down-regulated after stress exposure compared to controls. Due to the up-regulation of nCAM-mRNA in control animals after learning, which was observed for Nptn (DG) and SynCAM (CA1), it might be more suitable to call this effect an inhibition of up-regulation of nCAM-mRNA related to the CSS exposure.

Table 5: **Summary of mRNA-regulation patterns for NCAM, Nectins, SynCAM, Nlgn 1 and Nptn in young animals.** In the column on the left, all group effects, which were apparent after CSS, are listed, either in basal or in learn animals. The column on the right includes all condition effects, which appeared after learning, either in control or in stress animals. A very robust effect across conditions was the down-regulation of nCAM-mRNA in stressed animals after a learning experience. This effect appeared for NCAM (DG), Nec 1 (DG), Nec 3 (CA3, DG), SynCAM (DG) and Nlgn 1 (DG). The down-regulation of nCAM-mRNA after stress exposure in animals with learning experience was a striking effect between the groups; this was observed for SynCAM (CA1, CA3, DG) and Nptn (CA1, DG). For Nptn (DG) and SynCAM (CA1), it might be more appropriate to call this effect an inhibition of up-regulation, as in these cases the nCAM-mRNA in controls was up-regulated after learning (an upward arrow indicates a mRNA up-regulation; a downward arrow indicates a mRNA down-regulation).

nCAM	Group effect related to control animals	Condition effect related to basal animals
NCAM		
DG	Basal: ↑	Stress: ↓
Nec1		
CA1	Learn: ↑	Control: ↓; Stress: ↑
DG	---	Stress: ↓
Nec3		
CA3	---	Stress: ↓
DG	Learn: ↓	Stress: ↓
SynCAM		
CA1	Learn: ↓	Control: ↑
CA3	Learn: ↓	---
DG	Learn: ↓	Stress: ↓
Nlgn1		
DG	---	Stress: ↓
Nptn		
CA1	Learn: ↓	---
DG	Learn: ↓	Control: ↑

3.1.5 Expression levels of nCAM-proteins

The determination of nCAM expression on protein level was limited to those nCAMs with the most promising results on mRNA-level. This restricted set of nCAMs included Nectins, Nlgn 1 and Nptn. Nonetheless, the analysis revealed no significant group or condition effects for any of the candidate nCAMs. In total, the results on protein level were characterised by high standard variations with relatively small group sizes, which may have masked potential differences in the significant range. The results for Nec 3 and Nlgn 1 are illustrated as an example (see *Figure 40* and *Figure 41*).

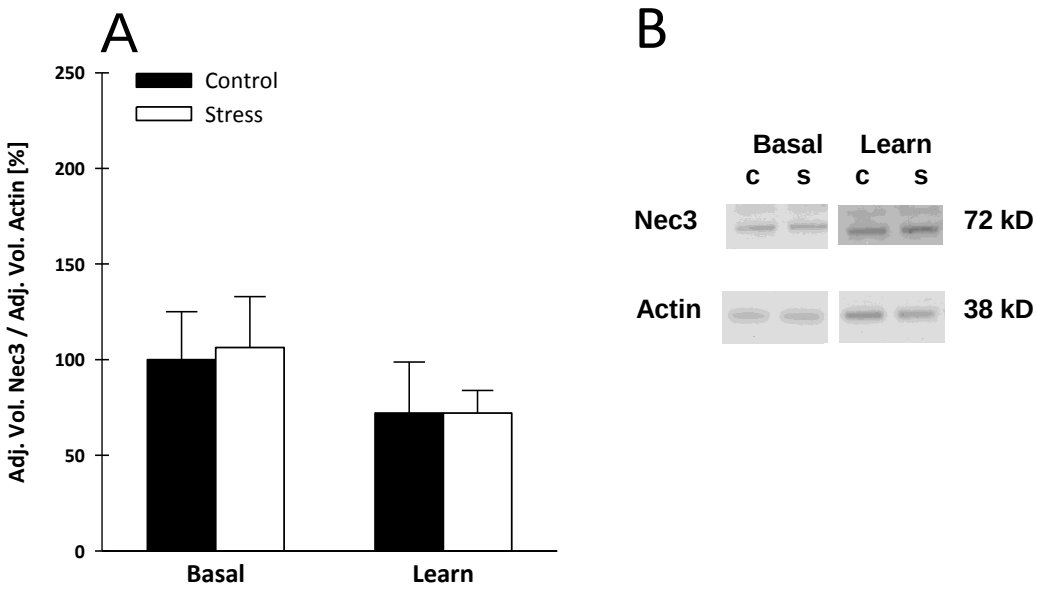

Figure 40: **Total Nec 3-protein-signal. (A)** Depicted are the adjusted volumes of Nec 3 divided by the adjusted volumes of the respective Actin. Values have been normalised to the amount of the respective protein content. By setting the basal controls to 100 %, the relative percentages of the other groups were calculated. No significant effects were identified. **(B)** Western blot autoradiographs of homogenised hippocampi derived from basal mice or mice after learning; animals were either stressed (s) or belonged to the control group (c).

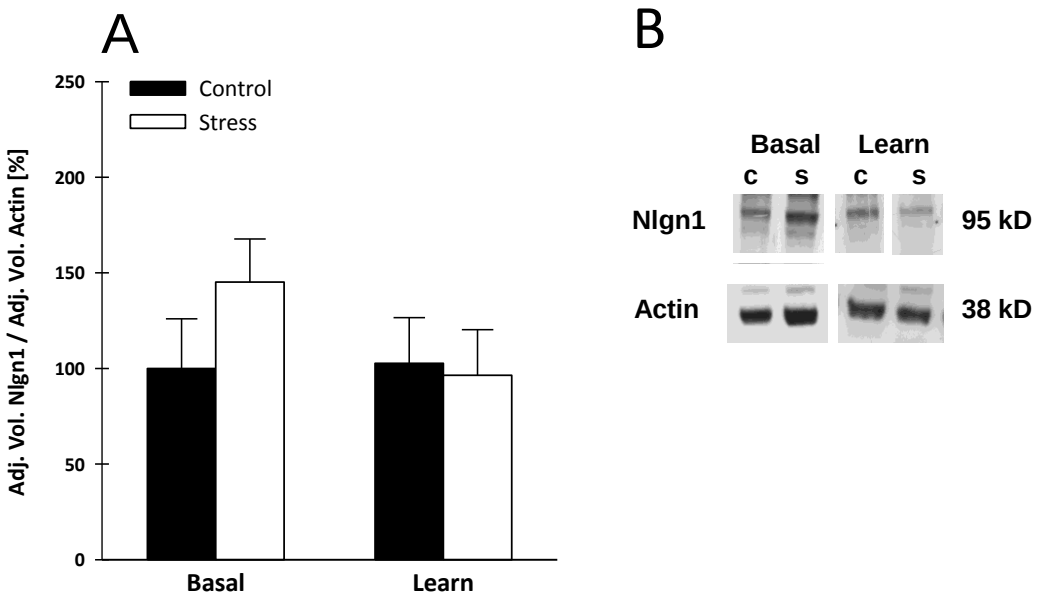

Figure 41: **Total Nlgn1-protein-signal. (A)** Adjusted volumes of Nlgn1 divided by the adjusted volumes of the respective Actin. Basal controls were set to 100 %, the relative percentages of the other groups were calculated. There were no significant differences. **(B)** Western blot autoradiographs of homogenised hippocampi derived from basal mice or mice after learning; animals were either stressed (s) or belonged to the control group (c).

3.1.6 Correlations

To elucidate the data further, we checked for correlations between the nCAM-mRNA expression levels and the performance during the OR test. The analysis revealed that there were no significant correlations within the group of stress animals only and within the combined group of controls and stress animals. Nonetheless, a significant Pearson correlation was found within the group of control animals only, namely between the SynCAM expression in the CA1 and the percentage of the time spent with the novel object (Pearson = -0.589; $p < 0.05$; see *Figure 42*). A control animal with a strong SynCAM-mRNA-signal in the CA1 spent most likely less time with the novel object, while a control animal with a lower SynCAM expression spent in all probability more time with the novel object.

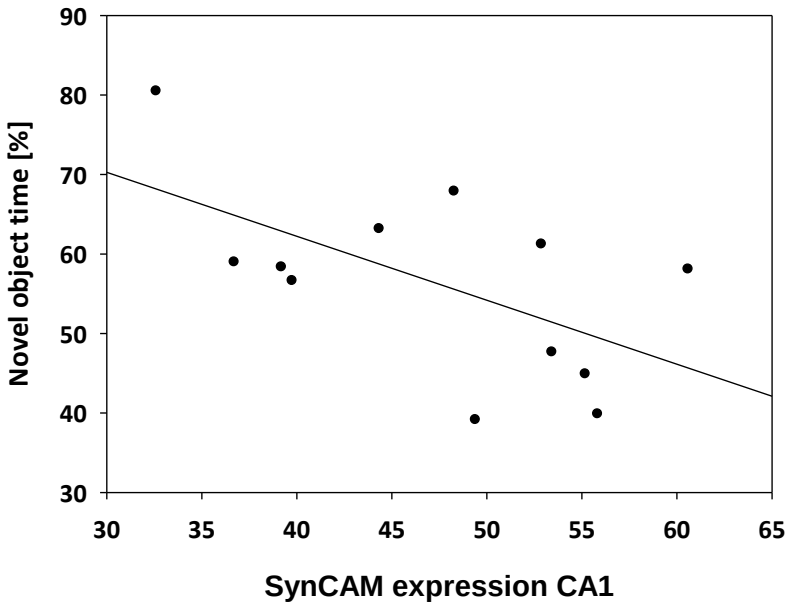

Figure 42: **Pearson correlation between SynCAM-mRNA expression in the CA1 and the novel object time.** There was a negative correlation stating that animals with a lower expression spend more time with the novel object, while animals with a higher expression spend less time with the novel object.

3.1.7 Summary

Adrenal weights were significantly elevated by the CSS procedure compared to adrenal weights of control animals. Additionally, the CSS procedure raised the corticosterone levels compared to corticosterone levels of controls. In general, learning experience increased the animals' corticosterone levels, both in control and in stress animals. CSS inhibited the animals' performance in the OR test: controls spent more time with the novel object than with the known object, while stressed animals did not differentiate between the two objects. In the MWM, controls, compared to stress animals, managed to reduce the escape latency only on spatial day 2. Several candidate nCAMs emerged to be regulated in distinct patterns after CSS and learning: NCAM (DG), Nec 1 (CA1, DG), Nec 3 (CA3, DG), SynCAM (CA1, CA3, DG), Nlgn 1 (DG) and Nptn (CA1, DG). There was one significant correlation between SynCAM-mRNA expression in the CA1 and the novel object time during the OR test.

3.2 Experiment 2: Effects of CSS in aged animals and the long-term impact on novel synaptic CAMs

Male CD1 mice were subjected to the CSS paradigm during adolescence. To be able to tap the long-term effects of CSS, they were then allowed to age for 12 months. Upon reaching the age of 15 months, the animals were behaviourally tested. Basal animals were decapitated 2 weeks after the last testing, while learn animals were decapitated 2 hours after the last test trial. Region-specific expression patterns of several candidate nCAMs on mRNA and protein level, as well as physiological, neuroendocrine and behavioural parameters were analysed.

3.2.1 Physiological data

Adrenal glands of overall 15 control and 16 chronic stress animals, which were sacrificed 1 year after CSS exposure, were dissected and weighed. ANOVA pointed to a condition effect ($F_{1, 30} = 4.668$; $p < 0.05$), but this finding did not withstand post-hoc t-testing. Independent t-tests revealed no significant differences between the groups (control and stress) in the absolute weight of the adrenal glands (controls compared to stress animals, basal condition: $T_{14} = 0.094$; $p = 0.927$, learn condition: $T_{13} = 0.519$; $p = 0.612$). Thymus glands were not dissected due to the thymus' natural tendency to shrink with increasing age.

3.2.2 Neuroendocrine data

To analyse neuroendocrine factors in basal animals, plasma corticosterone levels were determined in the morning during the circadian nadir, 2 weeks after the last testing day and 12 months after CSS exposure; independent t-tests did not identify a significant group effect between control and stress animals. Blood samples from animals with learning experience were acquired 2 hours after the last trial of behavioural testing (morning to midday) and likewise 12 months after the cessation of the CSS procedure. Within the batch of animals after learning, corticosterone levels did not differ significantly between control and stress animals. However, ANOVA revealed differences in corticosterone levels, based upon the condition of mice (basal or after learning; $F_{1, 28} = 19.663$; $p < 0.01$; see *Figure 43*).

Post-hoc t-tests confirmed that the corticosterone levels of animals after learning were increased compared to basal animals (control: $T_{12} = 3.432$; $p < 0.01$; stress: $T_{13} = 2.860$; $p < 0.05$; see *Figure 43*).

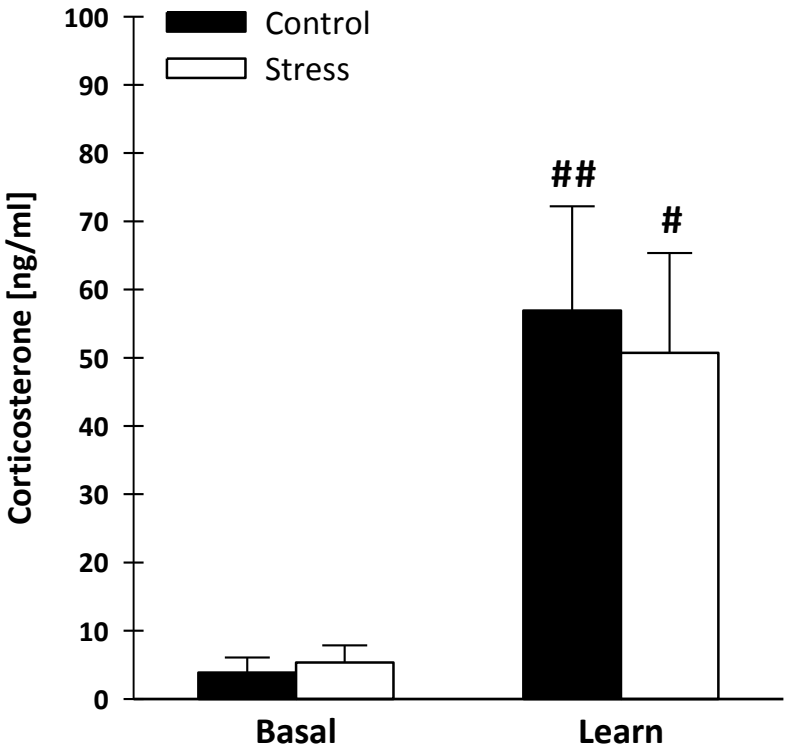

Figure 43: **Plasma corticosterone levels.** For both conditions, independent t-tests did not provide a significant difference within the groups. Animals after learning showed elevated corticosterone levels compared to basal animals (# significantly different from basal control animals, $p < 0.05$; ## significantly different from basal stress animals, $p < 0.01$).

3.2.3 Behavioural data

Behavioural tests were conducted to investigate the animals' general locomotion, cognitive performance and stress-coping behaviour. First, to observe hippocampus-independent short-term memory, the OR test was carried out. A significant difference was verified within the control group: control animals spent significantly more time with the novel object than with the known object ($T_{28} = 3.432$; $p < 0.01$; see *Figure 44*). Conversely, stress animals did not prefer one object over the other one; they showed no difference in the exploration of the objects. The analysis of the parameters time immobile and total distance travelled

disclosed that both groups were similarly active ($T_{29} = 0.153$; $p = 0.879$) and travelled an equal distance ($T_{29} = 0.306$; $p = 0.762$).

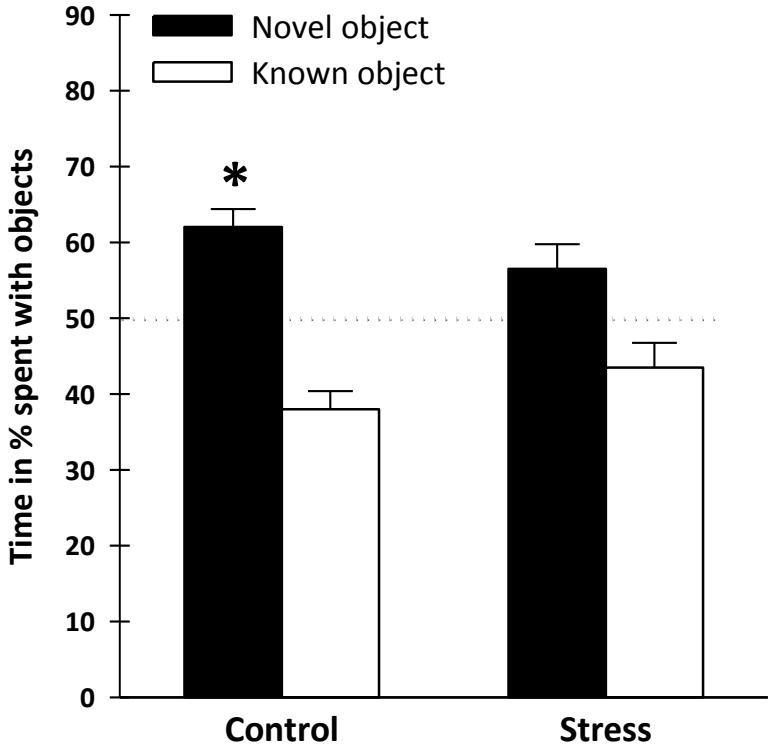

Figure 44: **Time spent with objects.** Control animals explored the novel object to a significantly greater extent than the known object. Stress animals showed no significant preference for one of the objects. The dotted line represents chance level at 50 % (* significantly different from known object time, $p < 0.05$).

Figure 45 revealed results for the control animals according to a normal distribution with the majority of values tending to cluster around a single mean. In contrast, the data for the stress animals were distributed according to a bimodal distribution with values grouped around two distinct peaks. The first group showed a rather weak OR performance and low occupation with the novel object; the second group spent a great amount of time with the novel object and thus seemed to be protected from the detrimental consequences of the CSS exposure.

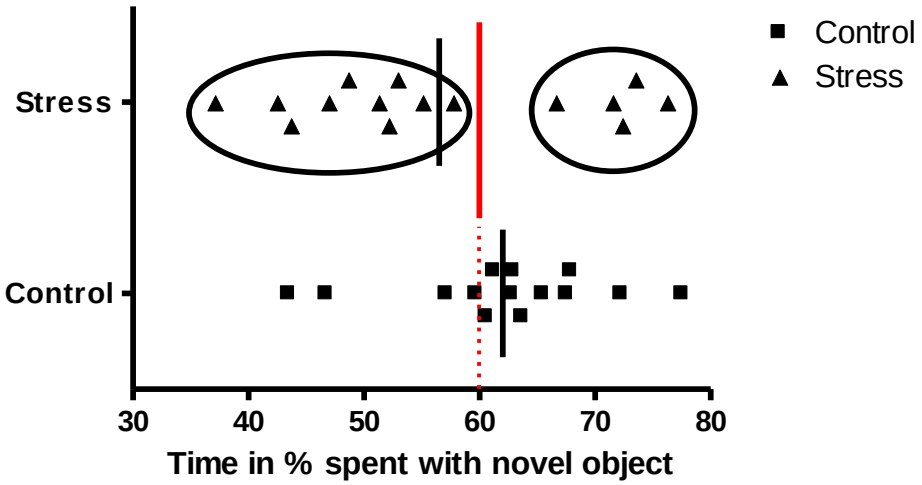

Figure 45: **Time spent with objects.** Unlike the controls', the stress animals' performance did not fit a Gaussian distribution. Within the stress group, there was a bimodal distribution revealing one stress group that was still affected by the CSS during adolescence (left circle) and a second group, which seemed to be protected from long-term effects (right circle). The red line represents the cut off criterion for the stress animals at 60 % time spent with the novel object.

Second, to determine hippocampus-dependent long-term memory, animals had to perform in the MWM. Over the course of the experiment, animals seemed to learn only partially. During the visual training, they reached the platform continually faster except for the last trial. However during the spatial training, the escape latencies remained relatively high and although the animals seemed to improve from the first to the fourth trial, after each completed spatial day, they started on the same level like the day before with escape latencies around 30 seconds. Only in the reversal learning, the animals managed to reduce the escape latency constantly from trial 1 to trial 4 with final escape latencies around 10 seconds. Additionally, the results revealed no significant differences between control and stress animals, except for the probe trial. Here, independent t-tests verified that the controls needed significantly less time to reach the former platform location compared to the stress animals ($T_{26} = 2.372$; $p < 0.05$; see *Figure 46*).

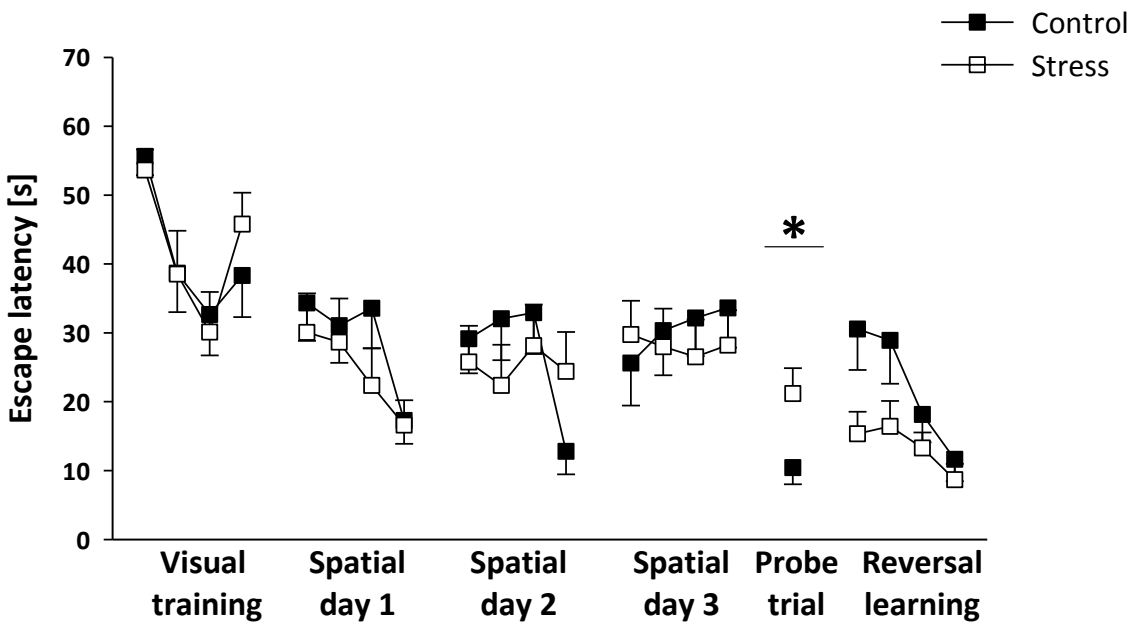

Figure 46: **Escape latencies in the MWM.** Animals exhibited reduced escape latencies only during the visual training and the reversal learning. A significant difference between the two groups (control and stress) was detected in the probe trial (* stress and control animals performed significantly different from each other, $p < 0.05$).

To measure the animals' preference for the target quadrant (the back-left quadrant), a probe trial without a platform was carried out. Control animals spent significantly more time in the non-target quadrants ($T_{28} = 3.055$; $p < 0.01$; see *Figure 47*) compared to the time in the former target quadrant. Stress animals exhibited no preference and searched equally long in the former target zone and the non-target zone. This effect within the controls received further support from the data set for distance travelled per quadrant; but here the data sufficed merely for a trend ($T_{28} = 1.987$; $p < 0.1$).

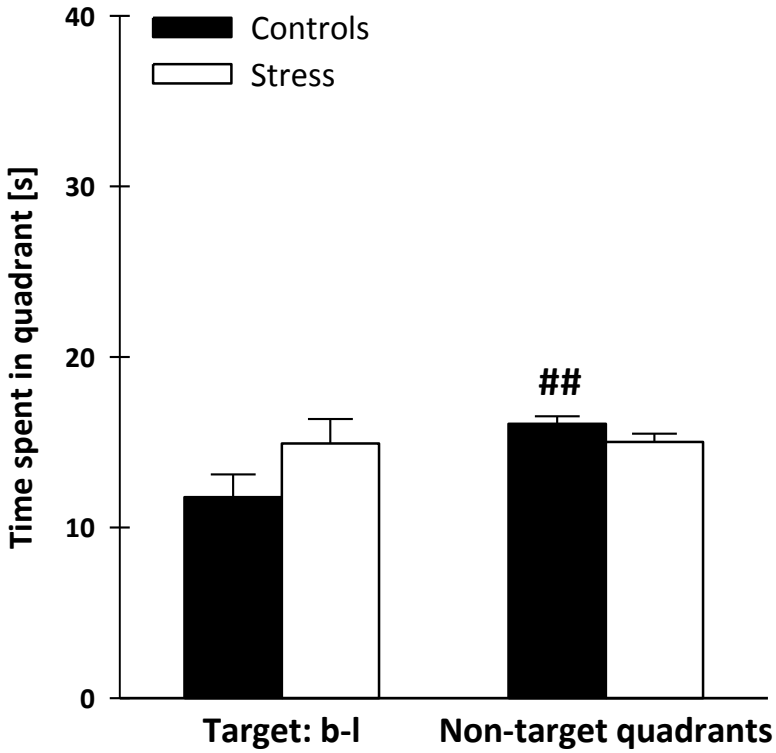

Figure 47: **Time spent in the quadrants of the MWM.** Control animals searched significantly longer in the non-target quadrants for the platform. Stress animals explored the former target quadrant and the non-target quadrants equally long (## significantly different from target quadrant, $p < 0.01$).

3.2.4 Expression levels of nCAM-mRNA

Expression patterns on mRNA-level were assessed for the same candidate nCAMs like in the young animals. Due to the poor results in the PFC of young animals, nCAM-mRNA levels of aged animals were investigated solely in the hippocampus. The following investigated nCAMs showed no significant regulations 1 year after CSS: NCAM, Nec 1, Nlgn 2 and Nlgn 1. Exemplary, the graph for Nlgn 1 expression in the CA3 is depicted in *Figure 48* (ANOVA, group: $F_{1, 30} = 0.05$; $p = 0.824$, condition: $F_{1, 30} = 0.505$; $p = 0.483$, interaction of group*condition: $F_{1, 30} = 0.000$; $p = 0.996$).

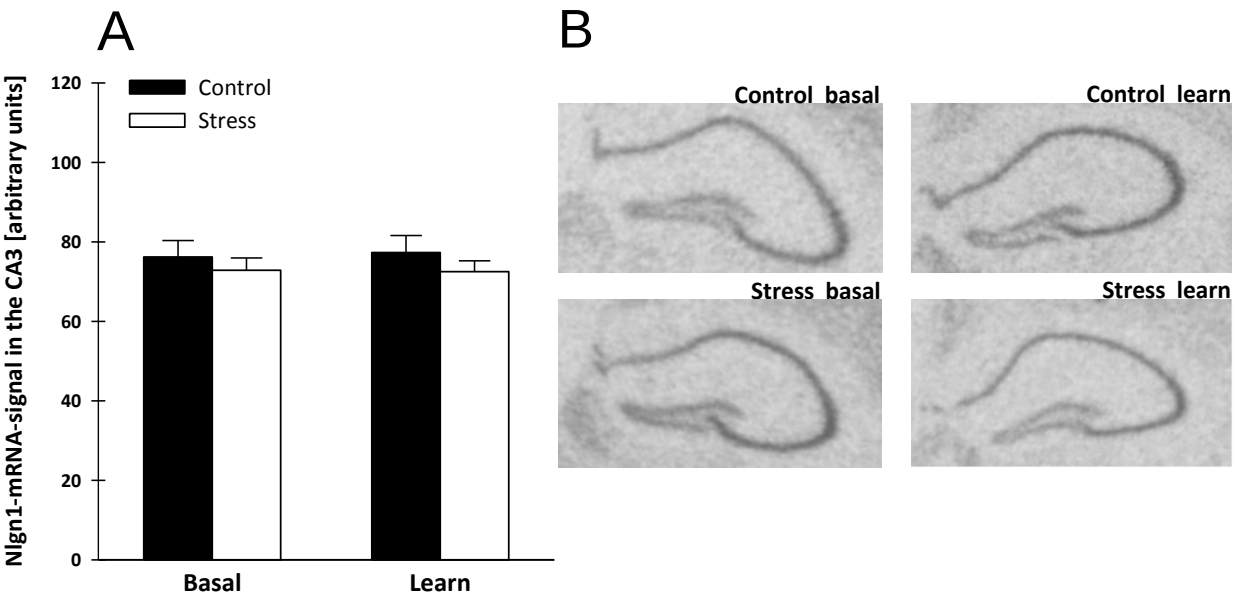

Figure 48: **Nlgn 1-mRNA-signal in the CA3. (A)** There were no significant differences in the CA3. This was also true for the CA1 and the DG. **(B)** Representative Nlgn 1-mRNA autoradiograms are depicted.

The majority of candidate nCAMs was not regulated 12 months after CSS and a recent learning experience (MWM). However, there were three nCAMs with significant differences even 1 year after CSS cessation: Nrxn-mRNA of different regions emerged to be similarly regulated in control and stress animals. Nec 3 showed a group effect solely in the basal batch, while Nptn exhibited a group effect only within the batch of animals after learning. For those two nCAMs, several condition effects were found as well.

For Nrxn, ANOVA pointed towards a condition effect both in the CA3 ($F_{1, 30} = 5.280$; $p < 0.05$) and the DG ($F_{1, 30} = 7.935$; $p < 0.01$). In the CA3, there was an up-regulation after learning within controls ($T_{13} = 2.306$; $p < 0.05$; see *Figure 49*). The same effect was found in the DG, but only in stress animals ($T_{14} = 2.193$; $p < 0.05$; see *Figure 50*).

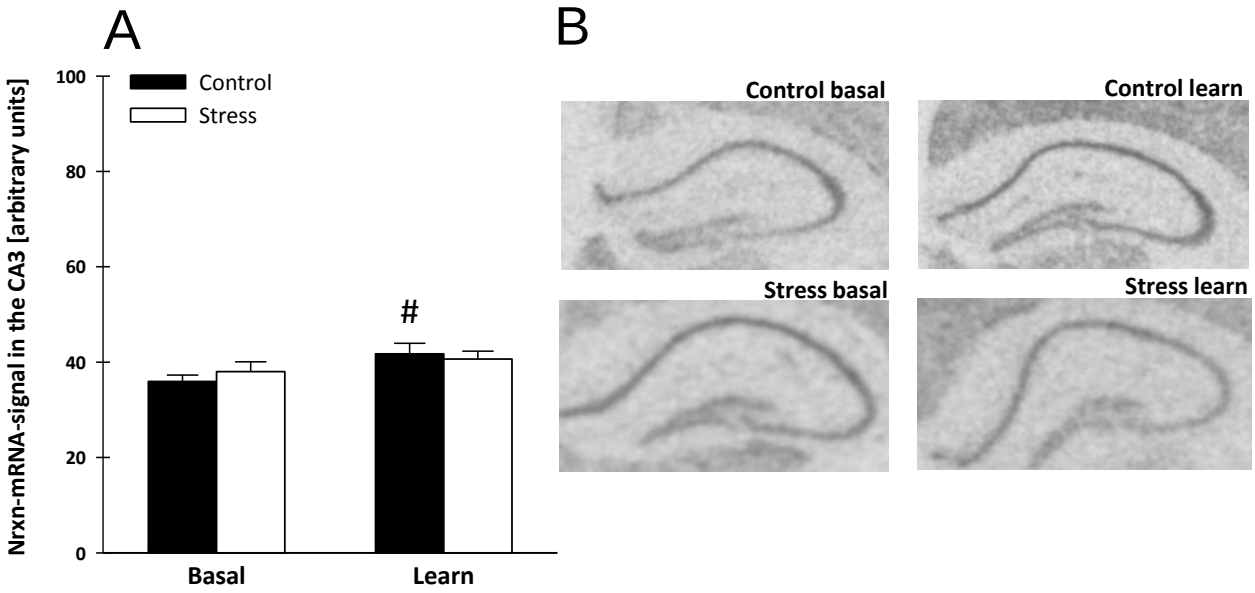

Figure 49: **Nrxn-mRNA-signal in the CA3. (A)** The Nrxn-mRNA-signal was significantly elevated in control animals after learning compared to basal controls (# significantly different from basal controls, $p < 0.05$). **(B)** Representative Nrxn-mRNA autoradiograms are depicted.

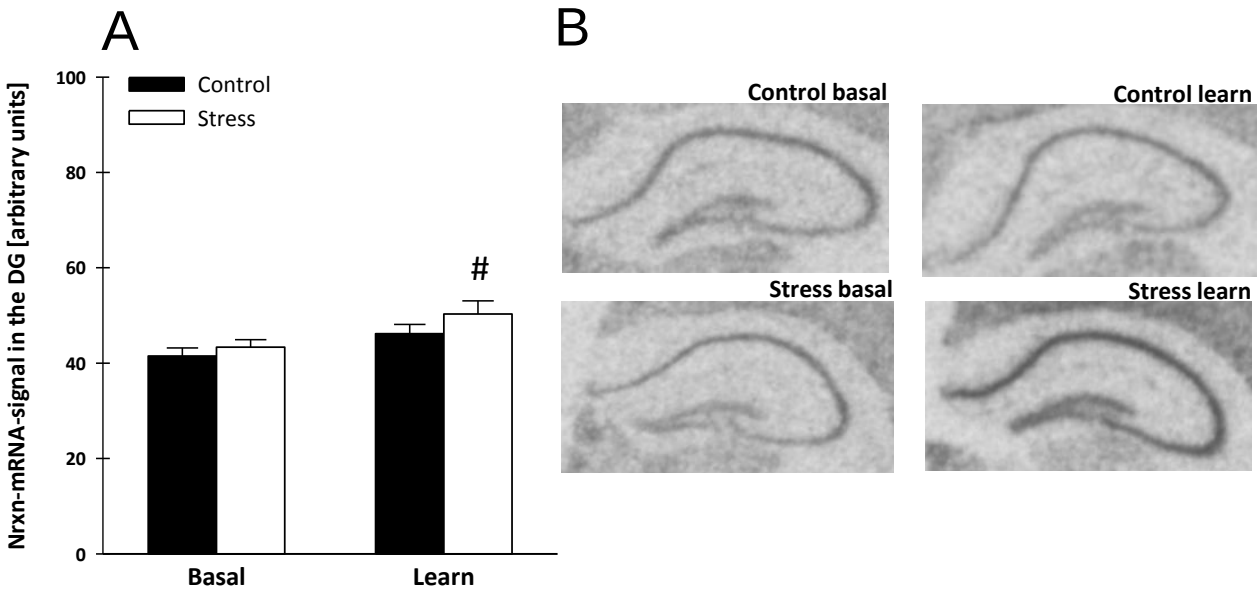

Figure 50: **Nrxn-mRNA-signal in the DG. (A)** The Nrxn-mRNA-signal was significantly elevated in stress animals after learning compared to basal stress animals (# significantly different from basal stress animals, $p < 0.05$). **(B)** Representative Nrxn-mRNA autoradiograms are displayed.

For Nec 3, ANOVA pointed to an interaction of group*condition in the CA3 ($F_{1, 29} = 7.146$; $p < 0.05$; see *Figure 51*). Post-hoc t-testing confirmed that Nec 3-mRNA was up-regulated in stress animals compared to controls within the batch of basal animals ($T_{14} = 3.038$; $p < 0.01$; see *Figure 51*). This effect between control and stress animals, reappeared in the DG ($T_{14} = 2.404$; $p < 0.05$; see *Figure 52*). For both regions, there were no significant effects within the batch of animals after learning. However, post-hoc t-tests confirmed an up-regulation of Nec 3-mRNA after learning within the control batch ($T_{12} = 2.369$; $p < 0.05$; see *Figure 51*) and a down-regulation after learning within the stress batch ($T_{14} = 2.407$, $p < 0.05$; see *Figure 51*), both in the CA3.

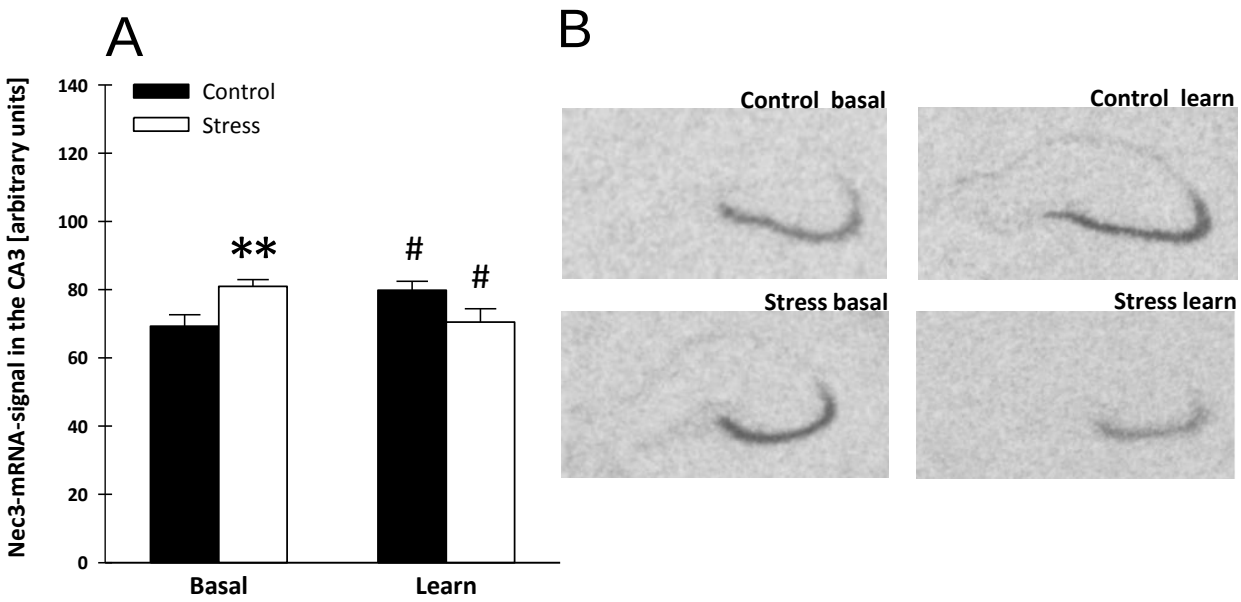

Figure 51: **Nec 3-mRNA-signal in the CA3. (A)** In basal animals, Nec 3-mRNA was up-regulated after CSS exposure. Additionally, there were condition effects both in control and in stress animals: in stress animals, Nec 3-mRNA was down-regulated after learning, while in controls, Nec 3-mRNA was up-regulated after learning (** within the basal batch, significantly different from control animals, $p < 0.01$; # significantly different from the respective basal animals, $p < 0.05$). **(B)** Representative Nec 3-mRNA autoradiograms are depicted.

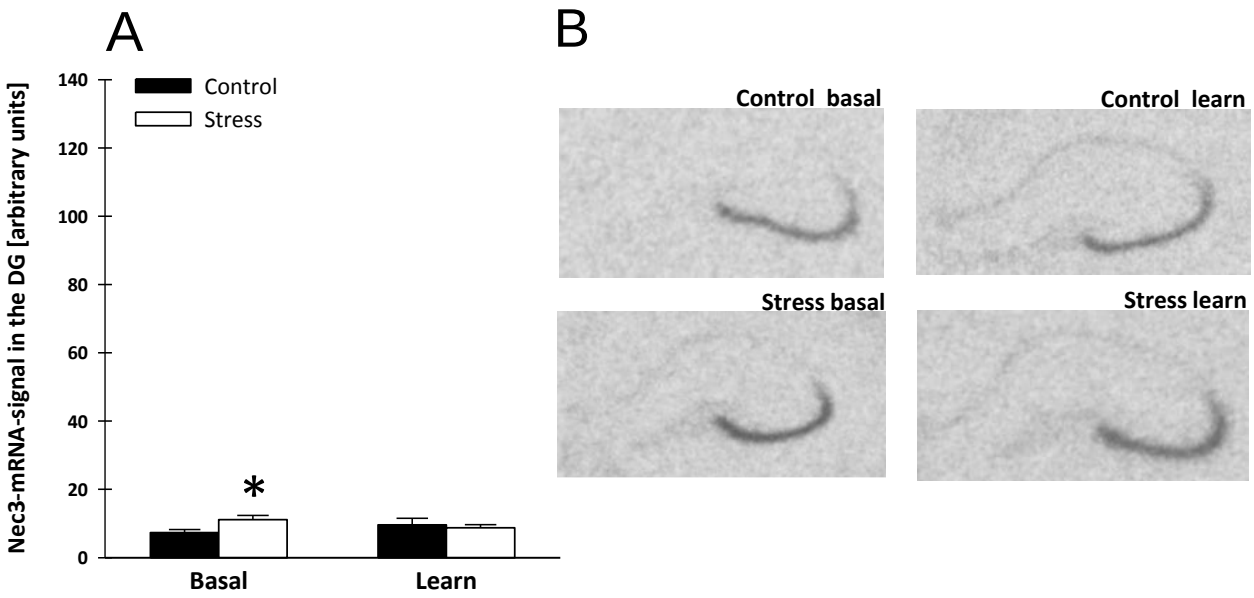

Figure 52: **Nec 3-mRNA-signal in the DG. (A)** There was a significant effect in basal animals: Nec 3-mRNA was up-regulated after CSS exposure (* significantly different from controls, $p < 0.05$). **(B)** Representative Nec 3-mRNA autoradiograms are depicted.

For Nptn, ANOVA indicated a group effect in the CA1 ($F_{1, 30} = 6.958$; $p < 0.05$) as well as in the CA3 ($F_{1, 28} = 23.650$; $p < 0.01$) and a condition effect in the DG ($F_{1, 30} = 4.387$; $p < 0.05$; see *Figure 53).* Nptn-mRNA showed a down-regulation in stress animals compared to controls within the learn batch. This effect between control and stress animals was verified by independent t-tests for the whole hippocampus (CA1: $T_{13} = 3.475$; $p < 0.01$; CA3: $T_{13} = 5.120$; $p < 0.01$; DG: $T_{13} = 3.451$; $p < 0.01$). Thus, the data for Nptn gene regulation in the DG are depicted as an example for the reoccurring down-regulation in stress animals after learning (see *Figure 53*). In the whole hippocampus, there were no significant effects within the batch of basal animals. However, Nptn-mRNA was up-regulated after learning within controls in the DG ($T_{13} = 2.471$, $p < 0.05$; see *Figure 53*).

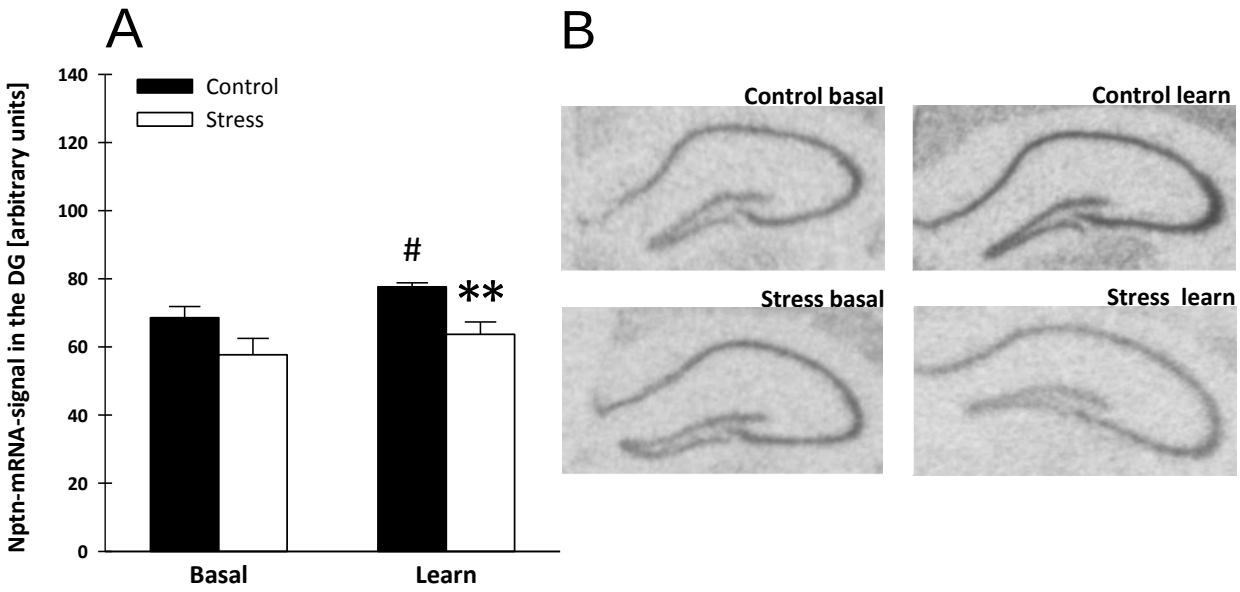

Figure 53: **Total Nptn-mRNA-signal in the DG. (A)** Nptn-mRNA was down-regulated after CSS exposure, but only in animals after learning. This affect appeared also in the CA1 and the CA3. Moreover in the DG, there was an effect across conditions in control animals: after learning Nptn-mRNA was up-regulated compared to basal controls (** within the learn batch, significantly different from controls, $p < 0.01$; # significantly different from basal control animals, $p < 0.05$). **(B)** Representative Nptn-mRNA autoradiograms are shown.

Table 6 gives an overview of all the previously presented nCAM-mRNA results of aged animals. All group effects in stress animals compared to controls are listed, as well as the condition effects in animals after learning related to basal animals: for Nrxn, a similar regulation across conditions was found, namely an up-regulation of mRNA after learning, both within stress (DG) and control animals (CA3). For Nec 3, there was a robust effect that appeared in the CA3 and the DG, but only in the basal animals. For Nptn, there was a robust effect in the opposite direction that was only valid in animals with learning experience. This effect was apparent in the whole hippocampus (CA1, CA3, DG). Similar to the gene regulation of Nrxn in the CA3 of controls, Nec 3- and Nptn-mRNA of control animals after learning were also up-regulated compared to basal controls. Furthermore, analysis revealed a down-regulation of Nec 3-mRNA in stress animals after learning compared to basal stress animals.

Table 6: **Summary of mRNA-regulation patterns for Nec3, Nptn and Nrxn in aged animals.** In the column on the left, all group effects, which were apparent after CSS, are listed, either in basal or in learn animals. The column on the right includes all condition effects, which appeared after learning, either in control or in stress animals. Nrxn was up-regulated after learning, both in control (CA3) and stress animals (DG). Nec 3-mRNA emerged to be up-regulated after stress exposure in the CA3 and the DG. This group effect appeared only in basal animals. In contrast, Nptn-mRNA was down-regulated after stress experience in the whole hippocampus (CA1, CA3, DG). This was only valid for animals after learning. Nec 3-mRNA was down-regulated after stress exposure and a recent learning experience (CA3) and up-regulated in controls after learning (CA3). Similarly, Nptn-mRNA was up-regulated in controls after learning (DG) (an upward arrow indicates a mRNA up-regulation; a downward arrow indicates a mRNA down-regulation).

nCAM	Group effect related to control animals	Condition effect related to basal animals
Nrxn		
CA3	---	Control: ↑
DG	---	Stress: ↑
Nec3		
CA3	Basal: ↑	Control: ↑; Stress: ↓
DG	Basal: ↑	---
Nptn		
CA1	Learn: ↓	---
CA3	Learn: ↓	---
DG	Learn: ↓	Control: ↑

3.2.5 Expression levels of nCAM-proteins

The analysis of nCAM expression on protein-level for aged animals was limited to the same set of candidate nCAMs like for the young animals; investigated were Nectins, Nlgn 1 and Nptn. In contrast to the analysis in young animals, there were only basal animals available for the analysis in aged animals. Similar to the results for the young animals, the analysis revealed no significant group effects for any of the candidate nCAMs. Overall, the results on protein-level were characterised by high standard variations with relatively small group sizes, which may have masked potential differences in the significant range. The results for Nec 3 are illustrated as an example (see *Figure 54*).

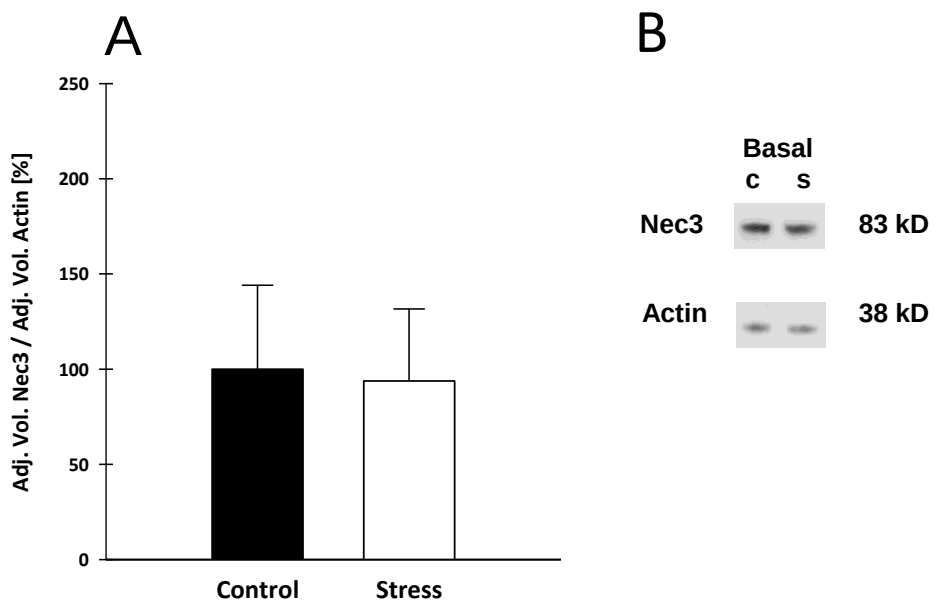

Figure 54: **Total Nec 3-protein-signal. (A)** Depicted are the adjusted volumes of Nec 3 divided by the adjusted volumes of the respective Actin. Values have been normalised to the amount of the respective protein content. By setting the control group to 100 %, the relative percentage of the stress group was calculated. No significant effects were identified. **(B)** Western blot autoradiographs of homogenised hippocampi derived from basal mice that were either stressed (s) or belonged to the control group (c).

3.2.6 Correlations

We checked for correlations between the nCAM-mRNA expression levels and the performance during the OR test of basal animals. The analysis revealed that there were no significant correlations within the group of control animals only. Nonetheless, several significant Pearson correlations were found within the group of stress animals only, namely for Nec 3 (DG: Pearson = 0.780; $p < 0.05$; see *Figure 55*), Nlgn 1 (DG: Pearson = 0.908; $p < 0.01$; see *Figure 56*), Nlgn 2 (CA3: Pearson = -0.760; $p < 0.05$; see *Figure 57*) and Nptn (CA1: Pearson = -0.874; $p \leq 0.01$; see *Figure 58*; CA3: Pearson = -0.817; $p < 0.05$; see *Figure 59*; DG: Pearson = -0.769; $p < 0.05$; see *Figure 60*). A positive correlation describes an animal spending the more time with the novel object the stronger the nCAM-mRNA-signal in a specific region of the hippocampus. A negative correlation describes an animal, which spends less time with the novel object the higher the nCAM-mRNA expression in a specific region.

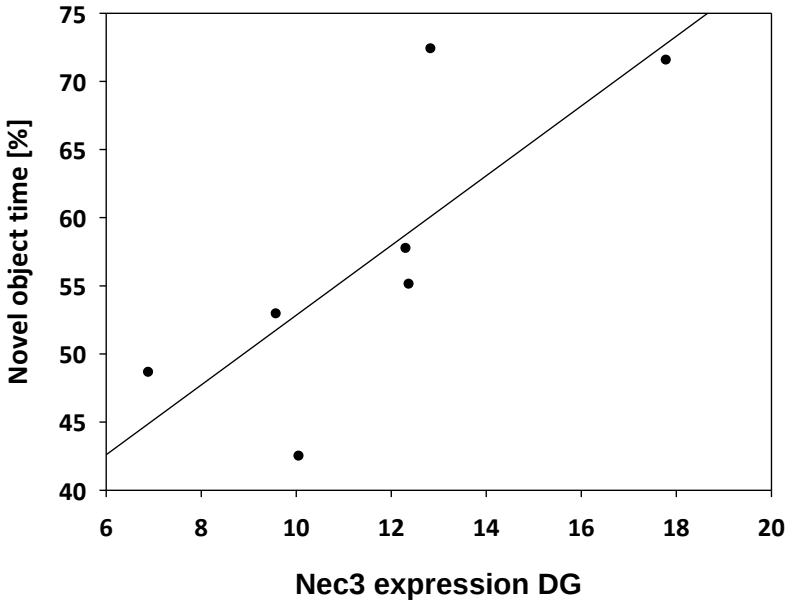

Figure 55: **Pearson correlation between Nec 3-mRNA expression in the DG and the novel object time.** There was a positive correlation stating that animals with a higher expression spend more time with the novel object, while animals with a lower expression spend less time with the novel object. This was only true for basal stress animals.

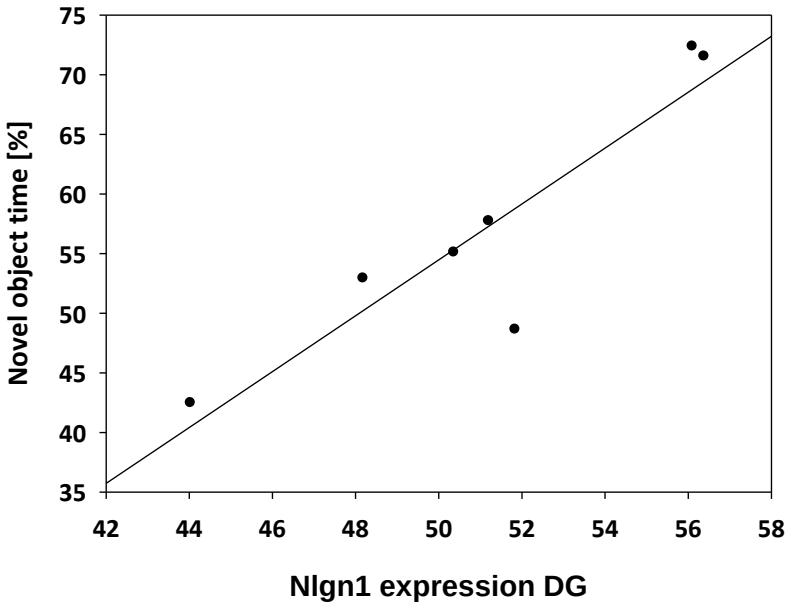

Figure 56: **Pearson correlation between Nlgn 1-mRNA expression in the DG and the novel object time.** There was a positive correlation for basal stress animals.

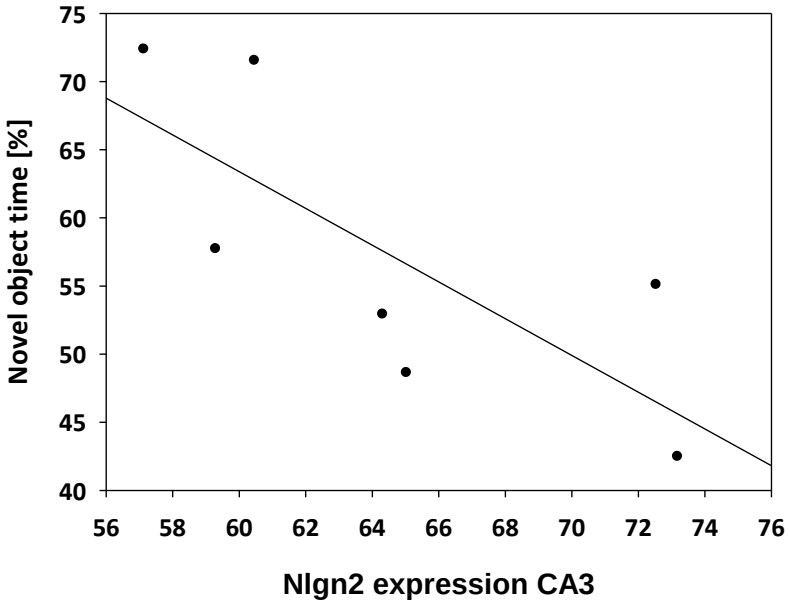

Figure 57: **Pearson correlation between Nlgn2-mRNA expression in the CA3 and the novel object time.** There was a negative correlation stating that animals with a lower expression spend more time with the novel object, while animals with a higher expression spend less time with the novel object. This was only valid in basal stress animals.

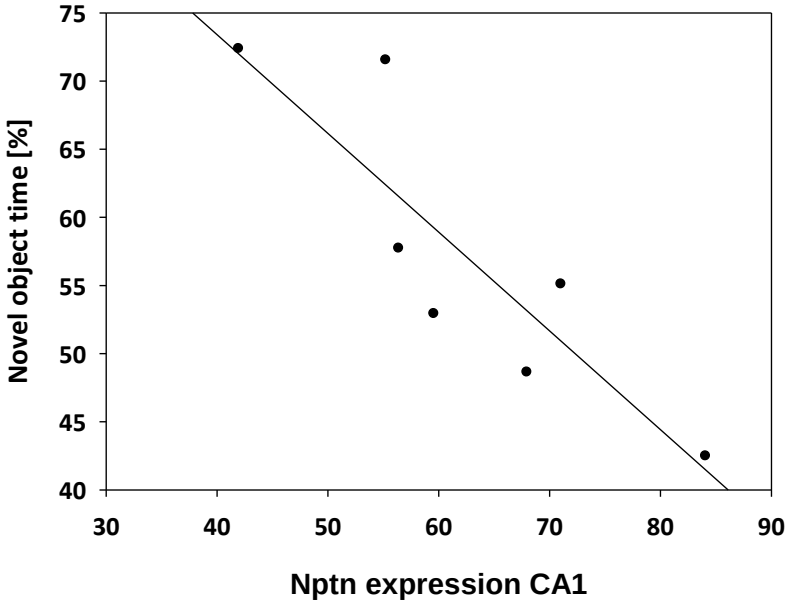

Figure 58: **Pearson correlation between Nptn-mRNA expression in the CA1 and the novel object time.** There was a negative correlation for basal stress animals.

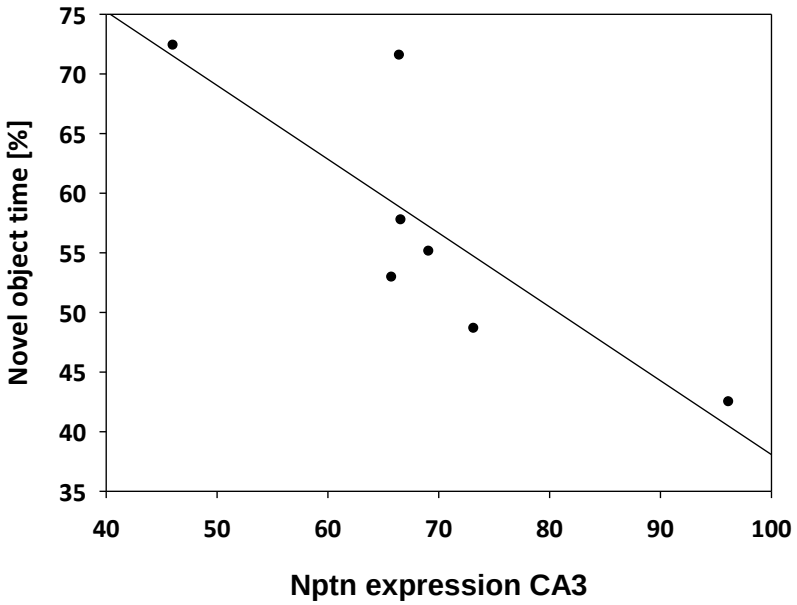

Figure 59: **Pearson correlation between Nptn-mRNA expression in the CA3 and the novel object time.** Similar to the situation for Nptn in the CA1, there was a negative correlation for basal stress animals in the CA3.

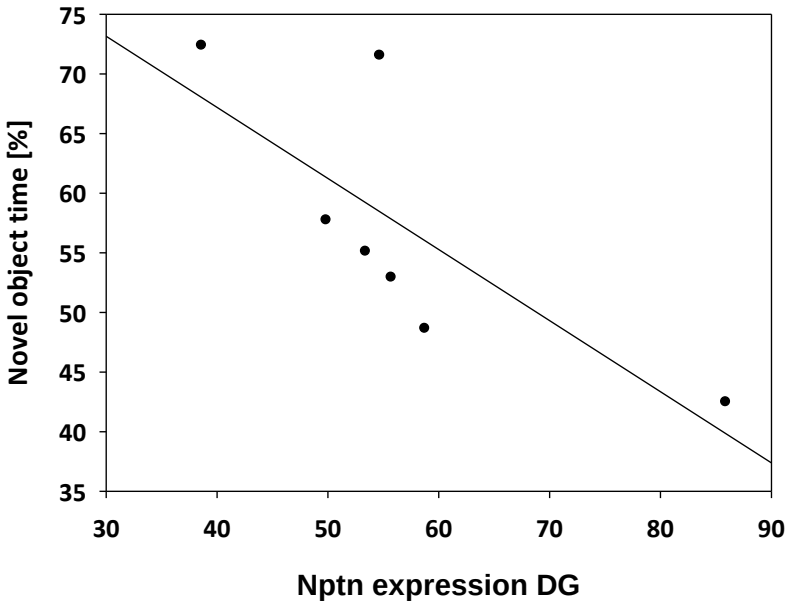

Figure 60: **Pearson correlation between Nptn-mRNA expression in the DG and the novel object time.** There was a negative correlation for Nptn in the DG as well.

Besides the correlations in the stress only group, there were also two significant findings in the combined group of control and stress animals: SynCAM-mRNA in the CA3 was negatively correlated with the novel object time in the OR test (Pearson = -0.516; $p < 0.05$; see *Figure 61*). For this correlation to come into effect apparently both groups, control and stress animals, were necessary as SynCAM was not regulated neither in the controls only nor in the stress group only. Moreover, Nlgn 1-mRNA in the DG was positively correlated with the novel object time during OR (Pearson = 0.497; $p \leq 0.05$; see *Figure 62*). This effect was mainly driven by the stress group, but was still apparent in combination with controls.

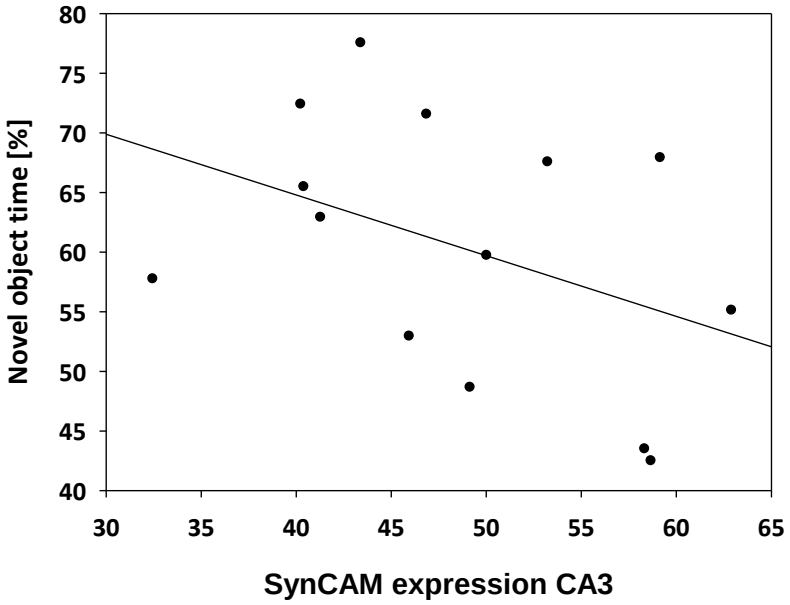

Figure 61: **Pearson correlation between SynCAM-mRNA expression in the CA3 and the novel object time.** There was a negative correlation for basal control and stress animals.

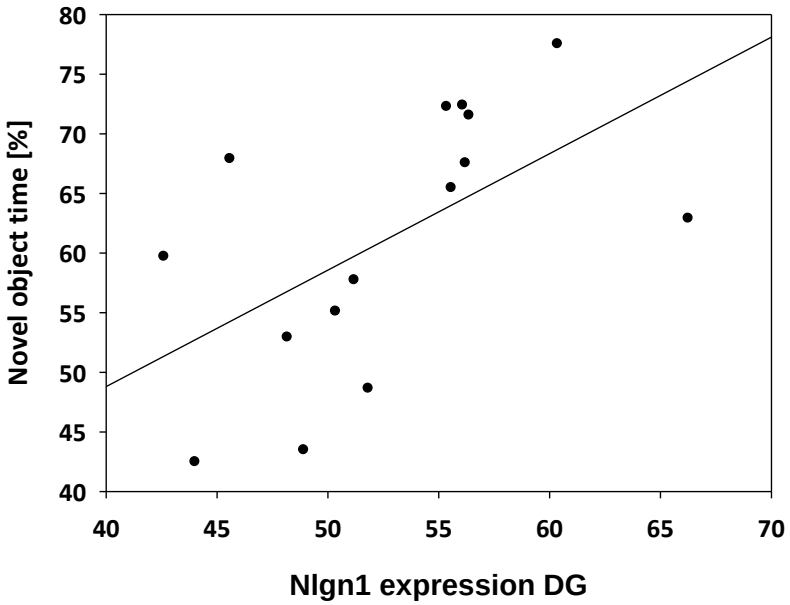

Figure 62: **Pearson correlation between Nlgn 1-mRNA expression in the DG and the novel object time.** There was a positive correlation for basal control and stress animals.

Although these findings confirmed distinct linear correlations between hippocampal expression levels of several nCAMs and the performance in the OR test, they provided no information about the cause-effect relationship of the investigated characteristics.

3.2.7 Summary

In general, the animals' corticosterone levels were elevated after learning both in control and in stress animals. CSS had long-term effects on the animals' performance during OR and inhibited the cognition of aged mice in the OR test: controls spent more time with the novel object than with the known object, while stressed animals did not prefer one object over the other. In the MWM, all animals did not learn very well. Controls, in comparison to stress animals, exhibited shorter escape latencies only during the probe trial, when the platform was removed from the maze. CSS was merely able to inhibit the performance in the MWM partially. As expected, only few candidate nCAMs were regulated 1 year after CSS and a recent learning experience: Nrxn (CA3, DG), Nec 3 (CA3, DG) and Nptn (CA1, CA3, DG). In contrast to the results of young animals, there was a variety of correlations between nCAM expression and the performance during OR for aged

animals: positive correlations were validated for Nec 3 (DG) and Nlgn 1 (DG), negative correlations were found for Nlgn 2 (CA3) and Nptn (CA1, CA3, DG).

3.3 Experiment 3: Regulation of novel synaptic CAMs by dexamethasone

Young mice (12 weeks of age) were injected subcutaneously with dex (controls with Ringer solution) to test whether novel nCAMs can be regulated directly via the GR. Due to the promising results from previous experiments, the following genes were chosen as candidate nCAMs for this experiment: Nec 1, Nec 3, Nlgn 1 and Nptn. Animals were sacrificed at five different time points after the injection.

3.3.1 Neuroendocrine data

Based on the data from the RIA, it was confirmed that the dex treatment worked successfully and that control animals demonstrated normal values according to their circadian rhythm. ANOVA indicated a group effect ($F_{1, 95} = 29.659$; $p < 0.01$), a condition effect ($F_{4, 95} = 15.449$; $p < 0.01$) as well as an interaction effect group*condition ($F_{4, 95} = 16.339$; $p < 0.01$). Both groups (dex and controls) displayed elevated corticosterone levels 1 h after the injection. This can be attributed to the handling during the injection procedure and the injection itself. However, the corticosterone levels of control animals were significantly higher than in the dex treated animals ($T_{16} = 2.399$; $p < 0.05$; see *Figure 63*). In the early morning, which means 2 h and 4 h after the injection, all animals appeared to have recovered from the injection procedure, as mice exhibited decreased corticosterone levels. This would be expected according to the diurnal rhythm of plasma corticosterone in rodents. For both time points, there were no significant differences between controls and dex animals. In the late afternoon, meaning 8 h after the injection, the animals approached the evening circadian peak that is typical for nocturnal mammals like mice. Here, controls showed strongly elevated corticosterone levels in comparison to the dex animals, where the corticosterone synthesis and distribution was inhibited by the dex treatment ($T_{18} = 4.818$; $p < 0.01$; see *Figure 63*). In the morning on the next day, meaning 24 h after the

injection, both groups seemed to have returned to baseline corticosterone levels, which were very low.

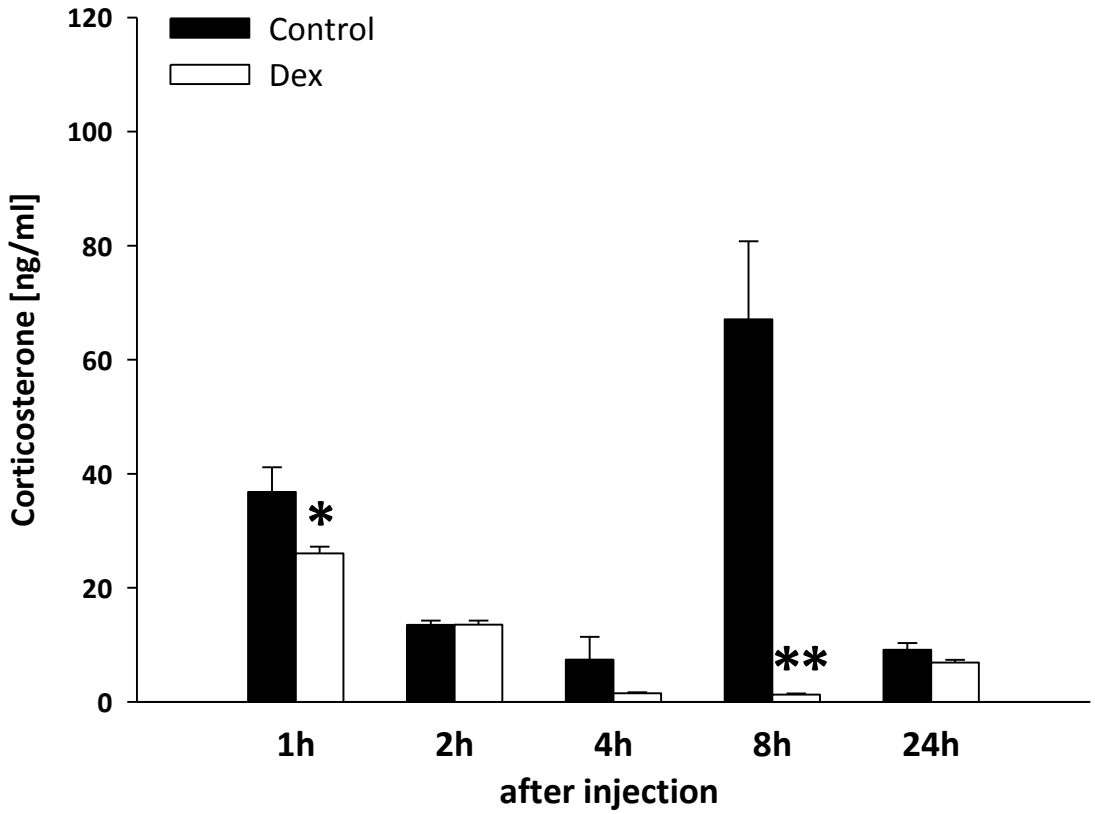

Figure 63: **Corticosterone levels over the course of the dex experiment.** Overall, control animals displayed expected levels according to their diurnal rhythm. 1 h after the injection, dex animals exhibited decreased corticosterone levels compared to control animals. 8 h after the injection, corticosterone levels of dex animals were strongly decreased. For the residual time points, there were no significant differences between the two groups (* significantly different from control animals, $p < 0.05$; ** significantly different from control animals, $p < 0.01$).

3.3.2 Expression levels of nCAM-mRNA

The analysis of nCAM-mRNA autoradiograms revealed that none of the candidate nCAMs was regulated by the dex treatment, as there were no significant differences in the total mRNA-signal between the controls and the dex treated animals. The results for mRNA expression 1 h after the injection for Nec 1 and 8 h after the injection for Nptn are depicted as an example (see *Figures 64* and *65*).

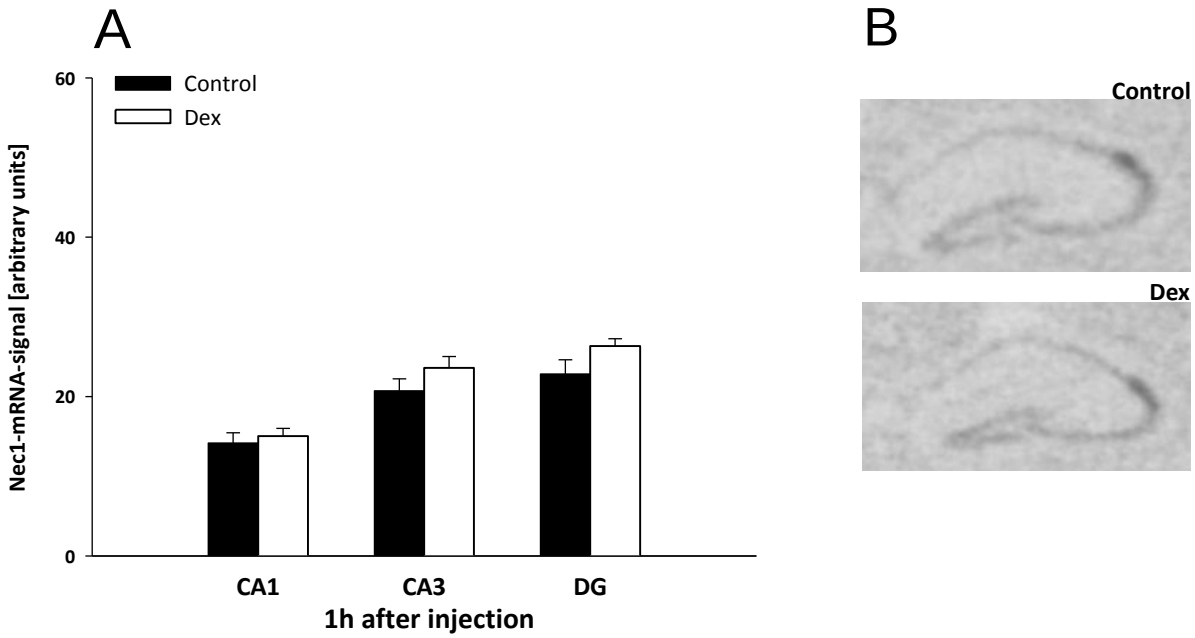

Figure 64: **Nec 1-mRNA-signal in the CA1, CA3 and the DG. (A)** There were no significant differences between the controls and the dex treated animals. **(B)** Representative Nec 1-mRNA autoradiograms are depicted.

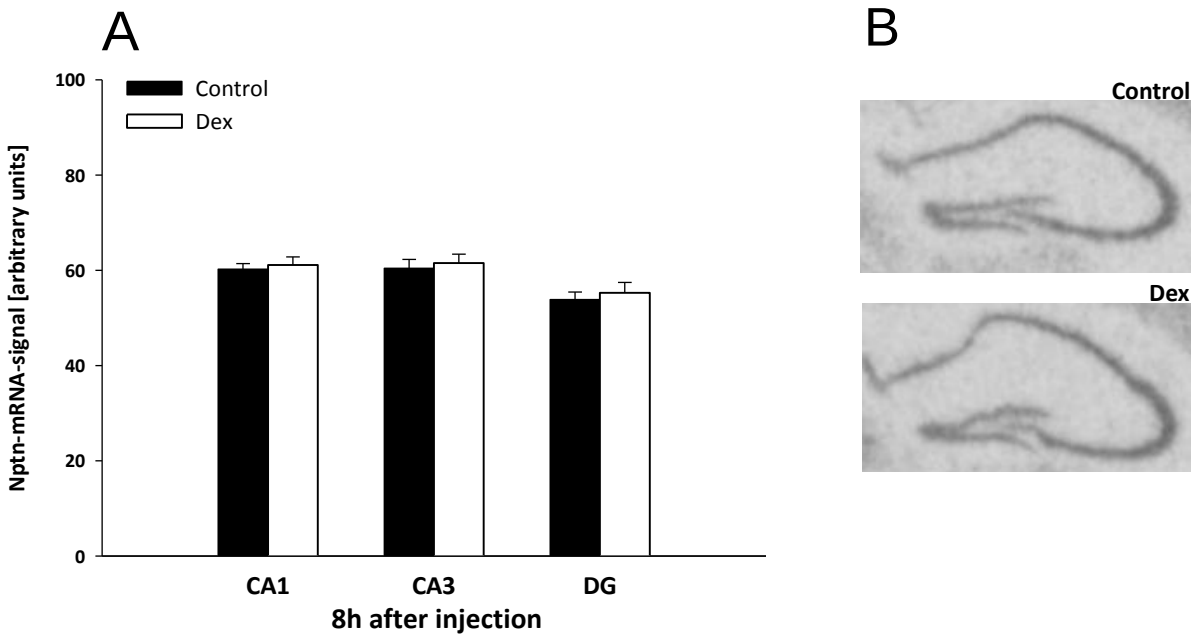

Figure 65: **Nptn-mRNA-signal in the CA1, CA3 and the DG. (A)** There were no significant differences between the controls and the dex treated animals. **(B)** Representative Nptn-mRNA autoradiograms are depicted.

3.3.3 Summary

The dex treatment was successful; control animals demonstrated normal values according to their circadian rhythm. None of the chosen nCAMs was verified to be regulated 1 h, 2 h, 4 h, 8 h, or 24 h after the dex injection.

3.4 Experiment 4: Acute treatment of young animals with mimetic peptides and the impact on cognition under basal conditions

3.4.1 Behavioural data

The first batch of animals received either the mimetic peptide Narpin or artificial cerebrospinal fluid as vehicle. The intrahippocampal injections were conducted before the training, that is 30 minutes before the acquisition trial, during which the animals were supposed to learn new information. During the OR test, Narpin injected animals spent significantly more time with the novel object than with the known object ($T_{10} = 2.742$; $p < 0.05$; see *Figure 66 A*). Vehicle injected animals did not prefer one object over the other and spent equal amounts of time with both objects (see *Figure 66 A*). During the spontaneous alternation test in the Y-maze, there were no significant differences between the two groups. Narpin injected animals as well as vehicle animals performed clearly above the chance level, but did not differ from each other (see *Figure 66 B*).

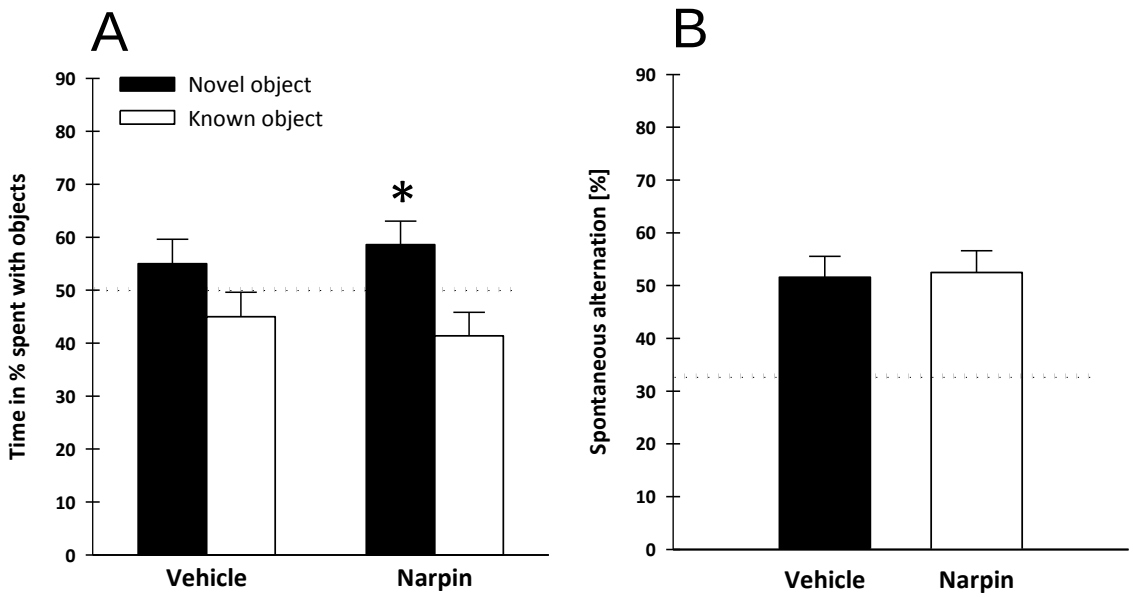

Figure 66: **Narpin injections 30 minutes before behavioural testing. (A)** During the OR test, Narpin animals preferred the novel object over the known object, while vehicle animals did not differentiate between the two. The dotted line represents the chance level at 50 % (* significantly different from known object time; $p < 0.05$). **(B)** Vehicle and Narpin treated animals exhibited an equal spontaneous alternation in the Y-maze. The dotted line represents the chance level at 33 %.

The second batch of animals received intrahippocampal injections of Enplastin or vehicle 30 minutes before the acquisition trial. During the OR test, both groups explored the novel object significantly longer than the known object (vehicle: $T_{20} = 4.175$; $p < 0.01$; Enplastin: $T_{22} = 6.844$; $p < 0.01$; see *Figure 67 A*). During the spatial Y-maze, vehicle animals did not prefer the novel arm over the known arms ($T_{22} = 0.524$; $p = 0.605$; see *Figure 67 B*). Only Enplastin treated animals showed a significant preference for the novel arm ($T_{20} = 2.328$; $p < 0.05$; see *Figure 67 B*). This effect was further supported by the data set for the arm entries, where again only Enplastin treated animals exhibited a preference to enter the novel arm more often than the known arms (vehicle: $T_{34} = 0.231$; $p = 0.819$; Enplastin: $T_{31} = 2.663$; $p < 0.05$).

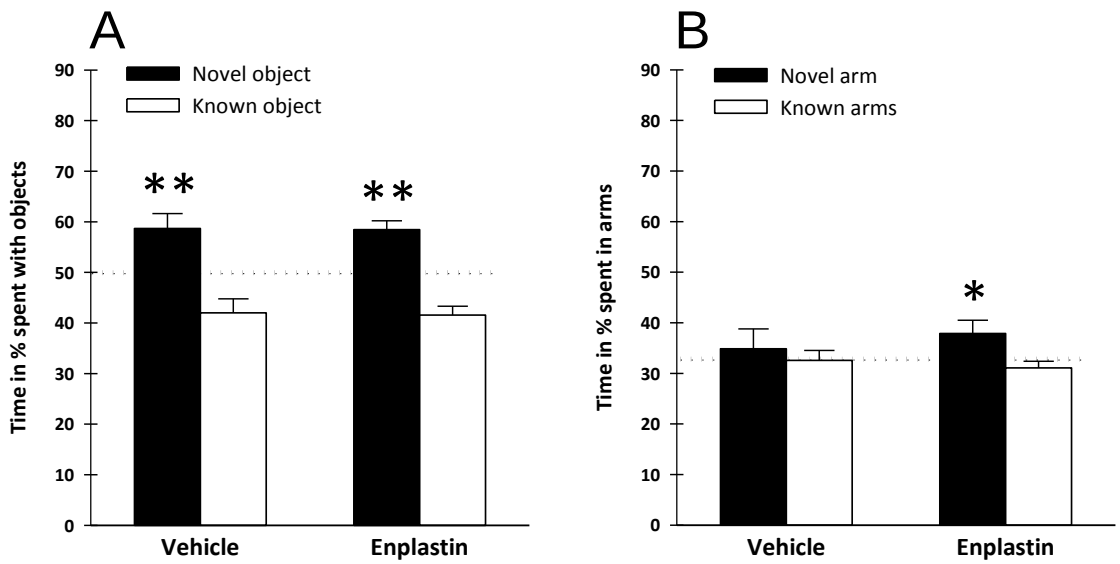

***Figure 67**:* **Enplastin injections 30 minutes before behavioural testing. (A)** During the OR test, both groups preferred the novel object over the known object. The dotted line represents chance level at 50 % (** significantly different from known object time; $p < 0.01$). **(B)** In the Y-maze, only Enplastin treated animals spent more time in the novel arm compared to the known arms. The dotted line represents chance level at 33 % (* significantly different from known arm time; $p < 0.05$).

The third batch of animals was subjected to the intrahippocampal injections of Narpin or vehicle during the behavioural training. Precisely, the injections were performed directly after the acquisition trial, which was 30 minutes before the retrieval trial, during which the animals were supposed to access the stored information. During the OR test, both groups explored the novel object significantly longer compared to the known object (vehicle: $T_{12} = 4.807$; $p < 0.01$; Narpin: $T_{18} = 3.479$; $p < 0.01$; see *Figure 68 A*). During the spatial Y-maze, Narpin treated animals entered the novel arm significantly more often than the known arms ($T_{28} = 2.372$; $p < 0.05$; see *Figure 68 B*). For vehicle animals, the tendency to enter the novel arm to a greater extent than the known arms remained only a trend ($T_4 = 2.753$; $p < 0.1$; see *Figure 68 B*). However, this effect may be attributed to the very small group size of only two animals. The analysis of the percentage of the time spent in the arms did not reveal any significant differences, neither for vehicle nor for Narpin treated animals (see *Figure 68 C*).

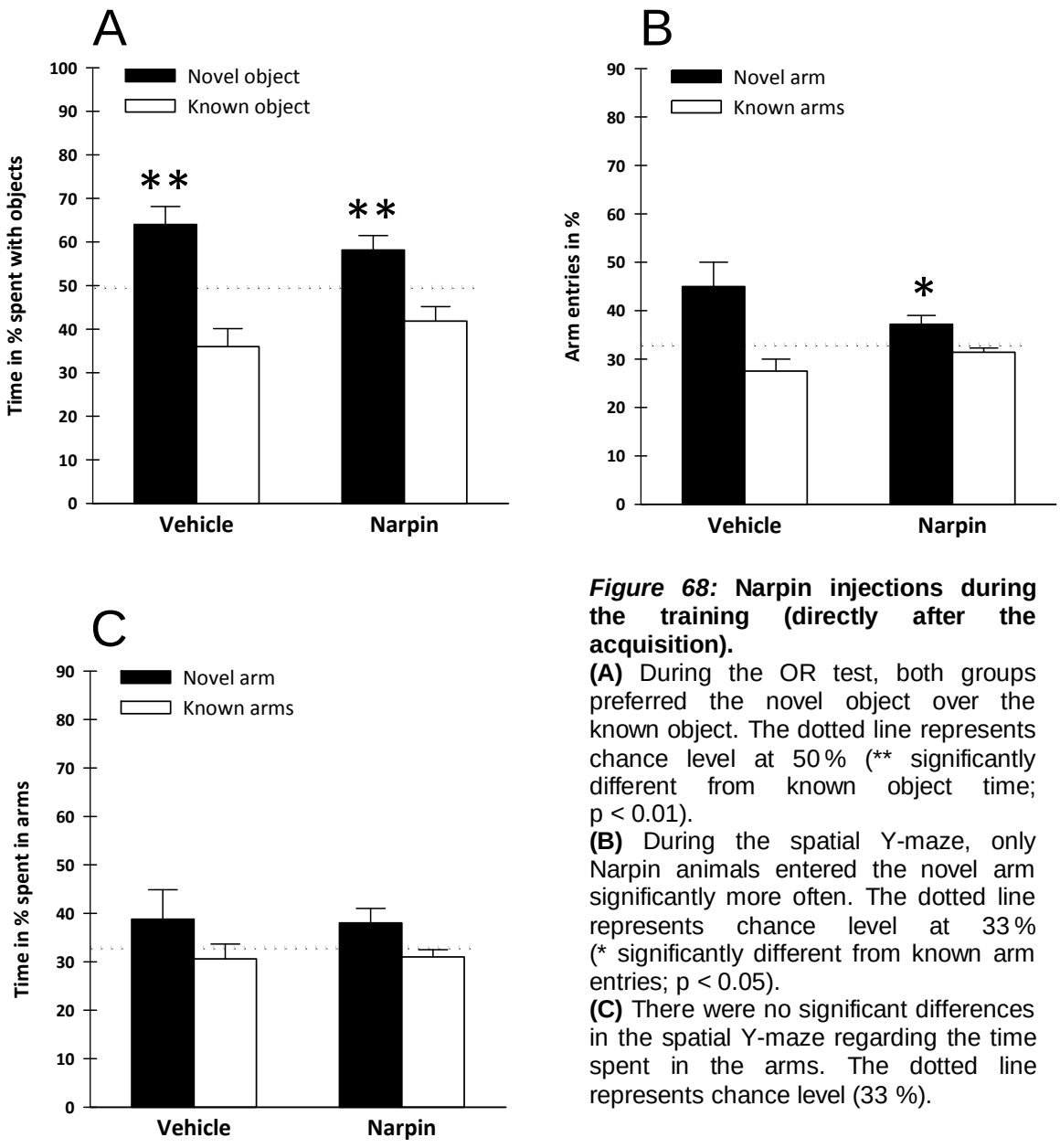

Figure 68: **Narpin injections during the training (directly after the acquisition).**
(A) During the OR test, both groups preferred the novel object over the known object. The dotted line represents chance level at 50 % (** significantly different from known object time; $p < 0.01$).
(B) During the spatial Y-maze, only Narpin animals entered the novel arm significantly more often. The dotted line represents chance level at 33 % (* significantly different from known arm entries; $p < 0.05$).
(C) There were no significant differences in the spatial Y-maze regarding the time spent in the arms. The dotted line represents chance level (33 %).

Directly after the acquisition trial, the fourth batch of animals was injected with either Enplastin or vehicle. During the OR test, both groups spent significantly more time exploring the novel object than the known object (vehicle: $T_{18}= 5.979$; $p < 0.01$; Enplastin: $T_{28} = 5.411$; $p < 0.01$; both see *Figure 69 A*). During the spatial Y-maze test, vehicle animals did not spent significantly more time in the novel arm compared to the known arms (see *Figure 69 B*). In contrast to Enplastin treated animals, which explored the novel arm significantly longer than the known arms ($T_{22} = 3.045$; $p < 0.01$; see *Figure 69 B*). However, the data set regarding the arm entries in %, revealed that both animal groups entered the novel arm significantly

more often than the known arms (vehicle: $T_{18} = 3.749$; $p < 0.01$; Enplastin: $T_{22} = 2.912$; $p < 0.01$; both see *Figure 69 C*).

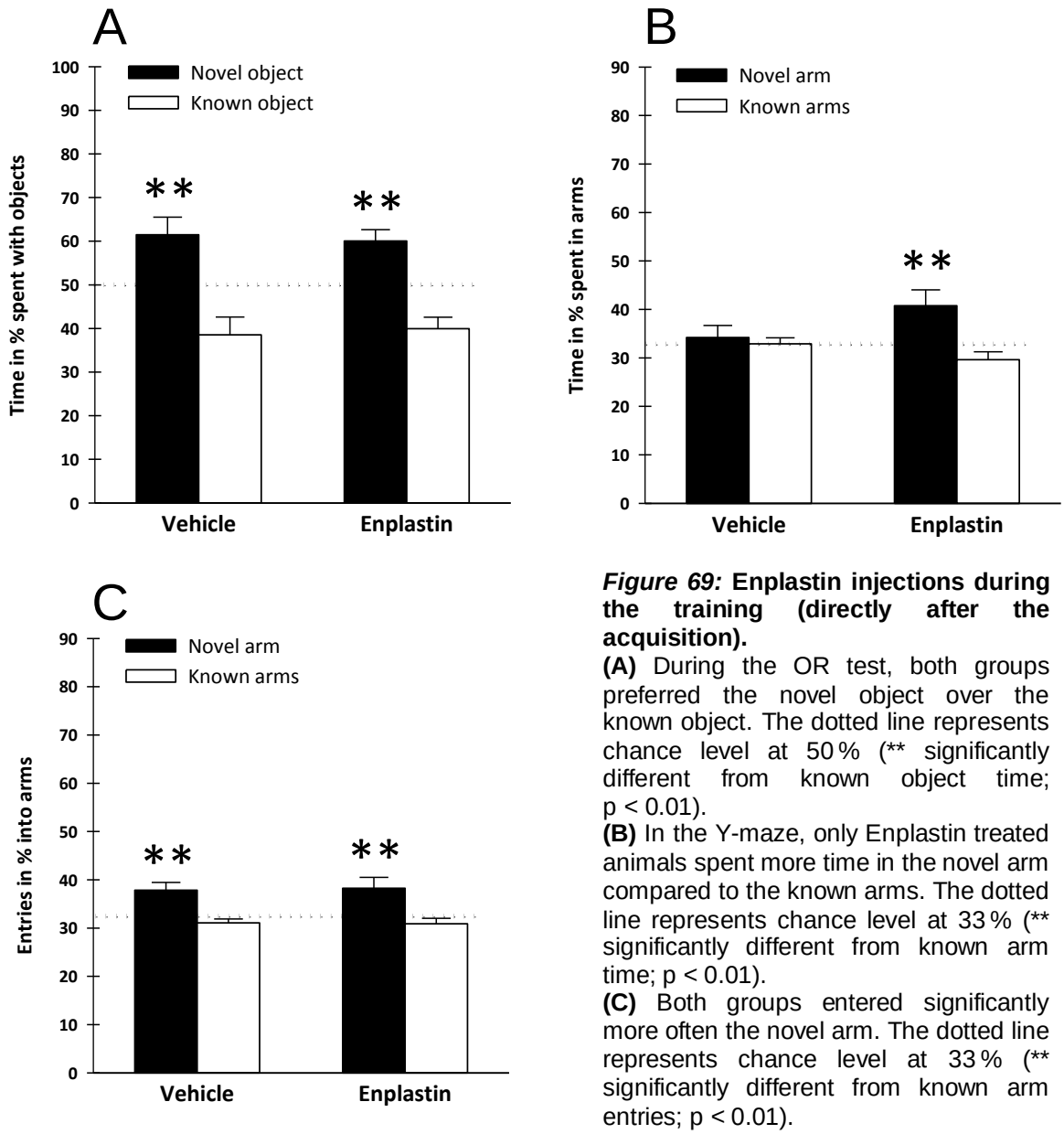

Figure 69: **Enplastin injections during the training (directly after the acquisition).**
(A) During the OR test, both groups preferred the novel object over the known object. The dotted line represents chance level at 50 % (** significantly different from known object time; $p < 0.01$).
(B) In the Y-maze, only Enplastin treated animals spent more time in the novel arm compared to the known arms. The dotted line represents chance level at 33 % (** significantly different from known arm time; $p < 0.01$).
(C) Both groups entered significantly more often the novel arm. The dotted line represents chance level at 33 % (** significantly different from known arm entries; $p < 0.01$).

Within each batch, mimetic peptide injected animals did not differ from vehicle treated animals in their general locomotion: the total time immobile (batch 1: $T_8 = 1.667$; $p = 0.134$; batch 2: $T_{10} = 0.096$; $p = 0.925$; batch 3: $T_{21} = 1.350$; $p = 0.191$; batch 4: $T_{27} = 1.797$; $p = 0.084$) and the total distance travelled (batch 1: $T_8 = 0.471$; $p = 0.650$; batch 2: $T_{10} = 1.087$; $p = 0.302$; batch 3: $T_{21} = 1.391$; $p = 0.179$; batch 4: $T_{27} = 1.722$; $p = 0.097$) were equal.

3.4.2 Summary

In the batch of Narpin animals with injections 30 minutes before behavioural testing, Narpin animals exhibited a preference for the novel object, while vehicle animals spent equal amounts of time with both objects. If Narpin was injected during the behavioural testing, both groups (vehicle and mimetic peptide treated) preferred the novel object over the known object. In the spatial Y-maze, only Narpin animals entered the novel arm significantly more often. Enplastin injections 30 minutes before behavioural testing did not alter the performance in the OR test: both groups explored the novel object longer than the known object. In the spatial Y-maze, only Enplastin animals preferred the novel arm (as measured by the arm time and the arm entries in %). Enplastin injections during the behavioural testing did not change the OR performance, vehicle and Enplastin treated animals both explored the novel object longer than the known object. While both groups entered the novel arm significantly more often than the known arms, only Enplastin treated animals preferred the novel arm in the spatial Y-maze as measured by the time spent in % in the arms. Overall, a modification of cognitive performance was possible: if vehicle animals already suffered from cognitive deficits and were unable to perform above the chance level, the mimetic peptides could counteract in some cases and raise the animals' performance to an anticipated level.

3.5 Experiment 5: Chronic treatment of young animals with mimetic peptides and the impact on cognition after CSS

To assess the consequences of a chronic treatment with mimetic peptides in young animals (11 weeks of age) on cognition, male young CD1 mice received s.c. injections of Enplastin, Narpin or vehicle (Ringer solution) on 6 consecutive days during the last week of the CSS paradigm. To record potential effects on cognition, animals were behaviourally tested. On these days, they received the injections 2 h prior to the testing. The aim was to investigate the possibilities of reversal or at least improvement of cognitive deficits that are elicited by CSS during adolescence.

3.5.1 Testing blood brain barrier permeability of mimetic peptides

To ensure that mimetic peptides crossed the blood brain barrier via s.c. injections and verify the validity of experiment 5, a preceding experiment was conducted. 12 weeks young, male CD1 mice were subcutaneously injected with biotylised Enplastin, biotylised Narpin or vehicle (Ringer); 1 h after the injection, the animals were perfused and brains extracted. The presence of mimetic peptides in the brain was visualised via immunofluorescence for free-floating brain sections (see chapter 2.6.2). Confocal imaging microscopy revealed that mimetic peptides indeed were able to cross the blood brain barrier and spread in the whole brain. Due to tissue damages in the brain slices, it was not possible to provide images of the hippocampus with satisfying tissue quality; instead *Figure 70* presents images of the cortex with layer 1 to 3 (40 x magnification). Nonetheless, the analysis clearly demonstrated the mimetic peptides' crossing of the blood brain barrier.

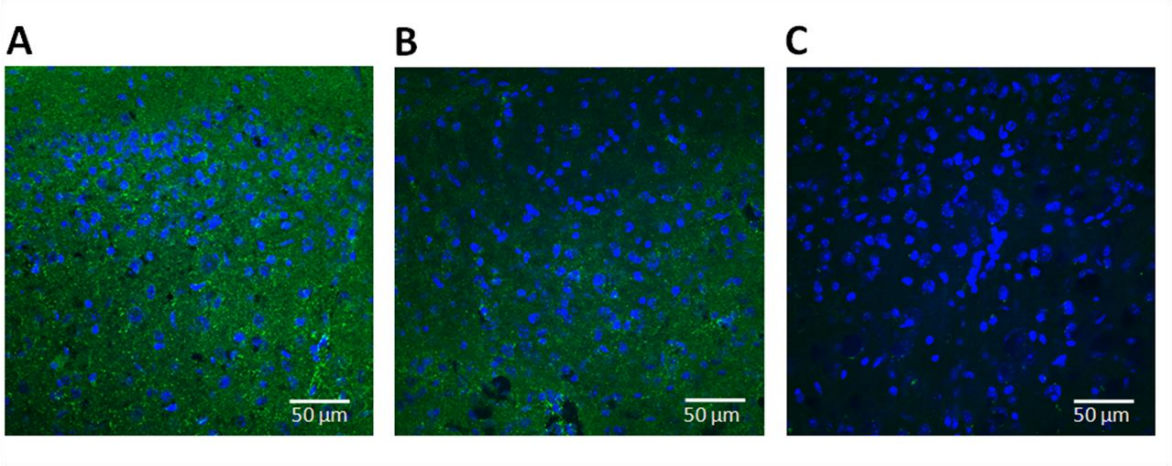

Figure 70: **Representative photomicrographs illustrating the presence/absence of mimetic peptides in the cortex of the mouse brain. (A)** Enplastin (dilution: 1:200). **(B)** Narpin (dilution: 1:200). **(C)** Negative control (vehicle) (blue: cell bodies, green: biotylised and antibody-bound mimetic peptide).

3.5.2 Physiological data

Animals were sacrificed 24 h after the last injection. Adrenals were extracted and the analysis revealed that CSS animals had enlarged and thus heavier adrenal glands compared to control animals (T_{22}= 3.389; $p < 0.01$; see *Figure 71*). Independent t-tests showed no significant differences in the final body weight of control and stress animals (T_{22}= 0.667; $p = 0.512$).

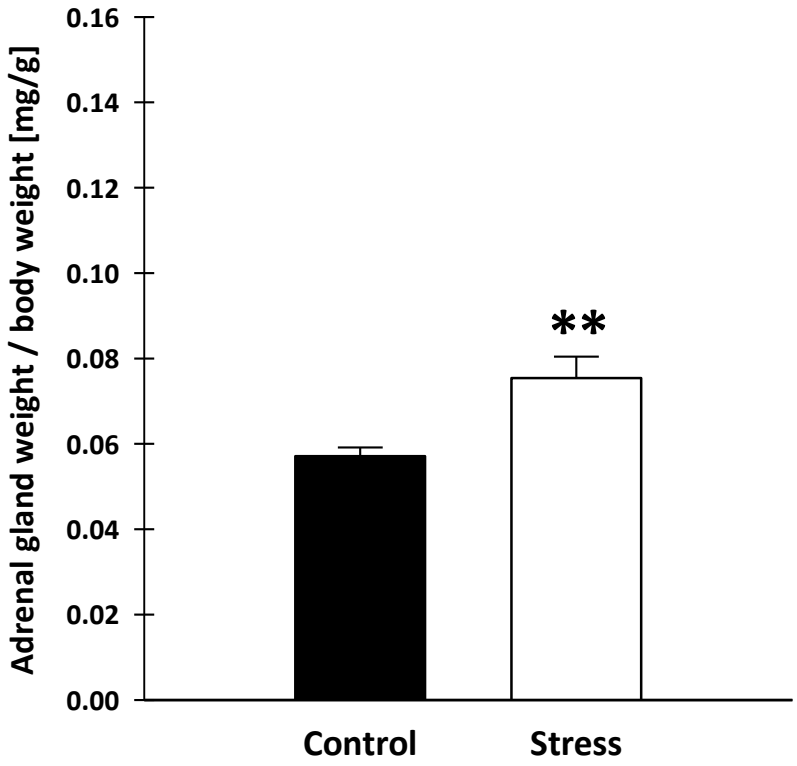

Figure 71: **Adrenal gland weight.** At the end of the CSS procedure, adrenal gland weights were significantly increased in stressed animals (** significantly different from the control group, $p < 0.01$).

3.5.3 Neuroendocrine data

Blood samples were collected simultaneously with the adrenals, hence 24 h after the last injection. RIA revealed increased plasma corticosterone levels in stressed animals ($T_{91}= 2.533$; $p < 0.05$; see *Figure 72*). In general, the plasma corticosterone values were relatively high, even for the control animals. This might be attributed to the daily injections and the handling of the mice.

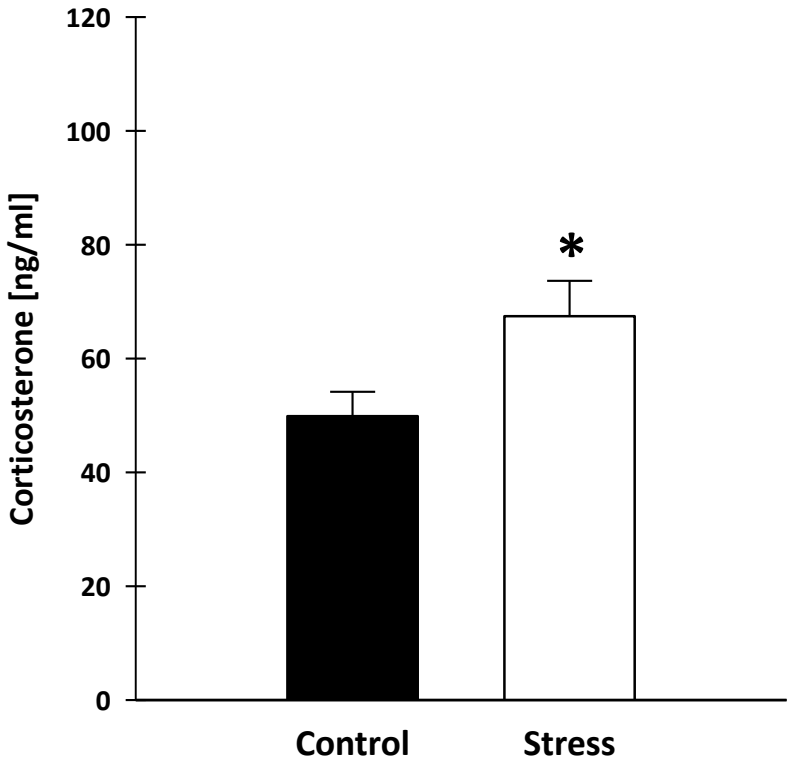

Figure 72: **Plasma corticosterone levels.** Stress animals revealed elevated corticosterone levels at the end of the CSS procedure compared to control animals (* significantly different from the control group, $p < 0.05$).

3.5.4 Behavioural data

No effects at all could be demonstrated in the OR test. All animals remembered the novel object very well and explored it to a great extent. The animals' performance exceeded the chance level by far. For all treatments (Narpin, Enplastin, vehicle) and all groups (control, stress), the average novel object time resided just below 70 % (see *Figure 73*).

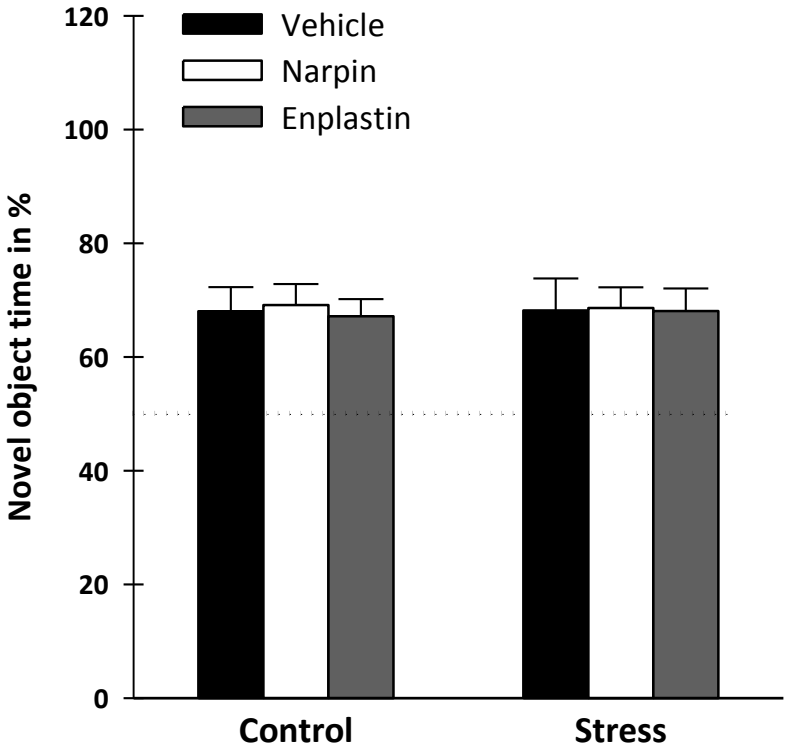

Figure 73: **Novel object time in % during the OR test.** All animals independently of treatment or group exhibited high novel object times. The dotted line represents chance level (50 %).

In the spatial Y-maze, all animals showed a performance clearly above chance level. Nonetheless, Narpin and Enplastin seemed to have differential effects: Narpin may have protected the animals from detrimental CSS consequences, whereas Enplastin did not change the performance for the better or for the worse compared to vehicle treated animals. ANOVA did not find significant differences for Enplastin treated animals (group: $F_{1, 46} = 0.314$; $p = 0.578$; treatment: $F_{1, 46} = 0.440$; $p = 0.511$; interaction group*treatment: $F_{1, 46} = 0.683$; $p = 0.413$), but indicated a treatment effect on the percentage of novel arm time between vehicle and Narpin treated animals ($F_{1, 46} = 11.210$; $p < 0.01$). Post-hoc t-test demonstrated that Narpin treated stress animals spent significantly more time in the novel arm compared to stressed vehicle animals ($T_{22} = 2.892$; $p < 0.01$; see *Figure 74 A*), while Enplastin treated animals spent equal amounts of time in the novel arm compared to vehicle animals, regardless of prior experience (see *Figure 74 B*). This result was further supported by the analysis of the novel arm entries, where Enplastin and vehicle treated animals again performed similarly and entered the novel arm equally often independent of the group. For Narpin treated

control animals, there was a trend that they had higher novel arm times than the vehicle treated controls (T_{21}= 1.890; p < 0.1; see *Figure 74 A*). The analysis of the novel arm entries for Narpin treated animals supported this trend in the control group: here, Narpin animals entered significantly more often the novel arm than the vehicle animals (T_{21}= 2.226; p < 0.05; see *Figure 75*). The analysis of the discrimination ratio confirmed that all animals performed above chance levels, that Enplastin did not alter the animals' performance in comparison to vehicle animals and that Narpin lead to a protection effect in animals after CSS, as they were the only group with an increased discrimination ratio compared to vehicle animals (T_{22} = 2.358; p < 0.05). Mimetic peptide treated animals did not differ from vehicle animals in their general locomotion: the time immobile (Enplastin batch: T_{45} = 0.012; p = 0.990; Narpin batch; T_{45} = 0.606; p = 0.547) and the total distance travelled (Enplastin batch; T_{44} = 1.002; p = 0.634; Narpin batch; T_{44} = 0.764; p = 0.449) were equal.

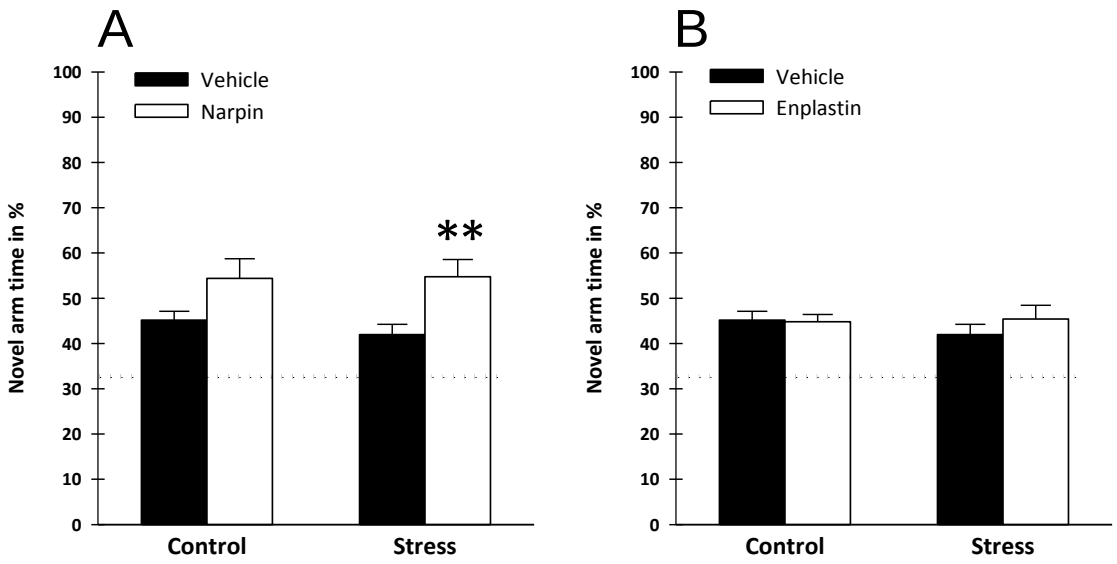

Figure 74: **Novel arm time in % in the spatial Y-maze. (A)** After CSS, Narpin seemed to have a protective effect and the novel arm time remained high. In controls, there was a trend that Narpin injections lead to higher novel arm times compared to the vehicle treatment (** within stress group, significantly different from vehicle treated animals; p < 0.01). **(B)** There was no significant effect of the Enplastin treatment, neither under control conditions nor after CSS. For both graphs, the dotted line represents chance level (33 %).

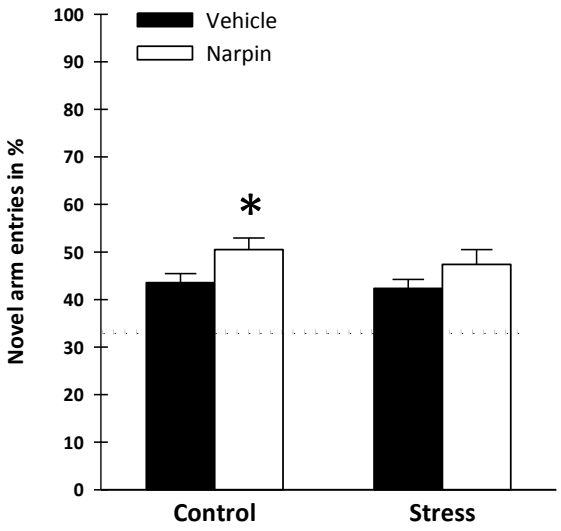

Figure 75: **Novel arm entries in % in the spatial Y-maze.**
In the control group, Narpin injected animals entered the novel arm more often than the vehicle injected animals. There was no effect in the stress group. The dotted line represents chance level at 33 % (* within control group, significantly different from vehicle treated animals; $p < 0.05$).

3.5.5 Summary

Preceding tests clearly demonstrated that mimetic peptides were able to overcome the blood brain barrier via s.c. injections. The validity of our CSS paradigm was once again confirmed: stress animals exhibited enlarged adrenal glands as well as elevated plasma corticosterone levels compared to controls. In the spatial Y-maze, all animals showed a performance clearly above chance level. However, Narpin and Enplastin had differential impacts on the animals' cognition: Enplastin did not alter the animals' performance compared to vehicle treated animals, while Narpin protected the animals from detrimental CSS consequences and the novel arm time remained high. Hence, a modification of cognitive performance via s.c. injections was possible, at least for Narpin treated animals.

3.6 Experiment 6: Chronic treatment of aged animals with mimetic peptides and the impact on cognition after CSS

To assess the consequences of a chronic treatment with mimetic peptides in aged animals on cognition, male CD1 mice received s.c. injections of Enplastin, Narpin or vehicle (Ringer solution) on 6 consecutive days. The injections were timed that upon starting behavioural testing, all animals had reached the age of 15 months. Animals were injected in the morning; on testing days, animals received the injections 2 h prior to the behavioural test. The aim was to investigate the possibilities of reversal or at least improvement of cognitive deficits that are elicited by CSS during adolescence and/ or aging.

3.6.1 Physiological data

Animals were sacrificed 24 h after the last injection. Adrenal glands were extracted for further analysis, but independent t-tests were not able to reveal any significant differences between control and stress animals ($T_{18} = 1.755$; $p = 0.096$).

3.6.2 Neuroendocrine data

Blood samples were collected simultaneously with the adrenals, hence 24 h after the last injection. As anticipated, RIA disclosed equal plasma corticosterone levels in control and stress animals due to the 12 months of undisturbed single-housing after the CSS procedure ($T_{55} = 0.306$; $p = 0.760$). Corticosterone values resided in the expected basal range just above 10 ng / ml.

3.6.3 Behavioural data

No effects at all could be demonstrated in the OR test. All animals remembered the novel object and their performance exceeded the chance level. For all treatments (vehicle, Narpin, Enplastin) and all groups (control, stress), the average novel object time resided just below 60 % (see *Figure 76*).

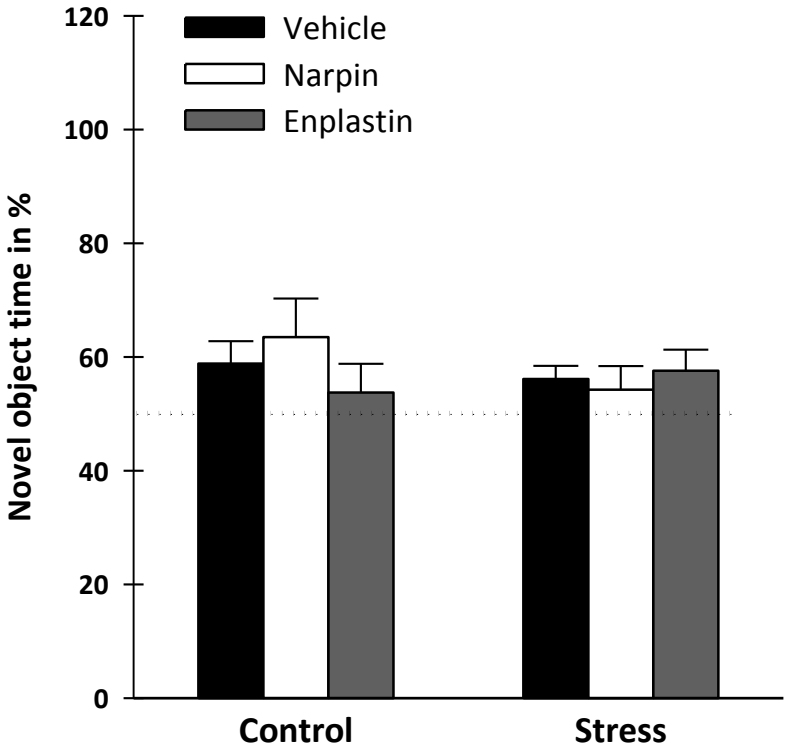

Figure 76: **Novel object time in % during the OR test.** All animals independently of treatment or group exhibited novel object times above chance level. The dotted line represents chance level (50 %).

In the spatial Y-maze, all animals showed a similar performance above or barely above chance level. There was neither a significant difference in the novel arm time between the different treatment groups (vehicle, Narpin, Enplastin) nor between stressed animals and controls (see *Figure 77*). This result was supported by the analysis of the novel arm entries, which also did not reveal any significant differences between treatments or groups.

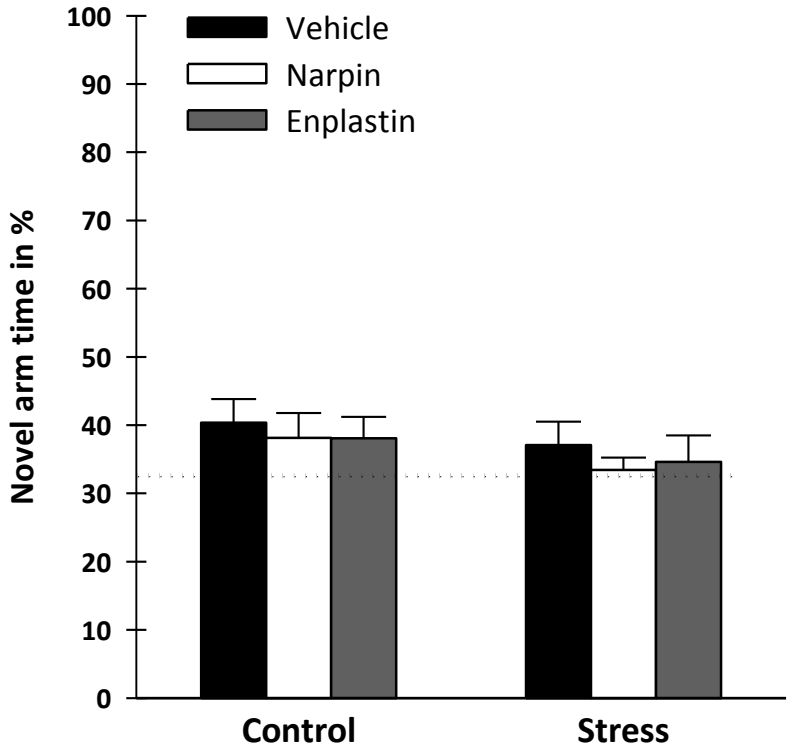

Figure 77: **Novel arm time in % in the spatial Y-maze.** No effects at all were demonstrated in the spatial Y-maze. Animals exhibited similar novel arm times independent of treatment or group. The dotted line represents chance level (33 %).

3.6.4 Summary

In this experiment, animals were 15 months old. Thus, they had been single-housed for 1 year after the cessation of the CSS paradigm. Consequently, there were no significant differences between control and stress animals regarding the physiological and neuroendocrine parameters. Moreover, behavioural testing did not reveal any differences, neither in the OR nor in the spatial Y-maze: all animals performed similarly independent of treatment or group. Hence, a modification of cognitive performance via s.c. injections in aged animals was not possible.

4 Discussion

The aim of this thesis was the investigation of recently identified synaptic CAMs and their function in processes of cognition, chronic stress and aging in male CD1 mice. Furthermore, the role of mimetic peptides as novel therapeutic targets was examined.

4.1 Physiology

Under control conditions, as well as directly after the CSS exposure or 1 year after CSS, physiological parameters were analysed in male CD1 mice by assessing body, adrenal gland and thymus weights.

CSS had no influence on body weight

The chronic activation of the HPA axis has been linked to changes in energy homeostasis (for example altered insulin actions), changes in food intake and hence, also to changes in body weight (Björntorp, 2001; Dallman et al., 2003; Dallman et al., 2004). Although these alterations are very well described, they are not fully understood. Human studies revealed that a disturbed HPA axis function is accompanied by abdominal obesity, an elevated body mass index and an increased waist-to-hip-ratio (Rosmond et al., 1998; Epel et al., 2000; Smith et al., 2005). In contrast, a variety of studies has shown that animal models of chronic stress, for example models that employ subordination, can be linked to a reduction in body weight, which was often related to a decreased food intake (Fuchs and Flügge, 2002; Rygula et al., 2005; Tamashiro et al., 2006; Tamashiro et al., 2007). On the other hand, several laboratories presented animal models of chronic stress that induced an increase in body weight and fatty tissue (Bartolomucci et al., 2004; Moles et al., 2006; Foster et al., 2006; Solomon et al., 2007).

Our CD1 mice did not exhibit any significant differences in body weight between control and stress animals, neither acutely nor in the long-term. Nonetheless, this finding corresponds to previous studies employing the CSS paradigm (Schmidt et al., 2007; Schmidt et al., 2008; Sterlemann et al., 2008; Schmidt et al., 2010).

Here, the experimental animals' body weight remained stable over the course of the experiment as well. Although CSS exposure did not have a direct effect on body weight, Schmidt and colleagues (2009) were able to show that the CSS paradigm led to a redistribution of body fat in mice in later life. This redistribution caused an unfavourable visceral fat / subcutaneous fat ratio, thereby possibly increasing the risk for metabolic disease.

Adrenal gland weight was increased shortly after CSS

It is generally accepted that a chronic activation of the HPA axis during a long-lasting stress phase leads to adrenal hyperactivity (Ulrich-Lai et al., 2006). Therefore, the adrenal weight is a regularly used parameter to assess the effectiveness of stress paradigms. Several studies associated chronic stress exposure with enlarged adrenals (Klein et al., 1992; Karst and Joels, 2003; Schmidt et al., 2007; Sterlemann et al., 2008; Zoladz et al., 2008), but Ulrich-Lai and colleagues (2006) specified these findings: hyperplasia (excessive cell division) and hypertrophy (inordinate cell growth) contributed simultaneously to the adrenal enlargement, but occurred in different adrenal sub-regions.

Our results are in line with these previous findings, as adrenal glands were consistently enlarged after CSS, thus implying an increased HPA axis activity and an adaptation of the HPA axis to chronic stress. However, these effects were only observed shortly after stress. A stress effect on adrenal glands in the long-term was not detected, as 1 year after the CSS exposure, adrenal glands of stress animals exhibited weights, which were equal to those of control animals. It is likely that due to adaptation mechanisms, the stress animals were able to recover from the stress experience with regard to this parameter. The adrenal gland as an endocrine organ is able to adapt to different demands (hyperplasia and involution). As the stimulus (CSS) was not present anymore, the hyperplasia was no longer needed and the organ size normalised. This is conform to a precedent long-term study conducted by Sterlemann and colleagues (2008).

CSS did not alter thymus weight

The thymus being a lymphoid organ plays a key role in initiating and supporting immune competence in mammals (Delima and Walford, 1975; Gomez-Sanchez, 2009). Hence, thymus weight is an indicator for the condition of the immune system, which in turn is strongly associated to stress experience (Frieri, 2003; Deak, 2008; Stojanovich, 2010). The thymus has a crucial role in the generation of T cells, even though there are extra-thymic T cells as well (Clegg et al., 1996; Abo, 2001). During the foetal and postnatal period, the thymic T cells are most active and have a high tendency to migrate from the thymus to the periphery after maturation. However, the thymus has been shown to involute during the aging process (Aspinall and Andrew, 2000). The thymic involution starts soon after birth. Thus, the thymus' ability to provide mature T cells is diminished throughout life until it reaches a minimum in the elderly (Fry and Mackall, 2002; Dominguez-Gerpe and Rey-Mendez, 2003; Rezzani et al., 2008). This is a normal age-related development and should not be equated with a stress-induced thymic atrophy (Pearse, 2006a). The thymus is known to be extremely sensitive to stress and often reacts towards GCs with apoptosis and therefore shrinkage (Klein et al., 1992; Karst and Joels, 2003; Taub and Longo, 2005). Nonetheless, the histological status of the thymus under both conditions (aging and stress) is similar. A reduced amount of cortical lymphocytes as well as the shrinkage of thymic lobules characterise both conditions (Schuurman et al., 1994). This similarity renders the distinction between aging-induced involution and stress-induced atrophy problematic. Furthermore, stress may accelerate immunosenescence, which is the term for aging-associated immunological alterations (Bauer et al., 2009). Consequently, thymus extraction in aged mice was not conducted. In young animals, our data did not reproduce the thymus atrophy after stress. However, this seems to be a finding that is not as consistent as the adrenal hypertrophy and hyperplasia following stress (Karst and Joels, 2003).

4.2 Neuroendocrinology

Corticosterone levels were increased shortly after CSS

To supply the body with energy necessary during periods of stress and high arousal, GCs are released as a main hormonal product of the HPA axis. Hence, elevated GC levels can be regarded as an endocrine correlate of a continued HPA axis hyperactivity. In the past, chronic stress has been frequently associated to pathologically elevated levels of circulating GCs and to a dysregulation of the HPA axis feedback mechanisms (Henry, 1992; Sapolsky, 1992; Keeney et al., 2001; Keeney et al., 2006; Schmidt et al., 2007; Sterlemann et al., 2008).

In accordance to those studies, significantly elevated morning corticosterone levels were found in stressed animals compared to controls directly after the stress exposure (in animals without learning experience). As expected, there were no significant differences between basal stress animals and basal controls 12 months after CSS cessation. As the stimulus (CSS) has been absent for 1 year, the corticosterone levels of stress animals were able to normalise. This is conform to our findings for adrenal gland weight, which revealed equal values for aged controls and stress animals. Furthermore, these results support the hypothesis of Qiu and co-workers (2007), who suggested that elevated basal corticosterone levels should be regarded as a side effect of enlarged adrenals.

Corticosterone levels were increased after learning in the MWM

Previous studies showed an up-regulation of corticosterone after learning. Behavioural paradigms, such as the MWM, are indeed designed to assess cognitive performance, learning and memory. However, these paradigms often include an aversive component and the testing procedure alone induces stress in the experimental animals, which can be measured as elevated corticosterone levels (McIlwain et al., 2001; Mifsud et al., 2011; Trollope et al., 2011). Additionally, GCs are strongly involved in the consolidation of memories, for example the acquired behavioural response. It is known that apart from the stress hormone, stress responses and learning procedures also share some signalling pathways (Sandi et al., 1997; Roozendaal et al., 2006; de Quervain et al., 2009). Our data confirmed these findings: control and stress animals after learning did not

differ significantly in their plasma corticosterone levels, but exhibited in general much higher corticosterone levels than animals without learning experience. This learning-induced increase of corticosterone levels was found in young as well as in aged animals. However, the conclusion that learning increases corticosterone levels might not be applicable generally, as both, the exposure to the stressful test paradigm (MWM) and the learning process itself, induce corticosterone levels to rise. It is difficult to distinguish the process of learning from the stressful experience in the test paradigm.

To summarise: (I) CSS acutely elevated corticosterone levels in animals without learning. (II) The increased corticosterone levels in animals after learning (control and stress animals) verified that the experimental animals were stressed by the MWM procedure. (III) The animals were able to recover from the stressful learning experience back to low corticosterone concentrations as seen in the basal animals from experiment 2. These animals also participated in the MWM, but were decapitated for trunk blood sampling only after a recovery period of 2 weeks.
As neuroendocrine alterations are major players in the metabolic system, a neuroendocrine response alone is not a sufficient indicator for the experience of stress (Koolhaas et al., 2011). Woodson and colleagues (2003) reported that predator exposure as well as the exposure to a female conspecific activated the HPA axis in rats to a similar degree. While the first event is highly probable to be perceived as stressful by the animals, this is unlikely for the latter. This was verified by the fact that only predator exposure impaired the animals' cognition. Nonetheless, if taken together, physiological and neuroendocrine parameters are valuable tools for the prediction of HPA axis activity and whether a specific event leads to stress perception or not. Thus in this thesis, the combined results of physiological and neuroendocrine analysis are sufficient to confirm the proper mode of action and the effectiveness of our CSS paradigm.

4.3 Cognitive testing

To investigate the influence of CSS and / or aging on learning and memory performance, cognitive testing was performed. To test short-term recognition memory, the OR test was used (Dodart et al., 1997; Ennaceur et al., 1997; Gaskin et al., 2003; Winters et al., 2004). To assess hippocampus-dependent short-term spatial memory, the Y-maze test was applied (Dellu et al., 1992; Olton and Markowska, 1994; Conrad et al., 1996). Finally, to analyse hippocampus-dependent long-term spatial learning and memory, mice were tested in the MWM test (D'Hooge and De Deyn, 2001; Alvin V. and Terry Jr., 2009).

In young animals, CSS inhibited recognition memory, while the spatial Y-maze performance remained unaffected

In experiment 1, young animals were tested in the OR test, the spatial Y-maze test and the MWM test. CSS clearly inhibited the animals' recognition memory. This finding is in line with the study from Scullion and colleagues (2009) and Li and co-workers (2008), who also reported impairments in object recognition following stress. However, the study by Wright and Conrad (2005) showed that chronic stress left novelty seeking behavior intact, while it impaired spatial recognition memory in the Y-maze. In general, object recognition has been specifically linked to the function of the prefrontal cortex in humans (Schendan, 2008) and animals (Bussey, 2000; Barker, 2008). It seems therefore likely that the exposure to chronic social stress in the current study had a negative impact on this brain region. In contrast to these results of the OR test, in the Y-maze test, both control and stress animals spent more time in the novel arm than in the known arms. Hence, it seemed that the CSS procedure did not affect the spatial memory performance of our mice in the Y-maze test. However, it is possible that the employed ITI of 30 minutes was too short to visualise potential differences between control and stress animals, as both groups showed intact spatial recognition. To model a chronic stress situation in young animals successfully and visualise impairing effects on cognition, apparently, further measures need to be taken in the Y-maze. Thus, in follow-up studies, a prolonged ITI between the acquisition and the retrieval trial, for example 1 hour instead of the previously

employed 30 minutes, could be applied. This more challenging task may reveal potential stress-related impairments in young animals in the Y-maze test.

In young animals, CSS partially inhibited spatial MWM learning and memory

Previous studies have often reported about strain specific and individual variation, particularly in the MWM (Crabbe et al., 1999; Gerlai, 2001; Wahlsten et al., 2003). Thus, individual variations within experimental animal groups are already known as a main impediment in behavioural research and this variability remains an issue even in genetically identical cohorts (Saab et al., 2011). Theories about the origin are broadly diversified. Epigenetics (Mager and Bartolomei, 2005), site specific interactions, for example via animal handling staff (Crabbe et al., 1999), the animals' individuality itself meaning the total collection of innate and acquired traits such as timidity or ignorance of risk (Lathe, 2004), early factors (foetus position, nutrition *in utero*) and postnatal effects (endocrine factors, social status) all potentially contribute to the individual variation in normal biology as well as in diseases. Despite this variety of hypotheses, it is generally accepted that individual variation is also a consequence of being unable to regulate all potential factors that might influence animal physiology and behaviour (Wahlsten et al., 2003; Saab et al., 2011).

Individual variation was also an issue in our experiment, which may have masked overall biological effects. During the probe trial of the MWM, when the platform was absent, controls tended to have shorter escape latencies than stress animals, but due to the large variability within the groups, this effect did not reach significance. The major source of variation probably originated from the different aptitudes of animals within the same group to learn the task. There were no differences in locomotion such as swim speed or total distance travelled. Thus, the individual variability in cognitive ability is much more likely to be the source of variation than the variability in the non-cognitive locomotor skills such as swimming. This is in accordance to the data by Saab and colleagues (2011).

During the spatial learning, all young animals were able to solve the task and managed to discover the escape platform faster and faster over time. Control animals exhibited a significantly reduced escape latency compared to stressed

animals, but only on spatial day 2. However, this stress effect was not robust and could not be replicated in a separate experiment.

In young animals, cognitive flexibility was reduced by CSS

Besides escape latency, it is common to measure also the time spent in the former target quadrant during the probe trial. Originally, the amount of time spent in the former platform quadrant was used as indicator for the strength of memory with a decreased amount of time being an indication for impaired cognition. However, recently other interpretations were considered, for example interpretations concerning the animals' cognitive flexibility. Cognitive flexibility is an important aspect especially for extrapolation from rodent to human studies. Extrapolation to human studies is also desirable for the findings from this thesis, but might be a long-term goal, which will not be easy to achieve. Humans constantly collect new memories, build up on preceding ones and recognise, when a memory is no longer valid (Saab et al., 2011). Cognitive flexibility enables individuals to remodel their memory stock and shift their focus. Several studies have shown that stress experience can lead to deficits in cognitive flexibility in humans (Renner and Beversdorf, 2010; Laing et al., 2010; Plessow et al., 2011) as well as in animals (Girotti et al., 2011; Nikiforuk and Popik, 2011).

This was confirmed by our findings during the probe trial, when stressed animals spent overall more time in the former platform quadrant searching for the target, while control animals spent more time in the non-target quadrants. One could hypothesise that due to their intact cognitive flexibility, the controls noticed quickly the platform's absence and started searching in alternative areas for it. Conversely, the cognitive flexibility of stressed animals seemed to be impaired and the animals failed to disengage from their previously learned strategy and adapt to a novel situation by applying a more fitting behaviour.

Overall, the animals showed an equal ability to apply a successful spatial strategy, but the stressed animals seemed to be affected to a certain degree in their cognitive flexibility. Nonetheless, this inhibition seemed to be in an initial phase, as this effect was not continued in the reversal learning phase and was not replicated in a separate experiment using another batch of young mice.

In total, CSS indeed led to decrements in the cognitive performance of young animals during recognition and spatial memory tasks. Nonetheless, the stress effects on spatial memory were not that robust due to several reasons. One could hypothesise that the observed stress effects acted in a selective manner and therefore had a stronger impact on PFC associated memory tasks than on hippocampus-dependent tasks. Here, the individual variability of different animals within a batch of mice might play a role. Another important aspect may be the timing of testing. In contrast to the OR test, MWM testing was performed after the cessation of the CSS paradigm. Hence, the animals were not tested directly under stress conditions, but had a few days to recover, which in turn might have attenuated the detrimental impact of CSS. In conclusion, there was an observable CSS phenotype, but it was not that pronounced and stress effects manifested themselves only as a mild cognitive impairment.

In aged animals, CSS inhibited recognition memory

Experiment 2 tested aged animals in OR and the MWM, i.e. 12 months after the CSS exposure. A recent study by Bergado and colleagues (2011) revealed that aged rats have an ability to detect novelty that is equal to that of young rats. The authors further postulated that aging indeed alters the total exploration time, but not the ability to differentiate between novel and known objects. Based on this and a study by Sterlemann and co-workers (2010), a long-term inhibition in cognitive functions due to the CSS procedure (but not due to aging) was expected, as they were able to demonstrate long-term cognitive impairments on the spatial memory performance of mice in the Y-maze test.

In line with these previous findings, our data revealed adverse long-term effects on the memory performance in the OR test due to the CSS procedure 12 months ago: controls preferred the novel object, while stress animals did not distinguish between the two objects.

Aged animals exhibited different vulnerabilities towards CSS

A study by Schmidt and colleagues (2010) discovered vulnerable and resilient individuals within a large group of animals that underwent the CSS procedure. Vulnerable mice were characterised by still markedly elevated basal corticosterone

levels even after a recovery period of 5 weeks. Conversely, resilient mice recovered quickly and could not be distinguished from controls after their recovery. Further analysis of our OR results also indicated different vulnerabilities within the group of stressed animals. The stress animals' memory performance, unlike the controls', did not fit a Gaussian, but a bimodal distribution with values grouped around two distinct peaks (see chapter 3.2.3). The two different peaks are likely to resemble two groups of stress animals with different susceptibilities towards the CSS exposure 12 months ago. The first group exhibited a weak OR performance and was clearly lastingly affected by the CSS. The second group explored the novel object longer, performed well above the cut off criterion and hence, seemed to be protected. This situation might run parallel to the situation in humans, where some individuals suffer from severe and adverse life events, but recover from it without any long-term damages, while vulnerable individuals may develop an affective disorder after comparably mild stress experience (Charney, 2004; Schmidt et al., 2008). This similarity to the human situation is another indication for the importance of extrapolation from rodent to human studies and should be considered in future experiments.

Aging inhibited spatial learning and memory in the MWM

A recent study by Bergado and co-workers (2011) showed that major aging-induced impairments in rats appear especially in the MWM test and that old rats learn slower than young ones. Similar results have been reported in other studies that employed the MWM test to evaluate spatial learning and memory (Rasmussen et al., 1996; Schulz et al., 2002; Topic et al., 2005). Hence, the experimental mice were expected to suffer from cognitive impairments in the MWM as well.

Our results confirmed this hypothesis: aged mice, stress as well as control animals, failed to acquire the task. During the spatial training, they did not exhibit an improvement in escape latencies that was stable from day 1 to day 3. Instead, the animals fell back to the level from the day before. At 16 months of age, the task according to our protocol might have been too challenging for them. Probably, an aged animal needs more training days than the young conspecific to show an improvement in the learning curve. Thus, a prolonged spatial learning phase is

suggested, for example 6 days of spatial learning instead of the previously applied 3 days. Aging-induced impairments in visual acuity or in the musculoskeletal system can be excluded as failure source, since during the visual training, the animals managed to locate and climb onto the platform continually faster.

In aged animals, cognitive flexibility was reduced by CSS

Cognitive flexibility has been linked to the PFC (Rasmussen et al., 1996; Chao and Knight, 1997; Head et al., 2008). The PFC is a brain area that was often overlooked in basic research of aging. Animal models of aging frequently have been focusing on cognitive deficits due to hippocampal malfunction (Gallagher and Rapp, 1997; Rapp et al., 1999; Small et al., 1999; Smith et al., 2000; Small et al., 2004). However, the PFC is also vulnerable to aging-induced, detrimental alterations for example in executive function (Salat et al., 2001; Salat et al., 2005; Nordahl et al., 2006; Shankar, 2010; Kaczorowski et al., 2011; Bloss et al., 2011).

In our experiment, control animals searched significantly longer in the non-target quadrants, while stress animals spent equal amounts of time in all quadrants. As already suggested for the young animals, this might point to a stronger cognitive flexibility in the controls, which persisted even at this age and enabled the controls to adapt to the novel situation faster than the stress animals. One could speculate that hippocampal aging was more advanced than PFC aging, as controls had an intact cognitive flexibility, but spatial learning was inhibited. Furthermore, the PFC has been suggested as a brain area able to compensate for some aging-induced cognitive deficits (Aine et al., 2006).

Additionally, controls exhibited reduced escape latencies during the probe trial. This might be an indicator that although all animals were cognitively challenged in the MWM due to their advanced age, control animals were able to remember the platform location after three training days. Intriguingly, this was not the case for animals that were exposed to CSS during adolescence, indicating long-lasting effects of the stress experiment. This is in line with a previous study by Sterlemann and colleagues (2010).

Aging affected spatial learning and memory, but not recognition memory

Previous studies have highlighted the independence of age-related deficits across different cognitive and behavioural domains (Baxter, 2010; Burke et al., 2010) and dissociated object recognition and spatial navigation (Winters et al., 2004; Forwood et al., 2005). Instead of supporting the concept of a global and generalised mechanism for aging-induced cognitive decrements, the authors postulated multiple region-dependent mechanisms that underlie different aspects of aging.

This in accordance to our results: aging inhibited the spatial memory performance of all animals in the MWM, as they were not able to improve their learning curve during the spatial training, but did not alter recognition memory. Overall, it seemed that aging specifically affected hippocampus-dependent learning (MWM), while hippocampus-independent learning (OR) remained intact.

To summarise, CSS had acute and long-term effects in the OR test and inhibited recognition memory. There were no aging-induced decrements in hippocampus-independent recognition memory, as the aged controls exhibited a clear preference for the novel object. In the MWM, CSS partially impaired the spatial memory performance (young mice: spatial day 2; aged mice: probe trial). Furthermore, in both young and aged animals, stress animals seemed to have a limited cognitive flexibility compared to controls, but only during the probe trial.

4.4 Synaptic CAM dynamics on mRNA and protein level

As highlighted in the introduction, chronic stress has profound effects on brain structure and function. But how are stress responses translated into changes in neural circuits and finally lead to changes in behaviour and cognition? The underlying cellular and molecular mechanisms still remain elusive (Sandi, 2004). Since nCAMs of the Ig-SF mediate synaptic function and are able to remodel neuronal circuits, recent studies have highlighted their role in chronic stress-induced cognitive and neuronal alterations (Bisaz et al., 2011). However, this is a rather new field of research and only few laboratories have dealt with the mRNA or protein expression levels of nCAMs after CSS, learning and during aging yet.

During the screening process of mRNA expression patterns, three promising candidate nCAMs were found. Those nCAMs, namely Nptn, Nec 3 and Nrxn, exhibited altered mRNA levels even 12 months after CSS cessation. The discussion therefore focuses on these candidate nCAMs.

Learning as a crucial factor in nCAM dynamics

The most prominent findings for nCAM regulations on mRNA level were found in animals after learning. Hereby, CSS was often involved as well. Basal effects, if any, seemed to be of little importance. It can be thus concluded that learning is a crucial event in the regulation of nCAM dynamics and that learning processes alter the flexibility of the whole nCAM system. It is known that synapses form the foundation for learning processes: first, there is a change in the electrical properties of a synapse, then, second messenger molecules interfere and finally, synaptic proteins are modulated (Bear et al., 2007). As nCAMs bridge the synaptic cleft, they are very likely to be influenced by learning-induced changes at the synapse. This can be confirmed by previous studies for all three of our candidate nCAMs. Electrophysiological changes related to learning led to modulations in Nptn dynamics: in hippocampal cell culture, Np 65 was increased by LTP, while antibodies against Np 65 impaired hippocampal LTP (Owczarek, 2011). A specific mouse viral Nec 3 knockdown *in vivo* indicated a link between this nCAM and cognitive function (Wang et al., 2011). Several Nrxn mutant mice revealed cognitive deficits in spatial memory and learning (Blundell et al., 2010), impaired hippocampal LTP (Dahlhaus et al., 2010) and a reduced synaptic transmission (Etherton et al., 2009). In conclusion, learning is suggested as an important regulating event in nCAM dynamics, although it is very likely that it is only one of several mechanisms involved in the interplay of nCAM regulations.

Potential interactions of aging and learning in nCAM dynamics

The changes that occur shortly after learning at the synapse may be converted into permanent ones by altering the synapse structure lastingly. Hence, storage of long-term memory is accompanied by the synthesis of new proteins and the modulation of already existing proteins (Bear et al., 2007). These modulations take place in the cell body, but also locally at the synapse, where new gene products,

presumably also nCAMs, lead to structural modifications and growth of new connections (Kandel, 2001; Bailey et al., 2004; Bailey and Kandel, 2008; Choi et al., 2011). This is in line with our results, which also showed long-term effects on the mRNA expression of specific nCMAs 12 months after CSS and a recent learning experience. Due to these observable long-term effects on nCAM dynamics, one could speculate that aging and cognition interact to regulate nCAM expression patterns. Besides structural alterations at the synapse, epigenetic processes or altered signal cascades are also conceivable mechanisms that regulate nCAMs in the aging brain and after learning.

Synaptic CAMs were differentially regulated in dependence of the type of stressor

In this thesis, a prominent finding was the up-regulation of specific nCAM mRNAs after learning as well as the inhibition of this up-regulation by CSS.

Intriguingly, other stress paradigms resulted in different effects: a study by Wang and colleagues (2011) assessed the impact of early life stress (ELS) on Nptn-mRNA levels in conditional forebrain CRHR1 knockout and wild-type mice. However, there were no significant differences in the regulation patterns of Nptn after ELS, neither in the wild type nor in the CRHR1 knockout. The results merely sufficed to indicate a trend that Nptn might be down-regulated after ELS. Since Wang and colleagues did not measure after learning, it might still be plausible that ELS alters nCAM dynamics, but nobody has looked at this yet. The discrepancy among this study and the present thesis also underlines the importance of the time window during which the stressor is applied, as CSS and ELS are 2 distinct types of stress with different durations (7 weeks vs. 7 days) (Rice et al., 2008; Lupien et al., 2009), different timings (adolescence vs. first postnatal week) and thus also different developmental conditions of the experimental animals (fully organised hippocampus vs. a hippocampus in growth). This difference in the developmental status also plays a role for Nptn, as Np 65 is not expressed until PD 14 (Marzban et al., 2003), while Np 55 is already expressed in the embryonic brain (Buckby et al., 2004). This suggests that Np 65 was not even present at the time of ELS, while both isoforms were fully expressed during the CSS paradigm. Nonetheless, the 2 isoforms are not distinguishable by our ISH. For future experiments, it might be

interesting to design distinct Nptn ribonucleotide probes for the 2 isoforms, which enable the differentiation between Np 65 and Np 55 regulations.

Dissociation of nCAM mRNA and nCAM protein findings

In contrast to differences observed in the regulation of nCAM mRNA, the analysis of nCAM expression on protein-level did not reveal any significant differences between the groups. This was the case for young animals as well as for aged mice. Several reasons are conceivable. It might be that potential differences were masked due to the high variations within a relatively small group. Thus, it could be useful to repeat the experiment with an extended group size to reduce the effect of individual variability. Another important aspect may be that the analysis on protein level, in contrast to the analysis on mRNA level, was conducted for the whole hippocampus. This might have masked effects that appeared in separate hippocampal compartments or sub-regions. Nonetheless, a technique that allows the protein analysis of several separate hippocampal regions is difficult to implement and hence also imprecise. One could even speculate that protein regulations appear not only in separate hippocampal compartments, but in a synapse specific manner. To visualise a synapse specific localisation, a different methodical approach is needed. Finally, it is also possible that despite distinct effects on mRNA level, these effects simply are not sufficient to be clearly noticeable on protein-level. Overall, the absence of a significant difference between control and stress animals on nCAM protein level does not diminish the validity of the observed mRNA results.

Learning is stressful, but the GR was not sufficient to regulate nCAM dynamics

As behavioural tests, such as the MWM, lead to stress experience in the experimental animals and increase corticosterone levels (see chapter 4.2), it was important to exclude the possibility that nCAMs were regulated due to the release of GCs during the applied learning paradigms. Therefore in experiment 3, dex injections in mice were conducted to test whether the effects observed in nCAM regulation appeared due to a direct regulation via the GR. Dex is a GR agonist and has emerged from its extensive usage in the dexamethasone suppression test

(DST). Dex has become a valuable and reliable tool to study HPA axis activity and affective disorders related to stress (Carroll et al., 1981; Holsboer, 1983; APA Task force, 1987; Cole et al., 2000; Coryell et al., 2006; Steimer et al., 2007).
For the purposes of this thesis, the dex injections had the aim to mimic a stressful situation and induce suppression robust GR activation. Afterwards, nCAM-mRNA regulation patterns were screened for potential differences between controls and dex treated mice. To flood the body with the synthetic glucocorticoid and ensure the drug's effectiveness, the animals were subcutaneously injected with high doses of dex (10 mg / kg body weight). The dex injections activated the physiological feedback process and drastically reduced the amount of endogenous corticosterone, especially 8 h after the injection, when the mice approached the evening circadian peak that is typical for nocturnal mammals. This is in accordance to previous findings in humans indicating that the suppression of plasma cortisol by dex persists for at least 24 h (APA Task force, 1987). The reduction of the endogenous corticosterone levels in our mice confirmed that the drug was able to overcome the blood brain barrier in a sufficient manner. Subsequent analysis of nCAM-mRNA autoradiograms revealed that there were no differences in the hippocampal mRNA expression patterns (CA1, CA3, DG) between the control and the dex group at any of the five tested time points. As no research group has looked into this matter yet, this finding provides a novel insight within the scientific field of novel nCAMs and their role in stress and learning.

In total, dex clearly induced the GR, similar to the activation during a stressful learning paradigm, but nCAM expression was not solely regulated by dex. Hence, for the mRNA effects seen so far, it is very unlikely that the GR alone was a major player. A direct regulation via glucocorticoids can be excluded, while the regulation of nCAM mRNA after learning was a consistent finding. Consequently, the presented mRNA regulations most likely developed due to the experience of both stress and learning.

Alterations in nCAM dynamics could be implicated in stress- or aging-induced cognitive impairments

At the moment, a distinct causal link between nCAM regulations and cognitive decrements cannot be established. However, in this thesis, it was shown that nCAM regulation is strongly associated to chronic stress, learning and aging. Chronic stress and aging both diminish cognitive function and increase the vulnerability towards adverse life events such as disease (see chapter 1.2.2). Due to the strong association between nCAMs, chronic stress and aging, nCAMs are suggested to be potential regulators in stress- and aging-related alterations that are accompanied by symptoms such as cognitive impairment. Nonetheless, conclusive human data on nCAM regulations and the implication for cognitive diseases are missing in the literature. For Np 65, it is known that the distribution in the human brain is remarkably different as compared to rodents. Np 55 expression could not be detected at all until now in the human brain. Bernstein and colleagues (2007) therefore proposed different cellular functions of Nptn in different species. Nec 3 has been revealed to influence axodendritic adhesion and plasticity (Honda et al., 2006), but until now only in the mouse model. Dysregulation of the human Nrxn-Nlgn complex has been shown to play a role in schizophrenia, autism and mental retardation (Kim, 2008; Yan, 2008; Rujescu, 2009). Besides these findings for Nrxn-Nlgn, human data are lacking, but still it is very likely that other nCAMs also play a crucial role in cognitive alterations related to stress-induced diseases or aging. This remains an important issue for future studies.

4.5 Mimetic peptide treatment

4.5.1 Specificity of mimetic peptides

The differentiation between Np 65 and Np 55 was not possible for the analysis of mRNA results, as the ISH ribonucleotide probe was designed to recognise both isoforms. Hence, the mRNA results discussed before cannot be attributed to one specific Nptn isoform. However, for the effects on cognitive performance after mimetic peptide application, this was not an issue: the mimetic peptides were each derived from one isoform only, Narpin from Np 55 and Enplastin consequently from

Np 65. Thus, the effects elicited by one mimetic peptide can be assigned to a specific isoform and thus may indicate a potential implication in cognition or aging.

4.5.2 Acute vs. chronic mimetic peptide treatment

After acute administration, both peptides were able to improve the spatial memory performance in the Y-maze test, although for Narpin, this effect was not that robust and resulted only in a mild improvement. One could speculate that a single treatment, such as an intrahippocampal injection, was in this case not sufficient to initiate changes that underlie the augmentation of cognitive functions. These changes might be for example structural alterations, modifications in certain signalling pathways or the synthesis of new proteins. It is also likely that a specific interplay of several processes and events is needed. A limiting factor could be that the time span between the acute administration and the time point of testing was too short to allow these restructuring processes. Animals received the mimetic peptide either 30 minutes before the behavioural testing or during the ITI. They were decapitated on the same day approximately 2 hours after the retrieval trial, which limits the time window for restructuring processes to less than 4 hours. Narpin might be a substance that needs more time to have a beneficial effect on cognition. This is in line with the findings for the chronic Narpin treatment, where animals received overall 6 injections within 1 week. Here with a time window of 7 days from the first injection until testing, Narpin clearly improved the spatial memory performance in the Y-maze.

After chronic treatment, Enplastin was not able to interfere with the spatial memory. It is possible that at the time point of testing, the concentration of Enplastin was too low after even after several s.c. injections. Enplastin, in contrast to Narpin, might be a substance that needs to be present in high concentrations to act beneficially, while the time window might be less important. This is in line with the findings after acute administration, where Enplastin was injected directly into the hippocampus via intrahippocampal cannulas, which ensured a high concentration directly at the target area. Here, Enplastin clearly improved spatial memory performance in the Y-maze test. A prior experiment indeed showed that both mimetic peptides were able to cross the blood brain barrier via s.c. injections.

However, the concentration at the time point of testing after an intrahippocampal administration is likely to be higher than after a single s.c. injection, as the peptide administration is specifically limited to the hippocampus, while after s.c. injections the mimetic peptide distributes through the whole body to enter the brain. To which extent Narpin or Enplastin penetrate the blood brain barrier and then target specifically the hippocampus remains unclear. Hereby, degradation processes influence the biological half-life of the substance, but it is not known how or when a mimetic peptide is decomposed after an s.c. injection.

4.5.3 Mimetic peptides and hippocampus-independent learning

Narpin seems to be a substance that is able to improve spatial memory particularly after chronic treatment, while Enplastin is only active in a beneficial way after acute application. Both were able to improve spatial memory performance in the Y-maze test, whereas they did not alter recognition memory in the OR test. It is conceivable that due to a “ceiling-effect”, the peptides could not further increase recognition memory: all animals already performed so well (novel object time of 70 %) that an additional increase in the amount of time spent with the novel object might be difficult due to the natural tendency of mice to explore their surrounding environment. Another important aspect may be the involvement of different brain regions for the different behavioural tests: the OR test according to our protocol is a mostly hippocampus-independent test, while the Y-maze test mainly depends on the hippocampus. As the target area for the mimetic peptides was the hippocampus, the finding that a mimetic peptide treatment did not modulate hippocampus-independent recognition memory seems convincing. However, it remains unclear to which extent s.c. injections specifically target the hippocampus, thereby creating the possibility that mimetic peptides reached also brain regions related to object recognition such as the PFC. This leads to the consideration that Enplastin and Narpin might be involved only in hippocampus-dependent learning and simply cannot interfere with recognition memory even if the mimetic peptides were present in the responsible brain regions. Nonetheless, these findings do not automatically allow the conclusion that Np 65 and Np 55 are not involved in hippocampus-independent learning. Enplastin and Narpin are not synthesised as

exact copies of Nptn, they do not fully mimic Nptn function but rather act as partial agonists (Owzcarek, 2010; Owzcarek 2011). For example, Enplastin is likely to cluster several Np 65 molecules on its membrane due to its dendrimeric form, which might lead to an increased activation of Np 65-triggered signalling pathways. Another difference between this mimetic peptide and the nCAM from which it is derived is the evoked pattern of synaptic Ca^{2+}-release. Due to these differences, the effects elicited by the mimetic peptides can only indicate a potential function of the associated nCAMs.

4.5.4 Limitations of the study

Interestingly, a relatively frequent finding was that vehicle treated animals did not reach a spatial memory performance clearly above chance level, in contrast to the animals treated with mimetic peptides. One could speculate that these decrements in the Y-maze test emerged as a side effect of the rather stressful intrahippocampal injection procedure. Conversely to the s.c. injections, when the animals were merely handled for a few seconds, during the intrahippocampal injection, the mice had to be hand-held and fixed for at least 4 minutes to allow a slow administration and ensure the diffusion of the substance. The hypothesis of an impaired cognition due to the stressful injection procedure is supported by the fact that in case of the less stressful s.c. injections, animals showed an anticipated performance above chance level in the Y-maze as well as in the OR test.

In conclusion, the beneficial effect of our mimetic peptides after acute treatment seemed to be able to take place mainly due to the impaired cognition of vehicle animals. It is suggested that the mimetic peptides could counteract the already underlying cognitive deficits in the vehicle animals and raise their performance to a “normal” and anticipated level.

4.5.5 Memory could not be modulated in aged animals

In aged animals, both mimetic peptides failed to alter spatial memory performance in the Y-maze as well as recognition memory in the OR test. It is conceivable that a beneficial effect on cognition needs certain resources or processes to come into

action. These resources, for example proteins, enzymes and hormones are probably limited and less dynamic in aged animals. An example for a crucial process, which might be impaired in aged individuals, is synaptic plasticity. Previous animal studies dating back to the late 1970s have shown that age-related neurocognitive changes are linked to age-dependent alterations in synaptic plasticity (Barnes, 1979; Freitas et al., 2011). It is also likely that these aging-related limitations have a broadly diversified impact, for example they might affect the flexibility of circuits as well as the reaction rate of signalling cascades and the availability of certain crucial agents. Thus, one could speculate that also nCAM dynamics are more rigid and less plastic than in younger animals. Hence, to achieve a modulation of memory in aged animals, additional measures need to be taken to overcome the boundaries set by the aging process. For follow-up studies, it is feasible to apply higher concentrations of the mimetic peptides. However, intrahippocampal injections, which provide an accurate mimetic peptide application in high dosages, are difficult to be employed in aged animals due to the increased risk of mortality after a surgery. For this reason, it is suggested to implement the increase in concentration via s.c. injections and apply several dosages for example 15, 20 and 25 mg/kg body weight instead of the prior used dosage of 10 mg/kg body weight. Another important aspect might be the total duration of the treatment, which could be easily prolonged from the previously employed 7 days to 14 or 21 days. Finally, one could even hypothesise that learning processes in the aging brain do not depend on the same pathways and mechanisms as in the young brain and that nCAMs are not at all involved or play only a minor role. This needs to be tested in future experiments.

4.6 Summary

Overall, nCAM mRNA expression was altered particularly after learning and the modulation of cognitive performance or learning via nCAM derived mimetic peptides was possible. The 2 findings together further underline the importance of learning processes, when analysing nCAMs and their implication for cognition. However, it is important to note that an increased nCAM expression, for example more Nptn homophilic binding, not necessarily leads to synaptic and structural

remodelling and finally to cognitive enhancement. A good example in this respect is NCAM: it has been shown that the inhibition of NCAM homophilic binding via polysialylation might serve as a protection mechanism against overstimulation induced by chronic stress (Sandi, 2004) (see chapter 4.8). A beneficial effect on cognition is dependent on a variety of factors such as timing, type of administration and concentration. Thus, the conclusion “the more, the better” is likely not applicable for the sophisticated interplay in nCAM dynamics. However, evidence was provided that mimetic peptides are able to modulate memory. Altogether, these findings confirmed nCAMs as an important novel therapeutic target in the search of drugs against stress-induced or age-related cognitive impairments.

4.7 Conclusion

Based on the multiple levels of evidence, which have been provided in the previous chapters of this thesis, the following conclusions can be drawn:

1. CSS affects basal HPA axis activity.
2. CSS has acute and long-term adverse effects on hippocampus-dependent (MWM) and hippocampus–independent (OR) learning and memory.
3. Aging impairs spatial learning and behavioural performance.
4. CSS, learning and aging differentially affect nCAM-mRNA regulation patterns.
5. Regulations of nCAM expression are not influenced by a direct regulation via the GR. Learning experience plays an essential role in this context.
6. Enplastin and Narpin are both Nptn derived peptides, which are able to improve cognition under certain conditions.
7. Alterations in nCAM dynamics play an important role in learning processes.
8. Alterations in nCAM dynamics could be causal for stress-induced and / or age-related differences in cognitive performance.

In conclusion, the findings described in this thesis support the hypothesis that stress and aging induce cognitive deficits and suggest synaptic CAMs as potential agents regulating the underlying molecular mechanisms. Synaptic CAMs were

confirmed to be promising molecular targets to modulate cognition and treat cognitive impairments.

4.8 Future perspectives

In contrast to already well characterised synaptic CAMs like NCAM, the nCAMs that are discussed in this thesis may be considered as a novel group, as they just have been recently identified. Hence, there are still a lot of issues that need to be clarified.

One aspect might be whether specific nCAMs act in a hierarchical manner or if several nCAMs work together, acting in parallel (Washbourne et al., 2004). SynCAMs and Nrxns may be an example for different adhesion systems that are connected to each other, as both contain a binding site for the same cytoplasmic scaffolding protein, which activates identical downstream effectors (Dean et al., 2003). This common scaffold may be able to integrate signals from different extracellular pathways and organise them in a certain ranking order. Thus, it is conceivable that there are “early” nCAM systems, which are positioned upstream of others. These early nCAMs might be essential for initial synaptic events, for example during synaptogenesis, while “late” nCAMs contribute to the stabilisation and maturation of synapses. Nonetheless, it remains elusive whether different adhesion systems communicate with each other and how that could contribute to synapse formation and organisation.

Another important issue that needs to be addressed is how the activity of nCAMs is regulated. Washbourne and co-workers (2004) suggested an activity-dependent mechanism, which leads to alternative splicing of nCAM transcripts as response to a specific event. These events may be stress or learning experience and lead to different activities that regulate nCAM expression. A stress-dependent mechanism that regulates nCAM expression has already been reported by Sandi and colleagues (2004) for NCAM. NCAM-140, which is a specific isoform of NCAM, has been proposed to have a decisive role in the stress-induced hippocampal dendritic atrophy that particularly affects CA3 pyramidal neurons. NCAM-140 expression is diminished after stress, since chronic stress exposure leads to a reduced synaptic efficacy, synapse elimination and finally structural shrinkage.

Pre-existing synapses may be disconnected and the interaction between NCAM and FGFR might be hampered, which is responsible for neurite outgrowth. A similar mechanism has been suggested for polysialic acid NCAM (PSA-NCAM), which is supposed to be part of a neuroprotective circuit and increases plasticity. The process of polysialylation is a posttranslational modification that diminishes the adhesive properties of NCAM (Tsoory et al., 2008). Due to its "slippery" conformational characteristics, PSA-NCAM reduces the binding to other proteins (Rutishauser, 2008). Several diseases are linked to increased PSA-levels, for example Alzheimer's disease (Mikkonen et al., 1999), chronic neuropathic pain (El Maarouf et al., 2005) and temporal lobe epilepsy (Mikkonen et al., 1998). Their pathology often involves the loss of cellular elements or an abnormal circuitry with cells that need to be replaced or circuits that need remodelling. The increase in PSA-levels could be seen as an attempt to accelerate and improve the repair processes. Stress also induces an up-regulation in PSA-NCAM, disconnecting synapses and thereby protecting them from overstimulation and potential damages exerted by excessive glutamatergic input. More nCAM activity-regulating mechanisms are also conceivable for other nCAMs. However, this has to be verified in future studies.

A further important aspect for future studies might be the choice of the mouse strain. Strain-specific differences in behavioural, biochemical and molecular properties are well described in the literature (Bothe et al., 2005; Brooks et al., 2005; Nesher et al., 2011). In this thesis exclusively male, CD1 mice were used. CD1 is a genetically heterogeneous outbred mouse strain, which provides individuals with varying innate traits such as resilience or vulnerability towards stress experience (Rice and O'Brien, 1980; Chia et al., 2005). An inbred mouse strain exhibits a lower genetic variability, for example mice from the highly emotional BALB/c strain in general show elevated levels of anxiety (Dulawa et al., 2004), while C57BL/6 mice are known to have a non-anxious phenotype (Ohl et al., 2003). Palumbo and colleagues (2009) found different effects of chronic stress on learning and memory in 2 genetically different strains of mice; the behaviour as well as neurodevelopmental and neurochemical parameters revealed strain-specific properties. Hence, dependent of the strain, mice experiments might

provide varying insights into the regulation patterns of nCAMs and their role in stress, learning and aging.

The specific effects of chronic stress exposure emerge as a function of the timing and the duration of the stress experience (Lupien et al., 2009). Thus, it is very likely that the choice of the stress paradigm might lead to different results regarding behaviour and nCAM regulation. Our CSS paradigm interferes with the adolescent phase for 7 weeks long and employs a social stressor. Stress in adolescence is known to decrease neurogenesis in the DG (Gould et al., 1997), reduce hippocampal volume (McEwen, 2000b) and induce dendritic atrophy in hippocampal CA3 pyramidal neurons (Magarinos and McEwen, 1995). In contrast, prenatal stress has “programming” effects on the HPA axis and the brain (Barker, 1991), as maternal GCs are able to at least partially pass through the placenta and reach the foetus (Seckl, 2008). This may also have an impact on foetal early expressed nCAMs and alter nCAM activity and regulation patterns in the long-term. Postnatal stress, such as maternal deprivation, has long-term effects beyond the HPA axis: for example, the density of CRH binding is increased in the PFC, hippocampus, cerebellum, amygdala and the hypothalamus (Anisman et al., 1998). The intensity of these long-term effects is dependent on the duration of the pups’ separation from the dam and of the age of the pups (de Kloet and Oitzl, 2003). Again, this may influence nCAM expression and involved signalling pathways. Besides applying stress paradigms that have an impact on life stages other than the adolescence, it would be also interesting to test mice in stress paradigms that indeed interfere with the adolescent phase, but employ a different type of stressor than our CSS paradigm. Consequently, chronic restraint stress or chronic social defeat stress might be conceivable for future studies as well.

Reference List

Abbas, L., (2003) Synapse formation: let's stick together. Curr. Biol 13, R25-R27.

Abo, T., (2001) Extrathymic pathways of T-cell differentiation and immunomodulation. Int. Immunopharmacol 1, 1261-1273.

Aine, C.J., Woodruff, C.C., Knoefel, J.E., Adair, J.C., Hudson, D., Qualls, C., Bockholt, J., Best, E., Kovacevic, S., Cobb, W., Padilla, D., Hart, B., Stephen, J.M., (2006) Aging: compensation or maturation? NeuroImage 32, 1891-1904.

Alfarez, D.N., Joels, M., Krugers, H.J., (2003) Chronic unpredictable stress impairs long-term potentiation in rat hippocampal CA1 area and dentate gyrus in vitro. Eur. J. Neurosci 17, 1928-1934.

Alvin V. and Terry Jr., (2009) Spatial Navigation (Water Maze) Tasks. 2nd,

Anisman, H., Zaharia, M.D., Meaney, M.J., Merali, Z., (1998) Do early-life events permanently alter behavioral and hormonal responses to stressors? Int. J. Dev. Neurosci 16, 149-164.

Aoki, J., Koike, S., Asou, H., Ise, I., Suwa, H., Tanaka, T., Miyasaka, M., Nomoto, A., (1997) Mouse homolog of poliovirus receptor-related gene 2 product, mPRR2, mediates homophilic cell aggregation. Exp. Cell Res 235, 374-384.

APA Task force, (1987) The dexamethasone suppression test: an overview of its current status in psychiatry. The APA Task Force on Laboratory Tests in Psychiatry. Am J Psychiatry 144, 1253-1262.

Aplin, A.E., Howe, A., Alahari, S.K., Juliano, R.L., (1998) Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin-cell adhesion molecules, and selectins. Pharmacol. Rev 50, 197-263.

Aricescu, A.R. and Jones, E.Y., (2007) Immunoglobulin superfamily cell adhesion molecules: zippers and signals. Curr. Opin. Cell Biol 19, 543-550.

Arndt, S.S., Laarakker, M.C., van Lith, H.A., van der Staay, F.J., Gieling, E., Salomons, A.R., van't Klooster, J., Ohl, F., (2009) Individual housing of mice--impact on behaviour and stress responses. Physiol. Behav 97, 385-393.

Aspinall, R. and Andrew, D., (2000) Thymic atrophy in the mouse is a soluble problem of the thymic environment. Vaccine 18, 1629-1637.

Bailey, C.H. and Kandel, E.R., (2008) Synaptic remodeling, synaptic growth and the storage of long-term memory in Aplysia. Prog. Brain Res 169, 179-198.

Bailey, C.H., Kandel, E.R., Si, K., (2004) The persistence of long-term memory: a molecular approach to self-sustaining changes in learning-induced synaptic growth. Neuron 44, 49-57.

Barnes, C.A., (1997) Memory deficits associated with senescence: a neuro-physiological and behavioral study in the rat. J Comp Physiol Psychol 93, 74-104

Barker, D.J., (1991) The foetal and infant origins of inequalities in health in Britain. J Public Health Med 13, 64-68.

Barker, G.R., Warburton, E.C., (2008) NMDA receptor plasticity in the perirhinal and prefrontal cortices is crucial for the acquisition of long-term object-in-place associative memory. J Neurosci. 28, 2837-2844

Bartolomucci, A., Palanza, P., Sacerdote, P., Ceresini, G., Chirieleison, A., Panerai, A.E., Parmigiani, S., (2003) Individual housing induces altered immuno-endocrine responses to psychological stress in male mice. Psychoneuroendocrinology 28, 540-558.

Bartolomucci, A., Palanza, P., Sacerdote, P., Panerai, A.E., Sgoifo, A., Dantzer, R., Parmigiani, S., (2005) Social factors and individual vulnerability to chronic stress exposure. Neurosci Biobehav Rev 29, 67-81.

Bartolomucci, A., Pederzani, T., Sacerdote, P., Panerai, A.E., Parmigiani, S., Palanza, P., (2004) Behavioral and physiological characterization of male mice under chronic psychosocial stress. Psychoneuroendocrinology 29, 899-910.

Baudouin, S. and Scheiffele, P., (2010) SnapShot: Neuroligin-neurexin complexes. Cell 141, 908, 908-908, 908.

Bauer, M.E., Jeckel, C.M.M., Luz, C., (2009) The role of stress factors during aging of the immune system. Ann. N. Y. Acad. Sci 1153, 139-152.

Baxter, M.G., (2010) "I've seen it all before": explaining age-related impairments in object recognition. Theoretical comment on Burke et al. (2010). Behav. Neurosci 124, 706-709.

Bear, M., Connors, B., Paradiso, M., (2007) Molecular mechanisms of learning and memory. Third edition,

Becker, C.G., Artola, A., Gerardy-Schahn, R., Becker, T., Welzl, H., Schachner, M., (1996) The polysialic acid modification of the neural cell adhesion molecule is involved in spatial learning and hippocampal long-term potentiation. J. Neurosci. Res 45, 143-152.

Bergado, J.A., Almaguer, W., Rojas, Y., Capdevila, V., Frey, J.U., (2011) Spatial and emotional memory in aged rats: a behavioral-statistical analysis. Neuroscience 172, 256-269.

Bernstein, H.G., Smalla, K.H., Bogerts, B., Gordon-Weeks, P.R., Beesley, P.W., Gundelfinger, E.D., Kreutz, M.R., (2007) The immunolocalization of the synaptic

glycoprotein neuroplastin differs substantially between the human and the rodent brain. Brain Res 1134, 107-112.

Biederer, T., (2006) Bioinformatic characterization of the SynCAM family of immunoglobulin-like domain-containing adhesion molecules. Genomics 87, 139-150.

Biederer, T., Sara, Y., Mozhayeva, M., Atasoy, D., Liu, X., Kavalali, E.T., Südhof, T.C., (2002) SynCAM, a synaptic adhesion molecule that drives synapse assembly. Science 297, 1525-1531.

Birmingham and Grad, (1954) Peptidase activity in the thymus of a normal and a leukemic strain of mice during growth and aging. Cancer Res 14, 352-359.

Bisaz, R., Schachner, M., Sandi, C., (2011) Causal evidence for the involvement of the neural cell adhesion molecule, NCAM, in chronic stress-induced cognitive impairments. Hippocampus 21, 56-71.

Bishop, N.A., Lu, T., Yankner, B.A., (2010) Neural mechanisms of ageing and cognitive decline. Nature 464, 529-535.

Bizon, J.L. and Gallagher, M., (2005) More is less: neurogenesis and age-related cognitive decline in Long-Evans rats. Sci Aging Knowledge Environ 2005, re2-re2.

Björntorp, P., (2001) Do stress reactions cause abdominal obesity and comorbidities? Obes Rev 2, 73-86.

Blanchard, D.C., Spencer, R.L., Weiss, S.M., Blanchard, R.J., McEwen, B., Sakai, R.R., (1995) Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. Psychoneuroendocrinology 20, 117-134.

Bloss, E.B., Janssen, W.G., Ohm, D.T., Yuk, F.J., Wadsworth, S., Saardi, K.M., McEwen, B.S., Morrison, J.H., (2011) Evidence for reduced experience-dependent dendritic spine plasticity in the aging prefrontal cortex. J. Neurosci 31, 7831-7839.

Blundell, J., Blaiss, C.A., Etherton, M.R., Espinosa, F., Tabuchi, K., Walz, C., Bolliger, M.F., Südhof, T.C., Powell, C.M., (2010) Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. J. Neurosci 30, 2115-2129.

Bolliger, M.F., Pei, J., Maxeiner, S., Boucard, A.A., Grishin, N.V., Südhof, T.C., (2008) Unusually rapid evolution of Neuroligin-4 in mice. Proc. Natl. Acad. Sci. U. S. A 105, 6421-6426.

Borcel, E., Perez-Alvarez, L., Herrero, A.I., Brionne, T., Varea, E., Berezin, V., Bock, E., Sandi, C., Venero, C., (2008) Chronic stress in adulthood followed by intermittent stress impairs spatial memory and the survival of newborn hippocampal cells in aging animals: prevention by FGL, a peptide mimetic of neural cell adhesion molecule. Behav Pharmacol 19, 41-49.

Bothe, G.W.M., Bolivar, V.J., Vedder, M.J., Geistfeld, J.G., (2005) Behavioral differences among fourteen inbred mouse strains commonly used as disease models. Comp. Med 55, 326-334.

Bremner, J.D., (1999) Does stress damage the brain? Biol. Psychiatry 45, 797-805.

Breslau, N., (2001) Outcomes of posttraumatic stress disorder. J Clin Psychiatry 62 Suppl 17, 55-59.

Brooks, S.P., Pask, T., Jones, L., Dunnett, S.B., (2005) Behavioural profiles of inbred mouse strains used as transgenic backgrounds. II: cognitive tests. Genes Brain Behav 4, 307-317.

Brown, G.W. and Prudo, R., (1981) Psychiatric disorder in a rural and an urban population: 1. Aetiology of depression. Psychol Med 11, 581-599.

Brümmendorf, T. and Rathjen, F.G., (1995) Cell adhesion molecules 1: immunoglobulin superfamily. Protein Profile 2, 963-1108.

Brydon, L., Magid, K., Steptoe, A., (2006) Platelets, coronary heart disease, and stress. Brain Behav. Immun 20, 113-119.

Buckby, L.E., Mummery, R., Crompton, M.R., Beesley, P.W., Empson, R.M., (2004) Comparison of neuroplastin and synaptic marker protein expression in acute and cultured organotypic hippocampal slices from rat. Brain Res. Dev. Brain Res 150, 1-7.

Buckner, R.L., (2004) Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate. Neuron 44, 195-208.

Buitrago, S., Martin, T.E., Tetens-Woodring, J., Belicha-Villanueva, A., Wilding, G.E., (2008) Safety and efficacy of various combinations of injectable anesthetics in BALB/c mice. J. Am. Assoc. Lab. Anim. Sci 47, 11-17.

Buiza, C., Etxeberria, I., Galdona, N., Gonzalez, M.F., Arriola, E., Lopez de Munain, A., Urdaneta, E., Yanguas, J.J., (2008) A randomized, two-year study of the efficacy of cognitive intervention on elderly people: the Donostia Longitudinal Study. Int J Geriatr Psychiatry 23, 85-94.

Burke, S.N. and Barnes, C.A., (2006) Neural plasticity in the ageing brain. Nat. Rev. Neurosci 7, 30-40.

Burke, S.N., Wallace, J.L., Nematollahi, S., Uprety, A.R., Barnes, C.A., (2010) Pattern separation deficits may contribute to age-associated recognition impairments. Behav. Neurosci 124, 559-573.

Bussey, T.J., Duck, J., Muir, J.L., Aggleton, J.P., (2000) Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of the perirhinal and postrhinal cortices in the rat. Behav. Brain Res. 111, 187-202

Cambon, K., Hansen, S.M., Venero, C., Herrero, A.I., Skibo, G., Berezin, V., Bock, E., Sandi, C., (2004) A synthetic neural cell adhesion molecule mimetic peptide promotes synaptogenesis, enhances presynaptic function, and facilitates memory consolidation. J. Neurosci 24, 4197-4204.

Cannon, W.B., (1932) The wisdom of the body.

Carroll, B.J., Feinberg, M., Greden, J.F., Tarika, J., Albala, A.A., Haskett, R.F., James, N.M., Kronfol, Z., Lohr, N., Steiner, M., de Vigne, J.P., Young, E., (1981) A specific laboratory test for the diagnosis of melancholia. Standardization, validation, and clinical utility. Arch. Gen. Psychiatry 38, 15-22.

Cavallaro, U. and Dejana, E., (2011) Adhesion molecule signalling: not always a sticky business. Nature Reviews. Molecular cell biology 12, 189-197.

Chandola, T., Brunner, E., Marmot, M., (2006) Chronic stress at work and the metabolic syndrome: prospective study. BMJ 332, 521-525.

Chao, L.L. and Knight, R.T., (1997) Prefrontal deficits in attention and inhibitory control with aging. Cereb. Cortex 7, 63-69.

Chapman, T.R., Barrientos, R.M., Ahrendsen, J.T., Maier, S.F., Patterson, S.L., (2010) Synaptic correlates of increased cognitive vulnerability with aging: peripheral immune challenge and aging interact to disrupt theta-burst late-phase long-term potentiation in hippocampal area CA1. J. Neurosci 30, 7598-7603.

Charney, D.S., (2004) Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. Am J Psychiatry 161, 195-216.

Chen, C.S., Tan, J., Tien, J., (2004) Mechanotransduction at cell-matrix and cell-cell contacts. Annu Rev Biomed Eng 6, 275-302.

Chen, Y., Bender, R.A., Frotscher, M., Baram, T.Z., (2001) Novel and transient populations of corticotropin-releasing hormone-expressing neurons in developing hippocampus suggest unique functional roles: a quantitative spatiotemporal analysis. J. Neurosci 21, 7171-7181.

Chen, Y., Rex, C.S., Rice, C., Dube, C.M., Gall, C.M., Lynch, G., Baram, T.Z., (2010) Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling. Proc. Natl. Acad. Sci. U. S. A 107, 13123-13128.

Chia, R., Achilli, F., Festing, M.F.W., Fisher, E.M.C., (2005) The origins and uses of mouse outbred stocks. Nat. Genet 37, 1181-1186.

Choi, Y.B., Li, H.L., Kassabov, S.R., Jin, I., Puthanveettil, S.V., Karl, K.A., Lu, Y., Kim, J.H., Bailey, C.H., Kandel, E.R., (2011) Neurexin-neuroligin transsynaptic interaction mediates learning-related synaptic remodeling and long-term facilitation in aplysia. Neuron 70, 468-481.

Chothia, C. and Jones, E.Y., (1997) The molecular structure of cell adhesion molecules. Annu. Rev. Biochem 66, 823-862.

Chubykin, A.A., Atasoy, D., Etherton, M.R., Brose, N., Kavalali, E.T., Gibson, J.R., Südhof, T.C., (2007) Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2. Neuron 54, 919-931.

Church, D.M., Goodstadt, L., Hillier, L.W., Zody, M.C., Goldstein, S., She, X., Bult, C.J., Agarwala, R., Cherry, J.L., DiCuccio, M., Hlavina, W., Kapustin, Y., Meric, P., Maglott, D., Birtle, Z., Marques, A.C., Graves, T., Zhou, S., Teague, B., Potamousis, K., Churas, C., Place, M., Herschleb, J., Runnheim, R., Forrest, D., mos-Landgraf, J., Schwartz, D.C., Cheng, Z., Lindblad-Toh, K., Eichler, E.E., Ponting, C.P., (2009) Lineage-specific biology revealed by a finished genome assembly of the mouse. PLoS Biol 7, e1000112-e1000112.

Clapcote, S.J. and Roder, J.C., (2004) Survey of embryonic stem cell line source strains in the water maze reveals superior reversal learning of 129S6/SvEvTac mice. Behav. Brain Res 152, 35-48.

Clegg, C.H., Rulffes, J.T., Wallace, P.M., Haugen, H.S., (1996) Regulation of an extrathymic T-cell development pathway by oncostatin M. Nature 384, 261-263.

Cole, M.A., Kim, P.J., Kalman, B.A., Spencer, R.L., (2000) Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. Psychoneuroendocrinology 25, 151-167.

Coligan, J.E., (2001) Amino acids. Curr Protoc Immunol Appendix 1, Appendix-Appendix.

Comoletti, D., Flynn, R.E., Boucard, A.A., Demeler, B., Schirf, V., Shi, J., Jennings, L.L., Newlin, H.R., Südhof, T.C., Taylor, P., (2006) Gene selection, alternative splicing, and post-translational processing regulate neuroligin selectivity for beta-neurexins. Biochemistry 45, 12816-12827.

Conrad, C.D., Galea, L.A., Kuroda, Y., McEwen, B.S., (1996) Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. Behav. Neurosci 110, 1321-1334.

Conrad, C.D., LeDoux, J.E., Magarinos, A.M., McEwen, B.S., (1999) Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. Behav. Neurosci 113, 902-913.

Conrad, C.D., (2006) What is the functional significance of chronic stress-induced CA3 dendritic retraction within the hippocampus? Behav Cogn Neurosci Rev 5, 41-60.

Conrad, C.D., (2008) Chronic stress-induced hippocampal vulnerability: the glucocorticoid vulnerability hypothesis. Rev Neurosci 19, 395-411.

Coons, A.H. and Kaplan, M.H., (1950) Localization of antigen in tissue cells. J Exp Med. 91 (1), 1-13.

Coplan, J.D., Andrews, M.W., Rosenblum, L.A., Owens, M.J., Friedman, S., Gorman, J.M., Nemeroff, C.B., (1996) Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. Proc. Natl. Acad. Sci. U. S. A 93, 1619-1623.

Coryell, W., Young, E., Carroll, B., (2006) Hyperactivity of the hypothalamic-pituitary-adrenal axis and mortality in major depressive disorder. Psychiatry Res 142, 99-104.

Crabbe, J.C., Wahlsten, D., Dudek, B.C., (1999) Genetics of mouse behavior: interactions with laboratory environment. Science 284, 1670-1672.

Cremer, H., Lange, R., Christoph, A., Plomann, M., Vopper, G., Roes, J., Brown, R., Baldwin, S., Kraemer, P., Scheff, S., (1994) Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. Nature 367, 455-459.

Cryan, J.F. and Holmes, A., (2005) The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4, 775-790.

D'Hooge, R. and De Deyn, P.P., (2001) Applications of the Morris water maze in the study of learning and memory. Brain Res. Brain Res. Rev 36, 60-90.

Dahlhaus, R., Hines, R.M., Eadie, B.D., Kannangara, T.S., Hines, D.J., Brown, C.E., Christie, B.R., El-Husseini, A., (2010) Overexpression of the cell adhesion protein neuroligin-1 induces learning deficits and impairs synaptic plasticity by altering the ratio of excitation to inhibition in the hippocampus. Hippocampus 20, 305-322.

Dallman, M.F., (2005) Fast glucocorticoid actions on brain: back to the future. Front Neuroendocrinol 26, 103-108.

Dallman, M.F., La Fleur, S.E., Pecoraro, N.C., Gomez, F., Houshyar, H., Akana, S.F., (2004) Minireview: glucocorticoids--food intake, abdominal obesity, and wealthy nations in 2004. Endocrinology 145, 2633-2638.

Dallman, M.F., Pecoraro, N., Akana, S.F., La Fleur, S.E., Gomez, F., Houshyar, H., Bell, M.E., Bhatnagar, S., Laugero, K.D., Manalo, S., (2003) Chronic stress and obesity: a new view of "comfort food". Proc. Natl. Acad. Sci. U. S. A 100, 11696-11701.

Dalm, S., Schwabe, L., Schachinger, H., Oitzl, M.S., (2009) Post-training self administration of sugar facilitates cognitive performance of male C57BL/6J mice in two spatial learning tasks. Behav. Brain Res 198, 98-104.

Dalva, M.B., McClelland, A.C., Kayser, M.S., (2007) Cell adhesion molecules: signalling functions at the synapse. Nat. Rev. Neurosci 8, 206-220.

de Kloet, E.R., Joels, M., Holsboer, F., (2005) Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci 6, 463-475.

de Kloet, E.R., (2003) Hormones, brain and stress. Endocr Regul 37, 51-68.

de Kloet, E.R. and Derijk, R., (2004) Signaling pathways in brain involved in predisposition and pathogenesis of stress-related disease: genetic and kinetic factors affecting the MR/GR balance. Ann. N. Y. Acad. Sci 1032, 14-34.

de Kloet, E.R. and Oitzl, M.S., (2003) Who cares for a stressed brain? The mother, the kid or both? Neurobiol. Aging 24 Suppl 1, S61-S65.

De Kloet, E.R. and Reul, J.M., (1987) Feedback action and tonic influence of corticosteroids on brain function: a concept arising from the heterogeneity of brain receptor systems. Psychoneuroendocrinology 12, 83-105.

De Kloet, E.R., Vreugdenhil, E., Oitzl, M.S., Joels, M., (1998) Brain corticosteroid receptor balance in health and disease. Endocr. Rev 19, 269-301.

de Quervain, D.J.F., Aerni, A., Schelling, G., Roozendaal, B., (2009) Glucocorticoids and the regulation of memory in health and disease. Front Neuroendocrinol 30, 358-370.

Deak, T., (2008) Immune cells and cytokine circuits: toward a working model for understanding direct immune-to-adrenal communication pathways. Endocrinology 149, 1433-1435.

Dean, C., Scholl, F.G., Choih, J., DeMaria, S., Berger, J., Isacoff, E., Scheiffele, P., (2003) Neurexin mediates the assembly of presynaptic terminals. Nat. Neurosci 6, 708-716.

DeBold, C.R., Sheldon, W.R., DeCherney, G.S., Jackson, R.V., Alexander, A.N., Vale, W., Rivier, J., Orth, D.N., (1984) Arginine vasopressin potentiates adrenocorticotropin release induced by ovine corticotropin-releasing factor. J. Clin. Invest 73, 533-538.

Dedovic, K., Duchesne, A., Andrews, J., Engert, V., Pruessner, J.C., (2009) The brain and the stress axis: the neural correlates of cortisol regulation in response to stress. NeuroImage 47, 864-871.

Delima, M.G. and Walford, R.L., (1975) Effect of cortisone in delineating thymus cell subsets in advanced age. Proc. Soc. Exp. Biol. Med 149, 562-564.

Dellu, F., Contarino, A., Simon, H., Koob, G.F., Gold, L.H., (2000) Genetic differences in response to novelty and spatial memory using a two-trial recognition task in mice. Neurobiol Learn Mem 73, 31-48.

Dellu, F., Mayo, W., Cherkaoui, J., Le Moal, M., Simon, H., (1992) A two-trial memory task with automated recording: study in young and aged rats. Brain Res 588, 132-139.

Deng, W., Aimone, J.B., Gage, F.H., (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat. Rev. Neurosci 11, 339-350.

Deupree, D.L., Turner, D.A., Watters, C.L., (1991) Spatial performance correlates with in vitro potentiation in young and aged Fischer 344 rats. Brain Res 554, 1-9.

Dodart, J.C., Mathis, C., Ungerer, A., (1997) Scopolamine-induced deficits in a two-trial object recognition task in mice. Neuroreport 8, 1173-1178.

Dominguez-Gerpe, L. and Rey-Mendez, M., (2003) Evolution of the thymus size in response to physiological and random events throughout life. Microsc. Res. Tech 62, 464-476.

Dulawa, S.C., Holick, K.A., Gundersen, B., Hen, R., (2004) Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology 29, 1321-1330.

Dunn, J.D. and Orr, S.E., (1984) Differential plasma corticosterone responses to hippocampal stimulation. Exp Brain Res 54, 1-6.

El Maarouf, A., Kolesnikov, Y., Pasternak, G., Rutishauser, U., (2005) Polysialic acid-induced plasticity reduces neuropathic insult to the central nervous system. Proc. Natl. Acad. Sci. U. S. A 102, 11516-11520.

Empson, R.M., Buckby, L.E., Kraus, M., Bates, K.J., Crompton, M.R., Gundelfinger, E.D., Beesley, P.W., (2006) The cell adhesion molecule neuroplastin-65 inhibits hippocampal long-term potentiation via a mitogen-activated protein kinase p38-dependent reduction in surface expression of GluR1-containing glutamate receptors. J. Neurochem 99, 850-860.

Engelmann, M., Landgraf, R., Wotjak, C.T., (2004) The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. Front Neuroendocrinol 25, 132-149.

Ennaceur, A. and Delacour, J., (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav. Brain Res 31, 47-59.

Ennaceur, A., Neave, N., Aggleton, J.P., (1997) Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Exp Brain Res 113, 509-519.

Epel, E.S., McEwen, B., Seeman, T., Matthews, K., Castellazzo, G., Brownell, K.D., Bell, J., Ickovics, J.R., (2000) Stress and body shape: stress-induced cortisol secretion is consistently greater among women with central fat. Psychosom Med 62, 623-632.

Etherton, M.R., Blaiss, C.A., Powell, C.M., Südhof, T.C., (2009) Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. Proc. Natl. Acad. Sci. U. S. A 106, 17998-18003.

Fabre, S., Reymond, N., Cocchi, F., Menotti, L., Dubreuil, P., Campadelli-Fiume, G., Lopez, M., (2002) Prominent role of the Ig-like V domain in trans-interactions of

nectins. Nectin3 and nectin 4 bind to the predicted C-C'-C"-D beta-strands of the nectin1 V domain. J. Biol. Chem 277, 27006-27013.

Figueiredo, H.F., Dolgas, C.M., Herman, J.P., (2002) Stress activation of cortex and hippocampus is modulated by sex and stage of estrus. Endocrinology 143, 2534-2540.

Fluttert, M., Dalm, S., Oitzl, M.S., (2000) A refined method for sequential blood sampling by tail incision in rats. Lab. Anim 34, 372-378.

Fogel, A.I., Akins, M.R., Krupp, A.J., Stagi, M., Stein, V., Biederer, T., (2007) SynCAMs organize synapses through heterophilic adhesion. J. Neurosci 27, 12516-12530.

Forwood, S.E., Winters, B.D., Bussey, T.J., (2005) Hippocampal lesions that abolish spatial maze performance spare object recognition memory at delays of up to 48 hours. Hippocampus 15, 347-355.

Foster, M.T., Solomon, M.B., Huhman, K.L., Bartness, T.J., (2006) Social defeat increases food intake, body mass, and adiposity in Syrian hamsters. Am. J. Physiol. Regul. Integr. Comp. Physiol 290, R1284-R1293.

Fox, G.B., O'Connell, A.W., Murphy, K.J., Regan, C.M., (1995) Memory consolidation induces a transient and time-dependent increase in the frequency of neural cell adhesion molecule polysialylated cells in the adult rat hippocampus. J. Neurochem 65, 2796-2799.

Fox, J.G., Anderson, L.C., Loew, F.M., Quimby, F.W., (2002) Laboratory Animal Medicine. 2nd Edition,

Frank, E. and Landgraf, R., (2008) The vasopressin system--from antidiuresis to psychopathology. Eur. J. Pharmacol 583, 226-242.

Freitas, C., Perez, J., Knobel, M., Tormos, J.M., Oberman, L., Eldaief, M., Bashir, S., Vernet, M., Peria-Gomez, G., Pascual-Leone, A., (2011) Changes in cortical plasticity across the lifespan. Front Aging Neurosci 3:5

Frieri, M., (2003) Neuroimmunology and inflammation: implications for therapy of allergic and autoimmune diseases. Ann. Allergy Asthma Immunol 90, 34-40.

Fry, T.J. and Mackall, C.L., (2002) Current concepts of thymic aging. Springer Semin. Immunopathol 24, 7-22.

Fuchs, E. and Gould, E., (2000) Mini-review: in vivo neurogenesis in the adult brain: regulation and functional implications. Eur. J. Neurosci 12, 2211-2214.

Fuchs, E., (2005) Social stress in tree shrews as an animal model of depression: an example of a behavioral model of a CNS disorder. CNS Spectr 10, 182-190.

Fuchs, E. and Flügge, G., (2002) Social stress in tree shrews: effects on physiology, brain function, and behavior of subordinate individuals. Pharmacol. Biochem. Behav 73, 247-258.

Furay, A.R., Bruestle, A.E., Herman, J.P., (2008) The role of the forebrain glucocorticoid receptor in acute and chronic stress. Endocrinology 149, 5482-5490.

Gallagher, M. and Rapp, P.R., (1997) The use of animal models to study the effects of aging on cognition. Annu Rev Psychol 48, 339-370.

Gallagher, M., Bizon, J.L., Hoyt, E.C., Helm, K.A., Lund, P.K., (2003) Effects of aging on the hippocampal formation in a naturally occurring animal model of mild cognitive impairment. Exp. Gerontol 38, 71-77.

Gaskin, S., Tremblay, A., Mumby, D.G., (2003) Retrograde and anterograde object recognition in rats with hippocampal lesions. Hippocampus 13, 962-969.

Gass, P., Reichardt, H.M., Strekalova, T., Henn, F., Tronche, F., (2001) Mice with targeted mutations of glucocorticoid and mineralocorticoid receptors: models for depression and anxiety? Physiol. Behav 73, 811-825.

Gerges, N.Z., Aleisa, A.M., Schwarz, L.A., Alkadhi, K.A., (2004) Reduced basal CaMKII levels in hippocampal CA1 region: possible cause of stress-induced impairment of LTP in chronically stressed rats. Hippocampus 14, 402-410.

Gerlai, R., (2001) Behavioral tests of hippocampal function: simple paradigms complex problems. Behav. Brain Res 125, 269-277.

Giagtzoglou, N., Ly, C.V., Bellen, H.J., (2009) Cell adhesion, the backbone of the synapse: "vertebrate" and "invertebrate" perspectives. Cold Spring Harb Perspect Biol 1, a003079-a003079.

Gillies, G.E., Linton, E.A., Lowry, P.J., (1982) Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. Nature 299, 355-357.

Girotti, M., Donegan, J.J., Morilak, D.A., (2011) Chronic intermittent cold stress sensitizes neuro-immune reactivity in the rat brain. Psychoneuroendocrinology

Glisky, EL., (2007) Changes in cognitive function in human aging.

Gomez-Sanchez, C.E., (2009) Glucocorticoid production and regulation in thymus: of mice and birds. Endocrinology 150, 3977-3979.

Gould, E., McEwen, B.S., Tanapat, P., Galea, L.A., Fuchs, E., (1997) Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J. Neurosci 17, 2492-2498.

Graf, E.R., Zhang, X., Jin, S.X., Linhoff, M.W., Craig, A.M., (2004) Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. Cell 119, 1013-1026.

Green, B.L., Rowland, J.H., Krupnick, J.L., Epstein, S.A., Stockton, P., Stern, N.M., Spertus, I.L., Steakley, C., (1998) Prevalence of posttraumatic stress disorder in women with breast cancer. Psychosomatics 39, 102-111.

Grootendorst, J., Oitzl, M.S., Dalm, S., Enthoven, L., Schachner, M., De Kloet, E.R., Sandi, C., (2001) Stress alleviates reduced expression of cell adhesion molecules (NCAM, L1), and deficits in learning and corticosterone regulation of apolipoprotein E knockout mice. Eur. J. Neurosci 14, 1505-1514.

Grootendorst, J., Enthoven, L., Dalm, S., de Kloet, E.R., Oitzl, M.S., (2004) Increased corticosterone secretion and early-onset of cognitive decline in female apolipoprotein E-knockout mice. Behav. Brain Res 148, 167-177.

Gumbiner, B.M., (1993) Proteins associated with the cytoplasmic surface of adhesion molecules. Neuron 11, 551-564.

Gumbiner, B.M., (1996) Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell 84, 345-357.

Hassan, M., Li, Q., Brumback, B., Lucey, D.G., Bestland, M., Eubanks, G., Fillingim, R.B., Sheps, D.S., (2008) Comparison of peripheral arterial response to mental stress in men versus women with coronary artery disease. Am. J. Cardiol 102, 970-974.

Head, D., Rodrigue, K.M., Kennedy, K.M., Raz, N., (2008) Neuroanatomical and cognitive mediators of age-related differences in episodic memory. Neuropsychology 22, 491-507.

Heffelfinger, A.K. and Newcomer, J.W., (2001) Glucocorticoid effects on memory function over the human life span. Dev. Psychopathol 13, 491-513.

Henry, J.P., (1992) Biological basis of the stress response. Integr Physiol Behav Sci 27, 66-83.

Herman, J.P. and Cullinan, W.E., (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 20, 78-84.

Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C., Cullinan, W.E., (2003) Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front Neuroendocrinol 24, 151-180.

Herman, J.P., Ostrander, M.M., Mueller, N.K., Figueiredo, H., (2005) Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Prog. Neuropsychopharmacol. Biol. Psychiatry 29, 1201-1213.

Holmes, S.D., Krantz, D.S., Rogers, H., Gottdiener, J., Contrada, R.J., (2006) Mental stress and coronary artery disease: a multidisciplinary guide. Prog Cardiovasc Dis 49, 106-122.

Holsboer, F., (1983) The dexamethasone suppression test in depressed patients: clinical and biochemical aspects. J. Steroid Biochem 19, 251-257.

Holsboer, F., (2000) The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477-501.

Honda, T., Sakisaka, T., Yamada, T., Kumazawa, N., Hoshino, T., Kajita, M., Kayahara, T., Ishizaki, H., Tanaka-Okamoto, M., Mizoguchi, A., Manabe, T., Miyoshi, J., Takai, Y., (2006) Involvement of nectins in the formation of puncta adherentia junctions and the mossy fiber trajectory in the mouse hippocampus. Mol. Cell. Neurosci 31, 315-325.

Hynes, R.O., (1999) Cell adhesion: old and new questions. Trends Cell Biol 9, M33-M37.

Hynes, R.O. and Lander, A.D., (1992) Contact and adhesive specificities in the associations, migrations, and targeting of cells and axons. Cell 68, 303-322.

Irie, K., Shimizu, K., Sakisaka, T., Ikeda, W., Takai, Y., (2004) Roles and modes of action of nectins in cell-cell adhesion. Semin. Cell Dev. Biol 15, 643-656.

Ising, M., Horstmann, S., Kloiber, S., Lucae, S., Binder, E.B., Kern, N., Künzel, H.E., Pfennig, A., Uhr, M., Holsboer, F., (2007) Combined dexamethasone/corticotropin releasing hormone test predicts treatment response in major depression - a potential biomarker? Biol. Psychiatry 62, 47-54.

Issa, A.M., Rowe, W., Gauthier, S., Meaney, M.J., (1990) Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. J. Neurosci 10, 3247-3254.

Jacobson, L. and Sapolsky, R., (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocr. Rev 12, 118-134.

Jamain, S., Quach, H., Betancur, C., Rastam, M., Colineaux, C., Gillberg, I.C., Soderstrom, H., Giros, B., Leboyer, M., Gillberg, C., Bourgeron, T., (2003) Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat. Genet 34, 27-29.

Joels, M., Pu, Z., Wiegert, O., Oitzl, M.S., Krugers, H.J., (2006) Learning under stress: how does it work? Trends Cogn. Sci. (Regul. Ed.) 10, 152-158.

Kaczorowski, C.C., Davis, S.J., Moyer, J.R., Jr., (2011) Aging redistributes medial prefrontal neuronal excitability and impedes extinction of trace fear conditioning. Neurobiol. Aging

Kakunaga, S., Ikeda, W., Itoh, S., guchi-Tawarada, M., Ohtsuka, T., Mizoguchi, A., Takai, Y., (2005) Nectin-like molecule-1/TSLL1/SynCAM3: a neural tissue-specific immunoglobulin-like cell-cell adhesion molecule localizing at non-junctional contact sites of presynaptic nerve terminals, axons and glia cell processes. J. Cell. Sci 118, 1267-1277.

Kandel, E.R., (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294, 1030-1038.

Karst, H. and Joels, M., (2003) Effect of chronic stress on synaptic currents in rat hippocampal dentate gyrus neurons. J. Neurophysiol 89, 625-633.

Keenan, P.A., Jacobson, M.W., Soleymani, R.M., Newcomer, J.W., (1995) Commonly used therapeutic doses of glucocorticoids impair explicit memory. Ann. N. Y. Acad. Sci 761, 400-402.

Keeney, A., Jessop, D.S., Harbuz, M.S., Marsden, C.A., Hogg, S., Blackburn-Munro, R.E., (2006) Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice. J. Neuroendocrinol 18, 330-338.

Keeney, A.J., Hogg, S., Marsden, C.A., (2001) Alterations in core body temperature, locomotor activity, and corticosterone following acute and repeated social defeat of male NMRI mice. Physiol. Behav 74, 177-184.

Kendler, K.S., Thornton, L.M., Gardner, C.O., (2000) Stressful life events and previous episodes in the etiology of major depression in women: an evaluation of the "kindling" hypothesis. Am J Psychiatry 157, 1243-1251.

Kim, H.G., Kishikawa, S., Higgins, A.W., Seong, I.S., Donovan, D.J., Shen, Y., Lally, E., Weiss, L.A., Najm, J., Kutsche, K., Descartes, M., Holt, L., Braddock, S., Troxell, R., Kaplan, L., Volkmar, F., Klin, A., Tsatsanis, K., Harris, D.J., Noens, I., Pauls, D.L., Daly, M.J., MacDonald, M.E., Morton, C.C., Quade, B.J., Gusella, J.F., (2008) Disruption of neurexin 1 associated with autism spectrum disorder. Am. J. Hum. Genet 82, 199-207.

Kirschbaum, C., Wolf, O.T., May, M., Wippich, W., Hellhammer, D.H., (1996) Stress- and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. Life Sci 58, 1475-1483.

Kiselyov, V.V., Skladchikova, G., Hinsby, A.M., Jensen, P.H., Kulahin, N., Soroka, V., Pedersen, N., Tsetlin, V., Poulsen, F.M., Berezin, V., Bock, E., (2003) Structural basis for a direct interaction between FGFR1 and NCAM and evidence for a regulatory role of ATP. Structure 11, 691-701.

Klein, F., Lemaire, V., Sandi, C., Vitiello, S., Van der Logt, J., Laurent, P.E., Neveu, P., Le Moal, M., Mormede, P., (1992) Prolonged increase of corticosterone secretion by chronic social stress does not necessarily impair immune functions. Life Sci 50, 723-731.

Koolhaas, J.M., Bartolomucci, A., Buwalda, B., De Boer, S.F., Flügge, G., Korte, S.M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Wöhr, M., Fuchs, E., (2011) Stress revisited: A critical evaluation of the stress concept. Neurosci Biobehav Rev

Koolhaas, J.M., De Boer, S.F., De Rutter, A.J., Meerlo, P., Sgoifo, A., (1997) Social stress in rats and mice. Acta Physiol Scand Suppl 640, 69-72.

Kramer, A.F., Bherer, L., Colcombe, S.J., Dong, W., Greenough, W.T., (2004) Environmental influences on cognitive and brain plasticity during aging. J. Gerontol. A Biol. Sci. Med. Sci 59, M940-M957.

Kumar, A. and Foster, TC., (2007) Neurophysiology of old neurons and synapses.

Laing, S.S., Ocampo, C., Harris, J.R., (2010) Evaluating the relationships among psychological distress, executive cognitive function and economic factors on mammography use in unaffected African American women at risk for breast cancer. Ethn Dis 20, 467-473.

Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., LeVine, R., McEwan, P., McKernan, K., Meldrim, J., Mesirov, J.P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J.C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Waterston, R.H., Wilson, R.K., Hillier, L.W., McPherson, J.D., Marra, M.A., Mardis, E.R., Fulton, L.A., Chinwalla, A.T., Pepin, K.H., Gish, W.R., Chissoe, S.L., Wendl, M.C., Delehaunty, K.D., Miner, T.L., Delehaunty, A., Kramer, J.B., Cook, L.L., Fulton, R.S., Johnson, D.L., Minx, P.J., Clifton, S.W., Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J.F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Gibbs, R.A., Muzny, D.M., Scherer, S.E., Bouck, J.B., Sodergren, E.J., Worley, K.C., Rives, C.M., Gorrell, J.H., Metzker, M.L., Naylor, S.L., Kucherlapati, R.S., Nelson, D.L., Weinstock, G.M., Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Smith, D.R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H.M., Dubois, J., Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Rowen, L., Madan, A., Qin, S., Davis, R.W., Federspiel, N.A., Abola, A.P., Proctor, M.J., Myers, R.M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D.R., Olson, M.V., Kaul, R., Raymond, C., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G.A., Athanasiou, M., Schultz, R., Roe, B.A., Chen, F., Pan, H., Ramser, J., Lehrach, H., Reinhardt, R., McCombie, W.R., de la Bastide, M., Dedhia, N., Blöcker, H., Hornischer, K., Nordsiek, G., Agarwala, R., Aravind, L., Bailey, J.A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., Brown, D.G., Burge, C.B., Cerutti, L., Chen, H.C., Church, D., Clamp, M., Copley, R.R., Doerks, T., Eddy, S.R., Eichler, E.E., Furey, T.S., Galagan, J., Gilbert, J.G., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L.S., Jones, T.A., Kasif, S., Kaspryzk, A., Kennedy, S., Kent, W.J., Kitts, P., Koonin, E.V., Korf, I., Kulp, D., Lancet, D., Lowe, T.M., McLysaght, A., Mikkelsen,

T., Moran, J.V., Mulder, N., Pollara, V.J., Ponting, C.P., Schuler, G., Schultz, J., Slater, G., Smit, A.F., Stupka, E., Szustakowski, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y.I., Wolfe, K.H., Yang, S.P., Yeh, R.F., Collins, F., Guyer, M.S., Peterson, J., Felsenfeld, A., Wetterstrand, K.A., Patrinos, A., Morgan, M.J., de Jong, P., Catanese, J.J., Osoegawa, K., Shizuya, H., Choi, S., Chen, Y.J., (2001) Initial sequencing and analysis of the human genome. Nature 409, 860-921.

Landfield, P.W., Baskin, R.K., Pitler, T.A., (1981) Brain aging correlates: retardation by hormonal-pharmacological treatments. Science 214, 581-584.

Langnaese, K., Mummery, R., Gundelfinger, E.D., Beesley, P.W., (1998) Immunoglobulin superfamily members gp65 and gp55: tissue distribution of glycoforms. FEBS Letters 429, 284-288.

Lathe, R., (2004) The individuality of mice. Genes Brain Behav 3, 317-327.

Laursen, P., (1997) The impact of aging on cognitive functions. An 11 year follow-up study of four age cohorts. Acta Neurol. Scand. , Suppl 172, 7-86.

Li, S., Wang, C., Wang, W., Dong, H., Hou, P., Tang, Y., (2008) Chronic mild stress impairs cognition in mice: from brain homeostasis to behavior. Life Sci. 82 (17-18), 934-942

Li, Z. and Sheng, M., (2003) Some assembly required: the development of neuronal synapses. Nature Reviews. Molecular cell biology 4, 833-841.

Lightman, S.L., Wiles, C.C., Atkinson, H.C., Henley, D.E., Russell, G.M., Leendertz, J.A., McKenna, M.A., Spiga, F., Wood, S.A., Conway-Campbell, B.L., (2008) The significance of glucocorticoid pulsatility. Eur. J. Pharmacol 583, 255-262.

Lopez, M., Aoubala, M., Jordier, F., Isnardon, D., Gomez, S., Dubreuil, P., (1998) The human poliovirus receptor related 2 protein is a new hematopoietic/endothelial homophilic adhesion molecule. Blood 92, 4602-4611.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., (1951) Protein measurment with the Folin phenol reagent. J Biol Chem 193, 265-275.

Luine, V., Martinez, C., Villegas, M., Magarinos, A.M., McEwen, B.S., (1996) Restraint stress reversibly enhances spatial memory performance. Physiol. Behav 59, 27-32.

Lupien, S.J., Gaudreau, S., Tchiteya, B.M., Maheu, F., Sharma, S., Nair, N.P., Hauger, R.L., McEwen, B.S., Meaney, M.J., (1997) Stress-induced declarative memory impairment in healthy elderly subjects: relationship to cortisol reactivity. J. Clin. Endocrinol. Metab 82, 2070-2075.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci 10, 434-445.

Lüthl, A., Laurent, J.P., Figurov, A., Muller, D., Schachner, M., (1994) Hippocampal long-term potentiation and neural cell adhesion molecules L1 and NCAM. Nature 372, 777-779.

Magarinos, A.M. and McEwen, B.S., (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. Neuroscience 69, 89-98.

Mager, J. and Bartolomei, M.S., (2005) Strategies for dissecting epigenetic mechanisms in the mouse. Nat. Genet 37, 1194-1200.

Majima, T., Ogita, H., Yamada, T., Amano, H., Togashi, H., Sakisaka, T., Tanaka-Okamoto, M., Ishizaki, H., Miyoshi, J., Takai, Y., (2009) Involvement of afadin in the formation and remodeling of synapses in the hippocampus. Biochem. Biophys. Res. Commun 385, 539-544.

Marques, A.H., Silverman, M.N., Sternberg, E.M., (2009) Glucocorticoid dysregulations and their clinical correlates. From receptors to therapeutics. Ann. N. Y. Acad. Sci 1179, 1-18.

Martinez, M., Phillips, P.J., Herbert, J., (1998) Adaptation in patterns of c-fos expression in the brain associated with exposure to either single or repeated social stress in male rats. Eur. J. Neurosci 10, 20-33.

Martinez-Rico, C., Pincet, F., Perez, E., Thiery, J.P., Shimizu, K., Takai, Y., Dufour, S., (2005) Separation force measurements reveal different types of modulation of E-cadherin-based adhesion by nectin-1 and -3. J. Biol. Chem 280, 4753-4760.

Marzban, H., Khanzada, U., Shabir, S., Hawkes, R., Langnaese, K., Smalla, K.H., Bockers, T.M., Gundelfinger, E.D., Gordon-Weeks, P.R., Beesley, P.W., (2003) Expression of the immunoglobulin superfamily neuroplastin adhesion molecules in adult and developing mouse cerebellum and their localisation to parasagittal stripes. J. Comp. Neurol 462, 286-301.

McEwen, B.S., (1998) Stress, adaptation, and disease. Allostasis and allostatic load. Ann. N. Y. Acad. Sci 840, 33-44.

McEwen, B.S., (2000a) Allostasis and allostatic load: implications for neuropsychopharmacology. Neuropsychopharmacology 22, 108-124.

McEwen, B.S., (2000b) Effects of adverse experiences for brain structure and function. Biol. Psychiatry 48, 721-731.

McEwen, B.S., (2001) Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. Ann. N. Y. Acad. Sci 933, 265-277.

McEwen, B.S., (2005) Stressed or stressed out: what is the difference? J Psychiatry Neurosci 30, 315-318.

McEwen, B.S. and Wingfield, J.C., (2003) The concept of allostasis in biology and biomedicine. Horm Behav 43, 2-15.

McIlwain, K.L., Merriweather, M.Y., Yuva-Paylor, L.A., Paylor, R., (2001) The use of behavioral test batteries: effects of training history. Physiol. Behav 73, 705-717.

McKinney, W.T., (1984) Animal models of depression: an overview. Psychiatr Dev 2, 77-96.

McLaughlin, K.J., Gomez, J.L., Baran, S.E., Conrad, C.D., (2007) The effects of chronic stress on hippocampal morphology and function: an evaluation of chronic restraint paradigms. Brain Res 1161, 56-64.

Meng, W. and Takeichi, M., (2009) Adherens junction: molecular architecture and regulation. Cold Spring Harb Perspect Biol 1, a002899-a002899.

Merck Index, (1989) Merck Index. 11th Edition, 1244-

Mifsud, K.R., Gutierrez-Mecinas, M., Trollope, A.F., Collins, A., Saunderson, E.A., Reul, J.M.H.M., (2011) Epigenetic mechanisms in stress and adaptation. Brain Behav. Immun

Mikkonen, M., Soininen, H., Kälviänen, R., Tapiola, T., Ylinen, A., Vapalahti, M., Paljärvi, L., Pitkänen, A., (1998) Remodeling of neuronal circuitries in human temporal lobe epilepsy: increased expression of highly polysialylated neural cell adhesion molecule in the hippocampus and the entorhinal cortex. Ann. Neurol 44, 923-934.

Mikkonen, M., Soininen, H., Tapiola, T., Alafuzoff, I., Miettinen, R., (1999) Hippocampal plasticity in Alzheimer's disease: changes in highly polysialylated NCAM immunoreactivity in the hippocampal formation. Eur. J. Neurosci 11, 1754-1764.

Milsum, J.H., (1985) A model of the eustress system for health/illness. Behav Sci 30, 179-186.

Missler, M., Fernandez-Chacon, R., Südhof, T.C., (1998) The making of neurexins. J. Neurochem 71, 1339-1347.

Miyahara, M., Nakanishi, H., Takahashi, K., Satoh-Horikawa, K., Tachibana, K., Takai, Y., (2000) Interaction of nectin with afadin is necessary for its clustering at cell-cell contact sites but not for its cis dimerization or trans interaction. J. Biol. Chem 275, 613-618.

Mizoguchi, A., Nakanishi, H., Kimura, K., Matsubara, K., Ozaki-Kuroda, K., Katata, T., Honda, T., Kiyohara, Y., Heo, K., Higashi, M., Tsutsumi, T., Sonoda, S., Ide, C., Takai, Y., (2002) Nectin: an adhesion molecule involved in formation of synapses. J. Cell Biol 156, 555-565.

Moles, A., Bartolomucci, A., Garbugino, L., Conti, R., Caprioli, A., Coccurello, R., Rizzi, R., Ciani, B., D'Amato, F.R., (2006) Psychosocial stress affects energy

balance in mice: modulation by social status. Psychoneuroendocrinology 31, 623-633.

Montaron, M.F., Drapeau, E., Dupret, D., Kitchener, P., Aurousseau, C., Le Moal, M., Piazza, P.V., Abrous, D.N., (2006) Lifelong corticosterone level determines age-related decline in neurogenesis and memory. Neurobiol. Aging 27, 645-654.

Morris, R., (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11, 47-60.

Muller, D., Wang, C., Skibo, G., Toni, N., Cremer, H., Calaora, V., Rougon, G., Kiss, J.Z., (1996) PSA-NCAM is required for activity-induced synaptic plasticity. Neuron 17, 413-422.

Munck, A., Guyre, P.M., Holbrook, N.J., (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev 5, 25-44.

Narita, H., Yamamoto, Y., Suzuki, M., Miyazaki, N., Yoshida, A., Kawai, K., Iwasaki, K., Nakagawa, A., Takai, Y., Sakisaka, T., (2011) Crystal structure of the cis-dimer of nectin-1: implications for the architecture of cell-cell junctions. J. Biol. Chem

Neiiendam, J.L., Kohler, L.B., Christensen, C., Li, S., Pedersen, M.V., Ditlevsen, D.K., Kornum, M.K., Kiselyov, V.V., Berezin, V., Bock, E., (2004) An NCAM-derived FGF-receptor agonist, the FGL-peptide, induces neurite outgrowth and neuronal survival in primary rat neurons. J. Neurochem 91, 920-935.

Nemeroff, C.B., Widerlöv, E., Bissette, G., Walleus, H., Karlsson, I., Eklund, K., Kilts, C.D., Loosen, P.T., Vale, W., (1984) Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science 226, 1342-1344.

Nesher, E., Peskov, V., Rylova, A., Raz, O., Pinhasov, A., (2011) Comparative Analysis of the Behavioral and Biomolecular Parameters of Four Mouse Strains. J Mol Neurosci

Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J., Monteggia, L.M., (2002) Neurobiology of depression. Neuron 34, 13-25.

Newcomer, J.W., Craft, S., Hershey, T., Askins, K., Bardgett, M.E., (1994) Glucocorticoid-induced impairment in declarative memory performance in adult humans. J. Neurosci 14, 2047-2053.

Neylan, T.C., (1998) Hans Selye and the field of stress research. Neuropsychiatry Classics 10, 230-230.

Niederkofler, V., Baeriswyl, T., Ott, R., Stoeckli, E.T., (2010) Nectin-like molecules/SynCAMs are required for post-crossing commissural axon guidance. Development 137, 427-435.

Nihonmatsu-Kikuchi, N., Hashimoto, R., Hattori, S., Matsuzaki, S., Shinozaki, T., Miura, H., Ohota, S., Tohyama, M., Takeda, M., Tatebayashi, Y., (2011) Reduced rate of neural differentiation in the dentate gyrus of adult dysbindin null (sandy) mouse. PloS One 6, e15886-e15886.

Nikiforuk, A. and Popik, P., (2011) Long-lasting cognitive deficit induced by stress is alleviated by acute administration of antidepressants. Psychoneuroendocrinology 36, 28-39.

Nordahl, C.W., Ranganath, C., Yonelinas, A.P., Decarli, C., Fletcher, E., Jagust, W.J., (2006) White matter changes compromise prefrontal cortex function in healthy elderly individuals. J Cogn Neurosci 18, 418-429.

O'Connell, A.W., Fox, G.B., Barry, T., Murphy, K.J., Fichera, G., Foley, A.G., Kelly, J., Regan, C.M., (1997) Spatial learning activates neural cell adhesion molecule polysialylation in a corticohippocampal pathway within the medial temporal lobe. J. Neurochem 68, 2538-2546.

Ogita, H., Rikitake, Y., Miyoshi, J., Takai, Y., (2010) Cell adhesion molecules nectins and associating proteins: Implications for physiology and pathology. Proc. Jpn. Acad. , Ser. B, Phys. Biol. Sci 86, 621-629.

Ohl, F. and Fuchs, E., (1999) Differential effects of chronic stress on memory processes in the tree shrew. Brain Res Cogn Brain Res 7, 379-387.

Ohl, F., Roedel, A., Binder, E., Holsboer, F., (2003) Impact of high and low anxiety on cognitive performance in a modified hole board test in C57BL/6 and DBA/2 mice. Eur. J. Neurosci 17, 128-136.

Ohlin, B., Nilsson, P.M., Nilsson, J.A., Berglund, G., (2004) Chronic psychosocial stress predicts long-term cardiovascular morbidity and mortality in middle-aged men. Eur. Heart J 25, 867-873.

Oitzl, M.S. and De Kloet, E.R., (1992) Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. Behav. Neurosci 106, 62-71.

Oitzl, M.S., Fluttert, M., De Kloet, E.R., (1994) The effect of corticosterone on reactivity to spatial novelty is mediated by central mineralocorticosteroid receptors. Eur. J. Neurosci 6, 1072-1079.

Oitzl, M.S., Reichardt, H.M., Joels, M., De Kloet, E.R., (2001) Point mutation in the mouse glucocorticoid receptor preventing DNA binding impairs spatial memory. Proc. Natl. Acad. Sci. U. S. A 98, 12790-12795.

Olton, D.S. and Markowska, A.L., (1994) Memory and hippocampal function as targets for neurotoxic substances. Neurotoxicology 15, 439-443.

Owczarek, S., Kiryushko, D., Larsen, M.H., Kastrup, J.S., Gajhede, M., Sandi, C., Berezin, V., Bock, E., Soroka, V., (2010) Neuroplastin-55 binds to and signals through the fibroblast growth factor receptor. FASEB J 24, 1139-1150.

Owczarek, S., Soroka, V., Kiryushko, D., Hald Larsen, M., Yuan, Q., Sandi, C., Berezin, V., Bock Elisabeth, (2011) Neuroplastin-65 and a mimetic peptide derived from its homophilic binding site modulate neuronal differentiation and synaptic plasticity.

Oxenkrug, G.F., McIntyre, I.M., Stanley, M., Gershon, S., (1984) Dexamethasone suppression test: experimental model in rats, and effect of age. Biol. Psychiatry 19, 413-416.

Palanza, P., (2001) Animal models of anxiety and depression: how are females different? Neurosci Biobehav Rev 25, 219-233.

Palanza, P., Gioiosa, L., Parmigiani, S., (2001) Social stress in mice: gender differences and effects of estrous cycle and social dominance. Physiol. Behav 73, 411-420.

Palumbo, M.L., Zorrilla Zubilete, M.A., Cremaschi, G.A., Genaro, A.M., (2009) Different effect of chronic stress on learning and memory in BALB/c and C57BL/6 inbred mice: Involvement of hippocampal NO production and PKC activity. Stress 12, 350-361.

Papusheva, E. and Heisenberg, C.P., (2010) Spatial organization of adhesion: force-dependent regulation and function in tissue morphogenesis. EMBO J 29, 2753-2768.

Pariante, C.M., Thomas, S.A., Lovestone, S., Makoff, A., Kerwin, R.W., (2004) Do antidepressants regulate how cortisol affects the brain? Psychoneuroendocrinology 29, 423-447.

Pavlides, C., Nivon, L.G., McEwen, B.S., (2002) Effects of chronic stress on hippocampal long-term potentiation. Hippocampus 12, 245-257.

Paxinos, G. and Franklin, K., (2001) The mouse brain in stereotaxic coordinates. 2nd Edition,

Pearse, G., (2006a) Histopathology of the thymus. Toxicol Pathol 34, 515-547.

Pearse, G., (2006b) Normal structure, function and histology of the thymus. Toxicol Pathol 34, 504-514.

Peters, L.L., Robledo, R.F., Bult, C.J., Churchill, G.A., Paigen, B.J., Svenson, K.L., (2007) The mouse as a model for human biology: a resource guide for complex trait analysis. Nat. Rev. Genet 8, 58-69.

Plessow, F., Fischer, R., Kirschbaum, C., Goschke, T., (2011) Inflexibly Focused under Stress: Acute Psychosocial Stress Increases Shielding of Action Goals at the Expense of Reduced Cognitive Flexibility with Increasing Time Lag to the Stressor. J Cogn Neurosci

Qiu, G., Helmeste, D.M., Samaranayake, A.N., Lau, W.M., Lee, T.M.C., Tang, S.W., So, K.F., (2007) Modulation of the suppressive effect of corticosterone on adult rat hippocampal cell proliferation by paroxetine. Neurosci Bull 23, 131-136.

Ramos-Vara, J.A., (2005) Technical Aspects of Immunohistochemistry. Veterinary Pathology Online 42, 405-426.

Rao, S.S. and Winter, J.O., (2009) Adhesion molecule-modified biomaterials for neural tissue engineering. Front Neuroengineering 2, 6-6.

Rapp, P.R., Stack, E.C., Gallagher, M., (1999) Morphometric studies of the aged hippocampus: I. Volumetric analysis in behaviorally characterized rats. J. Comp. Neurol 403, 459-470.

Rasmussen, T., Schliemann, T., Sörensen, J.C., Zimmer, J., West, M.J., (1996) Memory impaired aged rats: no loss of principal hippocampal and subicular neurons. Neurobiol. Aging 17, 143-147.

Reinvang, I., Deary, I.J., Fjell, A.M., Steen, V.M., Espeseth, T., Parasuraman, R., (2010) Neurogenetic effects on cognition in aging brains: a window of opportunity for intervention? Front Aging Neurosci 2, 143-143.

Renner, K.H. and Beversdorf, D.Q., (2010) Effects of naturalistic stressors on cognitive flexibility and working memory task performance. Neurocase 16, 293-300.

Reul, J.M., Sutanto, W., van Eekelen, J.A., Rothuizen, J., De Kloet, E.R., (1990) Central action of adrenal steroids during stress and adaptation. Adv. Exp. Med. Biol 274, 243-256.

Rezzani, R., Bonomini, F., Rodella, L.F., (2008) Histochemical and molecular overview of the thymus as site for T-cells development. Prog Histochem Cytochem 43, 73-120.

Ribeiro, S.C., Tandon, R., Grunhaus, L., Greden, J.F., (1993) The DST as a predictor of outcome in depression: a meta-analysis. Am J Psychiatry 150, 1618-1629.

Rice, C., Sandman, C.A., Lenjavi, M.R., Baram, T.Z., (2008) A novel mouse model for acute and long-lasting consequences of early life stress. Endocrinology 149, 4892-4900.

Rice, M.C. and O'Brien, S.J., (1980) Genetic variance of laboratory outbred Swiss mice. Nature 283, 157-161.

Ronn, L.C., Bock, E., Linnemann, D., Jahnsen, H., (1995) NCAM-antibodies modulate induction of long-term potentiation in rat hippocampal CA1. Brain Res 677, 145-151.

Roozendaal, B., Okuda, S., Van der Zee, E.A., McGaugh, J.L., (2006) Glucocorticoid enhancement of memory requires arousal-induced noradrenergic

activation in the basolateral amygdala. Proc. Natl. Acad. Sci. U. S. A 103, 6741-6746.

Rosales, C., O'Brien, V., Kornberg, L., Juliano, R., (1995) Signal transduction by cell adhesion receptors. Biochim. Biophys. Acta 1242, 77-98.

Rosmond, R., Dallman, M.F., Björntorp, P., (1998) Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J. Clin. Endocrinol. Metab 83, 1853-1859.

Rozanski, A., Blumenthal, J.A., Davidson, K.W., Saab, P.G., Kubzansky, L., (2005) The epidemiology, pathophysiology, and management of psychosocial risk factors in cardiac practice: the emerging field of behavioral cardiology. J. Am. Coll. Cardiol 45, 637-651.

Rujescu, D., Ingason, A., Cichon, S., Pietiläinen, O.P.H., Barnes, M.R., Toulopoulou, T., Picchioni, M., Vassos, E., Ettinger, U., Bramon, E., Murray, R., Ruggeri, M., Tosato, S., Bonetto, C., Steinberg, S., Sigurdsson, E., Sigmundsson, T., Petursson, H., Gylfason, A., Olason, P.I., Hardarsson, G., Jonsdottir, G.A., Gustafsson, O., Fossdal, R., Giegling, I., Möller, H.J., Hartmann, A.M., Hoffmann, P., Crombie, C., Fraser, G., Walker, N., Lonnqvist, J., Suvisaari, J., Tuulio-Henriksson, A., Djurovic, S., Melle, I., Andreassen, O.A., Hansen, T., Werge, T., Kiemeney, L.A., Franke, B., Veltman, J., Buizer-Voskamp, J.E., Sabatti, C., Ophoff, R.A., Rietschel, M., Nöthen, M.M., Stefansson, K., Peltonen, L., St Clair, D., Stefansson, H., Collier, D.A., (2009) Disruption of the neurexin 1 gene is associated with schizophrenia. Hum. Mol. Genet 18, 988-996.

Rutishauser, U., (2008) Polysialic acid in the plasticity of the developing and adult vertebrate nervous system. Nat. Rev. Neurosci 9, 26-35.

Rygula, R., Abumaria, N., Flügge, G., Fuchs, E., Rüther, E., Havemann-Reinecke, U., (2005) Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav. Brain Res 162, 127-134.

Saab, B.J., Saab, A.M.P., Roder, J.C., (2011) Statistical and theoretical considerations for the platform re-location water maze. J. Neurosci. Methods 198, 44-52.

Sachser, N., Dürschlag, M., Hirzel, D., (1998) Social relationships and the management of stress. Psychoneuroendocrinology 23, 891-904.

Sakisaka, T. and Takai, Y., (2004) Biology and pathology of nectins and nectin-like molecules. Curr. Opin. Cell Biol 16, 513-521.

Salat, D.H., Kaye, J.A., Janowsky, J.S., (2001) Selective preservation and degeneration within the prefrontal cortex in aging and Alzheimer disease. Arch. Neurol 58, 1403-1408.

Salat, D.H., Tuch, D.S., Hevelone, N.D., Fischl, B., Corkin, S., Rosas, H.D., Dale, A.M., (2005) Age-related changes in prefrontal white matter measured by diffusion tensor imaging. Ann. N. Y. Acad. Sci 1064, 37-49.

Sandi, C., (1998) The role and mechanisms of action of glucocorticoid involvement in memory storage. Neural Plast 6, 41-52.

Sandi, C. and Loscertales, M., (1999) Opposite effects on NCAM expression in the rat frontal cortex induced by acute vs. chronic corticosterone treatments. Brain Res 828, 127-134.

Sandi, C., Loscertales, M., Guaza, C., (1997) Experience-dependent facilitating effect of corticosterone on spatial memory formation in the water maze. Eur. J. Neurosci 9, 637-642.

Sandi, C., Merino, J.J., Cordero, M.I., Touyarot, K., Venero, C., (2001) Effects of chronic stress on contextual fear conditioning and the hippocampal expression of the neural cell adhesion molecule, its polysialylation, and L1. Neuroscience 102, 329-339.

Sandi, C. and Rose, S.P., (1994) Corticosteroid receptor antagonists are amnestic for passive avoidance learning in day-old chicks. Eur. J. Neurosci 6, 1292-1297.

Sandi, C., (2004) Stress, cognitive impairment and cell adhesion molecules. Nat. Rev. Neurosci 5, 917-930.

Sandi, C., (2011) Glucocorticoids act on glutamatergic pathways to affect memory processes. Trends Neurosci 34, 165-176.

Sandi, C. and Pinelo-Nava, M.T., (2007) Stress and memory: behavioral effects and neurobiological mechanisms. Neural Plast 2007, 78970-78970.

Sapolsky, R.M., (1985) Glucocorticoid toxicity in the hippocampus: temporal aspects of neuronal vulnerability. Brain Res 359, 300-305.

Sapolsky, R.M., (1992) Cortisol concentrations and the social significance of rank instability among wild baboons. Psychoneuroendocrinology 17, 701-709.

Sapolsky, R.M., (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch. Gen. Psychiatry 57, 925-935.

Sapolsky, R.M., Krey, L.C., McEwen, B.S., (1983) The adrenocortical stress-response in the aged male rat: impairment of recovery from stress. Exp. Gerontol 18, 55-64.

Sapolsky, R.M., Romero, L.M., Munck, A.U., (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev 21, 55-89.

Sapolsky, R.M., Uno, H., Rebert, C.S., Finch, C.E., (1990) Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J. Neurosci 10, 2897-2902.

Sara, Y., Biederer, T., Atasoy, D., Chubykin, A., Mozhayeva, M.G., Südhof, T.C., Kavalali, E.T., (2005) Selective capability of SynCAM and neuroligin for functional synapse assembly. J. Neurosci 25, 260-270.

Sarter, M., Bodewitz, G., Stephens, D.N., (1988) Attenuation of scopolamine-induced impairment of spontaneous alteration behaviour by antagonist but not inverse agonist and agonist beta-carbolines. Psychopharmacology (Berl.) 94, 491-495.

Satoh-Horikawa, K., Nakanishi, H., Takahashi, K., Miyahara, M., Nishimura, M., Tachibana, K., Mizoguchi, A., Takai, Y., (2000) Nectin-3, a new member of immunoglobulin-like cell adhesion molecules that shows homophilic and heterophilic cell-cell adhesion activities. J. Biol. Chem 275, 10291-10299.

Schendan, H.E., Stern, C.E., (2007) Where vision meets memory: prefrontal-posterior networks for visual object constancy during categorization and recognition. Cereb Cortex 18, 1695-1711

Schmidt, M.V., Czisch, M., Sterlemann, V., Reinel, C., Sämann, P., Müller, M.B., (2009) Chronic social stress during adolescence in mice alters fat distribution in late life: prevention by antidepressant treatment. Stress 12, 89-94.

Schmidt, M.V., Scharf, S.H., Sterlemann, V., Ganea, K., Liebl, C., Holsboer, F., Müller, M.B., (2010) High susceptibility to chronic social stress is associated with a depression-like phenotype. Psychoneuroendocrinology 35, 635-643.

Schmidt, M.V., Sterlemann, V., Ganea, K., Liebl, C., Alam, S., Harbich, D., Greetfeld, M., Uhr, M., Holsboer, F., Müller, M.B., (2007) Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. Psychoneuroendocrinology 32, 417-429.

Schmidt, M.V., Schmidt, M., Oitzl, M.S., Levine, S., de Kloet, E.R., (2002) The HPA system during the postnatal development of CD1 mice and the effects of maternal deprivation. Brain Res. Dev. Brain Res 139, 39-49.

Schmidt, M.V., Sterlemann, V., Müller, M.B., (2008) Chronic stress and individual vulnerability. Ann. N. Y. Acad. Sci 1148, 174-183.

Scholey, A.B., Rose, S.P., Zamani, M.R., Bock, E., Schachner, M., (1993) A role for the neural cell adhesion molecule in a late, consolidating phase of glycoprotein synthesis six hours following passive avoidance training of the young chick. Neuroscience 55, 499-509.

Schulz, D., Huston, J.P., Jezek, K., Haas, H.L., Roth-Härer, A., Selbach, O., Luhmann, H.J., (2002) Water maze performance, exploratory activity, inhibitory

avoidance and hippocampal plasticity in aged superior and inferior learners. Eur. J. Neurosci 16, 2175-2185.

Schuurman, H.J., Kuper, C.F., Vos, J.G., (1994) Histopathology of the immune system as a tool to assess immunotoxicity. Toxicology 86, 187-212.

Scullion, G.A., Kendall, D.A., Sunter, D., Marsden, C.A., Pardon, M.C., (2009) Central noradrenergic depletion by DSP-4 prevents stress-induced memory impairment in the object recognition task. Neurosci ence 164 (2), 415-423

Secher, T., Novitskaia, V., Berezin, V., Bock, E., Glenthoj, B., Klementiev, B., (2006) A neural cell adhesion molecule-derived fibroblast growth factor receptor agonist, the FGL-peptide, promotes early postnatal sensorimotor development and enhances social memory retention. Neuroscience 141, 1289-1299.

Seckl, J.R., (2008) Glucocorticoids, developmental 'programming' and the risk of affective dysfunction. Prog. Brain Res 167, 17-34.

Seeman, T.E., McEwen, B.S., Singer, B.H., Albert, M.S., Rowe, J.W., (1997) Increase in urinary cortisol excretion and memory declines: MacArthur studies of successful aging. J. Clin. Endocrinol. Metab 82, 2458-2465.

Selye, H., (1936) A syndrome produced by diverse nocuous agents. 1936. J Neuropsychiatry Clin Neurosci 10, 230-231.

Selye, H., (1950) Stress and the general adaptation syndrome. Br Med J 1, 1383-1392.

Selye, H., (1975a) Confusion and controversy in the stress field. J Human Stress 1, 37-44.

Selye, H., (1975b) Stress and distress. Compr Ther 1, 9-13.

Selye, H. and Fortier, (1950) Adaptive reaction to stress. Psychosom Med 12, 149-157.

Shankar, S.K., (2010) Biology of aging brain. Indian J Pathol Microbiol 53, 595-604.

Sheng, M. and Sala, C., (2001) PDZ domains and the organization of supramolecular complexes. Annu. Rev. Neurosci 24, 1-29.

Shimizu, K., Amagaya, S., Ogihara, Y., (1983) Analysis of corticosterone in the serum of mice and rats using high-performance liquid chromatography. J. Chromatogr 272, 170-175.

Shively, C.A., (1998) Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys. Biol. Psychiatry 44, 882-891.

Shors, T.J., (2001) Acute stress rapidly and persistently enhances memory formation in the male rat. Neurobiol Learn Mem 75, 10-29.

Small, S.A., Stern, Y., Tang, M., Mayeux, R., (1999) Selective decline in memory function among healthy elderly. Neurology 52, 1392-1396.

Small, S.A., Chawla, M.K., Buonocore, M., Rapp, P.R., Barnes, C.A., (2004) Imaging correlates of brain function in monkeys and rats isolates a hippocampal subregion differentially vulnerable to aging. Proc. Natl. Acad. Sci. U. S. A 101, 7181-7186.

Smalla, K.H., Matthies, H., Langnaese, K., Shabir, S., Böckers, T.M., Wyneken, U., Staak, S., Krug, M., Beesley, P.W., Gundelfinger, E.D., (2000) The synaptic glycoprotein neuroplastin is involved in long-term potentiation at hippocampal CA1 synapses. Proc. Natl. Acad. Sci. U. S. A 97, 4327-4332.

Smith, A.W., Baum, A., Wing, R.R., (2005) Stress and weight gain in parents of cancer patients. Int J Obes (Lond) 29, 244-250.

Smith, T.D., Adams, M.M., Gallagher, M., Morrison, J.H., Rapp, P.R., (2000) Circuit-specific alterations in hippocampal synaptophysin immunoreactivity predict spatial learning impairment in aged rats. J. Neurosci 20, 6587-6593.

Solomon, M.B., Foster, M.T., Bartness, T.J., Huhman, K.L., (2007) Social defeat and footshock increase body mass and adiposity in male Syrian hamsters. Am. J. Physiol. Regul. Integr. Comp. Physiol 292, R283-R290.

Song, J.Y., Ichtchenko, K., Südhof, T.C., Brose, N., (1999) Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. Proc. Natl. Acad. Sci. U. S. A 96, 1100-1105.

Sonntag, W.E., Goliszek, A.G., Brodish, A., Eldridge, J.C., (1987) Diminished diurnal secretion of adrenocorticotropin (ACTH), but not corticosterone, in old male rats: possible relation to increased adrenal sensitivity to ACTH in vivo. Endocrinology 120, 2308-2315.

Sorrells, S.F. and Sapolsky, R.M., (2007) An inflammatory review of glucocorticoid actions in the CNS. Brain Behav. Immun 21, 259-272.

Spear, L.P., (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24, 417-463.

Steimer, T., Python, A., Schulz, P.E., Aubry, J.M., (2007) Plasma corticosterone, dexamethasone (DEX) suppression and DEX/CRH tests in a rat model of genetic vulnerability to depression. Psychoneuroendocrinology 32, 575-579.

Sterlemann, V., Ganea, K., Liebl, C., Harbich, D., Alam, S., Holsboer, F., Müller, M.B., Schmidt, M.V., (2008) Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: implications for stress-related disorders. Horm Behav 53, 386-394.

Sterlemann, V., Rammes, G., Wolf, M., Liebl, C., Ganea, K., Müller, M.B., Schmidt, M.V., (2010) Chronic social stress during adolescence induces cognitive impairment in aged mice. Hippocampus 20, 540-549.

Sterling, P. and Eyer, J., (1988) Allostasis: a new paradigm to explain arousal pathology. 629-649.

Stojanovich, L., (2010) Stress and autoimmunity. Autoimmun Rev 9, A271-A276.

Stott, G.H., (1981) What is animal stress and how is it measured? J. Anim. Sci 52, 150-153.

Strike, P.C. and Steptoe, A., (2004) Psychosocial factors in the development of coronary artery disease. Prog Cardiovasc Dis 46, 337-347.

Südhof, T.C., (2008) Neuroligins and neurexins link synaptic function to cognitive disease. Nature 455, 903-911.

Tabuchi, K. and Südhof, T.C., (2002) Structure and evolution of neurexin genes: insight into the mechanism of alternative splicing. Genomics 79, 849-859.

Takai, Y., Ikeda, W., Ogita, H., Rikitake, Y., (2008) The immunoglobulin-like cell adhesion molecule nectin and its associated protein afadin. Annu. Rev. Cell Dev. Biol 24, 309-342.

Takai, Y. and Nakanishi, H., (2003) Nectin and afadin: novel organizers of intercellular junctions. J. Cell. Sci 116, 17-27.

Talebizadeh, Z., Lam, D.Y., Theodoro, M.F., Bittel, D.C., Lushington, G.H., Butler, M.G., (2006) Novel splice isoforms for NLGN3 and NLGN4 with possible implications in autism. J. Med. Genet 43, e21-e21.

Tamashiro, K.L.K., Hegeman, M.A., Nguyen, M.M.N., Melhorn, S.J., Ma, L.Y., Woods, S.C., Sakai, R.R., (2007) Dynamic body weight and body composition changes in response to subordination stress. Physiol. Behav 91, 440-448.

Tamashiro, K.L.K., Hegeman, M.A., Sakai, R.R., (2006) Chronic social stress in a changing dietary environment. Physiol. Behav 89, 536-542.

Tan, Y.F., Preston, E., Wojtowicz, J.M., (2010) Enhanced post-ischemic neurogenesis in aging rats. Front Neurosci 4,

Taub, D.D. and Longo, D.L., (2005) Insights into thymic aging and regeneration. Immunol. Rev 205, 72-93.

Thiery, J.P., Duband, J.L., Rutishauser, U., Edelman, G.M., (1982) Cell adhesion molecules in early chicken embryogenesis. Proc. Natl. Acad. Sci. U. S. A 79, 6737-6741.

Tichomirowa, M.A., Keck, M.E., Schneider, H.J., Paez-Pereda, M., Renner, U., Holsboer, F., Stalla, G.K., (2005) Endocrine disturbances in depression. J. Endocrinol. Invest 28, 89-99.

Tirelli, E., Laviola, G., Adriani, W., (2003) Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. Neurosci Biobehav Rev 27, 163-178.

Togashi, H., Miyoshi, J., Honda, T., Sakisaka, T., Takai, Y., Takeichi, M., (2006) Interneurite affinity is regulated by heterophilic nectin interactions in concert with the cadherin machinery. J. Cell Biol 174, 141-151.

Topic, B., Dere, E., Schulz, D., de Souza Silva, M.A., Jocham, G., Kart, E., Huston, J.P., (2005) Aged and adult rats compared in acquisition and extinction of escape from the water maze: focus on individual differences. Behav. Neurosci 119, 127-144.

Touyarot, K. and Sandi, C., (2002) Chronic restraint stress induces an isoform-specific regulation on the neural cell adhesion molecule in the hippocampus. Neural Plast 9, 147-159.

Trollope, A.F., Gutierrez-Mecinas, M., Mifsud, K.R., Collins, A., Saunderson, E.A., Reul, J.M.H.M., (2011) Stress, epigenetic control of gene expression and memory formation. Exp Neurol

Tronche, F., Kellendonk, C., Kretz, O., Gass, P., Anlag, K., Orban, P.C., Bock, R., Klein, R., Schütz, G., (1999) Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nat. Genet 23, 99-103.

Tsoory, M., Guterman, A., Richter-Levin, G., (2008) Exposure to stressors during juvenility disrupts development-related alterations in the PSA-NCAM to NCAM expression ratio: potential relevance for mood and anxiety disorders. Neuropsychopharmacology 33, 378-393.

Tsoory, M. and Richter-Levin, G., (2006) Learning under stress in the adult rat is differentially affected by 'juvenile' or 'adolescent' stress. Int. J. Neuropsychopharmacol 9, 713-728.

Ullrich, B., Ushkaryov, Y.A., Südhof, T.C., (1995) Cartography of neurexins: more than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. Neuron 14, 497-507.

Ulrich-Lai, Y.M., Figueiredo, H.F., Ostrander, M.M., Choi, D.C., Engeland, W.C., Herman, J.P., (2006) Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am. J. Physiol. Endocrinol. Metab 291, E965-E973.

Ulrich-Lai, Y.M. and Herman, J.P., (2009) Neural regulation of endocrine and autonomic stress responses. Nat. Rev. Neurosci 10, 397-409.

Uno, H., Tarara, R., Else, J.G., Suleman, M.A., Sapolsky, R.M., (1989) Hippocampal damage associated with prolonged and fatal stress in primates. J. Neurosci 9, 1705-1711.

Unverzagt, F.W., Gao, S., Baiyewu, O., Ogunniyi, A.O., Gureje, O., Perkins, A., Emsley, C.L., Dickens, J., Evans, R., Musick, B., Hall, K.S., Hui, S.L., Hendrie, H.C., (2001) Prevalence of cognitive impairment: data from the Indianapolis Study of Health and Aging. Neurology 57, 1655-1662.

van Haarst, A.D., Oitzl, M.S., De Kloet, E.R., (1997) Facilitation of feedback inhibition through blockade of glucocorticoid receptors in the hippocampus. Neurochem. Res 22, 1323-1328.

van Praag, H.M., (2005) Can stress cause depression? World J. Biol. Psychiatry 6 Suppl 2, 5-22.

Vaughn, D.E. and Bjorkman, P.J., (1996) The (Greek) key to structures of neural adhesion molecules. Neuron 16, 261-273.

Venero, C., Tilling, T., Hermans-Borgmeyer, I., Schmidt, R., Schachner, M., Sandi, C., (2002) Chronic stress induces opposite changes in the mRNA expression of the cell adhesion molecules NCAM and L1. Neuroscience 115, 1211-1219.

Vijg, J. and Campisi, J., (2008) Puzzles, promises and a cure for ageing. Nature 454, 1065-1071.

Vogel, C., Teichmann, S.A., Chothia, C., (2003) The immunoglobulin superfamily in Drosophila melanogaster and Caenorhabditis elegans and the evolution of complexity. Development 130, 6317-6328.

VonDras, D.D., Powless, M.R., Olson, A.K., Wheeler, D., Snudden, A.L., (2005) Differential effects of everyday stress on the episodic memory test performances of young, mid-life, and older adults. Aging Ment Health 9, 60-70.

Vorhees, C.V. and Williams, M.T., (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 1, 848-858.

Wahlsten, D., Metten, P., Phillips, T.J., Boehm, S.L., Burkhart-Kasch, S., Dorow, J., Doerksen, S., Downing, C., Fogarty, J., Rodd-Henricks, K., Hen, R., McKinnon, C.S., Merrill, C.M., Nolte, C., Schalomon, M., Schlumbohm, J.P., Sibert, J.R., Wenger, C.D., Dudek, B.C., Crabbe, J.C., (2003) Different data from different labs: lessons from studies of gene-environment interaction. J. Neurobiol 54, 283-311.

Wang, X.D., Chen, Y., Wolf, M., Wagner, K.V., Liebl, C., Scharf, S.H., Harbich, D., Mayer, B., Wurst, W., Holsboer, F., Deussing, J.M., Baram, T.Z., Müller, M.B., Schmidt, M.V., (2011) Forebrain CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendritic remodeling. Neurobiol. Dis 42, 300-310.

Wang, X.-D., (2011) Personal Communication.

Washbourne, P., Dityatev, A., Scheiffele, P., Biederer, T., Weiner, J.A., Christopherson, K.S., El-Husseini, A., (2004) Cell adhesion molecules in synapse formation. J. Neurosci 24, 9244-9249.

Weinstein, A.A., Deuster, P.A., Francis, J.L., Bonsall, R.W., Tracy, R.P., Kop, W.J., (2010) Neurohormonal and inflammatory hyper-responsiveness to acute mental stress in depression. Biol Psychol 84, 228-234.

Willner, P., (1990) Animal models of depression: an overview. Pharmacol. Ther 45, 425-455.

Willner, P. and Mitchell, P.J., (2002) The validity of animal models of predisposition to depression. Behav Pharmacol 13, 169-188.

Wilson, R.S., Evans, D.A., Bienias, J.L., Mendes de Leon, C.F., Schneider, J.A., Bennett, D.A., (2003) Proneness to psychological distress is associated with risk of Alzheimer's disease. Neurology 61, 1479-1485.

Wilson, R.S., Arnold, S.E., Schneider, J.A., Kelly, J.F., Tang, Y., Bennett, D.A., (2006) Chronic psychological distress and risk of Alzheimer's disease in old age. Neuroepidemiology 27, 143-153.

Wilson, R.S., Arnold, S.E., Schneider, J.A., Li, Y., Bennett, D.A., (2007) Chronic distress, age-related neuropathology, and late-life dementia. Psychosom Med 69, 47-53.

Winters, B.D., Forwood, S.E., Cowell, R.A., Saksida, L.M., Bussey, T.J., (2004) Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. J. Neurosci 24, 5901-5908.

Wittenmayer, N., Körber, C., Liu, H., Kremer, T., Varoqueaux, F., Chapman, E.R., Brose, N., Kuner, T., Dresbach, T., (2009) Postsynaptic Neuroligin1 regulates presynaptic maturation. Proc. Natl. Acad. Sci. U. S. A 106, 13564-13569.

Wofford, J.L., Loehr, L.R., Schwartz, E., (1996) Acute cognitive impairment in elderly ED patients: etiologies and outcomes. Am J Emerg Med 14, 649-653.

Woodson, J.C., Macintosh, D., Fleshner, M., Diamond, D.M., (2003) Emotion-induced amnesia in rats: working memory-specific impairment, corticosterone-memory correlation, and fear versus arousal effects on memory. Learn. Mem 10, 326-336.

Wright, R.L. and Conrad, C.D., (2005) Chronic stress leaves novelty-seeking behavior intact while impairing spatial recognition memory in the Y-maze. Stress 8, 151-154.

Yamagata, M., Sanes, J.R., Weiner, J.A., (2003) Synaptic adhesion molecules. Curr. Opin. Cell Biol 15, 621-632.

Yan, J., Noltner, K., Feng, J., Li, W., Schroer, R., Skinner, C., Zeng, W., Schwartz, C.E., Sommer, S.S., (2008) Neurexin 1alpha structural variants associated with autism. Neurosci. Lett 438, 368-370.

Zola-Morgan, S.M. and Squire, L.R., (1990) The primate hippocampal formation: evidence for a time-limited role in memory storage. Science 250, 288-290.

Zoladz, P.R., Conrad, C.D., Fleshner, M., Diamond, D.M., (2008) Acute episodes of predator exposure in conjunction with chronic social instability as an animal model of post-traumatic stress disorder. Stress 11, 259-281.

Figures

Tables

Abbreviations

ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
AVP	arginine vasopressin
bp	base pairs
CA	cornu ammonis (part of the hippocampal formation) e.g. CA3
CA	cell adhesion
CAM	cell adhesion molecule
CNS	central nervous system
cpm	counts per minute (a measure of radioactivity)
CRH	corticotropin-releasing hormone
CRHR 1	corticotropin-releasing hormone receptor type one
CSD	chronic social defeat
CSS	chronic social stress
dex	dexamethasone
DG	dentate gyrus (part of the hippocampal formation)
ELS	early life stress
FGFR	fibroblast growth factor receptor
FST	forced swim test
Fn3	fibronectin type III
G	gauge (corresponds to the diameter of hypodermic needles)
GC	glucocorticoid
GR	glucocorticoid receptor
HPA axis	hypothalamic-pituitary-adrenal axis

Ig	immunoglobulin
Ig-SF	immunoglobulin superfamily
IHC	immunohistochemistry
i.p.	intraperitoneal
ISH	in situ hybridization
ITI	intertrial interval
kD	kilodalton (atomic mass unit)
l x w x h	length x width x height
MR	mineralocorticoid receptor
MWM	Morris water maze
NaCl	sterile solution of sodium chloride
nCAM	neuronal cell adhesion molecule (a whole class of molecules)
NCAM	neural cell adhesion molecule (one specific CAM)
Nec 1	Nectin 1
Nec 3	Nectin 3
Necl	nectin-like protein
Nlgn	Neuroligin
Np 55	Neuroplastin 55
Np 65	Neuroplastin 65
Nptn	Neuroplastin
Nrxn	Neurexin
OF	open field
OR	object recognition
p38-MAPK	p38 mitogen-activated protein kinase
PFA	paraformaldehyde

PFC	prefrontal cortex
PD	postnatal day
POMC	pro-opiomelanocortin
PSA-NCAM	polysialic acid NCAM
PSD	postsynaptic density
PTSD	post-traumatic stress disorder
PAJ	puncta adherentia junction
PVN	paraventricular nucleus
RIA	radio immuno assay
rpm	revolutions per minute
s.c.	subcutaneous
SEM	standard error of the mean
SNS	sympathetic nervous system
SSC	standard saline citrate
V1b	arginine vasopressin receptor 1B
VBS	visible burrow system
WB	western blot

Acknowledgements

At first, I would like to thank PD Dr. Marianne Müller, who gave me the opportunity to conduct my PhD in her research group and work on this interesting project. Her constant support and input throughout my thesis is strongly appreciated.

Second, I am deeply grateful to PD Dr. Mathias Schmidt, who allowed me to continue this project in his research group. Thank you for being my supervisor in the past 3 and a half years, for giving me scientific advice, encouraging me, patiently answering my questions and supporting me all the time.

Especially, I would like to thank Prof. Dr. George Boyan for his unhesitating willingness to read and evaluate my thesis as second examiner.

Of course, I would also like to thank the rest of my review board: Prof. Lutz Wiegrebe, Prof. Dr. Ute Vothknecht, Prof. Dr. Charles David and Prof. Dr. Jürgen Soll.

I am very grateful to all the members of the RG Müller and the RG Schmidt, who created a great atmosphere to work in and made the job a little less job and a little more fun! It was a great pleasure to work in such a friendly and open-minded work environment. Thank you to Bianca Mayer, Jakob Hartmann, Klaus Wagner, Claudia Liebl, Sebastian Scharf, Christiana Labermaier, Sara Santirelli, Daria Marinescu, Xiao-Dong Wang, Yun-ai Chen, Yuji Kitaichi and most notably Christine Kohl and Daniela Harbich. I will miss you guys!
I also would like to give thanks to the large number of people from the institute, who were very helpful in discussing the minor and major questions regarding the lab and animal work. Or for just having a nice chat now and then.

Finally, I would like to say thank you to my friends, my parents, my brother and my Vassilaki for the never ending support, faith and love. I could not have done this without you.

Thank you!

.Publications

Sterlemann V, Rammes G, **Wolf M**, Liebl C, Ganea K., Müller MB, Schmidt MV
Chronic social stress during adolescence induces cognitive impairment in aged mice
Hippocampus 2010 Apr;20(4):540-9.

Wolf M, Schuchmann M, Wiegrebe L
Localization dominance and the effect of frequency in the Mongolian Gerbil, Meriones unguiculatus
J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2010 Jul;196(7):463-70. Epub 2010 May 21

Wang XD, Chen Y, **Wolf M**, Wagner KV, Liebl C, Scharf SH, Harbich D, Mayer B, Wurst W, Holsboer F, Deussing JM, Baram TZ, Müller MB, Schmidt MV
Forebrain CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendritic remodelling
Neurobiol Dis 2011 Feb 3 [Epub ahead of print]

Hartmann J, Wagner KV, Liebl C, Scharf SH, Wang XD, **Wolf M**, Hausch F, Rein T, Schmidt U, Touma C, Cheung-Flynn J, Cox MB, Smith DF, Holsboer F, Müller MB, Schmidt MV
The involvement of FK506-binding protein 51 (FKBP5) in the behavioral and neuroendocrine effects of chronic social defeat stress
Neuropharmacology. 2011 Aug 4. [Epub ahead of print]

Wang XD, Rammes G, Kraev I, **Wolf M**, Liebl C, Scharf SH, Rice CJ, Wurst W, Holsboer F, Deussing JM, Baram TZ, Stewart MG, Müller MB, Schmidt MV
Forebrain CRF1 Modulates Early-Life Stress-Programmed Cognitive Deficits.
J Neurosci, 2011 Sep 21; 31(38): 13625-13634

Assertion/Erklärung

Ich erkläre an Eides statt, dass ich die vorliegende Dissertation selbständig und ohne unerlaubte Hilfe angefertigt habe. Es wurden nur die in der Arbeit angegebenen Quellen und Hilfsmittel benutzt.

Des Weiteren erkläre ich, dass ich nicht anderweitig ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen. Die vorliegende Arbeit liegt weder ganz, noch in wesentlichen Teilen einer anderen Prüfungskommission vor.

_____ _____

Ort, Datum Unterschrift Miriam Wolf