Stress, emotional learning and AMPA receptors: from behavior to molecule
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1. Stress and stress hormones

1.1. What is Stress?
In biological systems, stress is generally defined as the subjective experience of any condition that disturbs the physiological and/or psychological homeostasis of the organism (Chrousos, 1998; de Kloet et al., 2005; Kim and Diamond, 2002). Exposure to stressful events activates intrinsic mechanisms that help respond to these challenging conditions (Bracha et al., 2004). The entire repertoire of behavioral and physiological responses includes (among others things): enhanced appraisal, arousal and alertness, heightened attention, suppression of sexual and feeding behaviors, re-direction of energy flow towards the places where energy is needed and cardiovascular changes (Bracha et al., 2004). Ultimately, these actions are aimed to restore the disturbed equilibrium (de Kloet et al., 2005; Kopin, 1995). The response to a stressful experience is therefore highly adaptive and might even be beneficial for the survival and evolutionary development of the whole species. On the other hand, there are examples that exposure to stressful experiences can reduce health of individuals in general and can be a risk factor for psychopathological diseases such as depression and post-traumatic stress disorder (PTSD) (Yehuda, 2009). In depression and PTSD, the intrinsic mechanisms that promote behavioral adaptation to stressful events often appear to be dysregulated (de Kloet et al., 2005).

1.2. Main stress pathways
There are two main neuro-endocrine pathways activated upon exposure to stressful events: the autonomous nervous system (ANS) and the hypothalamo-pituitary-adrenal (HPA) axis. Both the ANS and HPA-axis are regulated by input from higher cortical, limbic, visual, auditory, olfactory and visceral areas, and are also subject to regulation by several hormones and
cytokines (Chrousos, 1998). Importantly, these systems interact at multiple levels to promote physical and behavioral adaptation to stressful events (De Kloet et al., 1998; Pacak et al., 1995; Tsigos and Chrousos, 2002).

1.2.1. Autonomic Nervous System
Activation of the autonomic nervous system (ANS) leads to elevated circulating levels of adrenaline and noradrenaline (Smith and Vale, 2006; Tsigos and Chrousos, 2002) which are released from the adrenal medulla and presynaptic nerve terminals. These catecholamines trigger an elevation in heart and respiratory rate, increase blood pressure and promote energy mobilization (Ulrich-Lai and Herman, 2009). In the central nervous system (CNS), exposure to a stressful event rapidly activates the locus coeruleus (LC), where the majority of noradrenergic neurons are located. These neurons project to other brain areas, such as the prefrontal cortex, cerebellum, amygdala, and hippocampus (Foote et al., 1983) and supply these areas with noradrenergic signals via α- and β-adrenergic receptors. Peripherally released adrenaline can also stimulate the ascending vagal afferents at the nucleus of solitary tract (NTS), from which noradrenergic neurons directly innervate the basolateral amygdala and LC. Therefore the brain is a major target for catecholamines that are released upon exposure to stressful events.

1.2.2. Hypothalamo-Pituitary-Adrenal Axis
A second major system that is activated upon exposure to stressful events is the hypothalamo-pituitary-adrenal (HPA)-axis. At baseline activity of the HPA-axis, glucocorticoid hormones (corticosterone in rodents; cortisol in humans) are secreted from the adrenal cortex in discrete pulses so that plasma glucocorticoid levels exhibit both an ultradian and a circadian rhythm (Lightman and Conway-Campbell, 2010; Lightman et al., 2008).

The perception of a stressful event activates parvocellular neurons in
the paraventricular nucleus (PVN) of the hypothalamus. Activation of these neurons triggers the release of corticotrophin-releasing hormone (CRH) and arginine-vasopressin (AVP) which eventually stimulate the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland into the peripheral circulation. ACTH acts on the adrenal cortex and initiates the synthesis (including a series of enzymatic steps) and secretion of glucocorticoids (Charlton, 1990). Adrenal corticosteroid hormones are not stored and their secretion usually reaches maximal levels within 15 min after HPA-axis activation (De Kloet et al., 1998).

Due to their lipophilic properties, circulating glucocorticoids easily enter the brain, reach target brain areas and affect brain function. In addition, these hormones exert a negative feedback action at the level of the hypothalamus and pituitary (de Kloet et al., 2005; Gwinup, 1967) to restrain their own release and maintain a dynamic yet homeostatic equilibrium. The HPA-axis activity can also be modulated by other brain areas during stress, including the hippocampus (Jacobson and Sapolsky, 1991), amygdala (Allen and Allen, 1974) and prefrontal cortex (Diorio et al., 1993) (Figure 1).

1.3. Corticosteroid receptors

In the brain, corticosteroid hormones bind to both thus far characterized corticosteroid receptors: the glucocorticoid receptor (GR), which has a relatively low affinity for cortisol and corticosterone, and the mineralocorticoid receptor (MR), which has a higher affinity for these hormones (Joels et al., 2006; Reul and de Kloet, 1985).

Both receptors belong to a superfamily of nuclear receptors (Arriza et al., 1987; Hollenberg et al., 1985; Lu et al., 2006; Miesfeld et al., 1986), which classically act as transcription factors to regulate gene expression. The intracellular steroid receptors are part of a cytoplasmic multiprotein complex, consisting of one receptor molecule, several heat shock proteins (HSP) and an immunophilin (De Kloet et al., 1998). Upon binding to MRs or
GRs in the cytoplasm, a rapid chain reaction happens: first, the receptors dissociate from HSPs and immunophilins; second, the ligand-bound receptors are translocated from the cytoplasm to the nucleus (Jewell et al., 1995); third, the receptors, either in the form of homo- or hetero-dimers (Trapp et al., 1994), bind to glucocorticoid responsive elements (GREs) (Beato et al., 1996); finally, binding to GREs in a promoter region leads to enhanced or repressed transcription of the corresponding gene (De Kloet et al., 1998; Joels, 2006; Munck et al., 1990; Vreugdenhil et al., 2001).

**Figure 1. HPA-axis and ANS** (see the text in Section 1.2 for details)

Abbreviation: ACTH: adrenocorticotropic hormone; AMY: amygdala nuclei; ANS: autonomic nervous system; CRH: corticotrophin releasing hormone; HIPP: hippocampus; HYP: hypothalamus; LC: locus coeruleus; NA: noradrenaline; NTS:
nucleus of solitary tract; PFC: prefrontal cortex

Even though the DNA binding domains of both GRs and MRs are nearly identical, the final functional outcome of the binding of glucocorticoids to MRs or GRs varies tremendously. Several main differences between MRs and GRs are listed below:

1) Affinity: MRs and GRs display a different affinity for corticosterone; MRs exhibit a 10-fold higher affinity for corticosterone than GRs (Reul and de Kloet, 1985). Due to their lower affinity, GRs become fully occupied only when serum corticosterone level increase, e.g. after exposure to stress or during diurnal peaks. In contrast, most of the MR (nearly 90%) is already bound with endogenous corticosterone under basal condition (Reul and de Kloet, 1985).

2) Distribution: GRs are ubiquitously expressed throughout the brain while MRs are highly expressed in the limbic system (Arriza et al., 1988; De Kloet et al., 1998; Reul and de Kloet, 1985). However, MRs and GRs also co-exist in some of the brain areas, including hippocampal pyramidal cell fields except for the adult CA3 region which shows minimal GRs expression (de Kloet et al., 2005; Evans and Arriza, 1989). Both receptors can also be found in the same hippocampal pyramidal cells (Bohn et al., 1991), which enables a cross-talk between these two types of receptors that might lead to either enhancement (synergism) or repression (antagonism) of gene transcription in response to physiological or cellular changes (De Kloet et al., 1998).

3) Structure: MRs and GRs differ in alternative RNA splicing, translation initiation as well as post-translational modulation (Joels, 2006; Pascual-Le Tallec and Lombes, 2005; Zhou and Cidlowski, 2005).

In addition to these slow, genomic actions in the brain (Joels and de Kloet, 1994), it is now evident that glucocorticoids can also exert rapid, non-genomic effects via membrane-associated receptors which are present
in different species and in different brain areas (Orchinik et al., 1991, Venero and Borrell, 1999, Di et al., 2005, Karst et al., 2005, Groc et al., 2008, Karst et al., 2010). In the hippocampus, glucocorticoids rapidly and reversibly enhance the frequency of mEPSCs (miniature excitatory postsynaptic currents) via MRs that are presumably located in the membrane (Karst et al., 2005). A similar rapid MR-mediated increase in mEPSC frequency has been reported in the basolateral amygdala (Karst et al., 2010b). Interestingly, this increase in synaptic transmission could be rapidly reversed by a non-genomic effect that involves GRs (Karst et al., 2010b). It is important to note that the presence of membrane corticosteroid receptors yields a completely new pathway via which glucocorticoids can regulate cellular function. The resultant cellular responses to the pulses of corticosterone during the circadian rhythm and after exposure to a stressful event are therefore determined by the dynamic interaction between corticosteroid hormones and MRs/GRs via genomic and/or non-genomic actions (Figure 2).

It is important to note that both MRs and GRs are localized in regions that are important for memory formation such as the hippocampus, amygdala and the prefrontal cortex. In interaction with a number of other compounds – such as (nor) adrenaline, CRH, endocannabinoids – corticosteroid hormones via their receptors alter neuronal activity in areas that play central roles in the storage of relevant information and promote behavioral adaptation (Joels and Baram, 2009; Roozendaal et al., 2009c).

2. Learning and memory
Experiences modify the nervous system so that individuals can learn and further evolve. Once information has been acquired (learned), the information can be consolidated and stored as a long-lasting memory trace (McGaugh, 2000). To become operational, memories need to be retrieved. Interestingly, the cascade of molecular mechanisms that is required for the
storage of information is also required to consolidate information once being retrieved and updated (reconsolidation, see Box 1) (Nader et al., 2000b).

Memory can be divided in at least two types: declarative (explicit) and procedural (implicit) memory (Graf and Schacter, 1985; Squire, 2004). Declarative memory requires conscious recall, and can be divided further into 1) semantic memory (concerning the facts), and 2) episodic memory (concerning events, including time and space). In contrast, procedural memory refers to unconsciously recalled information such as skills (e.g. learning how to bike or skate). Spatial memory is another special type of memory, which contains the information of the spatial environment. It can be recalled as a semantic memory (e.g. as one recalls a map learned from a
book) or as an episodic memory (e.g. as one recalls a journey he just had in tropical jungle).

2.1. Stress hormones and learning and memory
Stress can have a major impact on memory performance (Joels et al., 2006; Roozendaal et al., 2009a). Numerous studies have suggested that both in humans and rodents the most vivid memories tend to be of emotional events, which are likely to be recalled more often and with more clarity and details than neutral events (Cahill and McGaugh, 1998; Joels et al., 2006; McGaugh and Roozendaal, 2002; Olff et al., 2005). This ability to acquire, store and retrieve emotionally charged information may be beneficial from the evolutionary viewpoint.

In our modern society along with the advancement of technology and economic growth, unfortunately human beings are more often exposed to psychological stress (global economic crisis, losing jobs, competitions and terrorism etc.). The notion that memories of stressful events are very robust and may even stay inappropriately present in some individuals has stimulated research on the molecular and cellular mechanisms that underlie the formation of fearful memories. Studies in humans and animals reveal that hormones that are released during aversive events promote memory formation of potentially relevant events. However, dysregulation of appropriate (endocrine) responses may on the other hand contribute to the formation of fearful memories (Joels et al., 2006). Post-traumatic stress disorder (PTSD) is an extreme example illustrating that stressful events can have a negative impact on humans. Patients suffering from PTSD can vividly recall the previous “aversive” life experiences (accidents, abuse and so on) no matter how long they have passed (Cahill and McGaugh, 1998; Shors, 2006). PTSD patients also avoid anything that is likely to remind them to the trauma and display heightened irritability (Gersons and Carlier, 1992).
Norepinephrine (NE) and corticosteroid hormones, via their receptors, play an important role in the memory enhancing effects of stress and emotion (Joels et al., 2006; Roozendaal et al., 2009a). NE enhances memory formation of emotional events via the brain β-adrenergic receptors (β-ARs) both in humans and rodents: post-training application of norepinephrine or β-ARs agonists promotes memory consolidation in various memory tasks such as the inhibitory avoidance task, fear conditioning and Morris water-maze (Hu et al., 2007; Roozendaal et al., 1993). Activation of α-adrenergic receptors also enhances memory, presumably by enhancing the actions of β-adrenergic actions (Ferry et al., 1999).

Corticosteroid hormones via MRs have been implicated in the appraisal of information and response selection (Oitzl and de Kloet, 1992; Sandi and Rose, 1994a). Via GRs these hormones have been reported to promote long-term consolidation of information (Jin et al., 2007; Oitzl and de Kloet, 1992; Pugh et al., 1997a; Quirarte et al., 2009; Quirarte et al., 1997; Roozendaal et al., 2001; Roozendaal and McGaugh, 1996a, b, 1997a, b; Roozendaal et al., 2009c; Roozendaal et al., 1999a; Roozendaal et al., 1996; Roozendaal et al., 2002b; Roozendaal et al., 1999b; Sandi and Rose, 1994b). Accordingly, a point mutation in the mouse GR (Oitzl et al., 2001) and inactivation of the mouse MR gene in the forebrain (Berger et al., 2006), are associated with impaired spatial memory performance. Recent evidence suggests that membrane-associated GRs also promote long-term memory in an object recognition task, via chromatin modification (Roozendaal et al., 2010). Thus, it is likely that both non-genomic as well as genomic actions of corticosteroid hormones promote the storage of relevant information. Besides these well-described effects of stress and glucocorticoids on consolidation processes, these hormones have also been reported to affect memory retrieval mechanisms (de Quervain et al., 2007; de Quervain et al., 2003; de Quervain et al., 1998; Pakdel and Rashidy-Pour, 2007;
Rashidy-Pour et al., 2009; Sajadi et al., 2006, 2007) and extinction processes (Bohus and de Kloet, 1981; Gourley et al., 2009; Yang et al., 2006). Taken together, there is ample evidence that corticosteroid hormones, via activation of MRs and GRs, have a repertoire of behavioural effects and promote the consolidation of relevant information, which facilitates behavioural adaptation (de Kloet et al., 2005; Schwabe et al., 2010). Recent studies further suggest that corticosteroids act in concert with other hormones such as norepinephrine (Roozendaal et al., 2001; Roozendaal et al., 2004a; Roozendaal et al., 2004b; Roozendaal et al., 2006a; Roozendaal et al., 2006b), CRH (Coste et al., 2000; Muller et al., 2003; Roozendaal et al., 2002a) and endocannabinoids (de Oliveira Alvares et al., 2010; Moreira and Lutz, 2008) for optimal memory performance both in rodents and humans (de Quervain et al., 2009).

3. Glutamate receptors, synaptic plasticity and learning and memory

An important question is how emotional memories are formed, what the underlying molecular and cellular mechanisms are and how these processes are modulated by stress hormones. Changes in synaptic connectivity are generally believed to underlie learning and memory processes (Bliss and Collingridge, 1993; Doyere and Laroche, 1992; Morris et al., 1990). Plasticity at synapses can be regulated at the presynaptic site (by changing the release of neurotransmitters), the postsynaptic site (by changing the function and number of receptors) or both (Malinow and Malenka, 2002). The most explored forms of plasticity at excitatory synapses are N-methyl-D-aspartic acid receptor (NMDAR)-dependent long-term potentiation (LTP) and long-term depression (LTD), which have been associated with changes in postsynaptic signaling (Bliss and Collingridge, 1993; Neves et al., 2008).

3.1. NMDA receptors
Long-term potentiation (LTP) reflects a long-lasting increase in synaptic connectivity (Neves et al., 2008) that can experimentally be elicited by high-frequency stimulation or by afferent stimulation in combination with post-synaptic depolarization (Bliss and Collingridge, 1993; Bliss and Lomo, 1973). NMDA receptors play a critical role in the induction of LTP. These receptors are composed of GluN1 and GluN2 subunits (latest nomenclature by NC-IUPHAR) (Collingridge et al., 2009). The NMDA receptor forms a heterotetramer of two GluN1 and two GluN2 subunits (GluN2A-D). Each receptor subunit has an extracellular domain, a membrane spanning domain and an intracellular cytoplasmic domain. The extracellular domain contains two globular structures: a modulatory domain and a ligand-binding domain. The transmembrane domain forms a channel pore which has a high-calcium permeability and voltage-dependent magnesium block. The extensive cytoplasmic domain can be modified by protein kinases and protein phosphatases, and interacts with and hence anchored at the synapses by a family of proteins named membrane-associated guanylate kinases (Leonard et al., 1999; Wenthold et al., 2003), including PSD-95 (Kornau et al., 1995), a highly abundant protein in the postsynaptic density (PSD). The NMDA receptor is a unique ligand-gated ion channels since activation requires binding of glutamate as well as membrane depolarization which is needed to release the magnesium block of the channel and to open the channel with high probability (Nowak et al., 1984). Therefore, the NMDA receptor functions as a coincidence detector that determines specificity and associativity of synaptic potentiation.

3.2. AMPA receptors

There is ample evidence that the dynamic regulation of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors or AMPARs - which mediate most of the fast excitatory synaptic transmission - can change synaptic function and regulate storage of information (Malinow and
Malenka, 2002; Rumpel et al., 2005). Controlling the number of AMPARs on the postsynaptic membrane is an essential mechanism to regulate synaptic strength and plasticity (Malenka, 2003; Malinow and Malenka, 2002; Plant et al., 2006).

AMPARs are heteromeric tetramer complexes formed of different combinations of GluA1-4 subunits (Hollmann and Heinemann, 1994; Keinanen et al., 1990; Tanabe et al., 1992; Wisden and Seeburg, 1993). Each AMPAR complex contains four subunits (Rosenmund et al., 1998), and the topology of each subunit is similar: an N-terminal extracellular amino domain, a ligand-binding domain, a membrane localized domain and an intracellular C-terminal domain (Hollmann, et al., 1994). The number and composition of AMPARs can vary substantially among different neuronal populations. In adult hippocampal pyramidal neurons for example, two main populations of AMPAR complexes are found: GluA1/GluA2 and GluA2/GluA3 containing AMPARs. Very few GluA1/GluA3 and some (up to 8%) homomeric GluA1 AMPARs are present (Wenthold et al., 1996).

While the extracellular and transmembrane regions of AMPAR subunits are very similar, their intracellular cytoplasmic tails are distinct: GluA1, GluA4 and an alternative splice form of GluA2 (GluA2L) have longer cytoplasmic tails. In contrast, the predominant splice form of GluA2, GluA3 and an alternative splice form of GluA4 that is primarily expressed in cerebellum (GluA4c) have shorter cytoplasmic tails (Malinow and Malenka, 2002). The differences in cytoplasmic carboxyl termini and binding to different intracellular proteins result in different synaptic function and dynamic transport of AMPARs.

AMPAR subunits are synthesized and then assembled into receptors in the rough endoplasmic reticulum of the neuronal cell body (Barry and Ziff, 2002) and reach their synaptic targets after a complicated journey involving multiple sorting and transport steps along different cytoskeleton structures and through various membrane compartments. The initial polarized sorting
of AMPARs into dendrites is controlled by the microtubule-dependent motor protein dynein (Kapitein et al., 2010). Once inside dendrites, trafficking of AMPARs to and from the synapse is regulated by two main processes: 1) exocytotic/endocytotic recycling between intracellular and membrane receptor pools (Gerges et al., 2006; Passafaro et al., 2001; Wang et al., 2008b); 2) surface diffusion between extrasynaptic and synaptic receptor pools (Adesnik et al., 2005; Ashby et al., 2006; Ehlers et al., 2007). Recent studies are beginning to identify the molecular players in the endosomal pathway that are essential for AMPAR recycling (Carroll et al., 1999a; Lee et al., 2004). The insertion, through exocytosis, of AMPARs into the plasma membrane is a key step in controlling the number of surface receptors. Although there is still a debate on the exact localization and nature of inserted AMPAR, live imaging experiments have shown that exocytotic events occur in the dendritic shaft and may also happen in dendritic spines (Makino and Malinow, 2009; Yudowski et al., 2007). The sites of AMPAR internalization, through endocytosis, can lie both close to the postsynaptic density (PSD) and in the extrasynaptic membrane (Petrini et al., 2009). A two-step model has emerged in which AMPARs first traffic from and to the plasma membrane mostly outside or lateral to synapses, and then diffuse at the neuronal surface from extrasynaptic sites to synaptic membrane compartments. In line with this model, recent studies have shown that the delivery of AMPARs from intracellular stores to the synapse depends on extrasynaptic receptor exocytosis (Petrini et al., 2009).

3.3. AMPA receptor trafficking in synaptic plasticity and learning and memory

Activation of NMDA receptors allows Ca^{2+} influx into dendritic spines of post-synaptic neurons which activates calcium-dependent enzymes, such as calcium/calmodulin-dependent kinase II (CaMKII), protein kinase A (PKA) and protein kinase C (Sheng and Kim, 2002). These kinases modulate
synaptic transmission, including regulation of the function of AMPARs. CaMKII phosphorylates the GluA1 subunit of AMPARs at serine831 and enhances channel function of AMPARs (Barria et al., 1997a; Mammen et al., 1997). Moreover, activated CaMKII reduces the probability of synaptic failures, indicating that the enzyme converts silent synapses into functional contacts (Lledo et al., 1995). Third, CaMKII helps to organize a structural process that leads to the incorporation of AMPARs-binding proteins into the PSD, followed by subsequent anchoring of additional AMPARs (Lisman and Zhabotinsky, 2001). PKA phosphorylates GluA1 subunit at Serine845 or S845 (Hu et al., 2007; Lee et al., 2000; Roche et al., 1996), while PKC phosphorylates GluA1 at ser818 (Boehm et al., 2006). The phosphorylation of PKA and PKC is crucial for synaptic incorporation of AMPAR (Ehlers, 2000; Esteban et al., 2003; Qin et al., 2005) and can regulate AMPAR mediated synaptic plasticity (Lee et al., 2003).

The link between AMPAR surface diffusion and cycling is evident in synaptic plasticity paradigms. Well-established protocols of synaptic potentiation induce massive exocytosis of AMPARs, which mostly originate from endosomal compartments. The exocytosed receptors, at extrasynaptic sites or near the spines, then diffuse and accumulate at postsynaptic density compartments. Together, the regulation of synaptic AMPAR numbers relies on a dynamic equilibrium between intracellular, extrasynaptic, and synaptic pools of AMPARs, and is regulated by the activity status of the neuronal network (Groc et al., 2008; Krugers et al., 2010; Makino and Malinow, 2009; Petrini et al., 2009).

Interestingly, the trafficking of AMPARs appears to be dependent on the subunit composition. The GluA1 carboxyl terminus mediates regulated delivery of AMPARs onto synapses upon synaptic activation while the GluA2 carboxyl terminus determines the continuous delivery of AMPARs onto synapses independent from synaptic stimulation (Shi et al., 2001). Upon LTP induction, GluA1-containing calcium-permeable AMPARs (GluA1
homomers) are rapidly and transiently incorporated into synaptic membrane from an intracellular reserve pool (Shi, 2001), and are replaced by GluA1-lacking calcium-impermeable AMPARs shortly after LTP induction (Plant et al., 2006). The GluA2/3 containing AMPARs represent a substantial proportion of endogenous AMPARs in cortical neurons (Lu and Ziff, 2005). They are highly mobile and involved in cell surface expression of AMPARs (Barry and Ziff, 2002). Functionally, these GluA1-lacking AMPARs (such as GluA2/3) are calcium-impermeable (Burnashev et al., 1992; Kauer and Malenka, 2006; Plant et al., 2006) and may play a role in maintaining synaptic strength (Kauer and Malenka, 2006; Malenka, 2003; Malinow and Malenka, 2002; Plant et al., 2006).

The role of AMPAR trafficking in experience-dependent plasticity has been well-studied in the developing rodent sensory cortex. Sensory stimulation in vivo induces an LTP-like increase in the strength of neocortical synapses, which is probably dependent on delivery of GluA1-containing AMPARs (Takahashi et al., 2003). The role of AMPAR trafficking in learning has also been extensively studied in limbic areas. In tone-cued conditioning, the trafficking of GluA1 containing AMPARs is essential for the formation of fearful memories (Rumpel et al., 2005). In contextual learning paradigms, the phosphorylation of GluA1 subtype AMPARs at S831 is enhanced and GluA1 and GluA2 protein levels are rapidly and transiently enhanced in synapses, indicating that such a cognitive task is accompanied by changes in AMPAR trafficking in hippocampal neurons (Whitlock et al., 2006). Moreover, aversive learning induces LTP-like changes (Whitlock et al., 2006) and disrupting once established synaptic potentiation also impairs learning and memory (Pastalkova et al., 2006). In addition, learning recruits newly synthesized AMPAR selectively to mushroom-type spines in adult hippocampal CA1 neurons 24 hours after fear conditioning (Matsuo et al., 2008). Studies in mutant mice confirm that GluA1 containing AMPARs are essential for spatial working memory (Reisel et al., 2002). Taken together,
there is ample evidence that AMPARs play a critical role in synaptic transmission, synaptic plasticity and learning and memory.

4. Corticosteroids and AMPA receptors: relevance for synaptic function and synaptic plasticity
The effects of glucocorticoids on synaptic plasticity are complex (Joels et al., 2006). Through activation of mineralocorticoid receptors (MRs), presumably via genomic actions, glucocorticoids maintain glutamatergic transmission as well as aminergic transmission in the hippocampus (Joels, 1999). Via activation of GRs, glucocorticoids enhance the calcium-dependent after-hyperpolarization (Joels and de Kloet, 1989) and interfere with (subsequently induced) synaptic potentiation (Joels et al., 2006). Evidence is accumulating that glucocorticoids affect various aspects of function and dynamics of AMPARs, both via slow, protein-synthesis-dependent (genomic) effects as well as rapid (non-genomic) actions (Figure 3).

4.1. Slowly developing effects of corticosteroids on AMPA receptors
After exposure to a stressful event, elevated plasma corticosteroid levels return slowly to their pre-stress level in about 2 hours (de Kloet et al., 2005). Nevertheless, these hormones exert - via a slow, genomic mode of action - long-lasting effects on neuronal function. For example, elevated glucocorticoid levels slowly increase the membrane expression and synaptic insertion of GluA2-containing AMPARs in hippocampal neurons (Groc et al., 2008; Martin et al., 2009). These effects are mediated via GRs, require the synthesis of new proteins, and most likely result from increased lateral diffusion and/or altered ratio of endocytosis/exocytosis of GluA2-containing AMPARs (Groc et al., 2008; Martin et al., 2009). Functionally, glucocorticoids also slowly increase the amplitude of evoked as well as spontaneous AMPAR-mediated synaptic currents in hippocampal primary cultures and hippocampal slices (Karst and Joels, 2005b; Martin et
al., 2009), thereby enhancing AMPAR-mediated synaptic transmission.

4.2. Rapid / non-genomic effects of corticosteroids on AMPA receptors
In addition to the slow genomic effect of glucocorticoids on AMPAR function, recently it has been shown that glucocorticoids also rapidly increase the frequency of mEPSCs in the hippocampus (Karst et al., 2005) and amygdala (Karst et al., 2010b). By using specific agonists, antagonists, and brain-specific inactivation of MRs, it was determined that these rapid effects are mediated by low affinity MRs (Karst et al., 2005), which are located in the membrane. Moreover, the rapid and reversible increase in the frequency of mEPSC after glucocorticoid exposure most likely results from an increase in the presynaptic release of glutamate (Karst et al., 2005a; Olijslagers et al., 2008). Later activation of GRs rapidly reduces the frequency of mEPSCs in the amygdala (Karst et al., 2010b). These studies highlight that glucocorticoids can rapidly (within minutes) regulate synaptic transmission. At the same time scale, corticosteroid exposure, via MRs which are located in the membrane, also rapidly increases the lateral diffusion of GluA2 subunits without altering the postsynaptic receptor numbers (Groc et al., 2008; Martin et al., 2009). Importantly, corticosteroids promote the synaptic insertion of GluA2-containing AMPARs after induction of chemical LTP, via activation of membrane MRs (Groc et al., 2008).

4.3. Corticosteroids and AMPA receptors in learning and memory
Current data suggests that glutamatergic neurotransmission is maintained under basal corticosterone levels. In addition, application of glucocorticoids, via a rapid, non-genomic mode of action, enhance mEPSC frequency (Karst et al., 2010b; Karst et al., 2005), promote the induction of long-term potentiation in vitro (Wiegert et al., 2006) and facilitate the synaptic insertion of AMPARs (Groc et al., 2008). These effects are mediated via MRs. Therefore, the data so far indicates that the surge of glucocorticoids can
rapidly increase the ability to encode information, which might therefore be relevant for the appraisal of a stressful event and/or the acquisition of information related to a stressful event (de Kloet et al., 1999; Oitzl and de Kloet, 1992; Schwabe et al., 2010).

Hours after exposure to a stressful event, slow genomic effects of GRs on synaptic transmission and synaptic plasticity emerge. These actions include: 1) enhanced mobility of GluA2-containing AMPARs (Groc et al., 2008), 2) enhanced surface expression (Martin et al., 2009) and synaptic insertion (Groc et al., 2008) of GluA2 containing AMPARs; 3) suppression of long-term potentiation (Alfarez et al., 2002); 4) facilitation of long-term depression (Coussens et al., 1997; Xu et al., 1997); and 5) an increase in endocytosis of synaptic AMPARs upon stimuli that weaken synaptic transmission (Martin et al., 2009). These effects might contribute to the storage of information in several ways. First, activity-dependent increased synaptic GluA1 containing AMPARs are replaced by GluA2-containing AMPARs (such as GluA2/3) (Hayashi et al., 2000; Plant et al., 2006; Shi, 2001), which might be important to maintain or stabilize the synaptic transmission in the absence of activity. Accordingly, GluA2-containing AMPARs are critical for the formation of fearful memories (Migues et al., 2010) and glucocorticoids promote learning and memory via GluA2-containing AMPARs (Conboy and Sandi, 2010). Second, glucocorticoids, via a genomic mode of action that involves GRs, reduce the ability to encode novel information (i.e. reduce the ability to elicit synaptic potentiation) (Alfarez et al., 2002). This might preserve overwriting of information that is present in the network, in a meta-plastic manner (de Kloet et al., 2005). Accordingly, corticosteroids occlude the activity-dependent increase in synaptic AMPARs (Groc et al., 2008) and prevent the activity-dependent increase in AMPAR-mediated synaptic transmission (Hui Xiong, personal communication). Third, GluA2-containing AMPARs are involved in spine formation (Passafaro et al., 2003; Saglietti et al., 2007):
alterations in synaptic AMPARs might therefore increase the capacity to store information.

Taken together, a picture emerges that glucocorticoids, via MRs, rapidly enhance the ability to encode information, which is consolidated via activation of GRs (Krugers et al., 2010).

![Figure 3. Differential effects of corticosteroid receptors on AMPAR trafficking.](image)

**Figure 3. Differential effects of corticosteroid receptors on AMPAR trafficking.**

a. GluA2-containing AMPARs traffic from and to the plasma membrane through endocytic and exocytotic recycling machinery between intracellular and membrane receptor pools. Surface AMPARs diffuse between extrasynaptic sites and synaptic membrane via lateral diffusion. b. MRs may influence AMPAR trafficking under basal condition (left part). During stress MRs regulate presynaptic release of glutamate and promote the synaptic insertion of AMPARs via rapid/non-genomic actions (middle part). GR activation - via slow/genomic actions - increases the synaptic incorporation and lateral diffusion of GluA2-containing AMPARs. Abbreviation: MR: mineralocorticoid receptor; GR: glucocorticoid receptor; PSD: postsynaptic density; GRE: glucocorticoid response element. Reproduced from Krugers et al., 2010 with the license granted by Nature Publishing Group.

5. **Outline of this thesis**

The overall aim of this thesis is to delineate the role of corticosteroid receptors in learning and memory formation, and better understand the role of AMPARs in this process.

Several studies have identified that activation of glucocorticoid
receptors (GRs) is critical for the consolidation of (emotional) information. In contrast, the role of MRs in the formation of fearful memories remains to be established. MRs might be extremely relevant in this respect since recent studies suggest that these receptors not only exert slow genomic actions on synaptic transmission but also rapidly regulate (enhance) synaptic efficacy (Karst et al., 2010b; Karst et al., 2005) and synaptic plasticity (Wiegert et al., 2006). We therefore examined in chapter 2 the role of both MRs and GRs in learning and memory using a fear conditioning paradigm.

Memories for fearful events become labile upon re-exposure (Nader et al., 2000b), which has opened a new avenue to potentially reduce fearful memories (Monfils et al., 2009; Schiller et al., 2010). Corticosteroids have been shown to participate in retrieval and extinction of conditioned fear memory via glucocorticoid receptors (Bohus and de Kloet, 1981; Gourley et al., 2009; Roozendaal et al., 2004b). On the other hand, mineralocorticoid receptors have been found important for the appraisal of stressful information and response selection (Bitran et al., 1998; Oitzl and de Kloet, 1992; Sandi and Rose, 1994b). More recently, we reported that blocking MRs during memory acquisition impaired contextual fearful memories (Zhou et al., 2010b). In chapter 3, we examined whether pharmacological blockade of MRs during memory retrieval was able to reduce retention of fearful information and if so, whether such reduction of fear memory was long-lasting.

AMPA receptor mediated synaptic modifications in the amygdala have been reported to sustain tone-cued fear conditioning (Rumpel et al., 2005). However, the hippocampal formation is also critically involved in fear learning (Kim and Fanselow, 1992). We therefore examined in chapter 4 whether fear conditioning is also accompanied by changes in AMPA receptor mediated synaptic transmission in the hippocampus. This was examined by recording spontaneous miniature excitatory postsynaptic currents (mEPSCs) in the hippocampal CA1 area after training in a fear
conditioning task. Memory retention as well as synaptic AMPAR expression after training were also examined in parallel.

Corticosteroid hormones have been reported to slowly enhance AMPAR-mediated synaptic transmission and surface expression. The underlying molecular mechanisms of this effect remain elusive. We examined several candidate proteins that could be involved in this effect. The majority of translational regulation of protein synthesis occurs at the level of translation initiation where the mammalian target of rapamycin (mTOR) plays an essential role (Hoeffer and Klann, 2009). Meanwhile, NSF/GluA2 dependent trafficking of AMPARs serves to maintain basal synaptic transmission (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998; Yao et al., 2008). We therefore in chapter 5 explored the involvement of the PI3K-mTOR pathway and NSF/GluA2 interaction in corticosteroid-regulation of AMPAR function and surface expression.

Stress hormones act in concert to promote learning and memory processes. In particular, glucocorticoids modulate memory formation (and synaptic plasticity) in synergy with arousal and activation of β-adrenergic receptors (Roozendaal et al., 2004a; Roozendaal et al., 2002b). We explored therefore in chapter 6 how glucocorticoids and β-adrenergic activation, alone and in concert, regulate surface labeling, phosphorylation and function of AMPARs in hippocampal neurons.

Results from the abovementioned experiments are combined and discussed in chapter 7 (General Discussion).
**Box 1. Fear Conditioning**

Fear conditioning is a learning paradigm to examine the formation of aversive and emotional memories. This paradigm has been successfully established across many species, from flies to human beings (LeDoux, 2000). In fear conditioning, an emotionally neutral conditioned stimulus (CS), such as a tone, context or picture is paired with aversive unconditioned stimulus (US), typically electrical foot shock for animals, or electrical shock (Marschner et al., 2008) / aversive noise (Sandin and Chorot, 1989) for human subjects. After successful association between the CS and US, individuals display behavioral (such as freezing), autonomic (such as elevated heart rate and blood pressure) and endocrine (such as hormone release) responses that are expressed in danger upon the re-representation of CS alone (which has become intrinsically aversive (LeDoux, 2000; Rodrigues et al., 2009; Sehlmeyer et al., 2009).

The amygdala is a key structure in the circuitry that underlies fear conditioning. The lateral nucleus of the amygdala (LA) is viewed as the sensory gateway to the amygdala and receives CS information (e.g. auditory input) from cortical and thalamic projections. The thalamo-amygdala pathway mediates rapid and raw information about the fear-provoking stimuli (LeDoux, 1995). In contrast, the cortico-amygdala pathway provides slower yet more detailed information about the CS, and facilitates conscious control and fine adjustment of fear responses (Rodrigues et al., 2009). Neurons in LA respond to both the CS and US information, which converge in the dorsal half of LA to form a CS-US association (Romanski et al., 1993; Sah et al., 2003). The central nucleus of the amygdala (CE) is viewed as the major output region of the amygdala, controlling the expression of fear reaction via projections to downstream targets, such as the central gray, hypothalamus and dorsal motor nucleus of the vagus to evoke behavioral, autonomic and endocrine responses.
(LeDoux, 1995, 2000; Rodrigues et al., 2009; Rodrigues et al., 2004). The basolateral amygdale (BLA) is considered to be important for the memory formation of emotional events.

Although the amygdala has been placed at the center of the fear circuitry, the hippocampus also plays an important role in contextual fear conditioning (Kim and Fanselow, 1992); in particular, the hippocampus provides input about the context of the fearful event. Thus, the amygdala-hippocampal network plays a pivotal role in fear conditioning and synchronization of theta activities in the amygdala-hippocampal network represents a neuronal correlate of conditioned fear that is involved in memory retrieval (Seidenbecher et al., 2003). Also the prefrontal cortex (PFC) is a crucial neural structure that is involved in the control of stress and fear responses. In general, the PFC exerts (inhibitory) control over the amygdala-mediated defensive behaviors. The interaction between the two structure is required for glucocorticoid effect on memory consolidation (Roozendaal et al., 2009c).

In several aversive learning paradigms (passive avoidance, fear conditioning) noradrenaline and corticosteroid hormones facilitate the memory for emotional events (Cahill et al., 1994; Pugh et al., 1997b; Roozendaal et al., 1993; Roozendaal and McGaugh, 1997b). These hormones have been reported to interact at the level of the basolateral amygdala to classical fear conditioning (Roozendaal et al., 2006c). However, norepinephrine and corticosteroid hormones can also influence synaptic plasticity in hippocampus that are involved in fear conditioning (Hu et al., 2007; Karst et al., 2005; Martin et al., 2009; Wiegert et al., 2006).
Box 2. Acquisition, (re)consolidation and extinction of information.

After the acquisition of CS-US association, newly formed memory is initially fragile, sensitive to disruption and requires time to consolidate (Dudai, 1996; McGaugh, 2000). During the consolidation phase, the association of CS-US is strengthened and short-term memory (seconds to hours after training) is converted into long-term memory (hours to months after training) (McGaugh, 2000; Nader et al., 2000b; Rodrigues et al., 2009), via a mechanism that requires protein synthesis (Schafe and LeDoux, 2000; Schafe et al., 1999). Disruption of this process can therefore potentially disrupt the formation of fearful memories (McGaugh, 2000).

Recent studies indicate that the molecular process that is required to consolidate information is also necessary to store information upon retrieval (reconsolidation) (Alberini, 2005). This might be extremely meaningful from an evolutionary point of view for individuals. A new round of storage of information upon retrieval provides a cellular and molecular mechanism to update information and integrate new information into the initial memory trace. Importantly this reconsolidation window (usually 6 hours after retrieval) (Monfils et al., 2009; Nader et al., 2000a) allows to potentially reduce the memory for fearful events (Dudai, 2006; Monfils et al., 2009; Nader et al., 2000a; Schiller et al., 2010).

Repeated exposure to conditioned stimuli in the absence of unconditioned stimuli reduces the amplitude and frequency of a conditioned response (Myers and Davis, 2002), a process called extinction. Extinction is generally considered as a new learning process, i.e. that exposure to a CS has no longer harmful consequences. Extinction is most often not permanent. The “extinguished” memory can come back under several conditions: 1) Spontaneous recovery, i.e. when certain amount of time has passed (Schiller et al., 2008); 2) reinstatement, i.e. when the original US is given unexpectedly (Bouton and Bolles, 1979) and 3) renewal, i.e. when CS
is given out of the extinction context (Effting and Kindt, 2007).
Box 3. The hippocampus

The hippocampus - named after its seahorse-like appearance - is located in the medial temporal lobe and is an elaboration of the edge of cerebral cortex. In typical coronal brain sections, the hippocampus appears as 2 C-shaped cortical plates interlocking with each other, with two major subfields that can be clearly distinguished: the dentate gyrus (DG) and Ammon’s horn (CA or hippocampus proper). The latter contains the CA3 and CA1 regions (with CA2 in-between). Anatomically, the hippocampus is closely connected to the adjacent structure, the enthorinal cortex (EC), which in turn is strongly and reciprocally connected with many other parts of the cerebral cortex. The EC therefore serves as the main source of hippocampal input and output and acts as an interface between the hippocampus and the rest of the brain.

The hippocampus is well-known for its intrinsic connectivity, namely the “tri-synaptic circuitry”. The information from EC is relayed via the perforant pathway to granular cells of the DG, from where - via the unmyelinated mossy fibers – information is relayed to CA3 neurons. CA3 neurons then send out their collateral axons (Schaffer collaterals) innervating massively the dendritic layer of CA1 pyramidal cells. These cells further project through the subiculum to the EC, from where the synaptic input originates. It should be mentioned though that the entire repertoire of intrahippocampal connections and connections between hippocampus and cortical areas are much more complex (van Strien et al., 2009). With this well-defined three-synaptic circuitry the hippocampus has become a very useful experimental substrate to study neuronal network functionality and synaptic transmission. This has led to fruitful achievements, including the discovery of long-term potentiation (LTP) (Lomo, 2003; Lømo, 1966).

Numerous studies have shown that the hippocampus also plays a major role in learning and memory processes (Anagnostaras et al., 2001; Mahut et al., 1982; Mizuno and Giese, 2005; Sanders et al., 2003; Scoville
and Milner, 1957). One well-known case is that of patient H.M., who had a long history of major and minor seizures uncontrollable by maximum medication of various forms at that time. Eventually a radical bilateral medial temporal-lobe (including the hippocampus, amygdala and several other temporal cortical structures) resection was carried out in order to relieve the symptoms. However, unanticipated memory deficits occurred immediately after the surgery, including the severe disability to form new memories (anterograde amnesia) as well as the inability to recall the memories formed shortly before the surgery (retrograde amnesia). Interestingly, older memories (prior to 19 months preceding surgery) remained largely intact (Scoville and Milner, 1957). Later, another patient, patient R.B. with bilateral lesions confined to the hippocampus (entire CA1 regions), developed severe anterograde amnesia with little retrograde amnesia (Zola-Morgan et al., 1986). These findings in human subjects, as well as in non-human primates (Mahut et al., 1982; Zola-Morgan et al., 1989), confirm the role of hippocampus in declarative memory formation.

Numerous studies have also revealed a crucial role for the hippocampus in spatial memory. It is believed that the hippocampus forms a spatial or topographic map representing the environment (Berthoz, 1997; O'Keefe and Dostrovsky, 1971). In support for a role of the hippocampus in spatial learning, London taxi drivers, who typically need to memorize huge amounts of navigational information, show increases in hippocampal volume which correlate with the years of navigation experience (Maguire et al., 2000). Similarly, rodents can be trained to remember where to find a submerged platform hidden in a water tank; lesions of hippocampus lead to profound and long-lasting place navigational impairment in these animals (Morris et al., 1982). Moreover, hippocampal place cells are involved in representation of the spatial environment (Nadel, 1991; O'Keefe and Dostrovsky, 1971) and dynamic self-location (Leutgeb et al., 2005) in the brain, working together with head-direction cells (Muller et al., 1996) and
grid cells (Bayer, 2010; Doeller et al., 2010) that are located in pre- and para-subiculum as well as medial entorhinal cortex.