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Stress, emotional learning and AMPA receptors: from behavior to molecule
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Citation for published version (APA):

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Summary

In biological systems, stress is generally defined as any condition that disturbs the physiological and/or psychological homeostasis of an organism. Stress is thus inherent to our daily life. The response of individuals to stressful experiences promotes physiological and behavioral adaptation to these potentially threatening events. Enhanced memory of stressful (relevant) information can be considered to be a highly adaptive behavioral response. Prolonged exposure to stressful experiences on the other hand has been reported to hamper memory formation. Failure to effectively cope with stressful events generates a variety of disorders such as post-traumatic stress disorder (PTSD) which decrease the quality of life.

Upon exposure to stressful events, several hormones and neuropeptides are released into the circulation and brain to help organisms cope with stressful events. Corticosteroids are released in increased amount from adrenal cortex and then activate their receptors in the brain - the low affinity glucocorticoid receptors (GRs) and high affinity mineralocorticoid receptors (MRs), which are differentially involved in stress-related learning and memory process. MRs appear to be involved in appraisal of stressful information as well as response / strategy selection. Activation of GRs plays an important role in memory consolidation and retrieval. While GRs have been well studied in memory formation process, the role of MRs in cognitive performance has been confirmed. One of the research questions that was addressed in this thesis was how MRs regulate fear memory formation.

To answer that question, in chapter 2 of this thesis, we applied antagonists against corticosteroid receptors (GRs or MRs) in mice shortly before training. In agreement with previous studies, we found that GR blockade impaired retention of contextual information. Interestingly, MRs
also play an important role in fear memory formation, since blocking MRs reduced encoding of contextual fearful information. This was confirmed in MR mutant mice in which forebrain MRs were depleted.

Previous studies have shown that memories become labile upon re-exposure and retrieval of information. Importantly, this provides a time window to interfere the previously-stored fear memory. Since pharmacologically blocking MRs before memory acquisition reduced subsequent fear memory expression (chapter 2), we went on examining whether blocking MRs prior to retrieval could also reduce fear expression later on. In chapter 3, by applying MR antagonist-spiroloactone shortly before retrieval (either 3 or 30 minutes), we observed a significant reduction in fear memory expression during brief (3 minutes) contextual retrieval session and also in the subsequent memory retention test (24 hours later). However, this reduction in memory expression was not present one month later, and also absent 1) if the re-exposure to the context was prolonged in time (30 minutes) and 2) in tone-cue fear memory. These findings highlight an important role for the MRs in retrieval of contextual information. However, blocking MRs before retrieval did not erase the original fearful memory trace. It will be important to test whether blocking GRs or both MRs and GRs before retrieval is more efficient in long-lastingly reducing the expression of contextual far.

The underlying mechanisms of how stress hormones regulate learning and memory are still far from being fully understood. One of the putative molecular substrates could be AMPA-type glutamate receptors, which are important for synaptic plasticity, fear memory formation and also, as reported recently, for fear memory erasure. Corticosteroids are known to modulate the trafficking of AMPA receptors (AMPARs) and AMPAR-mediated synaptic transmission. In this thesis we therefore further examined how fearful learning and stress-hormones regulate the function of AMPARs.
In *chapter 4*, we studied AMPAR-mediated synaptic transmission in vitro and synaptic expression of AMPARs in the hippocampus after acquisition of contextual fear memory in vivo. We found that the miniature excitatory postsynaptic current (mEPSC) frequency was largely though transiently enhanced after training, in contrast to a relatively long-lasting enhancement in mEPSC amplitude. Meanwhile, a significant increase of synaptic GluA2 subtype AMPAR expression was also found. These results, in line with previous findings in other brain regions (such as amygdala), suggest that hippocampal AMPAR-mediated signaling underlies the formation of contextual fear memories. These effects on AMPAR-mediated synaptic transmission were also accompanied by transient increase of plasma corticosterone level, and corticosterone therefore presumably plays important roles in this process.

Indeed, corticosteroid hormones have been found to modulate AMPAR-mediated synaptic transmission, AMPAR lateral diffusion, and synaptic as well as surface expression of AMPARs. Yet, how corticosteroids modulate AMPAR function and expression remains unclear. To that end we investigated the involvement of 1) the interaction between N-ethylmaleimide-sensitive factor (NSF)/GluA2 - which underlies the trafficking of AMPARs - and 2) the PI3K-mTOR pathway - which regulates translational control of synaptic scaffolding proteins, relevant for the anchoring of synaptic AMPARs – in the effects of corticosteroids on AMPAR function (*chapter 5*). Application of pep2m - a peptide that specifically blocks the interaction between NSF and the intracellular C terminus of GluA2 subtype AMPAR - to hippocampal primary cell cultures, fully prevented the corticosterone-induced increase in peak amplitude of mEPSCs. The PI3K-mTOR pathway is involved in protein translational control of synaptic plasticity, AMPAR surface expression as well as synaptic insertion during LTP. In line with these findings, by pre-incubation with various antagonists we were able to demonstrate the involvement of PI3K-mTOR pathway in
corticosterone-induced increase in AMPAR-mediated synaptic transmission as well as surface expression. Further studies, using biochemical tools, are planned to confirm the above-mentioned results.

Corticosteroids act in concert with other hormones (such as norepinephrine) and peptides to modulate fear learning and memory formation. In *chapter 6*, we examined whether both corticosteroid and noradrenergic hormone systems also act in concert to modulate the function of AMPARs. Therefore we used primary cultured hippocampal neurons to study the interaction of corticosterone and isoproterenol – a beta-adrenoceptor agonist on AMPAR function. In contrast to the previous study in *chapter 5*, in which we focused on the slow (genomic) effects of corticosterone, we focused here on the rapid effects of the two hormones (up to 15-min after hormones application). The results indicated that combined administration of corticosterone and isoproterenol (which were ineffective by themselves) rapidly increased phosphorylation of AMPAR GluA1 subunit at S845, increased GluA1 and GluA2 subtype AMPAR surface expression and increased frequency of AMPAR-mediated mEPSCs. Together with previous studies, this suggests that corticosterone, in interaction with noradrenergic activation, rapidly enhances AMPAR-mediated synaptic transmission and which might promote the ability to store information at the cellular level. These studies highlight the notion that instead of working alone, hormones and peptides actually work together in vivo to help produce a “symphonic” reaction towards stress. This opens a novel avenue to examine in more details how – and which – stress hormones interact to modulate molecular substrates that are involved in learning and memory.

In conclusion, the overall aim of this thesis was to delineate the role of corticosteroid receptors in learning and memory formation and to better understand the role of AMPARs in this process. By applying various approaches such as fear conditioning, electrophysiology and molecular
techniques, we were able to illustrate at the behavioral level that the stress hormone corticosterone acts not only via GRs but also via MRs to modulate the acquisition, consolidation and retrieval of fear memory. These studies provide novel insights for the interference and hopefully erasure of the mal-adaptive expression of fear.

At the molecular level, AMPARs mediate the effect of corticosterone on fear learning and contextual fear memory formation. We report that this effect requires the involvement of NSF/GluA2 interaction and PI3K-mTOR signaling pathway. Moreover, AMPARs are subject to modulation not only by corticosterone alone but also by its interaction with other stress hormones.