Contextual glucocorticoid signaling in-vivo: a molecular perspective

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Chapter 8:

Appendices

SUMMARY

What we collectively call "stress" is how we experience our body's reaction to a stressor. This response is aimed to deal with the current stressor and to prepare for recurrences in the future. The stress response is for an important part dependent on glucocorticoid hormones. By and large, the acute response to glucocorticoids is beneficial, but chronic exposure often becomes maladaptive. To improve prevention and treatment of disorders we can develop due to stress, it is important to better understand the effects and working mechanisms of glucocorticoids. While we already possess extensive knowledge regarding glucocorticoids and glucocorticoid receptor (GR) signaling (discussed in **chapter 1**), we introduced and studied five "aspects of context", which we felt address important current misconceptions or gaps of knowledge.

The role of the mineralocorticoid receptor

Glucocorticoids can activate two transcription factors, the GR and the mineralocorticoid receptor (MR). Many studies are conducted under the notion that MR is fully saturated at basal glucocorticoid levels, and therefore is not involved in the response to stressors. We previously showed that MR-specific DNA-binding occurs in the rat hippocampus in a dose-dependent manner. In **chapter 2** we followed-up on these findings and validated some of the associated MR-specific target genes in mice that lacked expression of the MR in the hippocampus. Subsequently, we showed that *Jdp2* – one of the validated MR-specific target genes – was responsive to restraint stress. Based on these findings we confidently concluded that we cannot simply ignore the transcriptional contribution of the MR in the context of the stress-response and GR function.

Duration of glucocorticoid exposure

Chronic stress is known to negatively impact our body, indicating that the duration of glucocorticoid exposure is of importance. To address this we followed-up on the finding that the transcription of glucocorticoid-responsive genes in the mouse liver became dependent on active androgen signaling after chronic exposure to glucocorticoids. In **chapter 3** we replicated the original finding and observed that chronic exposure to corticosterone extensively altered the hepatic transcriptome in a largely androgen-dependent manner. On the contrary, acute exposure altered the expression of far lesser genes in total, and the alterations that we observed were not dependent on androgen signaling. A direct interaction between the GR and the androgen receptor (AR) was not observed, but we did find an indication that chronic GR activation itself upregulated hepatic AR expression and thereby created the androgen-dependency over time. Based on these findings we concluded that duration of glucocorticoid exposure indeed strongly impacts the transcriptional- and likely also the functional outcome in the liver.

This is in line with the general notion that acute exposure to glucocorticoids is often beneficial and chronic exposure is linked to maladaptation.

Interaction with other transcription factors

Glucocorticoids are involved in many processes, but often interact with other factors to establish their effects. In the case of memory consolidation, glucocorticoids require the arousal-induced release of noradrenaline to do so. As transcription factors GR and pCREB are two downstream mediators of these signaling routes, a genomic interaction was hypothesized to be part of the underlying mechanism. In **chapter 4** corticosterone treatment was combined with object location training, and we studied the potential GR-pCREB interactions at the level of DNA-binding and subsequent transcriptome alterations. We found no evidence for an interaction between both transcription factors. The effect of corticosterone was largely comparable for both DNA-binding and gene expression irrespective of training status and we found no effect of training on pCREB DNA-binding or gene expression. Our study identified a set of novel, likely direct, GR-target genes that are candidate mediators of corticosterone effects on memory consolidation. These data indicated a role of non-neuronal cells in the effects of glucocorticoids on the memory consolidation process. Even though we could not confirm our starting hypothesis, this study did provide useful insights and actionable leads for follow-up studies.

The age of glucocorticoid exposure

Early life is a period especially sensitive to disruptive effects of stress. In humans, exposure to excessive and/or chronic stressors during this period is a well-established risk factor for stress-related mood disorders. In rodents, early life stress (ELS) can result in behavioural and cellular changes that are apparent at adulthood. These previously were shown to be in part reversible by intervention with the GR antagonist RU486 during adolescence. The molecular mechanism leading to the increased risk, or underlying the reversal of the associated impairments has remained unknown. In **chapter 5** we assessed the hippocampal DNA accessibility and transcriptome alterations after ELS to gain insight in these processes. We found that ELS can alter the transcriptome of the hippocampus, but found no evidence for changes in the accessibility of the hippocampal DNA. However, these transcriptome alterations where not consistent between multiple cohorts, and we did not observe any normalization of the gene expression changes after adolescent intervention with RU486. After investigation of two replication cohorts in which virtually all controllable sources of variations were eliminated (amongst other things the early life stress paradigm, the laboratory and the researcher), we now consider the long-lasting transcriptional effects of ELS of stochastic

nature. The mechanism underlying ELS-induced changes in behaviour during adulthood therefore remains as enigmatic as it initially was.

Type of GR-ligand

The many changes that are typically observed in comprehensive analysis of gene expression after glucocorticoid treatment make it hard to identify genes that are causal to the process being studied. In search of a way to separate the wheat from the chaff, we embraced the variance and similarity of GR-ligands. Exposure to glucocorticoids results in a longlist of differentially expressed genes. Of these, only a handful is likely to be directly involved/key to the process under investigation. This also applies for the enhancement of memory consolidation by glucocorticoids. In **chapter 6** we applied a powerful approach – pharmacological filtering – to investigate the transcriptional effects of GR aiming to identify a shortlist of GR-target genes associated to memory consolidation. We utilized the properties of selective GR modulators (CORT108297 and CORT118335), alongside the endogenous agonist corticosterone and the classical antagonist RU486, to pinpoint GR-dependent transcriptional changes. In our project, the pharmacological filtering approach reduced the longlist of differentially expressed genes to a manageable shortlist of nine GR-target genes likely involved in the enhancement of memory consolidation by glucocorticoids. Similar to the results described in **chapter 4**, these results suggest a role for GR in non-neuronal microglia cells.

Context matters

Corticosterone was at the centre of all the studies we performed, yet the eventual outcome of GR activation differed extensively in all experiments. In **chapter 7** we discussed the differences, the overlap and the implications thereof. The differences included dosage, type of tissue, activation of other transcription factors, duration and age of exposure. In other words, the context in which corticosterone exerts its effects matters, and it is to us as researcher to be aware of this when designing new studies and interpreting available data. In a sense, the line of reasoning that resulted in the conducted experiments is as valuable as the specific outcomes reported. Whilst our research merely addressed some specific processes, the lessons learned from these experiments can be applied much broader to the biology of glucocorticoid signaling and other nuclear family members.