Chapter 7

General discussion
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7.1 Introduction

As outlined in the general introduction, the significance of pulsatile glucocorticoid hormone secretion for physiology is not yet clear. Changes in these patterns are thought to compromise resilience and may thus be an important factor in the aetiology of stress-related diseases (Young et al. 2004, Lightman 2008). The overall objective of this thesis was therefore to assess the role of glucocorticoid pulsatile patterns in central glucocorticoid signalling and the neuroendocrine and behavioural responsiveness to stressful challenges.

Model system and experimental design

Practical limitations in the control of high frequency ultradian pulses in living animals have hampered progress in obtaining evidence for the functional consequences of glucocorticoid pulsatility. Furthermore, the lack of rhythmic synchronisation between animals necessitates control of the pattern of hormone release. In the current thesis we surgically or pharmacologically modulated pulsatile corticosterone hormone levels by exogenous steroid administration or automated steroid infusion either with or without automated blood sampling (Lightman et al. 2008). The technical development and availability of these methods has opened up the opportunity to examine how corticosterone pulsatility might interact with stress responsiveness and has indeed provided the first data beyond descriptive correlates as described in this thesis.

7.2 Glucocorticoid signalling

Glucocorticoid signalling mechanism

Accumulating evidence suggests that nuclear receptor access and binding to regulatory elements in the genome, but also methylation of DNA is highly dynamic (Metivier et al. 2003, Reid et al. 2003, Kangaspeska et al. 2008). With respect to GR, it is now being recognised that rapid ultradian oscillations in glucocorticoid hormone exposure are also crucial for transcriptional control (Desvergne & Heligon 2009, George et al. 2009, Stavreva et al. 2009). Upon glucocorticoid treatment, GR rapidly translocates to the nucleus in both cultured cells and hippocampal tissue (chapter 2 and 3). Irrespective of the administration paradigm, MR, however, is continuously retained in the nucleus of hippocampal cells (Conway-Campbell et al. 2007). Therefore we suggest that fluctuating levels of glucocorticoids which are conveyed into oscillatory activity of GR but not MR, result in differential recruitment of the receptors and coregulators which may involve different pathways in transrepression and transactivation of glucocorticoid target genes in the brain. This difference in ultradian re-
recruitment of MR and GR may simply reflect their different ligand binding affinities, kinetics and in vivo clearance rate (Reul & de Kloet 1985, Spencer et al. 1993). MR is extensively occupied at very low levels of circulating corticosterone and remains bound to ligand for much longer \( t_{1/2} = 45 \text{ min} \) than GR \( t_{1/2} = 5 \text{ min} \). GR thus rapidly associates and dissociates from its endogenous ligand and is recruited in an ultradian manner. Hence, we propose that disturbances in glucocorticoid pulsatility will therefore mainly affect this receptor rather than MR.

**Glucocorticoid signalling in the rat brain**

In relation to the brain, little information is available on how rapidly fluctuating glucocorticoid levels in vivo affect receptor signalling, transcriptional output and consequently target tissue sensitivity thereby determining the functional effects of glucocorticoid action and efficacy (Kino 2007). We have shown considerable changes in molecular markers for both chronic and acute glucocorticoid action in the rat hippocampus after disruption of endogenous pulsatile patterns (chapter 4). Overt hypercorticism (100% corticosterone) resulted in maximally sustained responses, most likely due to receptor saturation rendering GR non-responsive to additional hormone. This possibly creates a non-favourable situation in which the normal flexibility in glucocorticoid signalling is lost. Many studies described such sustained effects of chronic high corticosterone levels on the brain mediated via GR. For instance, feedback sensitivity of the HPA axis after stress is attenuated after chronic treatment with corticosterone (Akana et al. 1992), through occupancy of GR (Bradbury et al. 1994). Also, CA1 pyramidal cells seem to lose their potential to normalise enhanced activity after stress (Joels et al. 2007), e.g. as seen from the risk of calcium overloading (Karst & Joels 2007) and attenuation of functional 5-HT responses (Karten et al. 1999).

Clamping corticosterone levels at daily average levels (40% corticosterone), however, did not alter baseline expression of our rather sensitive expression markers. Remarkably, this treatment did still attenuate the transient response to a glucocorticoid challenge mimicking the stress response (chapter 4). The findings of this study suggest that pulsatile glucocorticoid release is required to maintain the normal ‘resilience’ in glucocorticoid target tissue responsiveness. Furthermore, subtle changes in the pattern of pulsatile exposure seem to induce changes at least as severe for glucocorticoid signalling as overt hypercorticism. This notion is strengthened by the normalisation in GR responsiveness after washout of constant 100% corticosterone levels but not in the 40% cort group. Using similar administration paradigms, previous studies have shown attenuated stress responsiveness (Akana et al. 1992) and suppressed levels of 5-HT1A receptor expression exclusively in the rat dentate gyrus (Meijer et al. 1997). These effects were interpreted as mediated via MR (Bradbury et al. 1994, Meijer et al. 2000). We and others now provide support for the additional involvement of GR in target tissue responses (Conway-Campbell et al. 2007, Stavreva et al. 2009),
as we show that particularly this receptor is sensitive to changes in the underlying pulsatile pattern of corticosterone.

**Conclusion**

Understanding the interplay between rapidly fluctuating glucocorticoid levels and receptor signalling could greatly contribute to our knowledge of cellular and tissue responses as changes in the pattern of glucocorticoid exposure could possibly contribute to dysregulated glucocorticoid receptor signalling (Kino 2007). We propose that the molecular response of target tissues induced by a corticosterone challenge is a resultant of the dynamic interplay between the corticosterone exposure regime (e.g. pulsatile or continuous) and steroid receptor signalling. The pattern of hormone exposure therefore seems a major factor in maintaining receptor responsiveness determining the adaptive capacity of target tissues in the face of acute stress.

### 7.3 HPA axis activity

Daily variations in glucocorticoid hormone concentrations are thought to be fundamental for the maintenance of physiology and well being as deviations from the normal release pattern are considered to enhance vulnerability to stress-related disease (Young et al. 2004, de Kloet et al. 2005, Herbert et al. 2006). However, the consequences of changes in pulse characteristics to HPA axis (re)activity are largely unknown. In the context of circadian rhythms, it has been shown that basal HPA axis activity and the neuroendocrine response to stress is clearly affected under conditions of flattened corticosterone (Akana et al. 1985, Akana et al. 1992, Dallman et al. 2004). However actual disruption of ultradian variation with respect to this pellet model remained to be proven. During chronic glucocorticoid exposure, the HPA axis via negative feedback adjusts circadian trough and peak concentrations resulting in steady-state corticosterone concentrations (Akana et al. 1985, Akana et al. 1992, Meijer et al. 1997). This consequently may disrupt episodic feedback signals crucial in maintaining ultradian feedforward-feedback oscillatory activity between the pituitary and adrenal gland (Walker et al. 2010). We have now demonstrated that besides circadian variation, also rapid ultradian glucocorticoid pulses can be effectively flattened around the daily average by subcutaneous implantation of 40% corticosterone pellets (chapter 5). This not only mimics some of the risk factors for stress-related disease, it also provides a good and easy to manipulate model to study the consequent disruption in feedback activity.

Until now it was unknown how fast the negative feedback action abates after cessation of constant exogenous corticosterone and when the endogenous pulses re-emerge. Termina-
tion of constant exogenous corticosterone exposure resulted in rapid progressive normalisation of ultradian pulse characteristics within approximately 6 hours after removal of the pellet (chapter 5). This is most likely due to a delayed feedback mechanism suppressing pulse amplitude at earlier time points (Walker et al. 2010). Thus, the half life time of the suppression of HPA axis reactivity that is brought about by 40% corticosterone pellet implantation (Akana et al. 1992), is significantly less than 24 hours. This is reminiscent of the acute effects of MR and GR ligands on the axis, that last in the order of hours (Ratka et al. 1989, van Haarst et al. 1996, Atkinson et al. 2008, Spiga et al. 2008).

The endocrine response to noise stress also rapidly restored after reoccurrence of basal pulsatility. Paradoxically, we still observed substantial changes in the behavioural response to stress (chapter 5, discussed elsewhere in this chapter). These results show a striking parallel with observations in humans with a history of changes in cortisol exposure which suggest that even after normalisation of hormone levels there are residual disturbances in the brain (Tiemensma et al. 2010). Washout after continuous corticosterone administration may therefore constitute a model to study these clinically relevant processes.

**Conclusion**

We conclude that HPA axis activity is remarkably sensitive to changes in the characteristic pulsatile pattern of corticosterone exposure but adjusts in a reversible fashion to these alterations. We propose that it is the pulsatile pattern, rather than the absolute concentrations, of corticosterone exposure that determines subsequent responsiveness to stress, a notion with obvious implications for understanding the pathogenesis of stress related disease.

### 7.4 Neuroendocrine stress responsiveness in relation to pulse characteristics

Glucocorticoids play a major role in regulation of HPA axis activity through negative feedback inhibition (Keller-Wood & Dallman 1984, Dallman et al. 1987, Sapolsky et al. 1990), mediated via MR and GR (Ratka et al. 1989, van Haarst et al. 1996, Hinz & Hirschelmann 2000, Atkinson et al. 2008, Russell et al. 2010). The physiological importance of rapid and adequate feedback is directly underlined by many clinical studies that, for instance as seen during major depression, attribute hyperactivity of the HPA axis to defective negative feedback action of glucocorticoids (Dinan 1994). As a result, maladaptive feedback can have many adverse consequences including cognitive dysfunction and mood alterations, immunodeficiency, cardiovascular disorders and energy metabolism disorders (Dallman et al.
1987, De Kloet et al. 1998). It is however not clear how the feedback mechanism interacts with ultradian glucocorticoid rhythmicity.

The data presented in this thesis shows that glucocorticoid negative feedback inhibition brought about by corticosterone infusion, here in the context of stress, depends on the pattern, phase- and amplitude of corticosterone pulses (chapter 6). Constant infusion of corticosterone resulted in a clear suppression of ACTH responsiveness and has been described in literature before (Akana et al. 1992). Interestingly, pulsatile exposure of corticosterone resulted in a larger, more flexible response in ACTH responsivity to noise stress. This sensitising effect of pulsatility was even more pronounced during the rising phase of an ultradian pulse than during the falling phase. In addition, animals infused with higher amplitude pulses showed smaller increments in stress-induced ACTH release. These data thus clearly show that the neuroendocrine response to stress depends on circulating glucocorticoids in both a phase and amplitude dependent manner. This is in line with previous studies that retrospectively correlated higher corticosterone responses to a stressor with the rising phase of an endogenous pulse and lower mean corticosterone responses with increased pulse frequency (Windle et al. 1998b, Windle et al. 2001, Atkinson et al. 2006). However, we originally hypothesised that negative feedback of glucocorticoids should be greatest when corticosterone levels are rapidly rising within physiological concentrations in a process termed rate sensitive feedback (Dallman & Yates 1969, Kaneko & Hiroshige 1978). Although this apparent rate-sensitivity was not specifically addressed here, we found no evidence for this phenomenon. Comparisons between studies are complicated by the difference in duration of corticosterone exposure. In our study measurements were made after several pulses rather than the single infusions performed previously. The underlying explanation therefore most likely hinges on differential activation of genomic and non-genomic effects of corticosteroids and will be discussed later in this chapter.

Conclusion

It has recently been demonstrated that rapidly oscillating feedforward and feedback loops between the pituitary and adrenal gland give rise to ultradian corticosterone rhythms (Walker et al. 2010). Therefore, ultradian pulses may represent rapidly alternating phases of HPA axis activation and inhibition (Windle et al. 1998b, Lightman et al. 2008). In that respect, we suggest and clearly demonstrate differential HPA axis responsiveness over the ultradian cycle as the relation of the onset of a stressor to the phase or amplitude of ultradian pulses is a major determinant in acute HPA axis responsiveness. Continuous exposure to glucocorticoids attenuates stress-induced ACTH release. Therefore, we propose that transient surges in glucocorticoid level as achieved during the pulses serve to prepare and maintain flexibility in HPA axis activity for adequate responses to stress.
While alterations in the basal pulse frequency and amplitude are known to be a major factor influencing neurendocrine reactivity to acute stressors (Windle et al. 1998a, Windle et al. 2001, Atkinson et al. 2006), very little data is available on the behavioural response to stress. The results presented in chapter 5 demonstrate persistent changes in behavioural responsiveness after a history of flattened corticosterone rhythms, even after normalisation of basal HPA axis activity. Similar results in humans describe highly prevalent emotional and cognitive effects in patients with long-term cured Cushing’s disease compared to matched controls (Tiemensma et al. 2010). These and our results suggest that changes in the history of HPA axis pulsatility of the individual has consequences for behaviour, emotion and cognition and may thus indicate possible irreversible effects of prolonged previous glucocorticoid excess on the brain. Future research should determine how persistent the consequences of a period of chronic glucocorticoid exposure are.

Besides the corticosteroid history of an animal (chapter 5), behavioural stress responsiveness also seems to depend acutely on the phase of ultradian pulses (chapter 6). In line with the previously described increased neuroendocrine response, we found that total activity and risk assessment were highest when the noise stressor was experienced during the rising phase of corticosterone infusion. Behavioural responsiveness has been correlated to ultradian corticosterone pulses before. In a model in which animals were divided post-hoc, others observed that the propensity to behave aggressively was increased during the rising phase of an ultradian pulse (Haller et al. 2000a). These data strongly suggest that brain centers such as the amygdala, normally activated and modulated by emotionally arousing experiences (McGaugh 2004, Roozendaal et al. 2009), may respond differently depending on the rapid fluctuations in corticosterone and thereby ‘pulse-dependently’ modulate the emotional and exploratory response to stress.

Though mostly examined under conditions of chronic exposure, glucocorticoids are indeed known to modulate, in different time domains, limbic regions such as the hippocampus and amygdala (Herman et al. 2003, Jankord & Herman 2008). This may influence learning and memory processes in both rodents and humans (Schwabe et al. 2008, Schwabe et al. 2009), most likely due to changes in the neuroarchitecture and plasticity (Bodnoff et al. 1995, Fuchs et al. 2006, Joels et al. 2007). Such changes may pertain to (m)any neurotransmitter system(s) and substantial changes in information processing after chronic corticosterone and stress (Fuchs et al. 2006, Joels et al. 2007). But we have also shown that genomic responses of particularly GR in the brain are sensitive to changes in hormone patterns and, in fact, remain significantly affected after removal of chronic corticosterone exposure (chapter 4). In addition, there is strong evidence for non-genomic (behavioural) actions of glucocor-

Conclusion

Our results suggest that the history as well as the actual phase of HPA axis pulsatility of the individual somehow are a determinant factor in the behavioural response to stress. We thus postulate that both the neuroendocrine and behavioural response to stress vary over the ultradian cycle as every hourly surge in corticosterone seem to open a window of enhanced susceptibility to stress. Understanding the significance of this hourly enhanced stressor susceptibility opens up interesting new perspectives for the role of ultradian rhythmicity in physiology and behaviour. Whether the reduced stress reactivity during a descending ultradian phase is in fact advantageous or detrimental is still open for investigation and may depend on the particular circumstances.

7.6 Differential activation of neuronal pathways

It is currently unclear to what extent central structures are sensitive to ultradian glucocorticoid regulation. The audiogenic stressor we used typically results in a wide pattern of brain activation (Campeau & Watson 1997, Burow et al. 2005). This includes many auditory structures but also specific brain circuits involved in the stress response to noise such as the PVN, amygdala, prefrontal cortex. We used c-fos immediate early gene expression as a marker for activation of neuronal pathways involved in the stress response in the context of ultradian pulses and is extensively discussed in chapter 6. As described previously in literature but also in our hands, the hippocampus does not seem to be responsive to audiogenic stimulation (Campeau & Watson 1997, Burow et al. 2005). Hence, this specific type of stressor may not be appropriate for analysis of the influence of the pattern, magnitude or phase of corticosterone pulses specifically in this area. On the other hand, we also clearly show that noise stress-induced c-fos expression in other brain circuits (i.e. PVN, pituitary and amygdala) are responsive to the magnitude and/or phase of ultradian pulses.

Conclusion

Glucocorticoid target tissues and the receptors located there are exposed to rapidly fluctuating levels of steroid hormone (Cook 2001, Droste et al. 2008). This, together with the impact of changes in ultradian pulse characteristics on behavioural stress responsiveness (chapter 5), suggests that the brain is indeed receptive to glucocorticoid pulsatility. We now
also demonstrate that neuronal pathways induced by noise stress respond selectively to different aspects of the ultradian cycle. A plausible explanation hinging on region-specific pulsatile effects of glucocorticoids most likely includes the local cellular receptor mechanism due to differential presence of MR and GR and (nuclear) cofactors in the different brain regions.

7.7 Possible mechanisms underlying glucocorticoid pulsatility

The present studies, together with recent literature on corticosteroid receptor action provides a framework for mechanistic understanding of the physiological effects of ultradian glucocorticoid pulses. MR and GR are involved themselves in the dynamic generation and maintenance of basal ultradian HPA axis activity (Spiga et al. 2007, Atkinson et al. 2008). As a consequence of rapidly changing ligand concentrations and differences in affinity, MR and GR may be differentially recruited along the duration of a single ultradian pulse. The results described in this thesis suggest that MR and GR expression and function also seem to depend on the pattern and concentration of corticosterone administration and are differentially regulated in different brain areas (chapter 3, 4 and 6).

Both receptors are also involved in stress-induced HPA axis activity by mediating in complementary fashion the feedforward and feedback modes of glucocorticoid feedback operation (Ratka et al. 1989, Bradbury et al. 1994, van Haarst et al. 1996, Hinz & Hirschelmann 2000, Russell et al. 2010). The time domain of some parameters measured in the current thesis (i.e. ACTH release and behavioural stress responsiveness in chapter 6) suggests a role for rapid non-genomic, rather than slow genomic actions of glucocorticoids, via putative low affinity membrane-bound variants of MR and GR (Orchinik et al. 1991, Hinz & Hirschelmann 2000, Di et al. 2003, Karst et al. 2005). The non-genomic MR in limbic structures enhances the presynaptic glutamate release probability and reduces postsynaptic hyperpolarisation via the ERK1/2 pathway and K+-conductance with the net result to enhance excitatory transmission (Karst et al. 2005, Olijslagers et al. 2008). On the other hand, non-genomic GR reduces net neuronal excitatory transmission via endocannabinoids and nitric oxide (Di et al. 2003, Di et al. 2009). It is currently believed that the initial rise in corticosterone (e.g. during stress or the onset of an ultradian pulse) triggers non-genomic actions via the membrane-bound receptors which over time activate the slower, genomic GR. This has evolved into a new concept of corticosteroid action in the brain in which fast MR-triggered excitability via the membrane receptor interplay with the slow MR and GR-mediated genomic events (Joels et al. 2008a).
In relation to our ACTH data (chapter 6), a ‘brake’ during the falling phase is suggested by decreased ACTH release, i.e. development of a classical genomic feedback signal during rising phase that becomes apparent within 20 minutes and is predicted to maintain reciprocity until the next pulse. Alternatively increased ‘drive’ during the rising phase could result in stimulation of ACTH release or behaviour through activation of a rapid feedforward signal. The latter would likely depend on non-genomic mechanisms. Similar mechanisms may also underlie the translation of ultradian corticosterone oscillations into adequate behavioural responses but also more fundamental processes such as synaptic transmission and neuronal excitability. The apparent pure ‘ascending phase’-dependent effect on c-fos expression in the amygdala in particular seems to call for an explanation involving a rapid, non-genomic component.

**Conclusion**

It seems that the mechanism underlying responsiveness to ultradian glucocorticoid pulsatility involves a balance between rapid non-genomic and slower genomic effects via membrane-bound and nuclear variants of MR and GR that rapidly change over the duration of a single ultradian pulse. This may differ depending on the area of the brain resulting in changing states of responsiveness to environmental input. The current model system in which we impose hourly ultradian pulses provides a unique position to address such mechanistic issues in future studies.

**7.8 Perspectives**

**Clinical relevance**

Until now, the clinical relevance of the pulsatile nature of glucocorticoids was poorly understood or sometimes even regarded as not important. As described in this thesis, disturbances in glucocorticoid pulsatile patterns and aberrant HPA axis functioning are well recognised signs in patients suffering from stress-related disorders. Deviations from the optimal pulsatile pattern are considered to precipitate disease as daily variations in glucocorticoid hormone concentrations are thought to be fundamental for the maintenance of physiological, metabolic, cognitive and behavioural well being (Dallman et al. 2003, de Kloet et al. 2005, Herbert et al. 2006). Newly acquired insights in glucocorticoid hormone pulsatility could therefore be of great importance for the elucidation of the aetiology and pathophysiology of stress-related disorders (Young et al. 2004, Lightman et al. 2008). In addition, this will also result in more insight into the consequences of long term glucocorticoid excess or deficiency as seen in many stress-related disorders.
Knowledge of pulsatility also has important implications for the therapeutic application of glucocorticoids. Transient release patterns of other hormones are considered crucial for physiology and well being (Belchetz et al. 1978, Thompson et al. 2003, Rothman & Wierman 2007), as manipulation of the temporal aspect is already a successfully used approach in clinical therapy for instance for growth hormones (Amato et al. 2000) and estrogen-replacement therapy in post-menopausal women (Shoupe 2001). However, in relation to glucocorticoids, no such administration protocols have been designed yet. Better understanding of pulsatile glucocorticoid release and the underlying nuclear receptor mechanism may therefore greatly contribute to the prognosis and treatment of disease.

Future directions

It is currently not known if disorganisation of rapid fluctuations in glucocorticoid levels are causally linked to the aetiology of stress-related disease or vice versa. Unravelling the mechanisms that underlie the secretory pattern of corticosterone release, but also under conditions of stress, is thus essential. The generation of MR and GR specific transgenic knockout models but also the development of infusion systems that allow to control ultradian pulses, now provides a unique position to address such mechanistic issues in future studies. Many scenarios come to mind and a few are outlined in some detail below.

As extensively discussed is the previous paragraphs, we suggest that the different components of ultradian pulses (i.e phase and amplitude) have different effects on stress-activated circuitry. It is thus very likely that distinctly different mechanisms are operating simply due to the difference in the duration of glucocorticoid exposure. In that respect, the balance between rapid non-genomic and slower genomic effects via membrane-bound and nuclear variants of MR and GR could rapidly change over the duration of a single ultradian pulse. Moreover, the recruitment of MR and/or GR during stress, superimposed on the ultradian and circadian pattern, would also depend on the phase of the pulse. One approach would be to use either pharmacological or genetic knock-out models to systematically study the role of MR and GR in basal and stress-induced HPA axis activity and behavioural activity as a function of these different pulse characteristics to unravel the underlying mechanism. A complicating factor is that genetic models tend to be based on mice, while the rapid sampling/infusion system that we used exists only for rats (and humans). Besides an endocrine and/or behavioural context, it would also be very interesting to study at a more fundamental level the involvement of (membrane) MR and GR in rapid modulation of synaptic transmission and neuron excitability by ultradian corticosterone rhythms.

Measurement of HPA axis secretagogues (i.e. AVP and CRH) may also be an appropriate approach to understand acute effects of ultradian glucocorticoid pulses. The primary action of AVP within the axis is the potentiation of CRH action on the pituitary (Gillies et al. 1982,
Rivier & Vale 1983). Thus increased release of AVP may sensitise corticotrophs to the pulsatile CRH signals reaching them. Besides potentiating the magnitude of each ACTH burst, pulsatile administration of AVP to corticotrophs also causes a more rapid rise in ACTH release during each CRH pulse (Evans et al. 1988). The measurement of these peptides may therefore provide more insight in supra-pituitary integration of glucocorticoid pulsatility. Furthermore, it has recently been demonstrated that the circadian rhythm in glucocorticoids is partly attributed to rhythmic steroidogenesis in the adrenal gland (Son et al. 2008), which could gate adrenal sensitivity to ACTH (Oster et al. 2006). It is however not known to what extent ultradian corticosterone secretion is explained by these observations and how ultradian ACTH release relates to changes in adrenal responsiveness.

The endocannabinoid system works as an important neuromodulatory system by negative modulation of basal and stress-induced HPA axis activity (Cota et al. 2007). Endocannabinoids are suggested to modulate CRH, ACTH and glucocorticoid levels and fast negative feedback via rapid non-genomic effects of glucocorticoids (Manzanares et al. 1999). Glucocorticoids induce retrograde signalling of endocannabinoids from CRH-containing neurons and thereby inhibit excitatory transmission via activation of presynaptic CB1 receptors in the PVN (Di et al. 2003). How endocannabinoids are exactly involved in ultradian glucocorticoid pulsatility and negative feedback is not known but it has been proposed that ultradian oscillations are generated by inhibitory ultra-short cycles in negative feedback inhibition within the hypothalamus (Windle et al. 1998b, Lightman et al. 2008), but also between the pituitary and adrenal gland (Walker et al. 2010). Endocannabinoids could underlie such a self-sustaining mechanism in ultradian fluctuations as the rapid rise in corticosterone levels during every secretory phase (but also during stress) could potentially generate a fast feedback signal. Consequently this may result in inhibition of corticosterone secretion and thus cyclic levels of glucocorticoids. In addition, endocannabinoids are known to be under negative glucocorticoid regulation in the hippocampus and striatum (Di et al. 2003, Hill et al. 2005b) but in fact increase activity in the limbic forebrain after glucocorticoid exposure which may contribute to the region-specific effects of glucocorticoids (Hill et al. 2005a).

Finally, ultradian glucocorticoid pulses induce consecutive waves in GR signalling and transcriptional initiation of target genes in cultured cells (Stavreva et al. 2009). However, just a few studies have addressed the consequences of ultradian corticosterone exposure for nuclear receptor signalling in the brain [(Conway-Campbell et al. 2007) and chapter 4]. In that respect large scale gene expression profiling studies of for instance the hippocampal transcriptome or analysis of single target genes, would be very valuable in understanding the significance of ultradian hormone fluctuations for MR and GR signalling in the brain. Also, the consequences for protein levels have not been thoroughly examined. Furthermore, as new technologies are rapidly developing, whole genome-wide receptor binding studies together with visualisation techniques could provide the means to unravel the continuous com-
petitive cycling between MR and GR together with the transcription and proteasome machinery on the chromatin of glucocorticoid response elements according to rapidly changing hormone levels which eventually determine transcriptional output.

7.9 General conclusions

The results presented in the current thesis have evolved in a new concept in glucocorticoid endocrinology. Even though the link between the different markers used here is uncertain, glucocorticoid signalling as well as behavioural and neuroendocrine responsiveness to stressors are all rapidly and transiently modulated by ultradian corticosterone pulses. We thus provide strong evidence for functional consequences of ultradian glucocorticoid pulsatility. Moreover, we have revealed that in particular the GR is affected when glucocorticoid pulsatility is disrupted and could thus provide an excellent target for therapy to normalise the downstream effects of disturbances in glucocorticoid rhythms in stress-related disease. It also provides a handle to tackle long term problems seen after withdrawal of high glucocorticoid exposure, for instance such as seen postoperatively in ‘cured’ Cushing’s disease. The newly acquired insights are thus of great importance for the understanding of glucocorticoid biology but also for the elucidation of the pathophysiology and aetiology of stress-related disorders. As continuous administration of glucocorticoids attenuates many of the investigated parameters, we conclude that the significance of glucocorticoid pulsatility lies in ‘priming’ the HPA axis and different brain circuits through rapidly alternating feedforward and feedback modes providing full responsiveness and ‘resilience’ in the coordination of stressful events.