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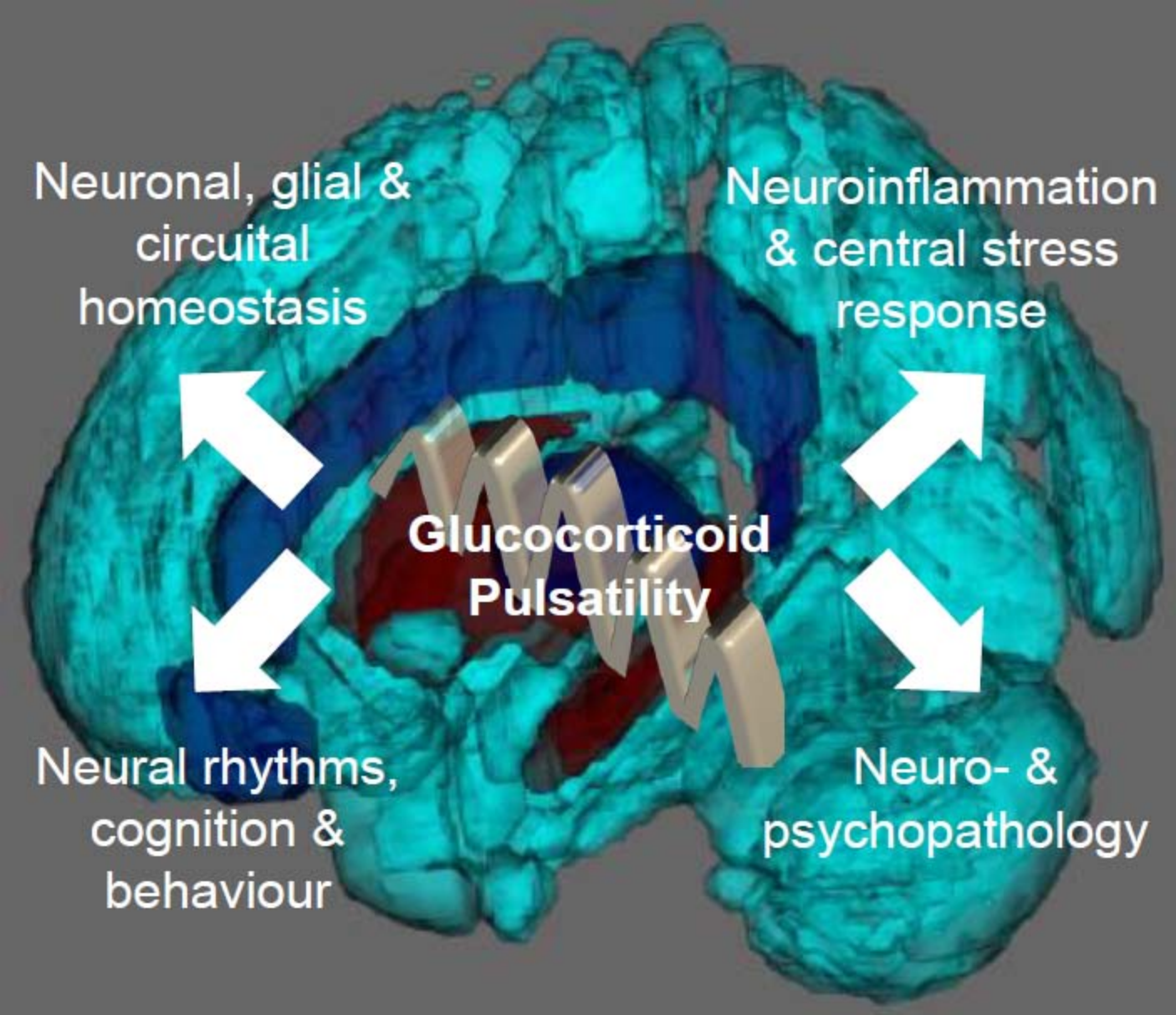
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Neuronal, glial &  
circuital  
homeostasis

Neuroinflammation  
& central stress  
response

**Glucocorticoid  
Pulsatility**

Neural rhythms,  
cognition &  
behaviour

Neuro- &  
psychopathology

- Glucocorticoids (GCs) have multi-level, sometimes conflicting effects in the brain
- GCs achieve complex neurodynamics, guided by their circadian and ultradian rhythmicity
- Ultradian rhythmicity leads to a spatial and temporal mosaic of GC-dependent actions in the brain
- The temporal relationship between GC pulses and other (physiological, pathological) stimuli lead to different effects
- GC rhythmicity needs to be taken into consideration for effective GC-based therapeutic strategies

## REVIEW ARTICLE

**Temporal control of glucocorticoid neurodynamics and its relevance for brain homeostasis, neuropathology and glucocorticoid-based therapeutics**

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**Abstract**

Glucocorticoids mediate plethora of actions throughout the human body. Within the brain, they modulate aspects of immune system and neuroinflammatory processes, interfere with

cellular metabolism and viability, interact with systems of neurotransmission and regulate neural rhythms. The influence of glucocorticoids on memory and emotional behaviour is well known and there is increasing evidence for their involvement in many neuropsychiatric pathologies. These effects, which at times can be in opposing directions, depend not only on the concentration of glucocorticoids but also the duration of their presence, the temporal relationship between their fluctuations, the co-influence of other stimuli, and the overall state of brain activity. Moreover, they are region- and cell type-specific. The molecular basis of such diversity of effects lies on the orchestration of the spatiotemporal interplay between glucocorticoid- and mineralocorticoid receptors, and is achieved through complex dynamics, mainly mediated via the circadian and ultradian pattern of glucocorticoid secretion. More sophisticated methodologies are therefore required to better approach the study of these hormones and improve the effectiveness of glucocorticoid-based therapeutics.

### **Keywords**

Glucocorticoid pulsatility; ultradian rhythmicity; glucocorticoid-based therapeutics; neuropsychopathology; glucocorticoid neurodynamics; mineralocorticoid receptors; glucocorticoid receptors

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## **1. Introduction: glucocorticoids and their clinical significance**

The hypothalamic-pituitary-adrenal (HPA) axis, whose significance in human and animal physiology and pathology has been extensively studied for many decades, is crucially

involved in regulating internal homeostatic mechanisms (many of which have a circadian pattern) and coordinating the organisms' stress responses. Many of these phenomena are regulated in man by one of the main end-products of the axis, the glucocorticoid (GC) cortisol, and to a lesser extent by corticosterone (which additionally constitutes the primary GC type in rodents and other non-human primates). These adaptation processes, which are characterized by great diversity, involve regulation of developmental (Allen, 1996; Jobe et al., 1998) and metabolic pathways (van Rossum and Lamberts, 2004), immune system components (Sorrells and Sapolsky, 2007) as well as modulation of human cognition and behaviour.

It is well known that GCs are biosynthesized for immediate release in the cortical *zona fasciculata* of the adrenal glands (AGs), and due to their lipophilic nature they rapidly diffuse across cell membranes, and are distributed via the systemic circulation predominantly - approximately 95% - bound to carrier proteins, mainly cortisol binding globin (CBG), and albumin, throughout the body (Lightman and Conway-Campbell, 2010) and cleared through liver (bile acids) and kidneys (urine) (Glantz et al., 1976). As indicated by these dynamic physicochemical properties as well as by more recent studies on endogenous GC dynamics (Hughes et al., 2010), GC abundance in the various tissues is primarily regulated by: (i) the pattern of their release into the systemic circulation from the adrenal cortex (i.e. the mode and integrity of activity of the HPA axis) (Henley et al., 2009a), (ii) the ratio of the circulating free to bound form (which is temperature-dependent and together with the concentration of CBG determines the availability of the biologically active cortisol) (Lentjes and Romijn, 1999), (iii) the tissue-specific existence of enzymes that locally modulate active GC levels (cortisol conversion to inactive cortisone and *vice versa*) (Tomlinson et al., 2004), (iv) the capacity of some tissues (for instance the brain) to locally produce/regenerate steroids (Mellon et al., 2001), (v) the activity of the P-glycoprotein (PGP) pump across the blood-

brain barrier (BBB) and (vi) the clearance rate of GCs from liver and kidneys. Processes (i), (ii) and (vi) determine the temporally-fluctuating, biologically active systemic GC concentrations.

The wide spectrum of GC-related biological actions, apart from indicating their generic significance in human (and many other animal species') physiology, has been exploited in the field of therapeutics of various disorders; natural or synthetic GCs are prescribed/used in several clinical conditions for instance inflammatory-oedematous diseases like serious allergies, asthma, serious bacterial infections (in combination with antibiotics) and primarily autoimmune disorders (Hill et al., 1990; Rhen and Cidlowski, 2005). Other therapeutic indications of GCs include conditions like chronic pain (in combination with first line pain killers under multi-drug schemes) and neoplastic lesions (again in combination with first line anti-neoplastic drugs under multi-drug schemes), as well as adrenal insufficiency (replacement therapy) (Crown and Lightman, 2005a).

Unfortunately, treatment with GCs is often only partial effective and also results in adverse effects (Boling, 2004; Crown and Lightman, 2005b). In the field of applied clinical neurosciences, GC-based therapeutics present two major challenges; the reduction of the neuropsychiatric adverse effects that accompany their high-dose or long-term use (Klein, 1992; Tavassoli et al., 2008; Ricoux et al., 2013) and good scientific evidence for their effectiveness (in neurological cases occasionally prescribed). To overcome these challenges, as well as to further explore possible applications of GCs in other neurological processes including diagnosis, discrimination between disease subtypes, prognosis, treatment strategies of neuropsychiatric conditions, it is important to conceptualize the multi-level regulatory dynamics of GCs in stress regulation and health preservation (Young et al., 2004).

The purpose of this article is to discuss how the new concept of HPA pulsatility can provide a methodological and clinically significant advance for our understanding of stress



physiology and pathology. We place GCs' effects on brain's functional phenotypes into the context of HPA rhythmicity, as well as highlight some important concepts related to GC neurodynamics in brain physiology and pathology.

## **2. Inconsistencies in our understanding of GC therapeutics in neurology**

There is a characteristic discrepancy between the preclinical evidence that support the utilization of GC-based therapeutics or prognostic markers in various pathological cases and the poor results in terms of their efficiency or appropriateness when they are actually applied in clinical practice. This is also the case in neurological conditions.

Association of GCs with stroke evolution and prognosis has for instance been highlighted in clinical terms, since cortisol levels were found high during the first post-stroke week and such concentrations were associated with higher prevalence of systolic blood hypertension and night-time blood hypertension 24-hours after stroke (Ahmed et al., 2004), and with increased dependency, delirium incidences, depression and mortality rates in post-stroke patients (though these conclusions are not necessarily independent of stroke severity and thus GC levels cannot be used as independent prognostic markers) (Barugh et al., 2014). Nevertheless, there is serious scientific confusion on whether GC levels can be used for short- and/or long-term prognosis of post-stroke patients, as well as at what stage of the post-stroke clinical evaluation these data should be acquired and interpreted (Christensen et al., 2004; Johansson et al., 2000; Marklund et al., 2004). There is also a debate on the causal origin of these raised GC concentrations, whether there is an alteration between total and free levels of circulating GCs in stroke patients, as well as whether there is a correlation between cortisol concentrations and its serum carrier protein levels; a recent study estimated an inverse and independent relationship between serum albumin and total cortisol levels in stroke patients, an observation also independent from stroke severity (Dziedzic et al., 2012).

Potential therapeutic responses of experimental neurovascular pathologies to GCs have been explored over the last 3 decades; GCs attenuate (along with mannitol and vitamin E) free radical-mediated peroxidation (Uenohara et al., 1988), increase endothelial NO synthase activity via the phosphatidylinositol-4,5-bisphosphate 3-kinase / protein kinase B pathway, and as such effectively augment regional cerebral blood flow and reduce cerebral infarct size (Limboung et al., 2002), while they could also control neuronal cell survival:death rates by indirectly influencing more complex neuroinflammatory signalling cascades (Takata et al., 2012). Nevertheless, when applied in actual clinical terms, GCs (or at least the clinical studies designed to justify their probable utility), fail to highlight any significant benefits; randomised trials comparing GC administration within 48 h of acute (presumed or definite) stroke onset with placebo or a control group didn't show any difference in the odds of death within one year, while treatment did not appear to improve functional outcome in survivors (Sandercock and Soane, 2011). Moreover, there is no evidence to support the routine use of GCs in patients with haemorrhagic stroke (Feigin et al., 2005).

At another level, recent clinical and pre-clinical research findings indicate a possible acute, transient suppression of the HPA axis in traumatic brain injury (TBI), (Taylor et al., 2013), which may offer valuable prognostic information (Hannon et al., 2013). Moreover, experimental treatment approaches that down-regulate neuronal / glial GR signalling exert neuroprotective features (Shi et al., 2014), while dexamethasone provides anti-oedematous / BBB-stabilizing effects in animal models of TBI (Thal et al., 2013). Lack of GCs (due to experimental adrenalectomy) has been shown to exert a similar and additive effect to experimentally-induced TBI (fluid percussion injury) on decreasing hippocampal mRNA expression levels of neurotrophin-3 (Grundy et al., 2004) and increasing brain-derived neurotrophic factor (BDNF) (Grundy et al., 2000), while it prevents the post-TBI-induced increase of nerve growth factor (Grundy et al., 2001). Some of these effects are reversed after

GC substitution. In clinical terms though, evidence strongly discourages the use of GCs as part of a therapeutic strategy in acute TBI; a large randomised placebo-controlled clinical trial (MRC CRASH trial) revealed not only an unchanged mortality rate but also an increased risk of death within 2 weeks post-TBI in patients receiving methylprednisolone (Roberts et al., 2004).

Similar discrepancies have been observed in a number of other conditions, like multiple sclerosis and neurodegenerative disorders. For example, increased plasma cortisol levels have been associated with more rapid disease progression in subjects with Alzheimer-type dementia (AD) (Csernansky et al., 2006). In contrast, a large post-mortem neuropathological examination of individuals receiving systemic GCs for various medical reasons revealed at least 50% less histological markers of AD pathology compared to non-treated subjects (Beeri et al., 2012). Table 1 summarizes our state of knowledge concerning GCs' involvement in the pathophysiology of a number of neurological conditions. These discrepancies highlight the importance of integrating the complex mechanisms underlying GCs' physiological mode of activity to the strategies for applying GC-based treatments or prognostic tools in routine clinical practice (Russell and Lightman, 2014).

### **3. The concept of HPA pulsatility and its relevance to brain homeostasis**

The contradictory results from preclinical and clinical studies concerning GC roles in normal and abnormal brain states introduce serious confounding parameters in our efforts to evaluate any possible valuable associations of GCs with clinical neurosciences from a therapeutic, prognostic, preventive and / or diagnostic point of view. Under which (intrinsic and extrinsic) conditions do GCs promote brain's effective, adaptive, physiological responses and what are the critical factors that transform GC influence to an ineffective, pathological insult? Under which terms could GCs be of any meaningful clinical use in solving neurological problems?

And how can we collectively evaluate the sometimes contrasting evidence from different experimental or clinical studies trying to approach this field? One crucial step towards answering these questions is to place them into the context of HPA (and GC) pulsatility.

### 3.1. Basic regulation of endogenous GC rhythmicity: HPA pulsatility

Schematically, we can classify the basic regulatory mechanisms that define GC pattern of daily systemic fluctuations into two main categories; principal and superimposed (Figure 1). GCs are secreted in a circadian rhythm, where the natural peak occurs just prior to the active period (in human at about 9 am), followed by a gradual fall during the day to reach their nadir levels at roughly midnight. These increased GC levels during the circadian peak are thought to mainly arise from an augmented corticotrophin-releasing hormone (CRH) drive resulting from reduced inhibitory input from the suprachiasmatic nucleus (SCN) to the paraventricular nucleus (PVN) and median eminence (Buckley and Schatzberg, 2005). GCs also inhibit the CRH-dependent stimulatory drive by a negative feedback loop at both pituitary and hypothalamic levels. Corticolimbic regions are also involved in this regulatory process, though it is worth noticing, that they do not directly innervate PVN. On the contrary they project via pathways like the bed nucleus of stria terminalis (BNST) to a number of basal forebrain, hypothalamic and brainstem cell populations that in turn innervate, the medial parvocellular part of this hypothalamic region (Herman et al., 2005). The hippocampus is also a target region for GC negative feedback, and it in turn exerts an inhibitory effect over HPA activity both at the circadian nadir and peak of secretion as well as at the onset and termination of the stress response (Jacobson and Sapolsky, 1991). The amygdala and prefrontal cortex (PFC) contribute to the regulation of the HPA functional status primarily after exposure to stressful conditions, with the amygdala contributing to these regulatory processes by enhancing the stress-related GC secretion in a region-specific manner; central

and medial amygdaloidal nuclei being susceptible to different stressful stimuli (intrinsic-inflammatory and extrinsic-environmental respectively) and contribute to the acute stress responses, while basolateral amygdala appears to have a role in the chronic stress integration. Medial PFC, on the other hand, has a regulatory role after acute psychogenic or systemic stress with an inverse relationship between chronic stress impact and PFC activity. Some of these PFC-related feedback mechanisms are characterized by laterality, with the right hemisphere being more important (Diorio et al., 1993; Jankord and Herman, 2008).

Underlying these mechanisms is a complex dynamic ultradian rhythm composed of individual pulses of GC secretion. These pulses vary in amplitude and duration throughout the day, but their origin seems to be the result of a self-sustaining feedforward and GR-dependent feedback oscillatory activity between the anterior pituitary (AP) and AGs - a sub-hypothalamic oscillatory mechanism (Waite et al., 2012; Walker et al., 2010; Russell et al., 2010). This results in a 24 h profile of circulating endogenous GCs, where pulses of adrenal GC secretion of varying amplitude and duration occur periodically, approximately every 60-145 min (Lightman et al., 2008; Gavrilu et al., 2003), in anticipation of cortisol's estimated half-life (around 90 min) (Rai et al., 2004; Depue et al., 1985).

Both the circadian and ultradian characteristics can be highly variable, both within and between individuals. They depend on genetic, age- and gender-specific variations (Van Cauter et al., 1996; Bartels et al., 2003; Spiga et al., 2014), intrinsic environmental factors and perceived stress responses that define the temporally fluctuating state of activity of feedback and feedforward mechanisms (Lightman et al., 2002). Moreover, any underlying neuropsychiatric pathology involving corticolimbic areas of the brain (for instance neurovascular or neurodegenerative insults), whose integrity is also crucial for effectively modulating stress responses, could alter the temporal pattern of GC circulation. A recent study in stroke patients with right-sided infarction (Lueken et al., 2009) observed an altered

tonic and phasic cortisol secretion and a damaged stress response compared to stroke patients with left-sided infarction or healthy age-matched controls, concluding that the asymmetrical (right hemisphere-coordinated) central regulation of stress system could be dysregulated by pathologies affecting these brain areas, leading to ineffective protection against disease and external challenges.

### 3.2. Complex dynamics of GCs reaching the brain

Although both the circadian and ultradian rhythms are preserved and synchronized within the systemic circulation, central nervous system (CNS) and subcutaneous tissue (Qian et al., 2012), the relationship between the patterns by which GCs are secreted from AGs and their effect on the modulation of brain physiology or pathology are clearly very complex, making the studies on the role of GC pulsatility in brain's physiology very challenging.

Schematically, endogenous GC dynamics are regulated at three different levels before reaching the level of brain-region specific cellular signalling. Firstly, at the level of their pulsatile secretion from AGs, as explained earlier, under the co-interacting feedforward and feedback mechanisms between and within corticolimbic brain areas (as well as possibly other unknown regulatory sites), brainstem, hypothalamus, anterior pituitary and adrenal cortex. Secondly, at the level of their systemic circulation, where the biologically active portion of GCs is distributed to various tissues. At this point, there are two important regulatory mechanisms; the amount and binding properties of GC-binding proteins (Henley and Lightman, 2011), and the clearance / metabolic rate of the biologically active portion of GCs from liver, kidneys and other sites (McKay and Cidlowski, 2003). Thirdly, at a brain-specific level, where GC dynamics could be effectively changed by processes like BBB penetration, resulting in alteration of the local cortisol to corticosterone ratio. Finally, at the brain region-specific cellular level, GC effects are determined from (i) the presence of enzymes with the

capacity to alter locally the active hormonal concentrations, (ii) the differential expression of the two target receptors of GCs (mineralocorticoid receptor or MR, and glucocorticoid receptor or GR) in the various cell types in that brain region (neuronal and glial populations), (iii) other neurotransmitters or neuromodulators that may synchronously co-influence the same brain area, (iv) intracellular interactions of the GC-sensitive receptors (like the phosphorylation of mitochondrial GR, affected by antidepressant fluoxetine) (Adzic et al., 2013) or GC-GR and GC-MR complexes that may regulate their signalling efficiency or cellular compartmentalization, and (v) the recruitment of other transcriptional co-activators or co-suppressors, as well as interaction with epigenetic mechanisms that define transcriptional selectivity (de Kloet et al., 2009; Biddie et al., 2012).

For instance, despite the fact that corticosterone is generally produced in much lesser degree compared to cortisol, as reflected by their systemic circulating concentrations, with a cortisol to corticosterone ratio (CCR) 93.5 : 6.5 (Raubenheimer et al., 2006), these dynamics change at a central level. There, PGP, a cellular membrane protein found (among others) in the endothelial cells of the BBB and responsible for releasing many substances out of the cells, shows a greater sensitivity in extruding cortisol rather than corticosterone, i.e. corticosterone is preferentially maintained in the human brain (de Kloet et al., 2009). Indeed, the CCR of the cerebrospinal fluid is substantially decreased at a ratio of 72 : 28 (Raubenheimer et al., 2006).

Another notable aspect of the complex neurodynamics of GCs is the ability of the brain to locally produce / regenerate / deactivate neurosteroids (Mellon and Griffin, 2002). Despite the fact that the enzymatic activity of Cytochrome P450 21-hydroxylase (P450c21), the main cytochrome 450 enzyme responsible for converting 17-OH-progesterone and progesterone to GC precursors (11-deoxycortisol and 11-deoxycorticosterone respectively) in AGs, is almost absent from CNS tissues (Mellon and Miller, 1989), various brain areas are

capable of locally altering the GCs levels via (i) cytochrome P450 2D (CYP2D) isoforms (like the CYP2D6 isoform in human brain, found in most corticolimbic areas, basal forebrain and cerebellum) (Miksys and Tyndale, 2004) which perform the steroid 21-hydroxylation (replacing P450c21 lack of activity within the brain) (Kishimoto et al., 2004), (ii) 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD), that either increase the turnover between active and inactive GCs (isoform 1, found in corticolimbic regions, hypothalamic areas, brainstem and cerebellum) or solely degrade active GCs to inactive molecules (isoform 2, found in GC-insensitive brain regions like the circumventricular organs) (de Kloet et al., 2009), (iii) cytochrome P450 11-beta-hydroxylase (P450c11 $\beta$ ) enzyme (found in neocortex) responsible for converting GC precursors to active hormones (cortisol and corticosterone respectively), and (iv) 5 $\alpha$ -reductase (found in hypothalamic areas, corticolimbic regions and circumventricular organs), which directs corticosterone precursors to other metabolic pathways (Mensah-Nyagan et al., 1999) (Table 2).

Thus, different brain areas are likely to be exposed to differential concentrations of GCs despite the fact that the pattern of systemically oscillating GCs levels as defined by the ultradian and circadian rhythm of HPA axis is preserved and synchronized throughout the brain. Moreover, the enzyme 11 $\beta$ HSD which is differentially expressed in different brain areas can alter the corticosterone to cortisol ratio initially established by the higher BBB permeability of corticosterone. For instance, circumventricular organs may be exposed to very low levels of GCs since they lack the ability to locally produce GCs, they additionally contain 11 $\beta$ HSD isoform 2 which degrades GCs to inactive molecules, and 5 $\alpha$ -reductase, which further depletes corticosterone precursors from that brain areas' microenvironment. On the contrary, corticolimbic areas like PFC are able to enhance the local presence of GCs by producing and regenerating active GCs (since they express enzymatically active forms of



CYP2D6, 11 $\beta$ HSD isoform 1 and P450c11 $\beta$ ), and especially cortisol (due to the presence of 5 $\alpha$ -reductase).

The ability of particular brain regions to locally enhance (or attenuate) the presence of GCs could offer a mechanism for increasing the efficiency of central stress response, i.e. to increase the chances that specific stress-related brain areas (like PFC) will recruit the (GR-dependent) neuronal mechanisms required for a successful behavioural adaptation to stress. Data indicating high degree of cellular co-localization between GRs, 11 $\beta$ HSD isoform 1 and P450c11 $\beta$  support this notion (Roland et al., 1995; Erdmann et al., 1996).

From another point of view, the ability of the brain to independently produce GC precursors mediated by a different enzyme (CYP2D6) compared to corresponding adrenal biosynthesis (P450c21) should probably be considered when trying to study the effects of GC deficiency in the brain due to P450c21 deficiency (congenital adrenal hyperplasia). In contrast, the drug metyrapone (which crosses the BBB if administered systemically) (Stith et al., 1976), used in clinical and preclinical GC research, blocks both the adrenal and central synthesis of GCs, because it inhibits the action of P450c11 $\beta$  which is a common enzyme in both tissues' steroidogenic pathways. In addition, metyrapone selectively blocks the activity of the 11 $\beta$ HSD subtype 1, thereby preventing the regeneration of active cortisol (Raven et al., 1995; Sampath-Kumar et al., 1997). Therefore, the experimental use of metyrapone is a reasonable strategy to induce cortisol (or/and corticosterone) deficiency in the brain (of animals or humans). Though it should be noted that metyrapone results to an increase in GC precursors (11-deoxycortisol and 11-deoxycorticosterone) which were recently shown to provoke particular neuro-modulatory effects (Kaminski et al., 2011).

Lastly, it is worth mentioning that CYP2D6 enzyme, apart from catalysing the synthesis of GC precursors within the brain, also metabolizes a series of CNS-affecting drugs like opioids, neuroleptics, antidepressants, beta-blockers, drugs of abuse, and neurotoxins

(Zanger et al., 2004). Moreover, CYP2D6 brain levels are significantly up-regulated in chronic smokers and alcoholics in a cell-type and brain region-dependent manner, while genetic variation of CYP2D6 has been associated with a number of neuropsychiatric conditions like AD or Parkinson's disease (PD) (Miksys and Tyndale, 2004). All these parameters need to be taken into consideration when trying to design a clinical study about the central effects of GCs and their contribution in neuropsychiatric pathology.

### 3.3. Molecular basis of GC actions within the brain

In accordance with the pluralism of actions that GCs exert in the rest of the body, cortisol and corticosterone modify brain's physiology at multiple levels, primarily through binding with MRs and GRs in the cytoplasm, nucleus, mitochondria, cellular and mitochondrial membranes. In addition, non-specific GC effects (possibly resulting from the physicochemical interactions of GCs with plasma and mitochondrial membranes) have also been characterized (Song and Buttgerit, 2006).

We now know that MRs exist in two isoforms within the human brain, as alternative splicing between exons 3 and 4 results in an MR mRNA variant encoding a receptor protein with four additional amino acids compared to the wild-type MR protein (Wickert et al., 2000). The region-specific ratio between the two isoforms could be changed under neurological conditions like epilepsy, although from a functional point of view these isoforms do not have substantial differences (Wickert et al., 2000). Two alternative transcripts of the hippocampal MR have also been identified in the rat (Castrén and Damm, 1993), while many more variants have been identified in aldosterone target tissues leading to differential responsiveness to mineralocorticoids (Pascual-Le Tallec and Lombès, 2005). Integrative research (by exploiting the binding properties of synthetic anti-mineralocorticoid [ $^3\text{H}$ ]ZK 91587 or applying immunohistochemical approaches like *in situ* hybridization for

investigating mRNA expression or Western blotting for protein level estimation) in many animal species including rats, guinea pigs, dogs and non-human primates like squirrel monkeys, indicate that most prominent MR-binding sites and sites of MR expression in CNS include hippocampus, lateral septum, amygdala, (Grillo et al., 1990; Patel et al., 2000) and to a lesser extent cerebral cortex, cerebellum, caudate-putamen complex, and hypothalamus (Patel et al., 2000; Agarwal et al., 1993) (Table 3).

The developmental stage of the individual dynamically alters this profile of MR distribution within CNS as MR expression fluctuates between pre- and postnatal development in a brain-region specific manner (Matthews, 1998; Diaz et al., 1998), while aging is associated with a significant decrease in the expression and substrate-binding capacity of MR in these brain areas (Rothuizen et al., 1993); a phenomenon that may contribute to the dysregulated feedback activity in the HPA axis observed in older individuals (Bohn et al., 1991). Moreover, chronic stress seems to down-regulate MR mRNA expression in hippocampus, not only in mammals but also in birds (Dickens et al., 2009). Generally, MR expression within the rat brain seems to be rapidly, inversely auto-regulated (Chao et al., 1998) responding to GCs' changing levels, as adrenalectomy increases MR protein expression within 12 hours while the substitution with corticosterone or aldosterone reverses this phenomenon. Moreover, chronic high levels of GCs reduce MR protein levels compared to normal controls (Kalman and Spencer, 2002).

GRs seem to be more resistant to aging-related alterations in their expression or binding capacity within the brain (Rothuizen et al., 1993), though this process may region-specific (Perlman et al., 2007). Areas of GR expression include cingulate cortex, hippocampus, PVN and supraoptic nucleus (Matthews, 1998; Kiss et al., 1988), lateral geniculate, lateral and medial amygdala, thalamus, cerebellum and cerebral cortex (Patel et al., 2000) (Table 3). GR expression fluctuates between pre- and postnatal development in a

brain-region specific manner (Matthews,1998; Diaz et al., 1998). Moreover, chronic stress down-regulates GR mRNA expression in the PVN, but not in the hippocampus (Dickens et al., 2009). Like MR, the developmental stage of the individual and various environmental factors (Meaney et al., 2013) dynamically alter this profile of GR expression within CNS, especially when these are accompanied with early-life stress (Pryce, 2008). Epigenetic phenomena play an important role on this matter (Kino and Chrousos, 2011) and could form a molecular basis for developing GR-mediated susceptibility to neuropsychiatric pathology (McGowan et al., 2009). Generally, GR expression within the rat brain is also inversely correlated, but seems to be less prone to GC changing levels, since GR protein levels fluctuate at a lesser degree even after longer periods after adrenalectomy (Kalman and Spencer, 2002). Moreover, the underlying regulatory mechanism is not only GR-dependent (auto-regulation) but also MR-dependent (Chao et al., 1998).

The mosaic of GC-sensitive receptors' distribution throughout the brain is not only region-dependent, as described above, but also cell type-dependent. Although neurons and glial cells express both kinds of GC-sensitive receptors (Bohn et al., 1991), the neuronal-to-glial density ratio of their expression differs between brain regions. An extensive study of multiple brain regions of healthy male rats based on computer-assisted morphometric and microdensitometric evaluation of the GR immunoreactivity (Cintra et al., 1994) revealed important variations between them. Areas like the frontal lobe, cingulate cortex, olfactory nuclei, basal forebrain, most parts of basal ganglia, thalamus and parietal cortex contain high levels of GR-expressing neurons and low GR-expressing glia (neuronal-to-glial density ratio 3-11 : 1), while areas like PVN and other hypothalamic nuclei, dorsolateral thalamus, the most internal layer of the parietal cortex, amygdala, retrosplenial cortex, locus coeruleus, lateral parabrachial nucleus and raphe nuclei share a more or less equal density of GR-expressing neurons and glia. In only a couple of brain areas (like dentate gyrus and solitary

tract) the density of GR-expressing glial cells is truly greater compared to neurons (neuronal-to-glial density ratio 1 : 3).

Thus, GCs may control a large number of CNS areas, but this influence is differentially mediated by glial cells and neurons in a brain-region dependent manner. On the contrary, there seems to be no fundamental variations in the manner of intracellular trafficking of GRs among different cellular types in vitro (Nishi et al., 1999). The particular interest about glial cells expressing GC-sensitive receptors lies on the fact that they represent a highly adaptive cellular part of CNS, and are involved in a series of fundamental histopathological processes like neuro-inflammation and neuro-protection. In this context, it has been highlighted that GCs control genomic pathways that could establish glial-specific mechanisms to process glutamate and thus protect injured tissue from glutamate-induced neurotoxicity (Vardimon et al., 1999). Moreover, it was recently observed that MR-expressing astrocyte (a glial cell type) migration is increased to the ischaemic core in 20-min middle cerebral artery occlusion mice models, and that blockage of MRs (by spironolactone treatment) led to significant suppression of superoxide production within the infarct area and to up-regulation in the expression of neuro-protective / angiogenic basic fibroblast growth factor and vascular endothelial growth factor (Oyamada et al., 2008). At another level the synchronous interplay between activated microglia and inflammatory agents under pathological (low) GC levels may contribute to the development of complex phenomena like hyperalgesia (Suarez-Roca et al., 2014).

#### 3.4. How is GC rhythmicity biologically perceived by the brain?

There is good evidence that GC rhythmicity is registered at the level of the brain. A recent study showed that the pattern of GCs pulses differentially regulates glutamatergic neurotransmission and long-term potentiation (LTP, an important neuronal mechanism

considered to underlie memory formation) induction in cultures of hippocampal neurons and dorsal hippocampal slices from rodent brains (Sarabdjitsingh et al., 2014), while a previous study has described the phenomenon of gene pulsing in rat hippocampus following pulses of GCs (Conway-Campbell et al., 2010).

In the context of GC actions within brain, GC rhythmicity could offer a mechanistic, molecular explanation for their diverse effects, because it provides a regulatory input which can be “read” differentially by different brain regions depending on the amount of GR or MR they express. Moreover, due to different affinities of membrane-associated and nuclear MRs and GRs for GCs, the pattern of GC rhythmicity will determine which GC- receptors will be activated and their duration of activation. This will result in differential cellular effects depending on the receptor population, their pattern of activation and the recent history of cellular activation.

In more detail, current state of knowledge indicates that MRs’ neuronal / glial activities may be mediated via either non-transcriptional mechanisms related to activation of membrane associated receptors or classic slower genomic pathways by activation of cytoplasmic MRs. The former, best described in hippocampal neurons, involve rapid (within minutes) effects (Roozendaal et al., 2010; Gutiérrez-Mecinas et al., 2011) resulting from activation of receptors in the cell membrane with subsequent activation of intracellular phosphorylation cascades. The affinity of these membrane-associated receptors is a factor of magnitude less than that for the nuclear MRs (Karst et al., 2005). This difference among affinity properties between nuclear- and non-nuclear-located MRs signifies the important role of GC pulsatility as a biological mechanism for modulating the initiation and duration of corresponding MR-dependent actions in relation to the physiological role that these actions exert. Nuclear MRs remain bound to DNA for much longer than GRs and circulating GC levels are at any time-point in the ultradian rhythm sufficient to preserve a continuous (=

tonic) occupation / activation of high-affinity nuclear MRs (90% at any time during the day which increases to approximately 100% under acute stress conditions) (Reul and de Kloet, 1985), while 10-fold lower-affinity non-nuclear MRs are activated only during the rising phases and peaks of the GC ultradian pulses (depending on the amplitude of each pulse) or under stress, leading to increased MRs' instability and thus proteasome-dependent MR degradation (Lightman et al., 2008).

Like MRs, GRs possess non-nuclear (rapid, non-genomic) and nuclear (genomic, delayed) actions. GR-dependent genomic effects may also be mitochondrial as well as the well-established nuclear (Scheller et al., 2000; Moutsatsou et al., 2001), and GCs can affect brain mitochondrial function *in vitro* (Morin et al., 2000). Nevertheless, GR-dependent effects, rapid or delayed, are mediated during periods of high GC concentrations, due to the comparatively low affinity of GRs towards cortisol and corticosterone.

It appears that MRs and GRs have been assigned, in evolutionary terms, to a different primary regulatory role, and their CNS-region specificity and mode of activation should follow that distinct role; nuclear MRs seem to possess a continuous, "background" activity that stabilises neuronal and glial functions, ensuring homeostasis (a process that evolves normally in a long-term basis, which is in accordance with the slow, genomic effects of nuclear MRs). Non-nuclear MRs, on the contrary, seem to be necessary for coordinating the initial brain response to stress (which is acute, and thus in accordance with the fast, non-genomic actions of non-nuclear MRs), while GRs at the same time initiate the (sub-acute or even chronic) processes responsible for attenuating, and eventually terminating, stress responses (re-establishing homeostasis) as well as performing vital neurobehavioral adaptations to increase effectiveness towards confronting future threats and noxious insults.

### 3.5. Altered HPA rhythmicity in stressful conditions and human pathology

In clinical terms, many stressful and pathological states have been correlated with a dysregulated 24h ultradian profile of circulating endogenous GCs, indicating an altered activity of HPA axis under these conditions, despite the fact that mean cortisol or corticotrophin (ACTH) levels do not necessarily differ from normal controls (Figure 2). For instance, the study of HPA axis ultradian rhythms in premenopausal, viscerally obese women revealed several abnormalities of ACTH pulsatile secretion (increased pulse frequency and reduced pulse amplitude) which were not accompanied by abnormal mean ACTH concentrations in peripheral blood (Pasquali et al., 1998). Another example is obstructive sleep apnoea; in a recent study, the deconvolution analysis of secretory pulses in the 24h systemic GC profiles of untreated patients revealed longer duration of ACTH and cortisol pulses compared to the same patients when they had been successfully treated with continuous positive airway pressure therapy (Henley et al., 2009b).

Analysis of the 24 h ultradian profile of circulating endogenous GCs could be a useful indicator of the aetiology of high or low mean cortisol levels in many pathological conditions. In the neuropsychiatric context, neurodegenerative disorders like AD and PD, or depression and post-traumatic stress disorder have been thoroughly studied. Apart from the disturbed circadian pattern (increased waking / morning levels and circadian amplitude) and the increased mean systemic cortisol levels observed in AD (Martignoni et al., 1990; Lei, 2010), analysis of the 24 h ultradian profile of AD (and PD) patients reveals that the hypercortisolemia observed in these subjects results from a raised mass of cortisol secreted per burst compared to healthy age-matched volunteers without any substantial alterations in the cortisol half-life, number of secretory bursts within 24 h, and the mean inter-secretory pulse interval (Hartmann et al., 1997). On the contrary, the possible existence of a dysregulated HPA axis in major depression (Pariante and Lightman, 2008) leading to hypercortisolemia, or fibromyalgia and chronic fatigue syndrome (Cleare, 2004; Calis et al.,



2004; Wingenfeld et al., 2008) leading to hypocortisolemia, is not accompanied by substantial changes in the ultradian pattern of circulating GCs in the majority of particular subgroup of patients, although further studies are required (Young et al., 2001; Crofford et al., 2004). The co-evaluation of the mean systemic cortisol levels with their ultradian profiles could differentiate between different conditions depending on the aetiology (like AD from depression or Cushing syndrome) and predict the causal involvement of GCs in the initiation and / or progression of neuropsychiatric disorders (Notarianni, 2013). It is worth mentioning that in the past, efforts based on the mean GC concentrations and their circadian characteristics did not achieve good discrimination between depressed patients and patients with other neuropsychiatric pathology (schizophrenia, AD and mania) (Christie et al., 1983).

#### **4. How does rhythmicity contribute to the diverse and frequently contradicting GC effects in the brain?**

GC pulsatility plays a crucial role in the expressed plurality of GCs actions, as it offers the biological mechanism to achieve a dissociation between the MR- and the GR-dependent actions, as well as an association of them, under differential extent, during particular time points or periods (Russell et al., 2015). This association or dissociation between GRs' and MRs' actions can be realized at various subcellular levels. For instance at a nuclear level; MRs and GRs, when both present and activated within brain cells, can form heterodimeric complexes with DNA-binding and transactivation properties different from those of the respective homodimers (Trapp et al., 1994). Furthermore, it can be realized at the level of subcellular trafficking of MRs and GRs, where a differential combinatory pattern between them has been recently observed in the rat brain after induction of behavioural stress, depending on the brain region and the time after stress (Caudal et al., 2004).

At a more macroscopic perspective, GC pulsatility discriminates the combinatorial pattern of GC-sensitive receptors' activation between the different brain regions. Under physiological conditions, there is a tonic (continuous) GC influence in nuclear MR-sensitive brain areas, while only a phasic (periodic) influence in non-nuclear MR- and GR-sensitive brain areas, though rapid and delayed respectively, creating a mosaic of GC-dependent effects within the brain that are receptor type-specific (MR or GR or MR-GR depending on which receptors are expressed and activated in a brain-region and cellular type dependent manner) and strictly temporally regulated (continuous or time-limited, acute or delayed). Under stressful conditions, the spatial mosaic of GC-dependent effects within CNS changes because the increased levels of GCs enhance activation events of non-nuclear MRs and GRs.

In relation to GC-sensitive receptor homeostasis, pulsatility offers a self-limiting method of controlling any membrane MR- or GR-dependent effects (and corresponding molecular cascades) that could be damaging in the long-term, but desired or necessary in the short-term or under acute stressful conditions. The significance of this self-limiting control of GC actions is lost in states characterised by a sustained dysregulation of the physiological ultradian pattern, such as chronic stress, various neuropsychiatric disorders or chronic treatment with high doses of GCs (Barnes and Adcock, 2009). These states result in prolonged high GC levels and thus the elimination of the recovery periods / self-limiting control of GR activation during the descending phase of the ultradian pulse cycles, leading to brain GC resistance (Meijer et al., 2003) followed by GR down-regulation and inductively reduced GR-dependent regulatory influences (Makino et al., 1995). For instance, rapid GR-dependent negative feedback regulation of ACTH release under basal conditions or acute stress (Russell et al., 2010) is reduced in major depression, a condition accompanied with an overactive HPA axis (Young et al., 1991). Other examples involve the reduction of immune system's sensitivity to GCs' immunosuppressive effects during chronic psychological stress

(Miller et al., 2002), or the selective down-regulation of hippocampal GRs under sustained stress in rodents and non-human primates (Brooke et al., 1994) or after the experimental induction of viral encephalitis in rats (Bener et al., 2007). Additionally, GC resistance is thought to contribute to neuropathological mechanisms related to AD (another condition accompanied with an upregulated ultradian pattern) (Hartmann et al., 1997) such as disrupted axonal transport in cortical areas (Dai et al., 2004).

#### 4.1. Neuroinflammation versus immunosuppression

Considering the importance of the temporal dimension of fluctuating GC concentrations within the brain, we can speculate that their short-term increase before or after an inflammatory insult may induce different effects. Indeed, acute increases of GCs have been shown to reduce certain types of inflammatory responses, especially of cytotoxic origin (attenuation of oxidative stress and cellular necrosis) if administered concurrently to or after an immune challenge (Nadeau and Rivest, 2003), but a major increase in GCs activity prior to an inflammatory insult (like exposure to lipopolysaccharide) can actually result in the exact opposite [exacerbation of neuronal / glial death, oxidative stress, potentiation of the glial-mediated inflammatory response by acting as pro-inflammatory chemokines, augmentation of interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumour necrosis factor- $\alpha$  (TNF $\alpha$ ), production and enhancement of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF $\kappa$ B)-related genomic actions] (Frank et al., 2010; Frank et al., 2012). Similarly, chronic exposure to high versus physiological GC concentrations could also result in varying immunological phenotypes. Indeed, in the long-term, under non-stress levels, GCs suppress cytokine (such as IL-1 $\beta$  and TNF $\alpha$ ) production, nitric oxide (NO) synthesis and transcription factors implicated in inflammatory activation (like NF $\kappa$ B), while during chronic stress pro-inflammatory mechanisms seem to be up-regulated (Sorrells and Sapolsky, 2007).

Given the temporal variation of the neurological effects of GCs and the multifactorial nature of the response, it is no surprise that increasing scientific evidence from preclinical and clinical studies suggests now that GCs may pharmacologically act in unpredictable ways in the context of multiple sclerosis patients, because the precise timing, dosage, duration, cellular exposure, and their background milieu may differentially affect the progression of the inflammatory response, BBB integrity and cellular viability (Krieger et al., 2014; Blecharz et al., 2010; Herold and Reichardt, 2013). Similarly, depression-like disruption of off-line motor memory consolidation has been observed in these patients under high-doses of corticosteroids (Dresler et al., 2010).

#### 4.2. Neurotoxicity versus neuroprotection

The temporal dimension of the dynamic regulation of GC concentrations, as achieved by their endogenous rhythmicity, seems to be crucial in creating optimal conditions both for neuronal and glial viability, and in altering their resilience to noxious stimuli. Physiological levels of GC concentrations offer a balanced environment for neuronal maintenance while both low and high concentrations of GCs may deviate this balance to the neurotoxic range (U-shape-like effect) (Abrahám et al., 2006). The hippocampus appears to be particularly vulnerable to these neurotoxic effects, with CA3 pyramidal neurons being particularly more sensitive compared to CA1 pyramidal cells (Levy et al., 1994). Moreover, combination of differential GC levels with various noxious stimuli [ $A\beta$ -toxicity, hypoxia, N-methyl-D-aspartate (NMDA)-induced excitotoxicity] leads to either an exacerbation of the neurodegenerative effects (when levels of GCs too low or too high) or an attenuation of the latter (under moderate corticosteroid levels) in a GC concentration-dependent manner (Abrahám et al., 2000). This latter effect has been also observed in animal models of cerebral ischemia, where chronic stress prior of neurovascular pathology was shown to increase stroke vulnerability,

likely through GC-related endothelial dysfunction, since this effect was reversed by a GR antagonist (mifepristone) (Balkaya et al., 2011). On the contrary, stress or corticosterone administration after neurovascular pathology (vasoconstriction-induced hippocampal ischemia) enhances cognitive recovery in rats (Faraji et al., 2009). In accordance with previous data, chronic stress and elevated GC levels correlate with A $\beta$  amyloid and tau accumulation (Green et al., 2006) as well as with alterations in hippocampal plasticity including dendritic remodelling, neurogenesis and LTP (Rothman and Mattson, 2010). On the contrary, administration of corticosterone in cortical co-cultures of neurons and astrocytes decreases cytosolic Ca<sup>2+</sup> levels in a calmodulin- and GR-dependent manner, counteracting glutamatergic cytotoxic effects due to calcium overload (Suwanjang et al., 2013).

#### 4.3. Enhancing versus attenuating systems of neurotransmission

The periodic nature of GC fluctuations within the brain gains further significance if we consider that dynamic processes of CNS function, like synaptic and circuit plasticity or neurotransmission, are influenced by GCs and need to be strictly controlled. For instance, GCs regulate the turnover of dopamine receptors (D1 and D2) as well as their sensitivity to their ligands (Biron et al., 1992), and prolonged treatment with corticosterone increases mRNA levels of D1 receptors in the striatum and nucleus accumbens and selectively up-regulates receptor-ligand binding potential in substantia nigra and ventral tegmental area (sites of dopaminergic neuronal bodies) in rats (Czyrak et al., 2003). On the other hand, metyrapone-induced pharmacological adrenalectomy has the opposite effects (Czyrak et al., 1997). Moreover, long-term high levels of GCs under specific conditions of genetic susceptibility could exert a long-term, epigenetic control of ventral tegmental area-originated dopaminergic neurons (Niwa et al., 2013) as well as promote stress-related, dopamine-dependent adaptive changes in dopaminergic neurons related with emotional and social

behavioural phenotypes (Barik et al., 2013), contributing to psychopathology. Furthermore, disruption of the circadian pattern of GCs' fluctuations and increased systemic GC levels in rats have been shown to increase dopamine release in the PFC possibly as a result of increased synthesis and vesicular storage, providing a mechanistic explanation for prefrontal dysfunction in bipolar and other affective disorders associated with GC dysrhythmia (Minton et al., 2009) (Figure 3).

A second example where the dynamic changes of GC concentrations impose rapid effects in a brain region-dependent manner is seen in glutamatergic neurotransmission; high levels of GCs increase glutamate release primarily from neuronal (and secondary from glial) populations in corticolimbic brain areas by increasing the number or the probability of vesicular exocytosis at the presynaptic level in a rapid, non-genomic MR-dependent manner (Karst et al., 2005), followed by an increased translocation of NMDA and, independently,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors from intracellular pools to the postsynaptic plasma membrane. Moreover, acute stress enhances a NMDA receptor-independent form of LTP by mobilising calcium-permeable AMPA receptors in a GC-dependent manner (Popoli et al., 2011). The duration of this MR-dependent up-regulation of glutamatergic neurotransmission is region-specific, since it's short-lasting at the hippocampal level but long-lasting in the basolateral amygdala, where subsequent acute stressful insults lead to a GR-dependent down-regulation of glutamatergic stimulation (Whitehead et al., 2013). In distinction to this there is a brain region-specific adaptation of glutamate release in relation to chronic stress or to acute insults after chronic stress, since in some parts of the corticolimbic system (i.e. hippocampus) neurotransmission remains constant, while in others (i.e. PFC) it gradually decreases. At the same time, there is also a PFC-specific down-regulation of both classes of glutamate receptors, an effect related to disrupted receptor trafficking and / or altered degradation or synthesis (Karst et al., 2010).

Moreover, GCs affect glutamate clearance from glial cells through glutamate transporter primarily expressed in these cellular populations; acute stress increases while chronic stress decreases glutamate uptake (clearance) and metabolism in the frontal cortex and hippocampus through GC-related pathways, although these observations need further experimental validation (Karst et al., 2010).

A third example where the pattern of GC fluctuations results in differential effects involves gamma aminobutyric acid mediated (GABAergic) neurotransmission; corticolimbic areas seem more susceptible in this modulatory effect, which is characterized by the increase in GABA<sub>A</sub>-receptor binding affinity at both low and high levels of GCs (Majewska et al., 1985; Majewska, 1987; Ong et al., 1987). High, acute stress-related GC levels result in NO release, which in turn stimulates GABA release from the GABAergic terminals in hippocampal inter-neuronal, inhibitory GABAergic populations; an effect that under chronic stress conditions could be proven detrimental for these neuronal networks (Hu et al., 2010). Daily administration of 1 mg/kg of corticosterone for 3 consecutive weeks in rats resulted in a down-regulation of GABA<sub>A</sub>-receptors' subunit  $\alpha_2$  expression, as well as in a corticolimbic area-specific (amygdala vs hippocampus) differential reduction of glutamate-to-GABA conversion (Lussier et al., 2013). On the contrary, much longer, chronic (1 year) cortisol exposure in primates resulted in significant increases in hippocampal calbindin (a  $\text{Ca}^{2+}$ -binding protein that buffers excess calcium), glutamic acid decarboxylase (GABA-synthesizing enzyme) and BDNF; an indication that this brain region could strengthen its GABAergic (inhibitory) influence under chronic stress conditions, trying to compensate the initial glutamate-releasing, excitatory (and the resulting glutamate-related cytotoxic) effects of GCs described earlier (McMillan et al., 2004).

#### 4.4. Stress induction and neurobehavioural adaptation

In terms of the stress systems' capacity for mobilisation, GC pulsatility offers a mechanism for preserving the ability of the individual to respond to stressful situations throughout the day and defines the time points of maximal effectiveness. The ascending phase of each GC pulse cycle (which gradually induces activation of membrane associated MRs and GRs) constitutes a preparatory stage for initiating an effective stress response (if required), while the descending phase of the pulse cycle is less prone to support an equally effective stress response, serving probably as a recovery period before the ascending phase of the next GC pulse. Indeed, research work has highlighted that exposure to noise stress induces a stronger ACTH release and behavioural reactivity when animals were stressed during the rising phase of an ultradian corticosterone pulse compared with animals exposed to the same stressor during the falling phase (Sarabdjitsingh et al., 2010). The actual mobilization, though, of a stress response requires the synergy of the "GC background" (defined by the phase of the pulse cycle at the moment of the stressful stimulus' occurrence) with a plethora of other neuro-hormonal phenomena such as the release of hypothalamic CRH (leading to an increase in the GC secretion) and other neuropeptides, the activation of the peripheral, sympathetic nervous system (adjusting the entire body's metabolic demands for confronting the stressful insult) as well as the central noradrenergic, dopaminergic and serotonergic circuits (which coordinate the behavioural adaptations during and after the stressful event) (Joëls et al., 2009).

The synchronous co-influence of GCs and other stress-coordinated neuro-hormonal stimuli within specific brain regions provides a mechanism for discrimination between the effects of high corticosteroid levels under baseline conditions (for instance during the peaks of the ultradian pulses) and stress. Such phenomena has been shown to mediate stress-dependent cognitive processes; for example, synergy between noradrenergic system and GCs leads to a strong deactivation of PFC areas during emotional encoding in human (van



Stegeren et al., 2010), while preclinical research has highlighted the crucial role of GCs-noradrenaline regulatory interactions at multiple levels: (i) at the level of basolateral amygdala for enhancing emotionally arousing-related memory consolidation (Roozendaal et al., 2006) and social behaviour (Roozendaal et al., 1996; Schwabe et al., 2010) by enhancing synaptic plasticity (Sarabdjitsingh et al., 2012), (ii) at the hippocampal level, where they alter the functional contribution of AMPA receptors to glutamatergic neurotransmission (Zhou et al., 2012), or (iii) at the level of hypothalamus, where they modulate feeding behaviour (Leibowitz et al., 1984; Jhanwar-Uniyal and Leibowitz, 1986; Roland et al., 1986). Collectively, these combined effects promote behavioural adaptation to stressful situations (Krugers et al., 2012).

## 5. Epilogue

GC rhythmicity which emerges as a natural consequence of the feedforward:feedback interactions between the pituitary and adrenal cortex, results in many physiological consequences (Table 4). Systemic GC concentrations should be perceived and studied not as a binary system (high versus low), but as a continuously changing system, whose impact on the brain depends on individual characteristics, the system's endogenous rhythms, brain region and cell type, as well as the temporal relationship between glucocorticoid fluctuations and application of other endogenous or exogenous, physiological or pathological stimuli. Any discrepancies about glucocorticoid effects on the brain or when trying to utilise them in the therapeutic or other clinical context become easier to comprehend if we consider the homeostatic importance of these continuous, dynamic, ultradian fluctuations.

A deeper understanding of HPA axis activity, its modulatory effects, and how physiological activity changes upon pathological activation, is a prerequisite for developing a rational system of glucocorticoid therapeutics. The defining, multi-level dependency of

human body homeostasis from GC homeostasis has been long ago recognised (Chrousos and Gold, 1992), as has the effect of disruption of GC homeostasis on biological processes including the developmental (*in utero*) determination of longevity, effective adaptation to environment and susceptibility to disease (Reynolds, 2013; Ter Wolbeek et al., 2015). Pulsatility offers a novel approach to understanding the diverse actions of GCs on neuroinflammatory responses, neuronal and glial metabolic properties and survival, CNS circuit dynamics, and behavioural and cognitive phenotypes. Furthermore it can provide insight into the reasons for the contradictory data found in studies of GC-brain interactions, justify the impressively wide range of the central GC actions, as well as promote a strong impetus for further studies into the therapeutic role of GCs in neuropsychiatric disorders. In addition, a better understanding of GCs' physiological and pathological responses in the brain could even allow the creation of algorithms to predict responses based on predefined biological and clinical parameters.

#### **List of abbreviations**

11 $\beta$ HSD: 11 $\beta$ -hydroxysteroid dehydrogenase

ACTH: corticotrophin

AD: Alzheimer disease

AGs: adrenal glands

AMPA:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AP: anterior pituitary

BBB: blood-brain barrier

BDNF: brain-derived neurotrophic factor

BNST: bed nucleus of stria terminalis

CBG: cortisol binding globin

CCR: cortisol to corticosterone ratio

CNS: central nervous system

CRH: corticotrophin-releasing hormone

CYP2D: cytochrome P450 2D

D1: dopamine receptor type 1

D2: dopamine receptor type 2

GABA: gamma-aminobutyric acid

GABA<sub>A</sub>: GABA receptor type A

GC: glucocorticoid

GR: glucocorticoid receptor

HPA: hypothalamic-pituitary-adrenal (axis)

IL-1 $\beta$ : interleukin 1beta

LTP: long term potentiation

MR: mineralocorticoid receptor

NF $\kappa$ B: kappa-light-chain-enhancer of activated B cells nuclear factor

NO: nitric oxide

P450c11 $\beta$ : cytochrome P450 11-beta-hydroxylase

P450c21: cytochrome P450 21-hydroxylase

PD: Parkinson disease

PFC: prefrontal cortex

PGP: P-glycoprotein

PVN: paraventricular nucleus

TBI: traumatic brain injury

TNF $\alpha$ : tumour necrosis factor alpha

SCN: suprachiasmatic nucleus

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## Conflict of Interest

The authors declare no conflict of interest.

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### Legends to the Figures

**Figure 1: Hypothalamic-pituitary-adrenal (HPA) axis and its interactions with brain regions under physiological or stressful conditions.** Glucocorticoids (GCs) are secreted from adrenal glands (AGs) into the systemic circulation in a pulsatile manner as the result of a self-sustained interplay between AGs and the anterior pituitary (AP). The characteristics of that interplay involve (i) a positive feedforward stimulation from AP to AGs (mediated via the hormone corticotrophin or ACTH) and (ii) a delayed negative feedback stimulation from AGs to AP (mediated via the GCs themselves). Certain hypothalamic nuclei, like the paraventricular nucleus (PVN), are able to modify the circadian characteristics of the GC pulses by secreting corticotrophin-releasing hormone (CRH), which promotes ACTH secretion at the pituitary level. At a supra-pituitary level, PVN constitutes a key neuroanatomical location being able to dynamically alter the mode of GC secretion (via CRH secretion) in response to a number of different stimuli, like: (i) inhibitory feedback signals

from circulating GCs, (ii) excitatory input from autonomic nervous system due to physical challenge, and (iii) inhibitory input from other hypothalamic nuclei and bed nucleus of stria terminalis (BNST) in response to psychological stress or pathological insults. The latter are encoded via complex interactions between corticolimbic regions of the brain, like amygdala, hippocampus and prefrontal cortex (PFC). These interactions, when dysregulated as a result of neuropsychiatric pathology, may affect the mode of HPA axis functioning and *vice versa*, a dysrhythmic HPA axis may facilitate the development of neuropsychiatric pathology. Green arrows: stimulatory effect, Red arrows: inhibitory effects, Grey arrows: mixed effect

**Figure 2: 24-h plasma cortisol and/or corticotrophin (ACTH) profiles.** Theoretical diagrams presenting normal 24-h plasma cortisol or/and ACTH profiles (dark blue) in comparison to other corresponding pathological profiles (light blue). (A): Abnormal cortisol profile is characterized by pulses of cortisol of increased amplitude, without any alterations in the overall number of pulses or their duration. Such a profile has been reported in patients with Alzheimer's or Parkinson disease, and leads to increased mean cortisol concentrations. (B): Abnormal ACTH profile is characterized by pulses of ACTH of decreased amplitude combined with an increase in the daily number of pulses. Such a profile has been observed in premenopausal, viscerally obese women. Mean cortisol concentrations may be normal. (C): Abnormal ACTH and cortisol profiles are characterized by pulses of longer duration. Such a profile has been reported in patients with obstructive sleep apnoea not treated with continuous positive airway pressure therapy. Mean cortisol concentrations could be increased.

**Figure 3: Glucocorticoids and psychopathology.** Reciprocal dysregulated interactions between various systems of neurotransmission, for instance the dopaminergic and/or the

serotonergic systems, with the hypothalamic-pituitary-adrenal (HPA) axis, alters susceptibility to different psychiatric phenotypes including depressive behaviour, addiction, psychosis, anxiety disorders, antisocial/aggressive behaviour and post-traumatic stress disorder (PTSD).

## Tables

**Table 1: Glucocorticoid (GC) involvement in neuropathology.**

GABA: gamma-aminobutyric Acid, HPA: hypothalamic-pituitary-adrenal, MS: multiple sclerosis

TBI: traumatic brain injury

<b><i>Multiple sclerosis</i></b>	<ul style="list-style-type: none"> <li>GC-based therapeutics for anti-inflammatory control in disease relapse management</li> <li>dysregulated HPA axis in MS with affective symptomatology</li> </ul>
<b><i>Stroke</i></b>	<ul style="list-style-type: none"> <li>stress-related GC-mediated signaling implicated in atherosclerotic development and endothelial dysfunction</li> <li>stress and up-regulation of HPA axis differentially affect stroke prognosis depending on the time of onset and duration</li> <li>GC-mediated reduction of oxidative stress and increase in penumbral rescue rate</li> <li>nevertheless, application of GCs, under current therapeutic schemes, does not attribute any benefit</li> </ul>
<b><i>Traumatic brain injury</i></b>	<ul style="list-style-type: none"> <li>acute, transient post-TBI HPA axis suppression.</li> <li>GC effects may counteract some of the damaging consequences of TBI, like oedema, blood-brain-barrier dysfunction and disruption of growth factors' homeostasis in certain brain regions.</li> <li>nevertheless, application of GCs is, under current therapeutic schemes, not beneficial or even harmful.</li> </ul>
<b><i>Alzheimer disease</i></b>	<ul style="list-style-type: none"> <li>hyperactive HPA axis</li> <li>stress-related GC-mediated signaling is involved in amyloidogenesis, tau hyper-phosphorylation and impaired hippocampal plasticity</li> </ul>

	phosphorylation and impaired hippocampal plasticity.
<b><i>Parkinson disease</i></b>	<ul style="list-style-type: none"> <li>hyperactive HPA axis.</li> <li>stress-related GC-mediated signaling is associated with inflammatory dopaminergic neurodegeneration.</li> </ul>
<b><i>Non-specific dementia</i></b>	<ul style="list-style-type: none"> <li>side effect of long-term and/or high-dose treatment with GCs.</li> <li>GCs interfere with glutamatergic, GABAergic, noradrenergic and cholinergic systems of neurotransmission, which are important in coordinating memory formation and consolidation, as well as glutamate-related cytotoxicity.</li> </ul>

**Table 2: Neuroanatomical distribution of enzymes involved in steroidogenic pathways.** 11 $\beta$ HSD: 11 $\beta$ -hydroxysteroid dehydrogenase, CYP2D: cytochrome P450 2D, P-45011 $\beta$ : cytochrome P450 11-beta-hydroxylase.

BRAIN REGIONS	5 $\alpha$ -reductase	11 $\beta$ HSD	P-45011 $\beta$	CYP2D
Brainstem		+		
Hypothalamus	+	+		
Thalamus	+			
Cerebellum		+		+
Striatum				+
Amygdala		+		
Hippocampus	+	+		+
Occipital lobe				+
Nucleus accumbens				+
Neocortex	+	+	+	+
Circumventricular organs	+	+		

**Table 3: Spatial pattern of distribution of glucocorticoid-sensitive receptors in the brain.**

GR: glucocorticoid receptor, MR: mineralocorticoid receptor

BRAIN REGIONS	MR dominance	Comparable quantities	GR dominance
Dorsomedial PFC			✓
Cingulate Cortex			✓
Hippocampus	✓		
Rest of PFC		✓	
Rest of Cerebral Cortex		✓	
Lateral Geniculate		✓	
Nucleus accumbens		✓	
Basal Ganglia	✓		
Amygdala		✓	
Thalamus			✓
Cerebellum		✓	
Hypothalamus			✓

**Table 4: Role of glucocorticoid (GC) pulsatility in brain function.**

GR: glucocorticoid receptor, MR: mineralocorticoid receptor

1. Temporal association/dissociation between MR- and GR-coordinated actions
2. Spatial mosaic of GC-dependent effects depending on the specific brain region and cell type

3. Importance for optimal cognitive and emotional function
4. Self-limiting control of damaging long-term GR-coordinated actions
5. Sustaining capacity of the stress system to effectively respond to insults throughout the day

## Figures

Figure 1

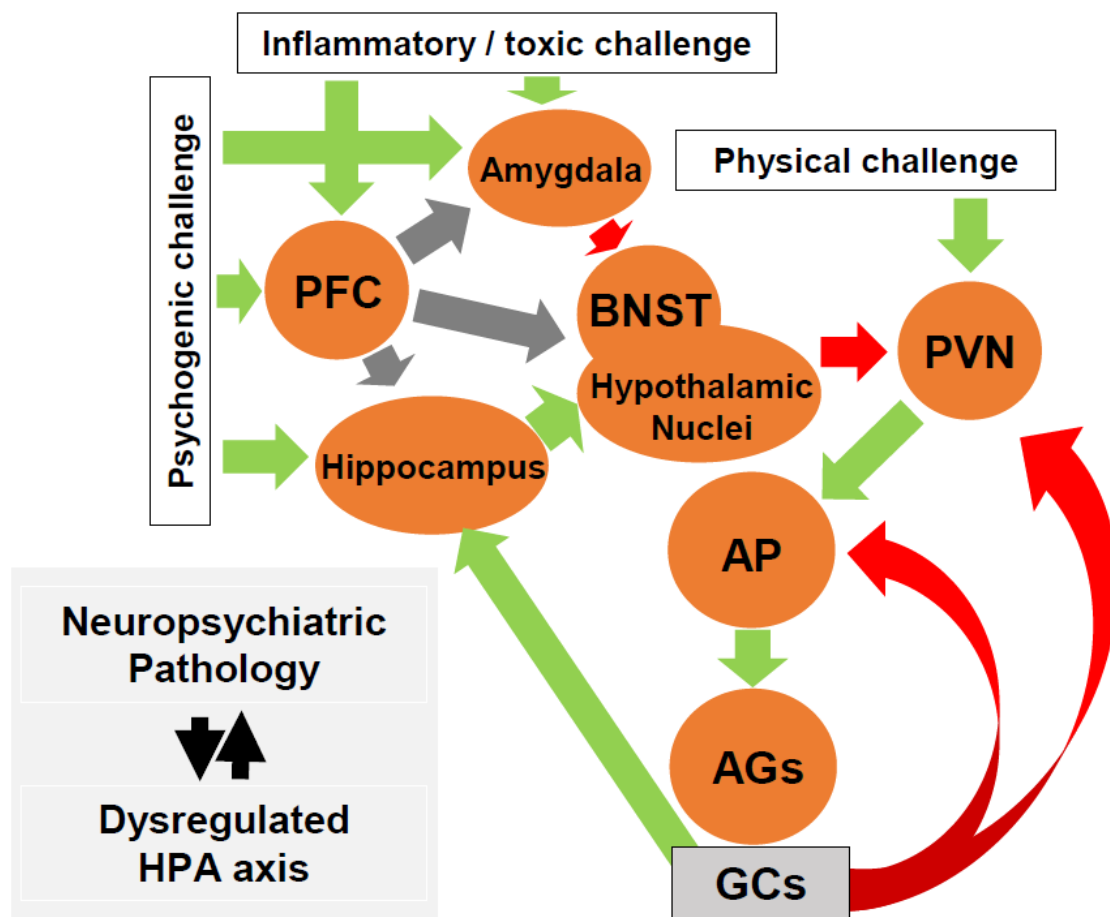


Figure 2

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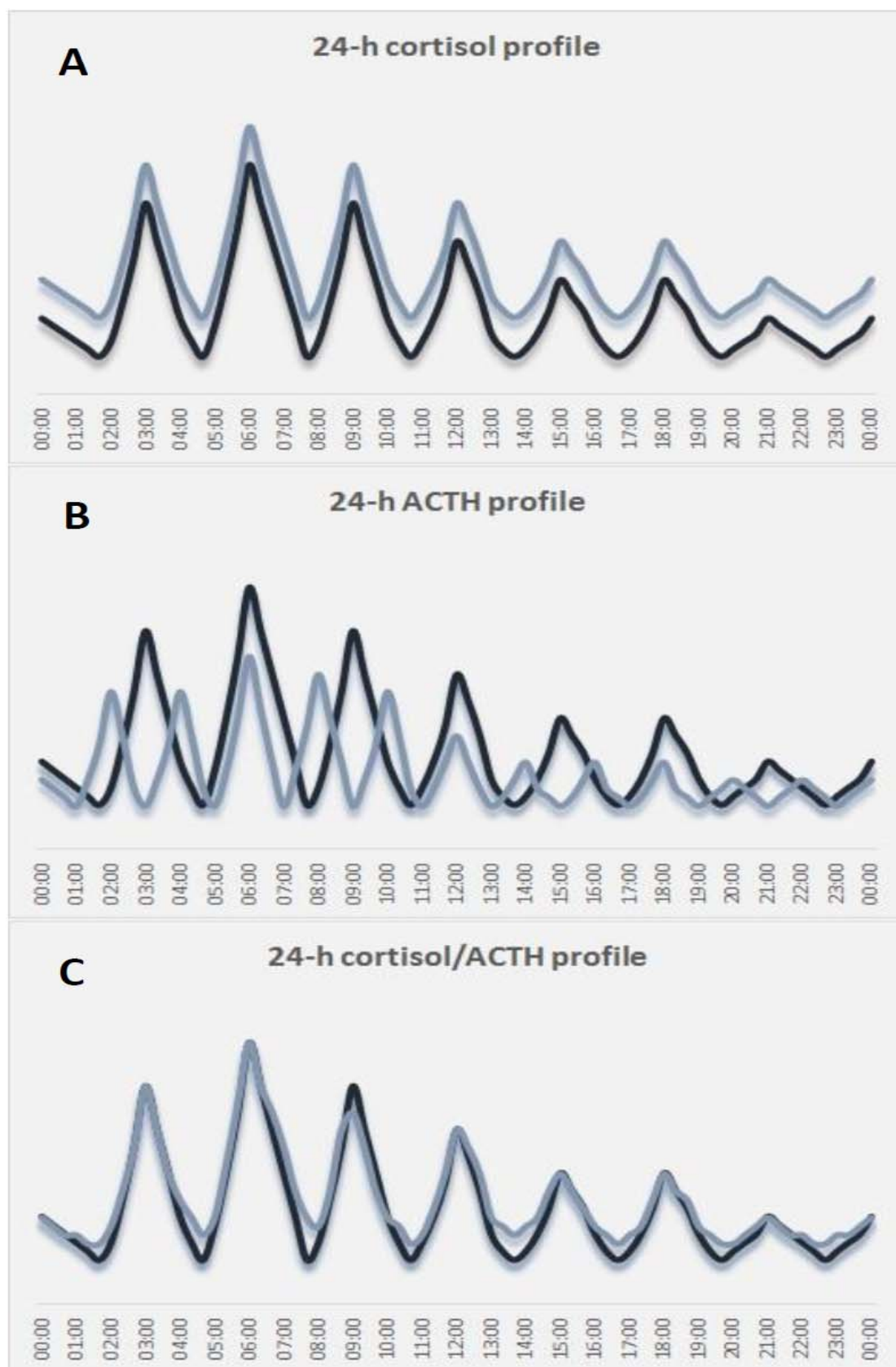


Figure 3

