Corticosteroids and the brain

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Abstract

The brain is continuously exposed to varying levels of adrenal corticosteroid hormones such as corticosterone in rodents and cortisol in humans. Natural fluctuations occur due to ultradian and circadian variations or are caused by exposure to stressful situations. Brain cells express two types of corticosteroid receptors, i.e. mineralocorticoid and glucocorticoid receptors, which differ in distribution and affinity. These receptors can mediate both rapid non-genomic and slow gene-mediated neuronal actions. As a consequence of these factors, natural (e.g. stress-induced) shifts in corticosteroid level are associated with a complex mosaic of time- and region-dependent changes in neuronal activity. A series of experiments in humans and rodents have revealed that these time- and region-dependent cellular characteristics are also reflected in distinct cognitive patterns after stress. Thus, directly after a peak of corticosteroids, attention and vigilance are increased, and areas involved in emotional responses and simple behavioral strategies show enhanced activity. In the aftermath of stress, areas involved in higher cognitive functions become activated allowing individuals to link stressful events to the specific context and to store information for future use. Both phases of the brain’s response to stress are important to face a continuously changing environment, promoting adaptation at the short as well as long term. We argue that a balanced response during the two phases is essential for resilience. This balance may become compromised after repeated stress exposure, particularly in genetically vulnerable individuals and aggravate disease manifestation. This not only applies to psychiatric disorders but also to neurological diseases such as epilepsy.

Variation in brain exposure to corticosteroid hormones

Humans or rodents that are exposed to potentially threatening situations (i.e. stressors, subjectively experienced as ‘stress’) are able to trigger a hormonal response that promotes adaptation. This hormonal response involves several well-described steps. Directly after stress, circuits involving the brainstem lead to activation of the sympathetic nervous system, causing enhanced release of adrenaline from the adrenal medulla (Wong et al. 2012). Indirectly, this results in increased release of noradrenaline from terminals in the brain. Slightly later, peptides such as corticotropin-releasing hormone and vasopressin are released from the median eminence, which evokes the secretion of adrenocorticotropic hormone from the anterior pituitary into the circulation (Herman et al. 2016). In the adrenal cortex, this causes
the synthesis and release of cortisol (the predominant corticosteroid hormone in humans) or corticosterone (in most rodents). These corticosteroid hormones reach many organs enabling the individual for example to have sufficient energy to face the challenge. The brain is also a prominent target of corticosteroid hormones. Through multiple pathways involving the pituitary and the hypothalamus (Tasker & Herman 2011, Dedic et al. 2018), the activation of this hormonal system is turned down. All in all, experiencing a stressful situation results in exposure of brain cells to waves of stress mediators, with specific yet overlapping time domains (Fig. 1).

Stress-induced waves of corticosteroids occur on top of diurnal fluctuations. Corticosteroid hormones, similar to many other hormones (Kalsbeek & Fliers 2013), show daily rhythms. In the case of cortisol and corticosterone, a diurnal peak is seen prior to awakening, which helps to coordinate bodily processes in anticipation of the active phase of the day (Russell et al. 2015). During the active phase, hormone levels gradually decline and reach a trough just before the onset of the inactive phase. Meticulous work over the past decades has demonstrated that the diurnal rhythm actually overarches multiple ultradian peaks, with inter-peak intervals of approximately 1h (Russel et al. 2015, Spiga & Lightman 2015). This cyclic pattern is caused by the delay between the activation and negative feedback of the hypothalamus–pituitary–adrenal axis, as was first predicted in a mathematical model and later demonstrated experimentally (Walker et al. 2012). The kinetic and dynamic properties of ultradian pulses are not static but are liable to changes for example in association with activation of the immune system and specific diseases (Spiga et al. 2017). It was shown that ultradian patterns in the circulation, at least in rodents, are maintained in the brain (Qian et al. 2012). The existence of ultradian pulses is important to achieve optimal transcriptional activity of neurons in response to corticosteroids (George et al. 2017) and modulates the degree to which organisms respond to stress (Sarabdjitsingh et al. 2010).

In sum, brain cells are not only exposed to stress-induced but also to diurnal and ultradian fluctuations in corticosteroid levels. In this review, we will mainly focus on changes in brain function related to shifts in corticosteroid hormones such as (may) occur after stress.
Rapid and delayed corticosteroid effects on neuronal activity

Exposure of brain cells to stress hormones will result in altered neuronal activity, after binding of the hormone to its receptor. The effect of monoamines and peptides is determined by the location of the terminals from which they are released in combination with the regional and subcellular distribution of the various receptor subtypes to which they bind as well as downstream signaling pathways (Joels & Baram 2009). Almost without exception, this involves G-protein-coupled receptors, which upon binding of the ligand mediate actions that develop within minutes and generally are short lasting, due to dissociation of the ligand from the receptor or other processes like internalization (Magalhaes et al. 2012). Still, secondary long-lasting actions frequently occur for example through the involvement of CREB (Chai et al. 2014).

In the case of corticosteroid hormones, the potential effects on neuronal activity are determined predominantly by the receptor distribution, since corticosteroids pass the blood–brain barrier quite well and basically reach all brain cells, although local enzymatic conversion and the degree of cell accessibility contribute to the actual intracellular concentration (Chapman et al. 2013). Corticosteroids bind to two receptor types in the brain, i.e. the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) (De Kloet et al. 2018). These belong to the family of nuclear receptors and act as transcriptional regulators. They either bind as homodimers – and possibly heterodimers (Nishi & Kawata 2007) – to specific gene sequences or they interact with other transcription factors, thus interfering with the function of these factors. Through both pathways, activated corticosteroid receptors alter gene expression profiles in a slow and persistent manner, targeting an estimated 1–2% of all genes at a time (Rubin et al. 2014). In this way, they affect brain function over the course of hours to days. The dwell-time of MRs in the nucleus is probably long lasting, whereas GRs are more rapidly bound and unbound to the DNA as well as liable to degradation by the proteasome (Conway-Campbell et al. 2012). This is not the only factor determining the duration of corticosteroid actions; the fate of the effector molecule is also important.

Transcriptional changes eventually are expected to alter the level of important molecules in brain cells, such as neurotransmitters, enzymes or receptors. This can be determined biochemically or with electrophysiological methods. Over the past decades, our group and others have studied the effects of corticosteroid receptor activation on electrical properties of brain cells, primarily in the hippocampus, but also in the basolateral amygdala (BLA) and prefrontal cortex (PFC). Most studies were carried out ex vivo, since detailed electrophysiological investigations to date are hard to perform in freely moving animals. For an overview, we refer to a comprehensive review on this topic (Joëls et al. 2012). Here, we will only highlight four general principles that emerged from this body of work.

First, in line with the genomically diverse targets, corticosteroid actions on electrical activity are pleiotropic, i.e. the hormone changes many cell properties at a time. This does not imply, though, that every cell property is equally sensitive to administration of corticosteroids. For example, a brief exposure of dorsal hippocampal CA1 pyramidal neurons to corticosterone increases particularly L-type calcium currents (Kerr et al. 1992, Chameau et al. 2007); an effect that develops with a delay of >1 h, requires DNA binding of GR homodimers and subsequent translational processing (Karst et al. 2000). Indirectly this also increases a calcium-dependent potassium current, which dampens firing frequency during bouts of excitatory input (reviewed in Joëls et al. 2012). This may contribute to normalization of earlier raised neuronal activity in the aftermath of stress. Yet, other voltage-dependent calcium currents, sodium currents or potassium currents that were tested turned out to be less susceptible to corticosteroid exposure.

Neurotransmitter signaling was also found to be altered by corticosteroids. For instance, hyperpolarization caused by activation of serotonin-1A receptors is increased after GR activation (Joëls et al. 1991), again involving receptor homodimers binding to the DNA (Karst et al. 2000). The effects of serotonin signaling were more pronounced than that on muscarinic receptor signaling (Joëls et al. 2012). Of note, none of the pathways affected by glucocorticoids has been fully resolved yet, that is, delineated all the way from the gene target to the functional consequences. The most extensively studied pathway (calcium signaling) suggests that the gene encoding for beta4 auxiliary subunits may form a preferential genetic target for GR homodimers (Chameau et al. 2007); enhanced translation of this subunit then promotes surface expression of L-type calcium channels, causing increased calcium current amplitude. But even in this well-investigated example, the evidence is still incomplete.

Second, the effects of corticosteroid hormones are region dependent. Again, calcium currents can provide a good example. While CA1 pyramidal neurons in the dorsal hippocampus show an increased L-type calcium current amplitude >1 h after a brief pulse of corticosterone,
this was not observed in granule cells of the dentate gyrus (Van Gemert et al. 2009). At the transcriptional level, neurons in the two areas responded in a comparable manner. Yet, at the protein and functional level, granule cells showed no effect of corticosterone exposure at all. This emphasizes that (i) signaling pathways downstream from the GR and (ii) the cellular context are important in determining the overall effect of corticosterone. Direct comparison of various brain areas with respect to the cellular responses to corticosteroids is often not possible based on the available literature, because very few studies focused on the same set of variables across various areas. The currently available information suggests that pyramidal neurons in the dorsal CA1 hippocampal area and in the ventromedial PFC respond in a comparable manner to GR activation, while principal neurons in the ventral hippocampus (Maggio & Segal 2012) and in the BLA show opposite effects.

Third, the dose dependency of corticosteroid actions seems to be related to the affinity of the receptors. Early binding studies demonstrated that the $K_d$ of MRs is in the subnanomolar range, whereas the $K_d$ of GRs is approximately tenfold higher. Low concentrations of corticosterone, such as circulate during non-stressful conditions at the circadian trough are sufficiently high to activate a substantial part of the MR, yet, too low to activate GR. When corticosteroid levels rise for example in anticipation of the active phase, they reach levels that are high enough to also activate GRs. Cellular studies in the dorsal hippocampal CA1 area have revealed that doses sufficient to activate MR but not GR generally result in effects that are opposite to those evoked by very high corticosterone concentrations. A U-shaped dose dependency was described for several cell properties that are sensitive to corticosteroids such as the amplitude of the L-type calcium current (Joëls 2006, Zoladz & Diamond 2008). Thus, in the absence of corticosterone, the amplitude of the L-type calcium current is high. Addition of low doses of corticosterone results in diminishment of the calcium current amplitude. When corticosteroid concentrations are increased, the calcium current amplitude increases again. Nearly all cells in the brain express GR, yet expression of MR is much more restricted. In those areas where MR expression is very low, the dose dependency of corticosteroid actions on cell function may be linear rather than U shaped (Joëls 2006).

Fourth, in addition to the slow, delayed effects of corticosterone, rapid actions have also been described. Early studies in the seventies and eighties already suggested that corticosterone can change cellular function within minutes (reviewed in Joëls et al. 2012). This was firmly proven by a series of experiments performed in the Tasker lab (Tasker et al. 2006). In parvocellular neurons of the paraventricular nucleus of the hypothalamus (PVN), corticosterone and dexamethasone were shown to decrease the release probability of glutamate-containing vesicles, via retrograde signaling involving the cannabinoid receptor-1. More recently, the involvement of GR rather than MR in these rapid corticosteroid actions was demonstrated, using conditional GR deletion (Nahar et al. 2015).

Dorsal hippocampal cells too can quickly respond to corticosterone (Karlo et al. 2005). However, the effects are in the opposite direction than found in the PVN and involve MR rather than GR. More specifically, corticosterone was shown to quickly and reversibly increase the frequency of miniature excitatory postsynaptic currents (mEPSCs), each of which represents the postsynaptic response to a (presynaptically) spontaneously released glutamate-containing vehicle. Interestingly, the concentration required to induce these rapid actions in the hippocampus is higher than that seen for gene-mediated actions via MR (Karlo et al. 2005). This suggests that MRs may function as a brain (or at least hippocampal) sensor for stress-dependent or ultradian shifts in corticosteroid concentration and translate these into functional changes. Indeed, dorsal hippocampal CA1 neurons accurately ‘follow’ a sequence of two high-amplitude pulses in corticosterone, i.e. show a brief increase in mEPSC frequency; the response to a third pulse was found to be somewhat attenuated, yet, application of a fourth pulse again induced a significant change in mEPSC frequency (Sarbajitsingh et al. 2016). Similar to what has been demonstrated for gene transcription, a sequence of hourly pulses may in fact be important to retain optimal (electrical) responsiveness to corticosterone (Sarbajitsingh et al. 2014). As is evident from the differences between dorsal hippocampal and PVN neurons, rapid actions by corticosterone are liable to regional differences, as was also found for the slow gene-mediated actions. In accordance, the rapid effects of corticosterone in principal neurons in the BLA are not identical to those seen in the dorsal hippocampus (Karst et al. 2010). In the BLA, mEPSC frequency is also rapidly enhanced by corticosterone, but the effects are long lasting. This changes the state of the cell such that a subsequent pulse of corticosterone now induces a different effect, that is, a decrease in mEPSC frequency. This phenomenon was called the ‘metaplasticity’ of corticosteroid actions.

Obviously, many more aspects will affect the overall effect of corticosterone on electrical properties of neurons.
For instance, the age and sex of animals may be important for the responsiveness to the hormone; the latter is relatively understudied. Similarly, life history and genetic background are likely to determine hormonal effects on neuronal activity. Examples illustrate that basal glutamate transmission in the hippocampus is altered when animals have a history of early life adversity (e.g. Loi et al. 2017). Also, many neuronal properties are influenced by chronic stress in adulthood (reviewed in Joëls et al. 2012).

The relevance of all these findings in very reduced preparations for neuronal circuits and behavior should be interpreted with caution. The ex vivo preparations lack most of their afferent fibers, which changes the context in which neurons respond. Moreover, a stress response involves many more hormones than only corticosteroids. These hormones not only change neuronal properties in their own right, but probably also influence the way in which some neurons respond to a wave of corticosterone. This was clearly demonstrated in BLA neurons, mimicking conditions of very mild-to-severe stress by consecutive waves of the β-adrenoceptor agonist isoproterenol and corticosterone (Karst & Joëls 2016; Fig. 2). Conditions mimicking low-to-moderate stress resulted in a brief increase in mEPSC frequency, followed by a suppression of mEPSC frequency for at least 2h. By contrast, application of very high doses of first isoproterenol and then corticosterone resulted in a long-lasting enhancement of the mEPSC frequency. Future studies will need to investigate the consequences of natural shifts in stress hormones for neuronal activity in freely moving animals.

**Balance between rapid and delayed effects**

The cellular studies so far underline that neuronal effects directly after stress – involving MR as well as receptors for monoamines and peptides that are released in response to stress – differ from genomic effects developing approximately 1 h later, which (as far as investigated) are mediated by GR. Whereas rapid effects seem particularly important for the activation of specific circuits in the brain, delayed effects may contribute to normalization of earlier enhanced activity. However, delayed actions of stress hormones do not only accomplish normalization of the earlier activated circuits, but also result in a specific set of actions that differs from the rapid effects.

Human neuroimaging studies have added insights to the cellular observations (obtained per region) by showing that shortly after stress energy resources seem to be redistributed within the brain, from areas involved in higher cognitive function toward the salience network and areas involved in habitual strategies, such as the caudate nucleus (Hermans et al. 2011, Schwabe 2017). Behavioral observations in (male) humans and rodents that addressed the rapid and delayed time domains after stress further elaborated this view. These studies showed that briefly (up to approximately 20min) after a peak in cortisol – related to stress or from exogenous
sources – emotional processing and distraction is enhanced, as is vigilance (Hermans et al. 2014; Fig. 3). The degree to which salient information is linked to the context is reduced and individuals revert to simple stimulus–response strategies in spatial learning tasks (Schwabe 2017). When exposed to reward-based decision-making situations, individuals act with a short-term perspective (Margittai et al. 2015) although this may be sex dependent (Van den Bos et al. 2009). When subjects are tested in the aftermath of stress, i.e. approximately one up to several hours after stress, their performance is entirely different; not only relative to the groups tested directly after stress, but also in comparison to controls, indicating that behavioral performance at that time not just reflects a restoration of the pre-stress state but taps into a separate repertoire. At this time, emotional processing and distraction are repressed; yet, contextualization is increased (Van Ast et al. 2013) and executive function facilitated (Hermans et al. 2014).

We argue that both phases of the cellular and behavioral stress response are necessary to optimally face challenges. When exposed to potentially threatening situations, individuals should be able to quickly act on salient stimuli and choose simple yet effective strategies for example to escape. This phase is linked to the classical fight-or-flight response. The second, later phase has received less attention, but is equally important: It literally helps to put salient situations in the right perspective, to rationalize events and choose more complex costly solutions that are beneficial (and remembered) in the long term.

The unbalanced stress system and disease

Dysfunction of either of these two behavioral phases may reduce the adaptive capacity of individuals. If one is not able to act directly in the face of a challenge, vulnerability may ensue; yet, when this initial response is too strong, the later phase may not be able to sufficiently contain it. Conversely, a ‘normal’ emotional response directly after stress may become a risk factor when not followed by a fully functional second phase, which normally would help to put things in the right perspective.

A dysfunctional secondary GR-dependent phase is predicted to occur for example in individuals with glucocorticoid resistance or when corticosteroid release after stress is attenuated (Flory & Yehuda 2015). This has been shown to occur in association with specific GR polymorphisms or haplotypes (DeRijk et al. 2011, Koper et al. 2014). This genetic predisposition may become more manifest in HPA axis disturbances when combined with multiple exposures to major life events, especially when these take place during development of the HPA axis or during brain development. Altered HPA-axis responsiveness has been described in relation to many psychiatric disorders, although the data are not entirely consistent and may depend on the diseased state. For instance, a meta-analysis of all studies reporting on cortisol release after experimental stress in psychiatric patient groups and healthy controls only revealed a significant attenuation in cortisol release of women with current major depressive disorder, but not in the remitted state (Zorn et al. 2017). Similar effects were observed in anxiety disorders. Interestingly, cortisol release tended to be altered in the opposite direction (in patients vs controls) in men as seen in women.

Figure 3
Effects of stress on cognition reported in literature have been measured with a range of cognitive tasks. (panel A) On the left, findings from 29 empirical research articles (yielding 35 observations) are summarized (gray circles). The effect sizes associated with stress induction or hydrocortisone administration reveal no systematic bias toward better or worse performance. This is also the case when data points are split for tasks related to executive control functions (EC, blue circles) or attentional vigilance-related functions (AV, red circles). When the timing factor is included (panel B), it is evident that the two systems are modulated in a reciprocal manner, with a negative rank order correlation over time for attentional vigilance functions and a positive correlation (at trend level) for executive control functions. In agreement with observations at the cellular level, trend lines cross after approximately 1 h (gray arrow), i.e. when genomic effects of corticosteroids start to develop. Reproduced, with permission, from Hermans et al. (2014).
Aberrant HPA-axis function is not only observed in association with psychiatric disorders, but also with a neurological disorder like epilepsy. It is well documented that a substantial proportion (approximately 50%) of individuals with epilepsy report more seizures in acutely stressful situations or periods of stress (McKee & Privitera 2017). In a large pediatric sample, we examined in what respect epilepsy patients with self-reported stress sensitivity of their seizure frequency differed from those who reported no link between seizure frequency and stress (Van Campen et al. 2012). Type of epilepsy, duration since onset, age and gender were all comparable between the groups. The only investigated factor that showed a difference after a multivariable analysis was the number of life events experienced so far. Since this concerned a pediatric sample, all life events took place during the vulnerable developmental period. Although not explicitly studied, these observations support that a potential (genetic) predisposition in a particular subgroup may amplify after several major stressors early in life.

A subsequent study revealed that children with stress-sensitive epilepsy – based on self-report or a 6-week diary – displayed a severely blunted cortisol release in response to psychosocial stress, not only compared to healthy controls but also to children with stress-insensitive epilepsy (Van Campen et al. 2015; Fig. 4). The blunted cortisol release could signify insufficient containment of the initial wave of activation following stress, increasing the probability for seizure development. Clearly, this requires more in-depth investigation. Data obtained in adults furthermore suggest that the brain may be very sensitive to cortisol in those having a stress-sensitive form of epilepsy, despite the presumably blunted cortisol response (Van Campen et al. 2016). Thus, interictal epileptiform activity was found to be positively correlated to salivary cortisol levels in this group, but not in epileptic patients with stress-insensitive seizure activity.

Although these and other studies support that HPA-axis abnormalities may amplify in vulnerable individuals over the course of life and occur in association with brain disorders, it has been much harder to prove causality for the latter. The current evidence falls into three categories. First, at the group-level, aberrant function of (components of) the HPA axis has been demonstrated prior to disease manifestation in those at risk. For instance, first-degree relatives of depressed individuals showed an aberrant dexamethasone-CRH response prior to the onset of any clinical symptoms (Friess et al. 2008). In the same vein, soldiers that developed PTSD after traumatic events differed in GR functionality prior to deployment from those that were resilient (Van Zuiden et al. 2012). Yet, predicting vulnerability at the level of a single individual based on genetic risk and/or environmental exposure is not yet reliable. The predictive power may increase with the advancement of our insight in critical factors.

A second line of evidence stems from the effectiveness of compounds acting on the HPA axis to reverse disease...
manifestation or impaired brain function. This evidence is particularly strong in rodent models of early life adversity or chronic stress. For instance, hippocampal neurogenesis is suppressed by chronic unpredictable stress in rats; yet, this is fully prevented by a brief treatment with GR antagonists (Hu et al. 2012, Zalachoras et al. 2012). Similarly, we recently showed that increased fear memory in adult mice exposed to adverse early life conditions was fully normalized by a 3-day treatment with the GR antagonist mifepristone just prior to the onset of puberty (Arp et al. 2016). Comparable effective normalization of early life associated abnormalities in rodents was demonstrated for CRH antagonists (Ivy et al. 2010). The effectiveness of such compounds in human disease is less convincing. Although early studies supported rapid actions by mifepristone (a GR antagonist) in psychotic depression, replication studies in larger cohorts failed to demonstrate significant effects, unless the concentrations of the drugs were very high (Moraitis et al. 2017).

Finally, there is growing body of evidence showing that (genes involved in) stress functionality may predict treatment responsiveness. This can be nicely illustrated by a recent translational study (Carrillo-Roa et al. 2017). In a murine model for depression, peripheral transcriptome profiles of good and poor responders to antidepressant treatment were compared, and this revealed a cluster of 259 differentially regulated genes. Based on the murine transcript signature, human orthologues were selected to examine differences in expression profiles of depressed individuals from baseline to week 12 of treatment. The selected set of genetic variants, which showed a significant enrichment of GR-regulated genes, allowed response prediction with an accuracy of 76%.

In conclusion, dysfunctional elements in (molecules involved in) the HPA axis, particularly related to the balance between MR- and GR-mediated events, may predispose individuals to psychiatric and neurological disease onset, a process than can be amplified by multiple life events. This predisposition is probably not restricted to brain disorders but most likely also applies to diseases involving other tissues reached by corticosteroid hormones, such as the vascular system or fat cells. Future prospective studies in large population cohorts, with data on genetic background and the exposome, may unravel the critical elements in this cascade and thus provide leads for potential prevention or treatment strategies.


