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REVIEWS

Glucocorticoid Receptor Mediated Stress Signaling in the Prefrontal Cortex

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Abstract

Stress reflects physiological or psychological displacement from homeostasis. In mammals, stressors activate the hypothalamic-pituitary-adrenal axis (HPA axis). HPA axis activation provides the nervous system with the required signals to respond to the stressor. In the brain, the response to HPA-axis activation is largely mediated by the glucocorticoid receptor (GR). GR orchestrates the transcriptional changes required for long term adaptation to the stressor in addition to ending the stress response via a negative-feedback circuit. This adaptation and feedback includes modulation of brain regions implicated in cognition and emotion. One of these brain regions is the prefrontal cortex (PFC). Acute and chronic stress are both known to affect PFC regulation of cognitive processes. Elucidating how the GR influences processing in the PFC is important for understanding the stress response; however, the mechanisms remain incompletely defined. This review presents current knowledge on the PFC and GRs as well as areas for future investigation into the PFC-GR interaction in regulation of cognition and emotion.

Keywords

Glucocorticoid receptor
Stress
Prefrontal cortex
Cognition
Emotion

The glucocorticoid stress response

The stress response. Stress reflects physiological or psychological displacement from homeostasis [1,2]. Encountering a "stressor" (either physical or psychological) can result in adaptive physiological changes known as the stress response. In mammals the stress response activates the hypothalamic-pituitary-adrenal (HPA) axis. The purpose of HPA axis activation is to maximize the energy resources needed to drive the response to the stressor. Neurons in the hypothalamus secrete corticotrophin releasing hormone (CRH) onto the anterior pituitary gland. CRH then binds to its receptor resulting in the release of adrenocorticotropin releasing hormone (ACTH) from the pituitary gland. In response to ACTH the adrenal cortex releases membrane permeable glucocorticoids (GCs) into the bloodstream. Once released from the adrenal gland GCs bind to either mineralocorticoid receptors (MRs) or glucocorticoid receptors (GRs) throughout the brain. Both MR and GR are transcription factors that normally reside in the cytosol. MRs have a high binding affinity for GCs and they are normally bound by the basal plasma GC levels making them less available for activation via stress induced rises in GC. However, GRs have a lower affinity for the ligand, and thus remain relatively unbound at basal GC levels. Since the MR's are already bound when GC levels rise, the stress response is believed to be primarily mediated by GRs[3,4]. GR is ubiquitously expressed; however, brain regions such as the PFC (see Fig. 1) contain a higher density of GR. Denser expression may be an indicator of the receptors importance in the PFC, particularly in the stress response.

The role of prefrontal cortex in the stress response

Prefrontal cortex function, anatomy, and homology. A large body of evidence implicates the PFC in the stress response. The PFC regulates cognitive and emotional processes by integrating past information from long term storage with current information before deciding on and initiating the optimal response via **top-down regulation**[a]. The ability to process past and present information has been coined "working memory"[6]. While the PFC is not the only brain region where cognitive functions are processed, a majority of human imaging studies indicate the PFC as the main site of working memory processing and decision making[7].

There is consensus that although the homologous regions in lower level primates are not as developed as those of humans, the type of information being processed is similar. For example, macaque monkeys trained to perform a modified version of the **Wisconsin Card Sorting Task**[b] (WCST) display PFC activation similar to that exhibited

a. **Top-down regulation**: Control of lower order process by higher order regions.

b. **Wisconson Card Sorting Task**: a classic rule based test of attention in which changes to the "rules" must be recognized by the participant. The participants score is determined by how long it takes them to learn the new rule.

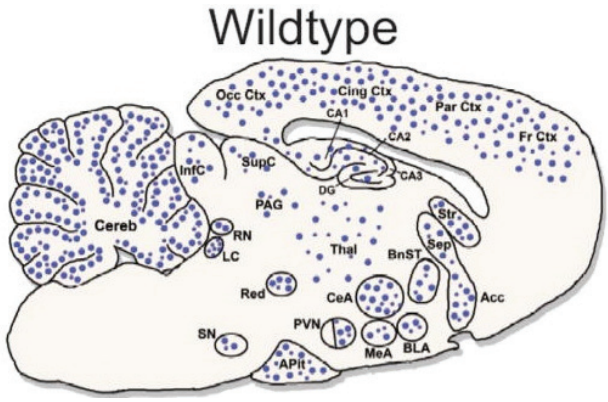

Figure 1. Regions of dense GR expression. Acc-nucleus accumbens; APit-anterior pituitary gland, BLA- basolateral nucleus of the amygdala; BnST- bed nucleus of the stria terminalis; CA1, CA2, CA3- hippocampal areas CA to CA3; InfC- inferior colliculus; LC- locus coeruleus; CeA- central nucleus of the amygdala; Cerb-cerebellum; Cing Ctx- cingulate cortex; DG- dentate gyrus; Fr Ctx- frontal cortex; PAG- periaqueductal gray; Par Ctx- parietal cortex; PVN- parventricular hypothalamic nucleus; Red- Red nucleus; Rn- raphe nuclei; Sep- septum; SupC- superior colliculus; SN- substantia nigra; Stri- striatum; Thal- thalamus. (Reproduced with permission[5])

in human controls[8]. For rodents however, the concept of a homologous PFC region has only recently begun to be accepted. By comparing the anatomical connectivity of the rodent and primate PFC areas, researchers have determined subdivisions in rodents that exhibit similar projection patterns[9]. Specifically, the granular medial portion of the rodent PFC has three distinct subregions which have the same connectivity as the primate PFC. The anterior cingulate cortex (ACC) is in the dorsal subdivision of the region and its projections are known to result in occulomotor movements. The ventral subdivision contains the prelimbic (PL) and infralimbic (IL) cortices which are implicated in cognitive and emotional regulation based on their connectivity with the amygdala, **mediodorsal thalamus**[c], **reunions nuclei**[d], and other **limbic system**[e] related structures[9,10]. While the ACC is considered part of the homologous PFC structure, this review will focus on the role of PL and IL in the stress response (each of which differentially regulate neuronal processes through their unique connectivity).

Early studies examined the functions of PL and IL by selectively lesioning one of the regions and examining the effects in stress-related behavioral assays. Lesions to PL diminish performance in tasks that involve delays (thus requiring functioning working memory) increase anxiety-like behaviors[11]. PL lesions do not however, affect performance on non-delayed tasks. Lesions to IL have the opposite effect on anxiety-related behaviors. Further distinctions between the two regions have been found using **fear conditioning**[f] **and extinction**[g] paradigms. Researchers found that inactivation of PL resulted in an inability for rats to express fear to stimuli that were previously paired with a shock[12]. However, PL inactivation did not diminish the response to innately feared stimuli. Further investigation revealed that IL inactivation impairs the ability to undergo extinction acquisition and develop an extinction memory[13]. IL has also been found to be important in the development of the stress resiliency that arises from environmental enrichment (EE)[14]. Lesions to IL prior to EE (although not after) prevented rats from developing the superior positive behavioral responses to chronic stress that were developed in control (but EE exposed) rats. Further differences between PL and IL have been demonstrated for autonomic responses. Under basal conditions, inactivation of neither PL nor IL produces cardiovascular changes[15]. However, when rats are administered restraint stress, rats with PL inactivation exhibit an elevated heart rate, while rats with IL inactivation exhibit a diminished response (compared to controls). It is possible that these PL- and IL- mediated stress-induced changes in behavior and physiology are regulated by GR activation.

The role of GR in the stress response

Activation of GR. When GC binds to GR the receptor undergoes a conformational change which results in its translocation to the nucleus. Once translocated, GR can affect the intracellular environment either as a dimer or a monomer. As a dimer it can bind the **glucocorticoid response element**[h] located on the promoter region of target genes leading to

c. **Mediodorsal thalamus**: Nucleus which plays a major role in relaying information from limbic regions to association cortices.

d. **Reunions nuclei**: Thalamic nucleus that relays signals from them PFC and the hippocampus

e. **Limbic system**: Network of brain regions that process and regulate cognition, memory and emotion -related stimuli

f. **Fear conditioning**: Learning paradigm in which a neutral stimulus is paired with a feared stimulus. Acquisition occurs when the neutral stimulus becomes feared.

g. **Fear extinction**: Learning paradigm in which the response a conditioned feared stimulus is learned to no longer be predictive of a noxious stimulus.

h. **Glucocorticoid response element**: region of a gene that activates its transcription when it is bound by GR.

transactivation[i] or **transrepression**[j][16,17]. As a monomer it can downregulate transcription via transrepression[18,19]. At numerous sites throughout the brain, binding of GCs to GRs facilitates adaptive changes to the stressor and restores the stress response to baseline[5] through negative-feedback on the HPA axis. Global and region specific manipulations of GR have been used to experimentally dissect the role of GR in the stress response.

Targeting GR in animal models. GR is encoded in the Nr3c1 gene. Of Nr3c1's 9 exons, exon 2 is the main transcriptional activation domain, and exons 3 and 4 are responsible for homodimerization and DNA binding[20]. Nr3c1 is ubiquitously expressed throughout the central and peripheral nervous system and early investigations revealed that prenatal global deletion of GR resulted in perinatal death as a result of GRs role in the periphery[21]. The first non-lethal deletion methods were developed in 1998[22]. One involved a point mutation in the DNA binding domain, and the other utilized the **cre-lox system**[k] and flanked Exon 3 with LoxP sites. In 2003, another method for non-lethal deletion of Nr3c1 was demonstrated[23]. In this model exon 2 of Nr3c1 was flanked with LoxP sites (Fig. 2). The cre-inducible methods allowed for local deletions dependent on where the cre enzyme was expressed. These cre-inducible methods of generating GR knockouts (GRKOs) along with several other methods have allowed for investigations into the function of the receptor.

Initial Studies on physiological effects GR activation. The initial animal models used to study the role of GR in the stress response involved gross expression of **antisense**[l] GR mRNA, gross expression of GR protein that lacked the DNA binding domain, or conditional knockouts (KO) using a region-specific promoter. Studies involving the antisense GR mRNA expression revealed depression-related cognitive deficits[24]. Mice with point mutations to Nr3c1 that prevented the formation of GR homodimers and DNA binding display diminished spatial memory capacity[25]. Using the cre inducible model (see Fig. 2), a forebrain GRKO mouse model was developed. Investigation of this model revealed a depression and despair phenotype[26, 27]. However, across most of these studies conflicting results concerning the interpretation of the anxiety-phenotype were reported with many of the mice exhibiting reduced anxiety-phenotypes in some behavior paradigms and heightened anxiety responses in others[3]. The confusion around the exact effect may result from the recently discovered fact that GR activation has different effects based on which region of the brain it is activated in and the conditions underlying the activation. Recent studies have begun to target GR in specific brain regions to elucidate its many roles.

Region specific GR studies. A majority of the region specific GR research has been performed in the hippocampus. Electrophysiological experiments have revealed that GR activation enhances **miniature excitatory postsynaptic currents**[m] (mEPSCs) in CA1 pyramidal neurons[28] for 2-4 hours post-administration. Increased amplitude of mEPSCs at the postsynaptic terminal of GR activated cells results in enhanced signaling, a correlate of **long term potentation**[n] (LTP). However GR also plays a role in decreasing the responsiveness of a cell. **Long term depression**[o] (LTD) in CA1 was found to be dependent on GR activation[29]. Under conditions of low synaptic input post-stress GR works to diminish the cell's responsiveness to incoming signals, as opposed to placing the synapse in a ready to receive state. How can GR accomplish both of these seemingly contradictory actions in CA1? **AMPA receptors**[p] (AMPARs) are mediators of both LTD and LTP. GR activation results in increased trafficking of AMPARs to the postsynaptic terminal[30]. If the synapse is receiving basal levels of input then this increase in AMPARs allows for increased synaptic efficacy. In addition to increasing surface AMPARs, GR activation decreases the threshold by which **NMDA receptors**[q] (NMDARs) initiate NMDAR dependent AMPAR endocytosis[31]. The decrease

i. **Transactivation**: biological process that results in increased rate the target genes expression

j. **Transprepression**: biological process that results in the decreased rate the target genes expression.

k. **Cre-lox system**: A molecular tool used to regulate gene transcription. In the presence of cre recombinase, DNA located between inserted lox P sites is excised.

l. **Antisense**: mRNA that contains the complementary sequence. The antisense mRNA binds the endogenous RNA, blocking translation.

m. **Miniature excitatory postsynaptic currents**: positively charged flow of ions in the absence of presynaptic depolarization.

n. **Long term potentiation**: Prolonged enhancement in signal transmission resulting for simultaneous stimulation of connected neurons

o. **Long term depression**: Prolonged reduction in signal transmission. It can be induced by numerous signal strengths dependent on the brain region the neurons are located in.

p. **AMPA receptors**: ionotropic glutamate receptor that allows cation intracellular influx. It is responsible for fast signaling at the synapse.

q. **NMDA receptors**: ionotropic glutamate receptor that allows cation intracellular influx. Its opening is also voltage dependent thus only signals above a threshold will activate the receptor.

in surface AMPARs is concomitant with LTD as the terminal is less responsive to incoming signals. Thus GR is able to generate a synaptic environment that optimizes either LTD or LTP depending on the nature of the incoming pre-synaptic signals. It is known that GC signaling leads to decreased viability of CA1 neurons[32]. With GR mediating both of these processes what would occur following a stressor if GR was not regulating the environment? Twenty-four hours following a traumatic brain injury, rats administered a GR antagonist show no loss of CA1 pyramidal neurons while control mice showed losses of ~30%[33]. Therefore GR is likely responsible for cell death in CA1 neurons following chronic stress. Thus, in the absence of synaptic input, GR activation bypasses LTD instead functioning to assist in the elimination of the inactive neuron.

GR research done in other limbic brain regions revealed that responses to GCs vary for each region[34]. Research into the effects of stress on **dentate gyrus**[r] (DG) pyramidal neurons revealed no change in calcium currents in response to 20 min GC exposure, while CA1 pyramidal neurons exhibited increased currents in response to the same stimulus[35]. GRs are present in high density in both the DG and CA1 indicating that it was not a difference in GR expression levels that caused the different responses in the DG and CA1. Rather, it was a difference in the stimulus induced expression of protein that resulted in the effect. While GR activation in CA1 neurons results in increased calcium channel Cav1.2 expression, in the DG GR does not upregulate transcription of that channel. In the basolateral amygdala (BLA), GR was found to enhance neuronal excitability over several hours, unlike the short term enhancement demonstrated in CA1[36]. In addition, under conditions of chronic stress, BLA GRs have the opposite effect on neuronal excitability. These physiologic responses to GR activation likely underlie behavioral responses. Kolber and colleagues found that deletion of CeA GRs results in decreased cFos expression and diminished fear conditioning[37]. The findings of GRs effects in these regions have important implications for its possible role in the PFC.

Known Role of GR in the PFC. Early investigations to elucidate the role of GR in the PFC revealed it to be a negative-feedback site. Dioro and colleagues found that lesions to the PFC result in elevated plasma GC levels in rats exposed to a 20 minute restraint stress[38]. Compared to the control group, the PFC lesioned rats exhibited a diminished ability to reduce the stress-induced rise in GC. A later study examined the effects of chronic stress on GR expression in the PFC. Rats were exposed to 4 weeks of chronic stress resulting in significant reductions in total GR mRNA expression compared to non-stressed controls[39]. Although overall mRNA was reduced, the researchers reported significant increases in nuclear GR and reductions in cytosolic GR.

Within the last decade, researchers have begun to examine the effects of GR activation on PFC structure, transcription, and function. Rats exposed to repeated restraint stress exhibit reduction in apical dendritic spine density, as well as apical dendritic length in PFC neurons[40,41]. Consistent with this finding, previous research has shown that chronic activation of GR (via **dexamethasone**[s]) results in behavioral dysfunction in working memory as well as atrophy and neuron loss in **layer II/III**[t] of PFC[42]. PFC neuronal expression of CRH mRNA has been shown to negatively correlate with chronic PFC GR activation[43]. Meng and colleagues were able to demonstrate a direct recruitment of the CRH promoter by GR and propose it as the mechanism by which activated GR reduces CRH mRNA expression. Similar to its actions in CA1, acute GR activation enhances the amplitude of NMDAR and AMPAR **excitatory postsynaptic currents**[u] in response to glutamate[44]. GR potentiation of those responses is responsible for the working memory enhancement associated with acute stress. However, chronic stress has the opposite effect on working memory. The working memory deficit in chronically stressed rats is correlated with diminished plasticity in hippocampal-PFC synapses, possibly through a similar NMDAR-AMPAR-GR regulated molecular signaling pathway[45].

Summary and future directions

The role of GR in the stress response has been heavily investigated. These studies have revealed that the long-lasting behavioral and cellular changes that result from GR activation are region specific[34]. It is possible that region specific GR mechanisms are responsible for proper regulation of each limbic system nucleus[13]. GR-mediated molecular pathways are currently being investigated and results demonstrate the involvement of a multitude of proteins including ERK1/2, MSK[46], and EGR1[47], which are which are not only regulated by classic **genomic**[v] GR activity, but also by rapid non-genomic GR mechanisms. For example, GR-

r. **Dentate gyrus**: Part of the hippocampal formation

s. **Dexamethasone**: a potent synthetic glucocorticoid receptor agonist

t. **Layer II/III**: largely responsible for intercortical signaling

u. **Excitatory postsynaptic currents**: influx of cations resulting from presynaptic depolarization.

v. **Genomic**: classical regulation through gene transcription (ie GR binding to GRE)

dependent **epigenetic**[w] modifications are being uncovered providing a mechanism by which GR can affect the cellular environment in a matter of minutes[46,48]. Ongoing research will determine how GR activation in the PFC can result in enhanced functioning, as well as how dysregulation of GR signaling leads to impairments in working memory such as those exhibited in many psychiatric illnesses associated with PFC dysfunction[49, 50].

References

1. Joels M and Baram TZ (2009). The neuro-symphony of stress. Nat. Rev. Neuro. 10: 459-466.
2. de Kloet ER, Joels M and Holsboer F (2005). Stress and the brain: From adaptation to disease. Nat. Rev. Neuro. 6: 463-475.
3. Kolber BJ, Wieczorek L and Muglia LJ (2008). HPA axis dysregulation and behavioral analysis of mouse mutants with altered GR or MR function. Stress. 11(5): 321-338.
4. Holsboer F and Ising, M (2010). Stress hormone regulation: Biological role and translation into therapy. Annu Rev Psychol. 61: 81-109.
5. Kolber BJ, (2009). Defining brain region-specific glucocorticoid action during stress by conditional gene disruption in mice. Brain Research 1293: 85-90.
6. Baddley A (2003). Working memory: Looking back and looking forward. Nat. Rev. Neuro. 4: 829-839.
7. Duncan J and Owen A (2000). Common regions of the human frontal lobe recruited by diverse cognitive demands. Trends in Neuroscience. 23: 475-483.
8. Nakahara K, Hayashi T, Konishi S and Miyashita Y (2002). Functional MRI of macaque monkeys performing a cognitive set-shifting task. Science. 295: 1532-1536.
9. Vertes RP (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse. 51:32-58.
10. Vertes RP (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience. 142:1-20.
11. Jinks AL and McGregor IS (1997). Modulation of anxiety-related behaviors following lesions of the prelimbic and infralimbic cortex in the rat. Brain Research. 772: 181-190.
12. Corcoran KA and Quirk GJ (2007). Activity in prelimbic cortex is necessary for the expression of learned but not innate fears. J. Neurosci. 27(4): 840-844.
This paper shows a distinct role for the PFC in fear learning. In addition, the researchers distinguish between the expressions of different types of fear.
13. Sierra-Mercado D, Padilla-Coreano N and Quirk GJ (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology. 36: 529-538.
14. Lehmann ML and Herkenham M (2011). Environmental enrichment confers stress resiliency to social defeat through an infralimbic cortex-dependent neuroanatomical pathway. J Neurosci. 31(16): 6159-6173.
15. Tavares RF, Correa FMA and Resstel LBM. (2009). Opposite role of infralimbic and prelimbic cortex in the tachycardiac response evoked by acute restraint stress in rats. Journal of Neuroscience Research. 87: 2601-2607.
16. Dahlman-Wright K., Wright A, Gustafsson J and Carlstedt-Duke J (1991). Interaction of the glucocorticoid receptor DNA-binding domain with DNA as a dimer is mediated by a short segment of five amino acids. J Bio Chem. 266(5): 3107-3112.
17. Schoneveld OJLM, Gaemers IC and Lamers WH (2004). Mechanisms of glucocorticoid signaling. Biochimica et Biphysica Acta. 1680: 114-128.
18. Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P, Schutz G (1998). DNA binding of the glucocorticoid receptor is not essential for survival. Cell. 93: 531-541.
19. Tuckermann JP, Reichardt HM, Arribas R, Richterm KH, Schutz, G and Angel P (1999). The DNA binding-independent function of the glucocorticoid receptor mediates repression of AP-1 dependent genes in skin. J Cell Bio. 147(7): 1365-1370.
20. Mittelstadt PR and Ashwell JD (2003). Disruption of glucocorticoid receptor exon 2 yields a ligand-responsive C-terminal fragment that regulates gene expression. Mol Endocr. 17(8): 1534-1542.
21. Cole TJ, Blendy JA, Monaghan A, Krieglstein K, Schmid W, Aguzzi A, Fantuzzi G, Hummler E, Unsicker K and Schutz G (1995). Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. Genes & Development. 9: 1608-1621.
22. Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, Bock R, Klein R and Schutz, G. (1998). Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nature Genetic. 23, 99-103.
23. Brewer JA, Khor B, Vogt SK, Muglia LM, Fujiwara H, Haegle KE, Sleckman BP and Muglia LJ (2003) T-cell glucocorticoid receptor is required to suppress COX-2-mediated lethal immune activation. Nature Medicine. 9(10): 1318-1322
24. Montkowski A, Barden N, Wotjak C, Stec I, Ganster J, Meaney M, Engelmann M, Reul JMHM, Landgraf R, Holsboer F (1995). Long-term antidepressant treatment reduces behavioral deficits in transgenic mice with impaired glucocorticoid receptor function. Journal of Neuroendocrinology. 7: 841-845.
25. Oitzl M, Reichardt H, Joels M and de Kloet ER (2001). Point mutation in the mouse glucocorticoid receptor preventing DNA binding impairs spatial memory. Proc. Natl. Acad. Sci. 98(22): 12790-12795.
26. Boyle MP, Brewer JA, Funatsu M, Wozniak DF, Tsien JZ, Izumi Y and Muglia LJ (2004). Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. Proc. Natl. Acad. Sci. 102(2): 473-478.
27. Boyle MP, Kolber BJ, Vogt SK, Wozniak DF and Muglia LJ (2006). Forebrain glucocorticoid receptors modulate anxiety-associated locomotor activation and adrenal responsiveness. J. Neurosci. 27(7): 1971-1978.
28. Karst H and Joels M (2005) Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal neurons. J Neurophysiol. 94: 3479-3486.
29. Xu L, Holscher C, Anwyl R and Rowan MJ (1998). Glucocorticoid receptor and protein/RNA synthesis-dependent mechanisms underlie the control of synaptic plasticity by stress. Proc. Natl. Acad. Sci. 92: 3204-3208.
30. Martin S, Henley JM, Holman D, Zhou M, Wiegert O, van

w. **Epigenetic**: alterations in gene expression without modifying the genes DNA sequence.