

REVIEW

## Hypothalamic–pituitary–adrenal axis dysregulation and behavioral analysis of mouse mutants with altered glucocorticoid or mineralocorticoid receptor function

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### Abstract

Corticosteroid receptors are critical for the maintenance of homeostasis after both psychological and physiological stress. To understand the different roles and interactions of the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) during stress, it is necessary to dissect the role of corticosteroid signaling at both the system and sub-system level. A variety of GR transgenic mouse lines have recently been used to characterize the role of GR in the CNS as a whole and particularly in the forebrain. We will describe both the behavioral and cellular/molecular implications of disrupting GR function in these animal models and describe the implications of this data for our understanding of normal endocrine function and stress adaptation.

MRs in tight epithelia have a long established role in sodium homeostasis. Recently however, evidence has suggested that MRs in the limbic brain also play an important role in psychological stress. Just as with GR, targeted mutations in MR induce a variety of behavioral changes associated with stress adaptation. In this review, we will discuss the implications of this work on MR.

Finally, we will discuss the possible interaction between MR and GR and how future work using double mutants (through conventional means or virus based gene alteration) will be needed to more fully understand how signaling through these two steroid receptors provides the adaptive mechanisms to deal with a variety of stressors.

**Keywords:** *Anxiety, depression, hippocampus, knockout, memory, over-expression*

### Introduction

Any imbalance in an organism's physical or psychological wellbeing creates a stress response that involves multiple systems and whose activation allows appropriate adaptation to the disturbance. Stress can be beneficial in that it can create a situation of increased arousal and emotional salience enabling the organism to appropriately respond to the stressor and ensure survival. However, under states of dysregulation or after chronic activation, stress can be maladaptive and can place the body in a state of increased susceptibility to illness or disease. For instance, early-life stress can induce changes in the endocrine stress response that lead later to increased incidence of depression

(Nemeroff 2004). Furthermore, severe stress in adulthood is associated with precipitation of the onset of psychiatric illness (Corcoran et al. 2003). Of the many systems involved in the mammalian stress response, one of the most important and intensely studied is the endocrine system comprising the hypothalamic–pituitary–adrenal (HPA) axis.

#### *The HPA axis*

During stress, the HPA axis is activated through afferent sympathetic, parasympathetic and limbic circuits that induce parvocellular neurons in the paraventricular nucleus (PVN) of the hypothalamus

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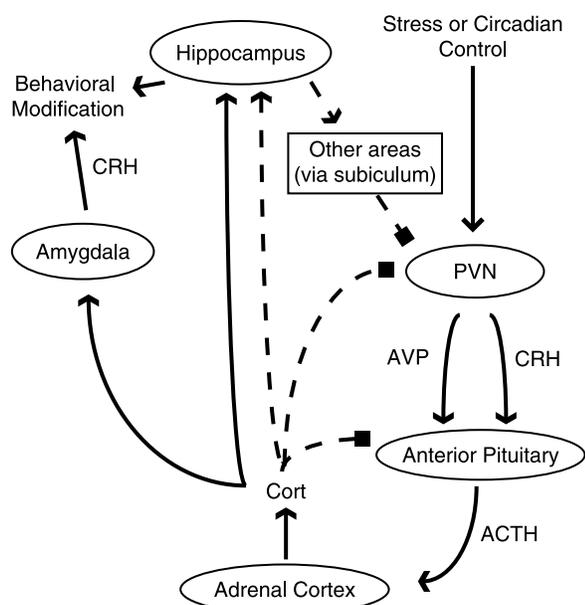


Figure 1. HPA axis activation and feedback control. Stressful stimuli and circadian gating stimulate neurons in the PVN of the hypothalamus to secrete CRH and AVP into the hypothalamic-pituitary portal system. Binding of these peptides to their receptors on the corticotrophs in the anterior pituitary gland, causes the release of ACTH, which then induces the release of corticosterone (Cort) from the adrenal cortex. Corticosterone then feeds back onto the anterior pituitary gland, and the CNS at the level of the PVN and the hippocampus, to inhibit HPA axis activation. In addition, corticosterone binds to additional GR populations in the amygdala and hippocampus to modulate a variety of behaviors. Negative feedback loop = ---; excitatory projection =  $\rightarrow$ ; inhibitory projection = ■.

to release two neuropeptides, corticotropin-releasing hormone (CRH) and vasopressin (AVP) which enter the hypothalamic-pituitary portal system (Figure 1). Binding of these neuropeptides to their receptors on corticotrophs in the anterior pituitary gland causes the secretion of adrenocorticotrophic hormone (ACTH), produced from pro-opiomelanocortin (POMC), into the general circulation. The binding of ACTH to receptors in the adrenal cortex causes the systemic release of cortisol/corticosterone (glucocorticoid). Glucocorticoid levels are controlled through a negative feedback loop when glucocorticoid binds to receptors at the level of the PVN, the anterior pituitary gland and the hippocampus causing a down-regulation of HPA axis activity.

A variety of stressors can activate the HPA axis causing a non-circadian release of glucocorticoid. Stressful circumstances force an organism to quickly adapt behavior and physiological processes to survive and to restore homeostasis. Chronic alterations in this adaptation are considered to promote a state of altered allostatic load that can result in the development of a maladaptive psychiatric and physical state (McEwen 2001). The HPA axis responds to two main types

of stressors, physiological and psychological stressors. Physiological or interoceptive stressors are usually homeostatic challenges sensed by the somatic, visceral or circumventricular organ pathways, while psychological or exteroceptive stressors are external challenges that contain species- and individual-specific characteristics.

In addition to the duration and type of stressor, timing of the onset of a stressor with respect to the circadian cycle can greatly influence the HPA response to stress. Glucocorticoid and ACTH secretion display dynamic patterns of release throughout the 24-hour period that are characterized by increased hormone levels prior to daily activity onset (circadian peak in the morning for humans and in the evening for many rodents, including mice and rats). In addition, the circadian pattern exhibits smaller pulses during the circadian trough and higher pulses during the circadian peak (Carnes et al. 1989; Windle et al. 1998). This ultradian pulsatile release of glucocorticoid has been shown to profoundly influence the glucocorticoid response to stress. When acute stress coincides with the rising phase of a pulse, resulting corticosterone concentrations exhibit a significantly greater increase than when the stress coincides with the falling phase (Windle et al. 1998). This suggests that the basal pulsatile activity of the HPA axis should be considered when interpreting the differential HPA responses seen after stress exposure.

A number of lines of evidence have indicated that hyperactivity of the HPA axis is an important correlate of mood disorders. Compared with healthy individuals, depressed patients often have elevated levels of plasma cortisol (Carpenter and Bunney 1971; Brown et al. 2004), enlarged adrenal glands (Nemeroff et al. 1992), increased PVN CRH content (Raadsheer et al. 1994; Blanchard et al. 2001) and impaired feedback inhibition of the HPA axis as measured by the dexamethasone suppression test (DST; Carroll et al. 1980; Holsboer et al. 1982). The DST interrogates the integrity of feedback inhibition on the HPA axis. In normal adults, a moderate (e.g. 1 mg) dose of dexamethasone, a glucocorticoid receptor (GR) agonist, will induce a dramatic reduction in circadian peak plasma cortisol level. However, many depressed patients often maintain elevated levels of cortisol in the DST, which implicates impaired negative feedback in depression. Furthermore, individuals afflicted with primary disorders of the HPA axis, such as Cushing's disease (i.e. hypercortisolemia), are at a much greater risk for developing depression (Sonino and Fava 2001), demonstrating that a dysregulation of the HPA axis may be involved in the pathogenesis of the disorder. Finally, successful antidepressant treatment is highly correlated with a reversal of HPA axis disruption (Pariante and Miller 2001).

Glucocorticoids released in response to a stressor act at a variety of locations throughout the body.

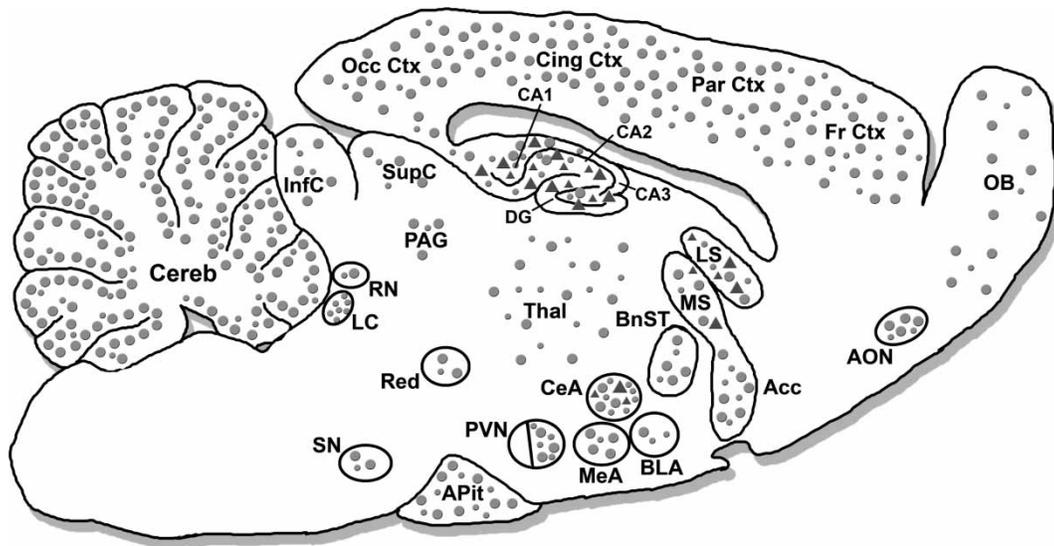


Figure 2. Schematic illustration of GR and MR expression in the rodent brain and anterior pituitary gland. GR is ubiquitously expressed throughout the brain, showing higher expression in a number of important limbic areas (e.g. CeA, PVN, hippocampus). MR expression is restricted to the hippocampus with minimal expression in amygdalar nuclei. Circles (●) represent GRs and triangles (▲) represent MRs. Abundance of receptors is given by the relative density of circles or triangles in an area. Acc-nucleus accumbens; AON-anterior olfactory nucleus; APit-anterior pituitary gland; BLA-basolateral nucleus of the amygdala; BnST-bed nucleus of the stria terminalis; CA1, CA2, CA3-hippocampal areas CA1 to CA3; CeA-central nucleus of the amygdala; Cereb-cerebellum; Cing Ctx-cingulate cortex; DG-dentate gyrus; Fr Ctx-frontal cortex; InfC-inferior colliculus; LC-locus coeruleus; LS-lateral septum; MeA-medial nucleus of the amygdala; MS-medial septum; OB-olfactory bulb; Occ Ctx-occipital cortex; PAG-periaqueductal gray; Par Ctx-parietal cortex; PVN-hypothalamic paraventricular nucleus; Red-red nucleus; RN-raphae nuclei; SupC-superior colliculus; SN-substantia nigra; Thal-thalamus. (Adapted from (Morimoto et al. 1996; Steckler and Holsboer 1999a; Kretz et al. 2001)).

Systemically, glucocorticoids have effects on metabolism, fat deposition, bone metabolism and immune responses. In the central nervous system, glucocorticoids have a variety of effects associated with different areas of the brain.

#### Corticosteroid receptors

Glucocorticoid binding in the hippocampus can alter HPA axis drive and under a variety of situations modulate behavioral and cellular function. Injection of glucocorticoid into the hippocampus can induce changes in cell number and morphology (Cameron and Gould 1994). During stress, glucocorticoid can alter learning and memory related processes such as long-term potentiation (LTP) and long-term depression (Avital et al. 2006). Glucocorticoid action in amygdalar nuclei induces changes in anxiety and emotionally relevant learning and memory (Roozendaal and McGaugh 1996; Shepard et al. 2000). It is likely that a combination of changes in corticosteroid receptor expression and activity (via changes in circulating cortisol) form the basis for the link between psychiatric disease and the HPA axis.

The secretion of glucocorticoids from the adrenal cortex can readily activate two types of corticosteroid receptors in target tissues: the Type I or mineralocorticoid receptor (MR) and Type II or glucocorticoid receptor (GR; Hollenberg et al. 1985; Arriza et al. 1987). MR and GR function as transcription factors

that reside within the cytoplasm in the ligand-free state. Once bound by ligands, they dimerize and translocate to the nucleus, allowing for transcriptional control over a variety of target genes. Transcriptional control is affected directly through positive or negative regulation of targeted genes or indirectly through modulation of other transcription factors via protein-protein interactions (Yang-Yen et al. 1990; Stocklin et al. 1996). Additionally, rapid non-genomic effects can occur with bound receptors functioning as cytoplasmic monomers that can interact with a variety of cellular proteins or through interactions with membrane-bound G protein-coupled receptors (Tasker et al. 2006). These rapid non-genomic effects appear crucial for generating the immediate behavioral and physiological responses needed to maintain homeostasis as glucocorticoid levels can increase significantly within minutes after exposure to a stressor (Reichardt et al. 2001).

One specific way in which GR may exert control over transcription is through chromatin remodeling. Psychological stress through novel environment exposure or forced swim (FST) exposure leads to increases in phospho-acetylation of histone H3 within the dentate gyrus, which can be prevented by GR antagonist treatment, but not MR antagonist treatment (Bilang-Bleuel et al. 2005; Chandramohan et al. 2007). It is known that chromatin remodeling allows activation of previously silenced genes through post-translational modification of the N-terminus of core

histone molecules. Therefore, this evidence suggests that GR, but not MR, can regulate gene expression via post-translational modification of histone H3.

Glucocorticoids activate both GR and MR, with MR having a 10-fold higher affinity for glucocorticoids than GR (Reul and De Kloet 1985). Additionally, another adrenal steroid, aldosterone, can activate MR. Specifically, within peripheral tissues, such as the tight epithelia in kidney and colon, only aldosterone can activate MR as glucocorticoids are enzymatically converted in these tissues by 11  $\beta$ -hydroxysteroid dehydrogenase Type II to inactive metabolites (cortisol to cortisone, corticosterone to 11-dehydrocorticosterone) (Funder et al. 1988; Seckl 1997). The localization of MR within these tissues is critical for the important role of aldosterone in sodium and water homeostasis.

In addition to differential ligand binding affinities of GR and MR, there is differential expression of the receptors throughout the brain (Figure 2). GRs are found in both neurons and glial cells, and are ubiquitously distributed throughout the brain with the highest density occurring in hypothalamic CRH neurons; there is also a high level of GRs in anterior pituitary corticotrophs (Reul and De Kloet 1985; Morimoto et al. 1996). Conversely, MRs have a more restricted distribution with the highest density in the limbic brain regions, such as the hippocampus and lateral septum and a minimal density at hypothalamic sites (Reul and De Kloet 1985; Kretz et al. 2001). Within the hippocampus, MRs are distributed throughout all pyramidal cell fields, while GRs are limited primarily to the CA1, CA2 and dentate gyrus (Van Eekelen et al. 1988).

It has been postulated that at basal glucocorticoid levels, MR is primarily activated, while GR becomes activated when glucocorticoid levels are elevated during the circadian peak or stress conditions. Therefore, the hypothesized interpretation is that MR modulates the tonic influences of glucocorticoid to maintain HPA axis basal activity, whereas GR modulates responses to increased glucocorticoid levels, as in responses to stress (De Kloet and Reul 1987; De Kloet et al. 1998). Evidence of the role of MR in modulating basal glucocorticoid levels is revealed by intracerebroventricular (icv) administration of an MR antagonist, which elevates circulating basal trough levels of glucocorticoid. Conversely, icv administration of a GR antagonist has no effect on basal trough levels of glucocorticoid (Ratka et al. 1989), but icv administration of the GR antagonist during the circadian peak increases basal HPA activity (Van Haarst et al. 1997). It is postulated that an imbalance in MR/GR may enhance the vulnerability to psychiatric disorders in individuals who are genetically predisposed (De Kloet et al. 1998; De Kloet 2000).

Over the last 15 years, a number of relevant genetic alterations in the corticosteroid receptor system have

been developed. In this review, we will discuss how the data from these alterations has modified understanding of corticosteroid signaling during and after stress.

### Genetic modification of GR in mice

Several researchers have used transgenic and knockout approaches to investigate the role of GR activity during stress. In this section, we will describe the targeting strategies of these various models and then summarize the major cellular, HPA axis (Table I) and behavioral (Table II) changes associated with each model to provide a novel framework with which to readily compare individual parameters of the various models.

#### *Targeting strategies for genetic GR manipulation*

The variety of GR models demonstrates the diversity of genetic approaches available for disruption of normal gene function. Researchers have generated knockouts, over-expressors and mutants with reduced or altered GR function.

*Antisense GR mutant.* The first published genetic model of glucocorticoid disruption involved the introduction of antisense GR cDNA into the mouse genome and is known as the antisense GR mouse (AGR; Pepin et al. 1992). A 1.8 kb fragment of the GR cDNA was inverted and placed under the control of the neurofilament promoter. This strategy was designed to force reduced expression of endogenous GR in the nervous system. However, inconsistent expression of the transgene induced differing amounts of reduced GR expression in neural (e.g. 50–70% decrease in the GR expression in the hypothalamus and cortex) and non-neural (e.g. 30–50% reduction in kidney and liver) tissue. Phenotypically, these mice exhibit increased weight and fat deposition with a concomitant reduction in food intake. Some of the important cellular changes seen in this model include an unexpected decrease in CRH expression in the PVN and median eminence (Dijkstra et al. 1998) as well as a decrease in LTP in the hippocampus (possibly a result of reduced MR expression; Steckler et al. 2001). Finally, although this model, as compared to a complete GR knockout, is more representative of human disease states in which GR expression in the brain is reduced, the varying consequences from cell-to-cell of variable amounts of GR expression limit the interpretation of the data.

*Conventional GR knockouts.* To investigate the effects of loss-of-function for the GR, two conventional knockout animals have been produced: Exon 2

Table I. Phenotypic consequences including HPA axis dysregulation in mice with targeted mutations of GR and MR.

Transgenic Line	Onset of Modulation	GR Brain Expression	MR Brain Expression	Hypothalamic CRH	Pituitary POMC/ACTH	Circadian HPA Axis				Non Circadian HPA axis				References #	
						Basal		Peak		Negative Feedback		Acute Restraint Stress			
						Plasma Cort	Plasma ACTH	Plasma Cort	Plasma ACTH	CRH/DST	DST	Peak	Return to baseline		Mild Stress
Mice with ↓ GR (AGR)	embryo	50–70% ↓	↓	↓		nc	nc	nc	nc		↑ cort		↑ ACTH; nc cort	Pepin, 1992; Montkowski, 1995; Barden, 1997; Dijkstra, 1998; Steckler, 2001;	
GR exon 2 Knockout (GR <sup>Hypo</sup> )	embryo	none *	nc	↑	↑	↑	↑						↑ cort	Cole, 1995; Heslen, 1996; Reichardt, 1996; Oitzl, 1997; Kretz, 1999; Ridder, 2005	
GR exon 3 Knockout (GR <sup>Null</sup> )	embryo	none													
GR exon 3 heterozygote (GR <sup>Null</sup> het)	embryo	70% ↓				nc		nc		↑ cort	↑ cort	↑ cort	delayed	Ridder, 2005	
GR DNA binding mutant (GR <sup>Dim</sup> )	embryo	nc **		nc	↑	↑	nc	↑					↑ cort	Derijk, 1997; Reichardt, 1998	
Panneural GR Knockout (GR <sup>NesCre</sup> )	embryo	none		↑	↑	↑	↓	↑				nc		Tronche, 1999	
Forebrain GR Knockout (FBGRKO)	young adult	none fore-brain	nc basal	nc basal		↑	nc	↑	↑		↑ cort	↑ cort	delayed	↑ cort; ↑ ACTH	Boyle, 2005; Boyle, 2006
Mice with ↑ GR (YGR)	embryo	50% ↑	30% ↓	↓	↓	***	***	***	***	nc	↓ cort	↓ cort	nc		Reichardt, 2000; Ridder, 2005
Forebrain GR Overexpressor (GRov)	1st post-natal week	75% ↑ forebrain	nc	nc	nc	nc	nc	nc	nc				nc		Wei, 2004

Table I – continued

Transgenic Line	Onset of Modulation	GR Brain Expression	MR Brain Expression	Hypothalamic CRH	Pituitary POMC/ACTH	Circadian HPA Axis				Non Circadian HPA axis			References #	
						Basal		Peak		Negative Feedback		Acute Restraint Stress		
						Plasma Cort	Plasma ACTH	Plasma Cort	Plasma ACTH	CRH/DST	DST	Peak		Return to baseline
MR Knock-out (MR <sup>Null</sup> )	embryo		none	↑	↑	↑								Berger, 1998; Bleich, 1999; Gass, 2000; Gass, 2001
Forebrain MR Knockout (MR <sup>CamKCre</sup> )	1st post-natal week	↑ HPC	none fore-brain	nc		nc		nc						Berger, 2006
Forebrain MR Over-expressor (MR <sup>Ov</sup> )		↓ HPC	20–25% ↑ forebrain	nc		nc		20–27% ↓ (p > 0.05)			↓ female nc male	nc	nc	Rozeboom, 2007
Forebrain MR Over-expressor (MR-Tg)		nc	> 25% ↑ forebrain			nc					nc	nc		Lai, 2007

Abbreviations: ↑ - increase compared to control mice; ↓ - decrease compared to control mice; nc = no change compared to control mice; ACTH - adrenocorticotrophic hormone; cort - corticosterone; CRH - corticotropin releasing hormone; DST - dexamethasone suppression test; HPC - Hippocampus.

\*GR<sup>Hypo</sup> mice express some aberrant GR; \*\* GR<sup>Dim</sup> mice express mutant GR to a similar level to wildtype GR in control mice; \*\*\* conflicting results in literature.

# References given by first author and year of publication.

Table II. Behavioral analysis of mice with targeted mutations of GR and MR.

Transgenic Line	Locomotion	Anxiety Tests			Despair Tests			Learning and Memory			References #
		Elevated Plus Maze/Elevated O Maze	Light:Dark Preference	Open Field	Forced Swim Test	Tail Suspension Test	Learned Helplessness	MWM	RAM	Novel Object	
Mice with ↓ GR (AGR)	↑ (EPM; open field)	↓ anxiety			↓ despair			↓ memory	↓ memory	*	Beaulieu, 1994; Montkowski, 1995; Rochford, 1997; Rouse, 1997; Steckler, 1999b & 2001
GR exon 2 Knockout (GR <sup>Hypo</sup> )	↑ (open field)			nc anxiety				↓ memory			Oitzl, 1997;
GR exon 3 heterozygote (GR <sup>Null</sup> het)	nc (open field)	nc	nc	nc	nc		↑ despair				Ridder, 2995
GR DNA binding mutant (GR <sup>Dim</sup> )	↓ (MWM); nc (open field)		nc	nc				↓ memory			Oitzl, 2001
Panneural GR Knockout (GR <sup>NesCre</sup> )	nc (open field)	↓ anxiety	↓ anxiety	nc	↓ despair**						Tronche, 1999
Forebrain GR Knockout (FBGRKO)	↑ (EPM); nc (open field)	↓ anxiety	↑ reactivity	nc	↑ despair	↑ despair					Boyle, 2005; Boyle, 2006
Mice with ↑ GR (YGR)	nc (open field)	nc	nc	nc	nc		↓ despair				Ridder, 2005
Forebrain GR Overexpressor (GRov)	nc (open field)	↑ anxiety	↑ anxiety	nc	↑ despair						Wei, 2004
MR Knockout (MR <sup>Null</sup> )		***	***	***							Gass, 2001
Forebrain MR Knockout (MR <sup>CamKCre</sup> )	↓ (MWM; L:D pref; EOM); nc (open field)	nc	nc	nc				↓ memory	↓ memory	↑ exploratory activity	Berger, 2006
Forebrain MR Overexpressor (MRov)	nc (open field)	↓ anxiety		↓ anxiety							Rozeboom, 2007
Forebrain MR Overexpressor (MR-Tg)	nc (open field)		↓ anxiety	↓ anxiety				↑ memory		↓ memory	Lai, 2007

Abbreviations: ↑ - increase compared to control mice; ↓ - decrease compared to control mice; nc - no change compared to control mice; EPM - elevated plus maze; MWM - Morris water maze; RAM - radial arm maze.

\* Conflicting data on this test in literature; \*\* show decreased immobility on 2nd day of testing; \*\*\* preliminary data suggests increased anxiety (but details are unpublished).

# References are given by first author and year of publication.

targeted GR<sup>Hypo</sup> (Cole et al. 1995) and Exon 3 targeted GR<sup>Null</sup> (Ridder et al. 2005). The GR<sup>Hypo</sup> mice were developed by inserting a PGK-Neo cassette into Exon 2 of the GR gene, a region involved in transactivation, while the GR<sup>Null</sup> mice were developed using mutant mice containing loxP sites surrounding Exon 3, a region involved in DNA binding. It has been reported that most of the GR<sup>Hypo</sup> mice and all of the homozygous GR<sup>Null</sup> mice died in the first hours of life from severe lung atelectasis. The finding that 5–10% of the GR<sup>Hypo</sup> mice survived to adulthood prompted researchers to carefully look for aberrantly truncated GR proteins (Cole et al. 2001). Analysis showed that GR<sup>Hypo</sup> mice on an outbred strain have a truncated GR with a ligand-binding domain that can bind the synthetic glucocorticoid dexamethasone. So, GR<sup>Hypo</sup> mice may have some remaining GR function that could limit interpretation of findings, particularly when differences in action are not found. Heterozygotes of both models survive into adulthood and have been used to study a variety of physiological, endocrine and behavioral factors (Hesen et al. 1996; Oitzl et al. 1997; Ridder et al. 2005). GR<sup>Hypo</sup> mice exhibit increased hypothalamic CRH and anterior pituitary gland POMC expression (Reichardt and Schutz 1996; Kretz et al. 1999). Both of these models, as heterozygotes, offer the opportunity to investigate the effect of reduced but not complete loss of neural GR activity in studies of stress-related questions.

*GR DNA binding mutant.* As mentioned previously, glucocorticoid binding to GR can induce cellular changes through dimerization-dependent and independent actions. To investigate these two types of GR activity on a variety of cellular processes, a GR mutant with a point mutation in Exon 4 was developed (GR<sup>Dim</sup>; Reichardt et al. 1998). The point mutation, A458T, had previously been shown to disrupt D loop formation causing a loss of GR dimerization and direct DNA binding (Heck et al. 1994). Interestingly, GR<sup>Dim</sup> homozygous mice are born at the normal Mendelian ratios from heterozygote to heterozygote pairings. Despite the lack of dimerization-dependent DNA binding in GR<sup>Dim</sup> mice, *in vitro* evidence suggests that GR with the GR<sup>Dim</sup> mutation can still bind DNA and directly influence the transcription of genes (Adams et al. 2003). This *in vitro* work is consistent with the observation that the mRNA levels of a GR-dependent gene are normal in GR<sup>Dim</sup> mice but are undetectable in GR<sup>Hypo</sup> mice (Cole et al. 1995; Reichardt et al. 1998). On a cellular level, CRH content in the median eminence is unaffected by the mutation, while POMC mRNA and ACTH expression are up-regulated in the anterior pituitary gland, demonstrating the importance of GR dimerization-dependent DNA binding effects on some but not all

aspects of GR-mediated feedback on the HPA axis (Reichardt et al. 1998).

All of the above mutants have a number of peripheral changes in metabolism and immune function associated with the conventional, whole organism targeting strategies used. Although, not fully characterized, these peripheral changes make it more difficult to derive specific conclusions about the role of GR in stress and nervous system function. To more precisely define the role of GR in the CNS, some research groups have produced pan-neural GR knockout (GR<sup>NesCre</sup>; Tronche et al. 1999) and forebrain-specific GR knockout (FBGRKO; Boyle et al. 2005) lines.

*Pan-neural GR knockout.* Tronche and colleagues (1999) generated mice with GR deleted throughout the brain, beginning prenatally using the Cre-loxP system. In this model, Exon 3 of the GR gene was flanked with loxP sites. Mating these mice with nestin-Cre recombinase mice results in offspring with deletion of GR in all CNS neurons and glial cells. GR<sup>NesCre</sup> mice have normal survival but exhibit a Cushing's syndrome-like phenotype. These mice have altered fat deposition with lowered weight gain and osteoporosis. GR<sup>NesCre</sup> mice show increased levels of CRH protein in the PVN and of POMC mRNA in the anterior pituitary gland. Investigation of GR downstream MAPK targets revealed a down-regulation of p-ERK1/2, Ras, Raf-1 and Egr-1 with potential implications for stress responsiveness and fear-based learning and memory (Revest et al. 2005). The GR<sup>NesCre</sup> mutants, as the first neural-specific line, provide an opportunity to investigate the role of both neurons and glial cells in understanding the effects of GR activation in the brain on HPA axis function and behavior.

*Forebrain GR knockout.* Recently, our group produced forebrain-specific GR disruption by mating mice containing a floxed GR Exon 1C through 2 with CamKII-Cre recombinase mice (Boyle et al. 2005). In this targeting strategy, promoter elements at the normal translation start sites for GR are deleted. The potential exists for the production of truncated GR regions analogous to the GR<sup>Hypo</sup> allele. However, such fragments have not been detected in tissues analyzed to date (Brewer et al. 2003). In these mice FBGRKO, the floxed GR region is progressively deleted from the age of 3–6 months in the hippocampus, cortex, basolateral nucleus of the amygdala (BLA) and nucleus accumbens, but GRs in the PVN, thalamus and central nucleus of the amygdala (CeA) are spared (Boyle et al. 2006). Associated with this specific disruption of GR, FBGRKO mice exhibit increases in basal PVN AVP and hippocampal (CA1 and DG)

CRH receptor-1 expression. The additional spatial specificity (i.e. forebrain only) and temporal aspects of deletion (i.e. deletion after 3 months of age) make the FBGRKO mice a particularly interesting model to investigate the role of extrahypothalamic sites of GR on basal and stress-induced HPA axis activity as well as the role of GR in limbic modification of behavior in the absence of non-specific developmental changes.

*General GR over-expressor.* To complement the loss-of-function studies, two models of GR over-expression have been generated. The first model (YGR) involved the addition of an extra copy of the full length GR gene (Reichardt et al. 2000). Although, insertion-site effects of the extra GR cannot be fully assessed, the inclusion of the known GR promoter and regulatory elements was predicted to induce over-expression in the normal areas of GR expression. Both GR mRNA and protein were only increased by about 50% despite the presence of two extra copies of the GR gene per mouse. In YGR mice, median eminence CRH protein, pituitary POMC mRNA and ACTH protein, hippocampal BDNF protein and MR expression are all decreased (Reichardt et al. 2000; Ridder et al. 2005). Despite the over-expression of GR throughout the nervous system and periphery of YGR mice, these mutants provide an interesting framework to study the effects of increased GR activation on stress-mediated adaptations.

*Forebrain GR over-expressor.* To improve the spatial specificity of GR over-expression, Wei and colleagues (2004) introduced a transgene containing the CamKII promoter driving expression of the GR cDNA. This model (GRov) exhibits about 78% over-expression of GR in the forebrain (including the cortex, hippocampus, CeA, BLA and nucleus accumbens) as well as the PVN, and possibly includes ectopic expression of GR within groups of neurons not normally expressing GR in the CNS such as the suprachiasmatic nucleus (SCN; Balsalobre et al. 2000). Importantly, expression excludes the cerebellum, thalamus and anterior pituitary gland as well as all peripheral organs. GRov mice show increases in CRH mRNA in the CeA and in expression of various neurotransmitter transporters (Wei et al. 2004). GRov mice offer the opportunity to investigate the role of increased GR in important limbic areas with the caveat that PVN over-expression of GR might make it difficult to disentangle hypothalamic vs. extra-hypothalamic GR modulation of HPA axis drive.

#### *HPA axis analysis from genetic models of GR alteration*

Change in HPA axis drive is one of the most important components of the link between psychiatric illness and

the endocrine system. Evaluating how GR activity alters normal circadian and stress-induced glucocorticoid release is crucial to understanding this link. The various GR models include a wide array of changes to the GR system, and when the various phenotypes from the strains are combined together the role of GR in modulating HPA axis activity can be more clearly understood.

*HPA axis circadian activity and feedback.* Glucocorticoid and ACTH secretion are normally modulated under non-stress conditions by the SCN, the center of circadian control. As previously mentioned, the circadian rhythmicity of glucocorticoid release is such that glucocorticoid levels are high at activity onset (lights off for mice) and low at the start of rest (lights on for mice). An important component of HPA axis activity during normal circadian glucocorticoid release or after HPA axis activation by stress is the negative feedback loop that returns the system to a state of homeostasis through the activation of GR in a variety of CNS areas (Figure 1). To assess feedback, two tests are used, the DST and the dexamethasone/CRH challenge test. Both of these tests measure suppression of glucocorticoid secretion in response to a GR agonist and have both been shown to be altered in psychiatric illness (Carroll et al. 1980; Holsboer et al. 1982). The use of these tests in the GR mutant models has led to a better understanding of the effects of each specific genetic alteration on basal HPA axis drive and feedback inhibition.

When the AGR mice were originally subjected to analysis of circadian changes in corticosterone production, initial results indicated an increase in circulating corticosterone level compared to control mice (Pepin et al. 1992). However subsequent and more in depth analysis failed to identify a difference between transgenic and control groups (Barden et al. 1997). While differences in circulating corticosterone level were not observed, the use of the DST showed reduced suppression of HPA axis activity consistent with the reduction but not elimination of GR activity in these mice.

Although, not analyzed with respect to circadian rhythmicity, GR<sup>Hypo</sup> mice on postnatal day 1 exhibit increased corticosterone (2.5-fold) and ACTH (15-fold) levels (Cole et al. 1995). While HPA axis activity for GR<sup>Null</sup> homozygotes has not been reported, GR<sup>Null</sup> heterozygotes exhibit normal corticosterone levels during the light and dark phases and reduced dexamethasone suppression, indicating impaired negative feedback (Ridder et al. 2005). Normal MR activity, even in the presence of reduced GR, may explain this lack of an abnormal circadian phenotype in the GR<sup>Null</sup> heterozygotes, at least at basal levels of HPA activity. Alternatively, the presence of one GR allele may be sufficient to maintain circadian

rhythmicity of HPA axis activity. However, when the system is challenged in the DST or during stress (as discussed below), the reduced GR expression may fail to fully control HPA axis activity causing elevated levels of corticosterone through impaired feedback inhibition.

GR<sup>Dim</sup> and GR<sup>NesCre</sup> mice demonstrate increased nadir (4- and 15-fold, respectively) and peak (1.5- and 4-fold, respectively) corticosterone levels compared to control mice (Tronche et al. 1999; Oitzl et al. 2001). Paradoxically, considering the increased corticosterone levels, both of these models exhibit unexpectedly low levels of ACTH, a 1.5-fold reduction in GR<sup>NesCre</sup> mice and no change in GR<sup>Dim</sup> mice. Interestingly, ACTH stimulation tests in the GR<sup>NesCre</sup> mice indicated that the sensitivity of the adrenal cortex is heightened in GR<sup>NesCre</sup> animals, a mechanism that could potentially account for the apparent discrepancy in ACTH levels (Tronche et al. 1999). The increase in corticosterone secretion in the GR<sup>Dim</sup> mice illustrates the relative importance of GR DNA binding-dependent vs. -independent mechanisms in the modulation of corticosterone-regulated negative feedback. It appears that while ACTH production in the pituitary gland is under transcriptionally-regulated control by GR, the actual secretion of ACTH is regulated by DNA binding-independent means.

FBGRKO mice exhibit increased circadian HPA axis activity, with a 2-fold increase in circulating corticosterone levels and a small but not statistically significant increase in ACTH at the nadir, and a 1.5-fold increase in corticosterone and ACTH levels at the peak (Boyle et al. 2005). These results suggest that forebrain GR may play an important role in basal inhibition of HPA axis activity, and that this inhibition is released when GR is deleted. In addition, FBGRKO mice show no dexamethasone suppression of corticosterone secretion. When compared to a reduction of suppression in AGR and GR<sup>Null</sup> heterozygotes, this result suggests that the hippocampus and other forebrain areas may comprise an important and necessary circuit in the negative feedback control of the HPA axis. Alternately, the chronic loss of GR in the forebrain may cause downstream changes in other components of the negative feedback system. More region-restricted genetic approaches should be useful in determining an essential role of forebrain GR in the DST.

At first glance, the two over-expression models appear to show different effects on HPA axis drive. GRov mice exhibit normal circadian rhythmicity of HPA axis drive (Wei et al. 2004). In contrast, initial evidence showed that YGR mice exhibit decreased nadir corticosterone (4-fold) but increased ACTH (2-fold) levels (Reichardt et al. 2000). However, it should be noted that the levels of basal corticosterone in the control mice in that experiment appear to be slightly elevated (40 ng/ml) compared to other

published values. Since the YGR mice exhibit a reduced corticosterone response to a strong stressor as compared to stressed control mice (discussed below), it may be that these basal levels are more likely to be representative of mild handling stress-induced corticosterone release. Moreover, subsequent analysis failed to find a difference in circadian corticosterone or ACTH levels but did reveal enhanced suppression in the DST (Ridder et al. 2005). The apparent lack of an abnormal HPA axis phenotype in GRov and YGR mice compared with the elevations in circadian corticosterone in FBGRKO mice may be explained by the possibility that the normal endogenous GR abundance is already at a level where further increases cause no changes.

Our findings with FBGRKO mice suggest that while resting levels of corticosterone primarily occupy MR, some of the GRs are also activated and mediate inhibition of the HPA axis. So, in both the FBGRKO and the GR<sup>NesCre</sup> mice, the lack of this small number of occupied GR releases the inhibition of the HPA axis causing increased corticosterone secretion. On the other hand, when GR is over-expressed in the forebrain, the increased amount of GR does not further potentiate this inhibition because of the limited amount of corticosterone. This interpretation of the role of forebrain GR in tonically inhibiting the HPA axis is further supported by the results from GR<sup>Null</sup> heterozygotes which exhibit reduced GR expression; this reduced level of GR may be sufficient to exceed the threshold for basal tonic feedback inhibition and consequently produce normal circadian corticosterone release. Previous observations that GR antagonists do not alter basal corticosterone secretion in wild-type animals (Ratka et al. 1989) could be explained on the basis that the above observations in GR mutant mice occur after weeks or months of altered GR expression/activity and may therefore be representative of a chronically altered HPA axis control system, or differences could be related to the fact that in the pharmacological experiments, GR agonists were delivered by the icv route.

*Stress induced activation of the HPA axis.* As a dynamic system, the HPA axis is activated in rodents to different degrees when the stress is mild (e.g. handling, needle stick, placement in the elevated plus maze (EPM)), moderate (e.g. swimming in a water maze) or more severe (e.g. acute or chronic restraint). Using these different types of stressors in GR mutant mice, investigators have been able to dissect the role of GR in stress-associated HPA axis activity.

Evaluation of mild stress (10 min in the EPM) in AGR mice revealed increased ACTH release but no difference in corticosterone secretion (Montkowski et al. 1995). In GR<sup>Hypo</sup> adult mice, there was evidence of a gene deletion dose effect on mild stress-induced

(novel environment) HPA axis activation (Hesen et al. 1996). GR<sup>Hypo</sup> homozygotes had the highest corticosterone levels followed by GR<sup>Hypo</sup> heterozygotes and wild-type animals.

In GR<sup>Dim</sup> mice, a moderate stressor (1 min of swimming) caused a greater increase than in controls in corticosterone release 30 min and 90 min after the stressor (Oitzl et al. 2001). However, the increase in corticosterone at these time points was about the same (2-fold increase) as the changes in basal corticosterone levels. Therefore, it cannot be concluded whether the HPA axis is more sensitive to stress or is just hyperactive overall.

The greater stress-induced increase in corticosterone secretion in GR<sup>Dim</sup> mice is in contrast to the lack of a difference from controls in GR<sup>NesCre</sup> mice after a more severe stressor (40 min restraint) (Tronche et al. 1999). However, the absence of a difference here may simply be the result of a ceiling effect. The notion of a ceiling effect is well illustrated with data from FBGRKO mice (Boyle et al. 2006). These mice show a greater increase in corticosterone and ACTH release after 5 min and 15 min of restraint but not after 30 min of restraint compared to control mice. Furthermore, FBGRKO mice show a greater increase in corticosterone release after a mild stressor (needle stick).

GR<sup>Null</sup> heterozygotes and YGR mice show increased and decreased corticosterone responses, respectively, after 30 min of restraint stress (Ridder et al. 2005). Finally, GRov mice exhibit no changes in corticosterone (or ACTH) responses to a mild stressor (5 min in the EPM) but have changes in HPA axis function with more severe stressors (Wei et al. 2004).

The HPA axis under stressful conditions appears to be modulated by density of active receptors with an inverse relationship between GR density and levels of corticosterone secretion. Mutants with reduced GR activity (GR<sup>Null</sup> heterozygotes, GR<sup>Dim</sup> and FBGRKO mice) show increases in stress-induced corticosterone while the YGR mice show reduced corticosterone after stress. The density of receptors probably modulates corticosterone abundance through altered feedback inhibition (i.e. increased GR density = increased negative feedback).

#### *Behavioral analysis from genetic models of GR alteration*

Thus far, we have discussed the physiological effects of genetic manipulations of GR as determined by alterations in HPA axis activity. In order to fully understand the role of GR in the whole organism's response to normal or pathologic stress, one must assess the behavioral correlates of these physiological alterations as well. In this section, we will describe the behavioral phenotypes of mice with GR alterations. These phenotypes are divided into one of two categories: behavior associated with anxiety

or depression and behavior associated with learning and memory (Table II).

*Changes in anxiety-related behavior in GR mice.* Common tests for anxiety include the open field test, the EPM, the elevated zero maze (EOM) and light:dark (L:D) preference. Although the open field provides a classic measure of anxiety-like behavior in rodents, it also offers data on general locomotor output that can significantly confound the interpretation of results from other behavioral tests. None of the mice tested (e.g. GR<sup>Hypo</sup>, GR<sup>Null</sup> heterozygotes, GR<sup>Dim</sup>, GR<sup>NesCre</sup>, FBGRKO, YGR nor GRov) show any alterations in open field anxiety-related behavior (Oitzl et al. 1997; Tronche et al. 1999; Oitzl et al. 2001; Wei et al. 2004; Ridder et al. 2005; Boyle et al. 2006). However, GR<sup>Hypo</sup> homozygotes and AGR mice exhibited increased locomotor activity compared to control mice which could lead to increased behavior in other tests being misinterpreted as an anxiety change when it is simply increased movement (Beaulieu et al. 1994; Oitzl et al. 1997). It should be noted that for the GR<sup>Hypo</sup> mice, this test occurred following Morris water maze (MWM) testing, which can be considered a moderate stressor.

Although, none of the transgenic mice tested showed alterations in anxiety behavior in the open field, a number of changes were observed in behavior in the EPM and EOM. GR<sup>NesCre</sup> mice show decreased anxiety-like behavior on the EOM; AGR and FBGRKO mice show an anxiolytic phenotype in the EPM (Montkowski et al. 1995; Rochford et al. 1997; Tronche et al. 1999; Boyle et al. 2006). However, for both the AGR and FBGRKO mice, other evidence complicates the interpretation of a simple anxiolytic phenotype. For instance, the AGR mice show increased anxiety on two other tests, the Thatcher Britton Novelty food test and the acoustic startle test (Rochford et al. 1997). In the EPM, diazepam (an anxiolytic) increased the anxiolytic phenotype (Rochford et al. 1997). These results suggest that the AGR mice exhibit an exaggerated exploratory response characterized by increased locomotor activity (in open field activity and in EPM total arm entries) that allows the animal to ignore potentially harmful stimuli (such as when on the open arm). In tests that do not involve a "safe" or "non-safe" choice, these mice have reduced anxiety-like behavior. Similarly, for the FBGRKO mice, the increased in anxiety-like behavior (e.g. more time on and entries into the open arms) was accompanied by an overall increase in activity in the closed and open arms and may represent exaggerated stress reactivity given that these mice show no alterations in open field locomotion. In contrast to the GR<sup>NesCre</sup>, AGR and FBGRKO mice, GRov mice show significant increases

in anxiety-related measures in the EPM (Wei et al. 2004). However, the observation that the YGR mice do not show any EOM differences complicates the interpretation of this finding that increased GR expression is correlated with increased behavior representative of an anxious state (Ridder et al. 2005).

The final major test for anxiety that has been thoroughly examined is the L:D preference test. This procedure pits a mouse's innate interest in exploring novel areas against its fear of open-illuminated environments. In this test, GR<sup>Dim</sup> mice show no differences from control mice indicating that direct DNA binding activities of GR dimers may not be central to the influence of GRs on anxiety-like behavior (Oitzl et al. 2001). GR<sup>NesCre</sup> mice show decreased latency to enter the light compartment and longer overall time in the light (both characteristic of decreased anxiety) indicating that decreased neural GR may reduce anxiety-like behavior (Tronche et al. 1999). FBGRKO mice show confusing behaviors in the L:D preference test. FBGRKO mice show decreased latency to enter the light and more entries into the light (both indices of anxiolytic responses; Boyle et al. 2006). However, in contrast to the GR<sup>NesCre</sup> mice, FBGRKO mice spent an equivalent amount of time in the light compared to controls. A closer analysis of the L:D preference data using reversed lighting (animals start in light rather than dark), stress and antidepressant treatment, shows that FBGRKO mice most likely exhibit increased stress reactivity and an enhanced flight behavior rather than more common changes in anxiety. Finally, as with the EPM/EOM, GRov but not YGR mice show significant increases in anxiety-related measures in the L:D preference test (Wei et al. 2004; Ridder et al. 2005). Since the GRov mice have no changes in circadian HPA axis activity or mild stress HPA axis activity, it appears that increased GR in the presence of a normal amount of corticosterone is sufficient to increase anxiety-like behaviors.

Overall, increased anxiety-like behavior appears to be associated with increased GR activity (GRov mice) while decreased anxiety (AGR, GR<sup>NesCre</sup>) and altered anxiety responsiveness (FBGRKO) are related to reduced GR activity. Conflicts between models such as the lack of an anxiety-like phenotype in the YGR mice coupled with the anxiogenic phenotype in GRov mice may be explained by the differences in the amount of GR over-expression (50% YGR vs. 78% GRov), or the general brain areas of over-expression (endogenous sites of GR expression in YGR vs. forebrain only in GRov).

*Changes in despair-related behavior in GR mice.* The forced swim test (FST; Kitayama et al. 1988), tail suspension and learned helplessness tests, all measures for despair-like behavior, help illustrate the

role of GR signaling in depression-like behavior. AGR mice exhibit reduced depression-like behavior in the FST, which is unexpectedly reversed by antidepressant treatment (Montkowski et al. 1995). This may suggest that the AGR mice have difficulty interpreting a potentially fearful situation and that antidepressant treatment can help bring the mice back to a state of increased awareness. Alternatively, this finding may simply reflect the elevations in locomotor activity seen in other tests (e.g. open field and EPM). Increased depression-related behavior in both the FBGRKO mice (in the FST, tail-suspension and sucrose preference tests) and GRov mice (in the FST) is reversed with antidepressant treatment (Wei et al. 2004; Boyle et al. 2005). Although, GR<sup>Null</sup> heterozygotes show no differences compared to control mice in the FST, they exhibit more depression-like coping strategies including increased escape latencies and more escape failures when compared to control mice in the learned helplessness paradigm (Ridder et al. 2005). Similarly, YGR mice show reduced depression-like behavior in the learned helplessness paradigm but have no observed changes in the FST (Ridder et al. 2005). Finally, GR<sup>NesCre</sup> mice show increased mobility on the 2nd day of a repeated FST (Tronche et al. 1999). While increased mobility is often thought of as a measure for reduced despair in the 2-day FST, it can also be a measure of cognitive impairment (Bilang-Bleuel et al. 2005). Interpreted in this fashion, the GR<sup>NesCre</sup> mice fail to learn that escaping from the FST is impossible while the control mice learn to conserve their energy and be immobile longer. It should be noted that some of the altered behavior in GR<sup>NesCre</sup> mice might be related to their Cushing's syndrome-like phenotype. GR<sup>NesCre</sup> exhibit osteoporosis and altered fat metabolism, which may affect buoyancy and locomotor behavior in water.

If one compares all of the models of GR alteration, there appear to be contradictory conclusions for depression-like behavior. For instance, both FBGRKO and GRov mice show an increase in despair-like behavior that is reversed with antidepressant treatment. While the different brain regions affected in these models may account for some of these conflicting results, the data also illustrate the idea that the effects of glucocorticoid action can be modeled on an inverted "U" shaped curve where either too little or too much GR activity can be harmful. An inducible system in which levels of GR expression could be titrated (e.g. with the tetracycline-inducible system) could be a useful method with which to more closely examine this model. Finally, differences in using knockout systems vs. systems that are prone to ectopic alterations (e.g. non-GR promoter-driven over-expression systems) could cause abnormal non-specific changes in despair-like behavior.

*Learning and memory behavior.* Stress and glucocorticoids have a well-established role in modifying learning- and memory-related behavior. Specifically, emotionally relevant memories have been shown to be altered by GR signaling (Roosendaal and McGaugh 1996). Interestingly, the effect of glucocorticoids on memory can be positive in some cases (e.g. acute stress) or detrimental in others (e.g. chronic stress, psychiatric illness). Due to their varying degrees and specificity of GR manipulation, the genetic models of GR alteration provide an interesting and informative framework for understanding this dynamic process of GR modification of cognition.

In terms of learning and memory-related behavior, spatial memory tests such as the MWM and working memory tasks are some of the most common and well-established procedures to evaluate memory in rodents. All of the animals tested in the MWM (AGR, GR<sup>Hypo</sup> and GR<sup>Dim</sup>) show deficits in various aspects of the test. AGR mice show deficits in location acquisition of both a submerged and a visible platform (Rousse et al. 1997). Furthermore, when the platform is removed, AGR mice show no preference for the target quadrant. While still performing worse than control animals, AGR mice do begin to show improvement from one session to the next when the incentive to find the visible platform is increased (by lowering the water temperature). GR<sup>Hypo</sup> homozygous and heterozygous mice both show deficits in probe trials (no platform) after training in the MWM (Oitzl et al. 1997). However, analysis of trials during training (submerged platform) or with a visible platform is difficult because the day-to-day variability is high. A similar deficit is seen when GR<sup>Dim</sup> mice are tested in the MWM (Oitzl et al. 2001). In this case, GR<sup>Dim</sup> mice exhibit clear deficits during training and during probe trials that are associated with decreased swimming speeds and an increase in corticosterone release after swimming. To account for differences in corticosterone levels, studies incorporated adrenalectomized GR<sup>Dim</sup> and control mice (Oitzl et al. 2001). After adrenalectomy, mice were tested on the MWM with or without corticosterone replacement. In the control group, corticosterone replacement significantly improved performance. However, both adrenalectomized GR<sup>Dim</sup> groups, regardless of corticosterone treatment, exhibited significant deficits. These experiments suggest that these spatial memory deficits in GR<sup>Dim</sup> mice are caused by the loss of GR dimerization-dependent DNA binding activity rather than increased corticosterone acting through altered GR or MR. In the radial arm maze test (RAM), a measure of working memory, AGR mice show more errors than control animals (Rousse et al. 1997). Finally, in two slightly different tests of object recognition, AGR transgenic mice exhibit either no difference or a deficit in novel object interaction compared to control mice (Steckler and Holsboer 1999b; Steckler et al. 2001).

In tests that involve more emotional processing (e.g. social recognition and conditioned fear) only a few of the GR mutants have been analyzed. In a social recognition task, AGR mice fail to recognize a previously encountered juvenile mouse (Montkowski et al. 1995). However, the AGR mice interact with the juvenile mouse less during the first exposure. So, it is unclear whether this is a memory deficit or a floor effect of interaction time. To evaluate the role of altering GR on fear-based learning and memory, YGR and GR<sup>Null</sup> heterozygotes were examined in a conditioned fear paradigm (Ridder et al. 2005). Despite significant changes in a learned helplessness paradigm, neither YGR nor GR<sup>Null</sup> heterozygotes mice exhibited abnormal contextual or cued fear learning.

Unfortunately, the low number of learning and memory studies has thus far prevented the development of more robust conclusions concerning the role of GR in various types (e.g. working, spatial, emotional) of learning processes. At this point, reduced GR activity appears to cause significant deficits in the MWM, a classic test of spatial memory. However, it is important to consider the fact that MWM is not a stress-free behavioral test. Immersion into water is likely to cause both physical and psychological stress that could confound the interpretation of any changes as being related strictly to spatial memory. All of the above mice with MWM deficits show increased corticosterone secretion when subjected to a mild stressor. Therefore, the deficits in spatial memory may be a consequence of increased corticosterone release and action. In the future, it will be important for more in-depth characterization of all the models of GR modulation. In addition, more specific targeting strategies that manipulate GR expression only in specific brain areas (e.g. the hippocampus only) should be valuable in addressing the role of GR in different types of memory formation and retrieval.

#### *Summary of HPA axis findings and behavioral phenotypes of GR mice*

Although, some variability between GR models exists, it can consistently be said that brain GRs are important for regulating basal and stress HPA axis function independent of pituitary GR activity. The spatial and temporal control of this central regulation will have to be further elucidated as genetic techniques progress, some of which are described below. GRs appear to be important for normal circadian HPA axis drive, with significant changes occurring only when DNA binding-dependent GR activity does not occur in the forebrain. Additional mechanisms of compensation that have not been fully appreciated include changes in MR expression or adrenal sensitivity to ACTH. Finally, although mouse behavior is at best a moderate

correlate for human affective disorder symptoms, it can be stated that GR contributes to behaviors characteristic of anxiety, despair and learning phenotypes. As more models of GR manipulation continue to be analyzed, the precise mechanisms of GR control (e.g. GR expression levels, areas of GR expression, types of GR modulation on cellular functions) will become clearer.

### Genetic modifications of MR in mice

Although, less well understood than GR, the role of MRs in stress-associated activity has begun to be investigated. A number of groups have now published data from transgenic and knockout animals for the study of MR function.

#### *Targeting strategies to manipulate MR expression*

**Conventional MR knockout.** Conventional MR knockout mice (MRKO) were developed by inserting a neo cassette into Exon 3 of the MR gene, a region involved in DNA binding (Berger et al. 1998). These mice develop pseudohypoaldosteronism, increases in plasma renin, angiotensin II and aldosterone, and hyperkalemia and hyponatremia, causing them to die around postnatal day 10. However, MRKO mice can be rescued by exogenous NaCl administration (Bleich et al. 1999). They display elevated CRH levels in the PVN and increased POMC and ACTH levels in the anterior pituitary gland (Gass et al. 2001). These mice display decreased granule cell density in the hippocampus suggesting a role for MR in neurogenesis. In addition, they exhibit increased plasma glucocorticoid levels (Gass et al. 2000) making it difficult to conclusively decipher whether the decreased neurogenesis in MRKO mice is a direct effect of knocking out MR or an indirect effect of increased GR activation. Interestingly, some evidence points to a potential role for MR as an anti-apoptotic modulator because adrenalectomy-induced apoptosis in the dentate gyrus can be rescued by low levels of corticosterone that would be sufficient for complete MR but not GR binding (Hassan et al. 1996). Although the MRKO mice can be rescued, their continual problems associated with salt balance, including chronic elevations in the renin-angiotensin system, make analysis of these mice difficult to interpret.

**Forebrain MR knockout.** The Cre-loxP system has been used to produce forebrain-specific MR knockout mice (MR<sup>CamKII-Cre</sup>; Berger et al. 2006). In this system, CamKII-Cre recombinase mice are mated to mice with an Exon 3 floxed MR allele. MR is completely deleted in these mice by postnatal day 12. These mice exhibit normal CRH mRNA expression in the PVN,

but up-regulated GR expression in the hippocampus. They also exhibit a histological alteration in mossy fiber morphology. The forebrain specificity of the knockout in these mice allows for a careful analysis of the role of MR in basal HPA axis control as well as in behavioral modulation.

**Forebrain MR over-expressor.** Two different laboratories have recently generated transgenic mice with over-expression of forebrain MR (MR-Tg, Lai et al. 2007; MRov, Rozeboom et al. 2007) under the control of the CamKII promoter. MRov mice exhibit over-expression at levels 20–25% above endogenous levels in the cortex and hippocampus (Rozeboom et al. 2007). MR levels in the amygdala and PVN are normal. MRov mice show decreased CA1 GR expression and an increase in CA1 5-HT<sub>1a</sub> level with no changes in PVN CRH. Lai and colleagues (2007) have generated MR-Tg mice using a similar system that exhibit over-expression at >25% above normal levels in the cortex, BLA and hippocampus. Unlike the MRov mice, MR-Tg mice show no change in GR levels in the hippocampus. Both of the MR over-expressors provide good models to investigate the hypothesized cellular role of MR as an anti-apoptotic factor and the systems level role of MR as a modulator of behavior.

#### *HPA axis analysis after MR alteration*

MR has been considered to be primarily responsible for basal HPA axis tone. The various models of MR mutation have been analyzed to investigate the role of MR in circadian HPA axis activity as well as in stress-induced activity (Table I).

MR<sup>CamKII-Cre</sup> mice show normal basal circadian HPA axis activity (Berger et al. 2006), implying that forebrain MR (e.g. MR in hippocampus, amygdala and septum) is not necessary for maintenance of the basal HPA axis activity, which would conflict with the increased corticosterone secretion seen in the conventional MRKO mice (Gass et al. 2000). A possible explanation is that increased corticosterone level in rescued MRKOs results from the chronic elevation in the renin-angiotensin system seen in these mice (Bleich et al. 1999). In addition, MR<sup>CamKII-Cre</sup> mice show no differences in corticosterone immediately after 40 min of restraint stress compared to control mice (Berger et al. 2006).

In both MRov and MR-Tg mice, basal HPA axis function appears unaffected; the corticosterone response to restraint stress is moderately suppressed in female, but not male MRov mice (Lai et al. 2007; Rozeboom et al. 2007). There is no change in corticosterone release in MRov mice in response to mild stress (5 min in the EPM; Rozeboom et al. 2007).

The fact that three of four models of MR modulation exhibit no change in circadian corticosterone argues that the classic model of MR control

of basal HPA axis drive should be revisited. Considering the results reviewed above for GR mutants along with the lack of an HPA axis phenotype in the MR mutants, a new model that emphasizes a role for partial occupation of forebrain GR in inhibiting basal HPA axis drive should be considered. Again, the fact that the above models represent chronic alterations in MR may help explain the lack of phenotype as more acute alterations, such as MR antagonist treatment, can increase HPA axis drive (Ratka et al. 1989). Future work involving more direct targeting (e.g. viral mediated modulation of genes) or inducible targeting (e.g. a tetracycline inducible system) of GR and MR populations within the forebrain should be useful in clarifying the roles of GR and MR in the basal control of HPA axis drive.

#### *Behavioral phenotype of mice with MR alteration*

Although, MR has been largely thought of as a mediator of hippocampal cellular plasticity and basal HPA axis drive, recent evidence suggests a role for MR activity in modulating cognition (Table II).

Preliminary studies in rescued MRKO mice suggest that these mice may display increased anxiety although no details have been published (Gass et al. 2001). In MR<sup>CamKCre</sup> mice, anxiety levels appear similar to controls in a variety of paradigms (e.g. open field, EOM, L:D preference), but decreased locomotor activity was seen in EOM and L:D preference (Berger et al. 2006). In contrast, MRov and MR-Tg mice display reduced anxiety-like behavior in an open field (both mutants), L:D preference (MR-Tg only) and EPM (MRov only; Lai et al. 2007; Rozeboom et al. 2007). The reduced anxiety phenotype may be partially explained by the increase in serotonin receptor 5HT-1a seen in MRov mice (Rozeboom et al. 2007).

MR<sup>CamKCre</sup> mice show memory impairments in the MWM with slower swim speeds and deficits in working memory in the RAM (Berger et al. 2006). In addition, hyper-reactivity toward a novel object in a familiar environment was seen, suggesting an increased drive for exploration. MR-Tg mice show enhanced spatial memory in the MWM and altered novel object recognition (Lai et al. 2007).

Increased MR activity in the forebrain is related to decreased anxiety and in some circumstances improved spatial memory capability. These anxiolytic effects of MR appear to only occur with MR over-expression as no alterations were seen in MR<sup>CamKCre</sup> mice and the anxiety-like behavior reported in conventional MR knockouts may be related to increased corticosterone acting on existing GR populations. This result suggests that GR may be sufficient for mediating the harmful effects of a dysregulated HPA axis on behavior as seen in some mood disorders. Additionally, targeted activation

of MR in the forebrain could serve as a novel therapeutic route to alleviating anxiety-related symptoms. The contrasting effects on spatial memory in MR<sup>CamKCre</sup> (deficit) vs. MR-Tg (enhancement) could be related to the role of MRs as an anti-apoptotic agent in the hippocampus. It will be interesting to see whether more in-depth characterization of the MR-Tg mice reveals any changes in hippocampal circuitry.

#### *Summary of MR phenotypes*

In the past, pharmacological and lesion studies suggested that MR activity could play a role in modulating HPA axis drive as well as behavior associated with anxiety, depression and learning and memory. However, the generation of transgenic MR mutants has identified the important role that signaling through MR has in modulating behavior while challenging the role of MR in basal HPA axis drive. As the role of MR in modulation of behavior is clarified, the interaction between MR and GR in each mutant background will have to be assessed. These studies should provide insight into the mechanism by which MR and GR function to regulate the HPA axis and behaviors associated with altered stress responsiveness. Indeed, this additional level of complexity in understanding the role of GR and MR in stress adaptivity could help disentangle some of the results generated thus far.

#### **Conclusions and future directions**

The use of transgenic and knockout genetic approaches has allowed a thorough and intriguing study of the role of both MR and GR in mediating HPA axis drive and stress-induced behavioral changes. These studies provide valuable insight into the link between the HPA axis, stress and psychiatric illness.

One important analysis that has only been partially characterized in a few of the GR and MR mutants is an evaluation of CRH and CRH receptor expression and function. While PVN CRH has a well-established role in activating the HPA axis, the role of CRH as a direct and crucial peptide modulator of behavior has emerged. An important component of this new role for CRH is in extrahypothalamic areas (including the amygdala, the bed nucleus of the stria terminalis and hippocampus). However, only GRov and FBGRKO mice have been analyzed for any changes in the extrahypothalamic CRH system. Additional analysis of the extrahypothalamic CRH system as well as other downstream targets of GR and MR activation should help to further clarify the role of corticosteroid receptor activity in the nervous system.

Taken together, the data from the GR and MR mutants indicates that GRs may play a role in the basal tonic inhibition that was previously attributed solely to MR activity. Under stressful conditions, GR seems

to be the predominant receptor in producing HPA axis feedback inhibition. With respect to behavior, increased GR expression is associated with increased anxiety- and despair-like behavior while decreased GR and increased MR activities are both associated with decreased behavior associated with anxiety and despair. The as yet incomplete characterization of behaviors associated with learning and memory in both sets of mutants hinders the formation of any strong conclusions but it is clear that the loss of either GR or MR can induce memory-like deficits while MR over-expression can facilitate spatial memory in the MWM.

While often mutually consistent, the various mouse strains analyzed sometimes show conflicting or contradictory phenotypes. Given the subtle differences in targeting strategies, peripheral effects and possible developmental adaptations, these differences in phenotypes likely reflect the dynamic nature in which the HPA system functions. Future work should include additional regional-specific and temporal targeting strategies. There is evidence for opposing roles of different region-specific GR populations in modifying behavior. If GR is deleted throughout the nervous system, or even within the entire forebrain rather than within a more restricted region, the observed phenotype may be a combination of these opposing roles and thus be less clear and informative. Future targeting strategies will include newly developed promoters targeting dopaminergic neurons (Lemberger et al. 2007) or amygdalar/PVN areas (Balthasar et al. 2005). In addition, work with viral based vectors and stereotaxic delivery methods could allow sub-region specific targeting of populations of GRs and MRs. Temporally specific manipulations using inducible systems, such as the tetracycline inducible system, should allow researchers to avoid developmental complications as expression can be turned on after critical periods of development. Additionally, the system allows modulation of gene expression in an effort to demonstrate reversibility of the phenotype. Finally, the use of double GR and MR mutant mice (knockouts or over-expressors) will help reveal the poorly understood cellular and systems-level interactions between these two important steroid receptors.

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