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Received: 26 June 2022 | Revised: 25 October 2022 | Accepted: 7 November 2022

DOI: 10.1111/jne.13213

REVIEW ARTICLE

Journal of Neuroendocrinology WILEY

Transcriptional glucocorticoid effects in the brain: Finding the relevant target genes

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Abstract

Glucocorticoids are powerful modulators of brain function. They act via mineralocorticoid and glucocorticoid receptors (MR and GR). These are best understood as transcription factors. Although many glucocorticoid effects depend on the modulation of gene transcription, it is a major challenge to link gene expression to function given the large-scale, apparently pleiotropic genomic responses. The extensive sets of MR and GR target genes are highly specific per cell type, and the brain contains many different (neuronal and non-neuronal) cell types. Next to the set "trait" of cellular context, the "state" of other active signaling pathways will affect MR and GR transcriptional activity. Here, we discuss receptor specificity and contextual factors that determine the transcriptional outcome of MR/GR signaling, experimental possibilities offered by single-cell transcriptomics approaches, and reflect on how to make sense of lists of target genes in relation to understanding the functional effects of steroid receptor activation.

KEYWORDS

corticosteroid, corticosterone, hippocampus, memory, stress

1 | INTRODUCTION

Glucocorticoid hormones are powerful regulators of brain processes. The circadian variation of corticosterone and/or cortisol over the day acts as a synchronizing signal for many tissues, including several brain regions,[1] and is important for daily activity and sleep.[2] The stress-induced elevations in glucocorticoids are essential for optimal adaptation, but may turn from "friend" into "foe" upon prolonged or out of context exposure.[3] Chronic hypercortisolemia not only is a risk factor for cognitive impairment and mood disorders, but also may increase the impact of neurodegenerative disease.[4]

The adverse consequences of excessive glucocorticoid exposure for mood and cognition are likely relevant in the context of chronic stress, but are perhaps most clear in patients with Cushing's disease[5] and in a subset of patients that are treated with high doses of synthetic glucocorticoids.[6] Strikingly, Cushing's patients display changes in brain structure even 10 years after remission, and this is reminiscent of the programming effects of early-life stress.[7] Of note, the long-lasting effects in Cushing's are perhaps most outspoken with regard to white matter, both in patients[8] and in Cushing's mouse models.[9,10] Even in a cross-sectional study using the UK biobank, the use of glucocorticoids (systemic *and* inhaled) was associated with

widespread changes in white matter integrity markers.[11] Although the functional consequence of such changes remains to be determined, the findings on white matter caution against overly neuron-centric thinking and emphasize the importance of evaluating all cell types of the brain.

Many of the effects of glucocorticoids are assumed to depend on changes in gene transcription that are mediated by mineralocorticoid and glucocorticoid receptors (MR and GR). Similar to other transcription factors, MR and GR have many different target genes, and these will only show limited overlap between cell types.[12] A major question in understanding the adaptive and maladaptive effects of glucocorticoids is: which gene or genes are responsible for which effect of the hormones? In some cells, such as the aldosterone-responsive cells in the kidney, the induction of a single target gene like *Sgk1* may come a long way to explain a major part of the hormone effect.[13] In the brain, regulation of the potent neuropeptide corticotropin-releasing hormone/corticotropin-releasing factor[14] likely is very important for the regulation of anxiety.[15] Yet, for lack of removal of the glucocorticoid sensitive regulatory component from a target gene, the link remains associative,[16] and there is in any situation a host of other MR and/or GR regulated genes.

Here, we discuss the principles of MR- and GR-mediated signaling, with a final focus on the challenge of the identification of relevant transcriptional targets in the face of widespread genomic effects that follow MR and GR activation, in different cell types and contexts.

1.1 | Two receptor types: Binding, localization and activity

The effects of the endogenous glucocorticoids are mediated by MR and GR. These can mediate rapid, non-genomic effects in the time scale of minutes, through only partially understood mechanisms. The rapid effects are relevant in the context of rapid changes in brain responsiveness that are associated with the ultradian peaks of hormone levels,[17] early phases of the stress response,[18] and rapid negative feedback of glucocorticoids on the pituitary and hypothalamus.[19] Of course, MR and GR also are well-characterized as transcription factors, acting to immediately change gene expression and, less well understood, to epigenetically modify chromatin. MR and GR show overlap *and* differences in their localization in brain regions and cell types, in their ligand binding, and in their effects on cellular function.[20] Below, we discuss the different processes involved in ligand binding and genomic action, with emphasis on classes of interacting proteins. Their presence and activity states can be strongly cell type ("trait") and context ("state") specific and this determines the final set of target genes that follow the binding of different ligands to MR and GR.

1.2 | Binding and efficacy

MR binds the endogenous glucocorticoids with a ten-fold higher affinity compared to GR, which implies a sequential occupancy as hormone levels increase from circadian trough levels to peak levels and then to stress-induced elevations. Of note, the *efficacy* (the concentration at which effects occur) does not necessarily follow these basal differences in binding affinity. The concentrations of hormone needed to exert non-genomic effects are typically higher than those needed for the transcriptional regulation of classical target genes.[19,21]

It is good to note that not only ligand binding affinity matters, but also the sensitivity of individual target genes of MR and GR differs substantially.[22] Indeed, it makes perfect sense that circadian 'maintenance' concentrations of cortisol should not activate genes that are necessary in the face of serious stressors. A genome-wide concentration–response experiment in the A549 cell line demonstrated orders of magnitude differences in efficacy between GR target genes, in which the circadian clock gene *PER1* stood out as highly sensitive.[23] Studies that addressed DNA binding in the rat hippocampus also suggest that, perhaps based on the affinity of chromatin loci, genomic responses differ for "high" versus "very high" concentrations of hormone.[24–26] For genes that can be induced via both MR and GR, such as FKBP5, the high affinity of MR leads to a very broad concentration range of cortisol, which covers three orders of magnitude.[27–29]

The ligand binding of MR and GR differs also for mineralocorticoids (aldosterone binds MR in cell types where cortisol is enzymatically degraded) and binding of synthetic glucocorticoids, in varying degrees.[30] For synthetic glucocorticoids with a very low MR affinity, this may have consequences for the neuropsychiatric side effects that these drugs may have in some individuals.[6] Drugs such as dexamethasone strongly suppress endogenous cortisol levels and lead not only to extensive GR activation, but also to an under-activation of brain MR.[31,32] In support of the relevance of MR under-activation, a clinical trial in patients with childhood leukemia suggests that co-treatment with low doses of cortisol may ameliorate some of the neuropsychiatric side effects of dexamethasone.[33] The protective effects of MR activation are in line with a series of studies suggesting that a genetic gain of function variant is protective against mood disorders.[34,35] Of note, although depletion of brain MRs may already occur at low levels of dexamethasone acting on the pituitary, the overactivation of GR (likely contributing to the central side effects) depends on sufficiently high doses because the blood–brain barrier partially excludes many glucocorticoids from penetrating the brain.[36]

1.3 | Localization

The ligand binding of MR and GR differs, as does their expression pattern in the brain. Ligand binding studies, mRNA studies, and immunohistochemistry show that cortisol-preferring MR has a limited expression that includes highly prominent expression in the hippocampus, as well as a presence in the prefrontal cortex, the amygdala complex, and, in the rat, the pre-autonomic neurons in the hypothalamus.[3,37] By contrast, GR is ubiquitously expressed. Most of the neuroanatomical localization is based on studies in mice and rats. The Allen Human Brain Atlas offers a comprehensive overview for the human brain. It is based on "bulk" gene expression from laser-microdissected brain areas human donors. It confirms the hippocampus as the site

with highest MR mRNA expression, with GR mRNA being low in the CA2 area and (surprisingly) modest in CA1. It also points to substantial MR expression in the amygdala and in a number of thalamic and brain stem nuclei.[38]

The recent technological advance of single-cell (or single-cell nucleus) sequencing has, at the RNA level, substantially expanded our knowledge about receptor expression. Mouse single-cell expression data of the cortex and hippocampus are now publicly available as a resource from the Allen Institute.[39] We recently used the mouse Allen Brain Atlas data to describe MR and GR gene expression in the four main types of glutamatergic neurons, the five main types of GABA-ergic neurons, and non-neuronal cells.[40] An overview of the expression of MR and GR in different neuronal and non-neuronal cell types is given in Figure 1. This analysis confirmed the predominance of MR over GR expression in glutamatergic cells, as has been repeatedly shown with other cellular anatomical approaches,[41,42] in the absence of significant sex differences.

CA2 pyramidal neurons have very high MR expression, which, in the mouse, has been convincingly linked to the identity of these cells.[43] Notably, mouse MR was also expressed at higher levels than GR in the hippocampal GABA-ergic neurons, and in the hippocampal astrocytes. By contrast, MR expression was absent in oligodendrocytes and microglia cells. This representation is very similar for the cell populations from the human cortex.[44] Although MR presence in GABA-ergic

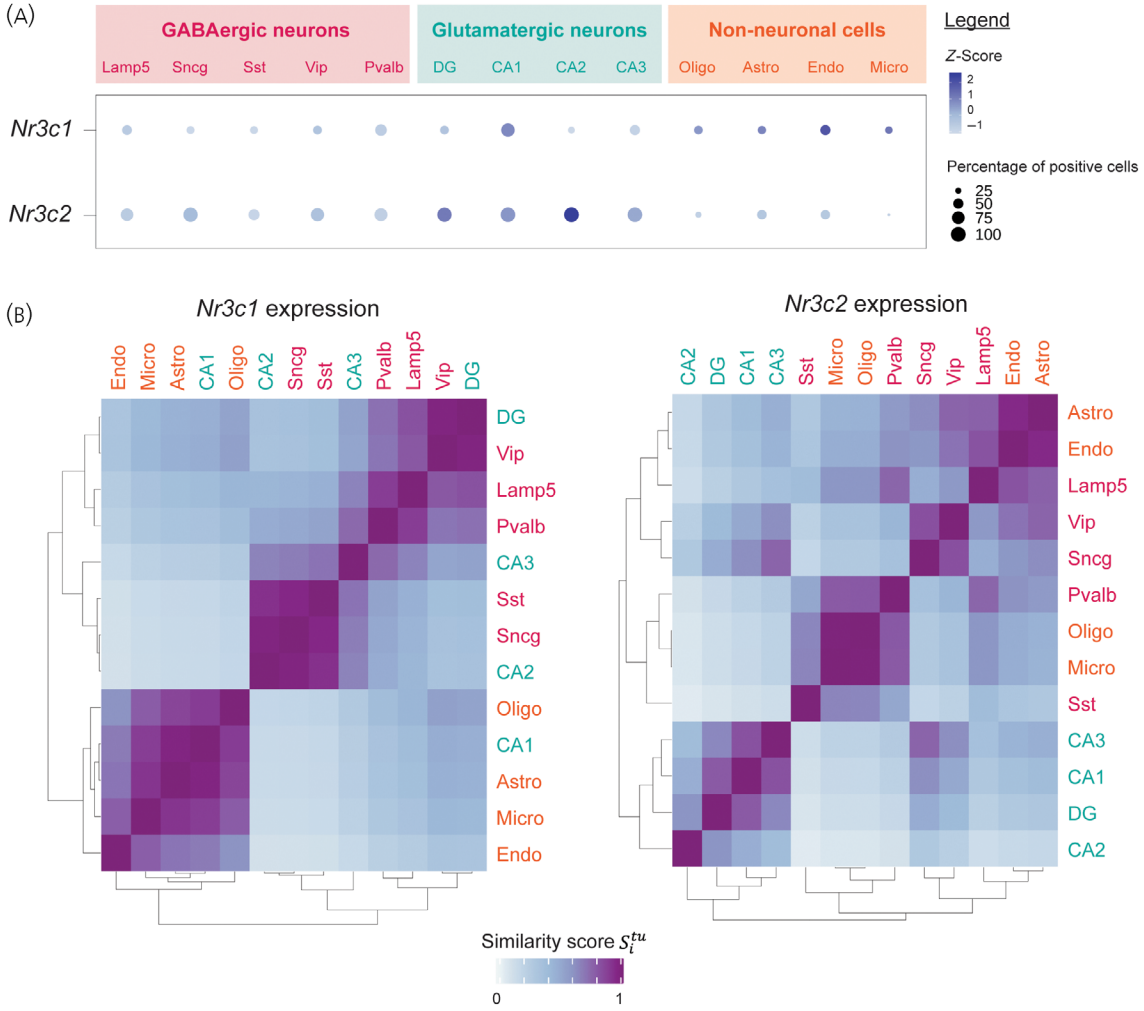

FIGURE 1 Corticosteroid receptor expression in the adult mouse hippocampal cell types. (A). Dotplot representation of *Nr3c1* and *Nr3c2* average expression across hippocampal glutamatergic and GABAergic neurons, and non-neuronal cells. The data were processed according to the standard Seurat pipeline (v.3.1.5),[105] as described previously.[40] The *Z*-score is the centered normalized average expression, and the dot size represents the percentage of cells positive for *Nr3c1* or *Nr3c2*. (B). Heatmap representation of the relative distance between cell types based on *Nr3c1* or *Nr3c2* expression. For each combination of cell types, the average expression was calculated for gene *i* (*Nr3c1* or *Nr3c2*) in cell type *t* (x_i^t) and in cell type *u* (x_i^u). For each gene, the score of similarity in expression between the two cell types was calculated as $S_i^{tu} = (x_i^t)/(x_i^u)$, where $x_i^t \leq x_i^u$. The similarity score (S_i^{tu}) varied between 0 (maximal distance) and 1 (minimal distance). Astro, astrocytes; Oligo, oligodendrocytes; Endo, endothelial cells; Micro-PVM, microglia/perivascular macrophages; Lamp5, lysosomal associated membrane protein family member 5; Vip, vasoactive intestinal peptide; Pvalb, parvalbumin; Sncg, synuclein gamma; Sst, somatostatin; DG, dentate gyrus; CA1, cornus ammonis 1; CA2, cornus ammonis 2; CA3, cornus ammonis 3

neurons and astrocytes needs to be confirmed at the protein level, the mRNA data point to the relevance of MR in a broader range of cell types than might be anticipated from classical visualizations. For example, autoradiograms may bias the interpretation towards the pyramidal and granule cells simply based on cell density. The data from these single-cell repositories are gathered from mice under generally undefined basal conditions and only six ("clinically unremarkable") human donors. Nevertheless, they are highly valuable, given their public availability, cross-species approach, and the fact that they should before long include cell types from all brain areas of the mouse and humans.

1.4 | Transcriptional mechanisms: DNA binding and interacting proteins

The effects of GR and MR as transcription factors depend on nuclear translocation and on interactions with other proteins that affect transcriptional regulation once the receptors are bound to the chromatin. These interactions in turn depend on post-translational modifications of the receptors.[45] All these aspects depend on the cell type and the context of cellular activity. For example, nuclear translocation is affected by the components of the chaperone complex of the cytoplasmatic GR,[46] and this may explain why nuclear localization differed between rat hippocampal cell types in absence of hormone.[41] Contextual activity was demonstrated elegantly in mouse cortical neurons, where synaptic activity can induce specific phosphorylation of the GR that is linked to transcriptional activity.[47]

The mode of DNA binding of MR and GR still is subject to debate. The receptors can bind as homo- or heterodimers to two inverted stretches of six nucleotides: the glucocorticoid response element (GRE[29]). One alternative mechanism is direct DNA binding to negative GREs (nGREs), a mechanism that seems unique to GR.[48,49] The last mode of binding is formed by the direct interaction of (in particular) GR with other transcription factors, which may or may not also involve the receptor binding to the DNA. This mechanism received much attention because of its promise to clinically separate anti-inflammatory effects from the side effects of such therapies.[50] However, this concept recently met with criticism because new approaches suggest that some form of direct DNA binding is occurring in all instances, and that previously reported protein–protein interactions may have involved "cryptic" GRE (half) sites.[51–53] Definitive answers on the relevance of the diverse mechanisms that involve non-GRE sites are still pending, even after 35 years of intense research.

In the rodent hypothalamic–pituitary–adrenal axis, the GR-mediated repression of hypothalamic *Crh* and pituitary *Pomc* may be regulated by nGREs as part of slow feedback via GR.[54,55] However, in the hippocampus (as assessed at the "bulk" level rather than the single-cell level), the predominant mode of DNA binding appears to be via GRE binding.[24–26,56] In chromatin contexts, this should occur in conjunction with other transcription factors that bind nearby.[57] These interactions likely play a role in the fact that MR and GR can have mutually exclusive binding to GREs in chromatin context, even if they also share GREs at many loci. For example, we found that exclusive MR binding co-occurred with the consistent presence of the binding motif for NeuroD transcription factors in the vicinity of the GRE. Indeed, NeuroD2 was detected at the DNA near the MR binding sites in hippocampal chromatin. In in vitro reporter assays, NeuroD factors could however potentiate both MR and GR-mediated transcription. Although other transcription factors play a role in the determination of MR/GR binding specificity, no exact mechanism has been resolved.[58] Nevertheless, enrichment of motifs for other transcription factors is consistently found, and these likely form a "code" for specific gene regulatory programs.

Of note, MR and GR dimers may form the basis for higher-order complexes, and transcriptional regulation may actually require tetrameric binding of the receptors.[59] This gives a new twist to the combined presence of MR and GR at the same GRE because there may be variable stoichiometry of MR and GR in higher-order complexes.[60] Although combined regulation of genes by MR and GR is clearly relevant for hormone sensitivity, the functional relevance of combined MR/GR presence is still unknown.

A next layer of MR/GR signaling takes place at the chromatin after DNA binding and consists of the recruitment of other proteins, that make up the actual "genomic" signal transduction of the receptors. The interacting proteins include transcription factors that bind nearby on the DNA,[58,61] and proteins that either form the bridge to the RNA polymerase II complex or that act as local chromatin remodelling factors: the nuclear receptor coregulators.[62] The protein complexes of steroid receptors and coregulators contain tens or hundreds of proteins.[63] Given the combinatorial nature of these complexes, cell-specific expression of individual factors can also be very important here. For example, two splice variants of steroid receptor coactivator (SRC)-1 differentially affect steroid receptor signaling[64] and, in combination with their differential distribution in the brain, this may account for the directionality of regulation of the *Crh* gene via GR that has been observed in both mouse and rat brain.[65,66]

Also for coregulators, genome-wide spatial[67] and single-cell[40] expression analysis revealed a substantial specificity of expression. One example is found in microglia cells, which, in the mouse single-cell data, uniquely seem to rely on SRC-2, rather than on SRC-1, as the predominant member of the SRC-coactivator family. This suggests that GR signaling in microglia is mechanistically different from all other brain cells, and indeed reminiscent of GR signaling in peripheral immune processes.[68] The coregulator diversity is all the more interesting because it may be targetable with some degree of selectivity via ligands known as selective GR (or MR) modulators (SGRMs and SMRMs[69,70]). Full agonists induce or stabilize a fully active conformation and antagonists prevent downstream signaling, whereas selective receptor modulators combine agonistic and antagonistic properties. A possible basis for these differences may lie in separating DNA binding from protein–protein interactions, but this notion is losing some of its popularity.[53,71] Rather, differences in coregulator recruitment may underlie selective receptor modulation.[72] For example, finding ligands that differentiate between SRC-1 and -2 may lead to more selective targeting of GR in microglia. As another example, it may be possible to differentiate between GR in the limbic brain and GR at negative

feedback sites based on differences in coactivator versus corepressor recruitment by GR that are induced after ligand binding.[73]

In summary, the single-cell type transcriptomes offer the possibility to define cross-talk partners for MR and GR, and this information can be linked to interactions that are induced by specific ligands. The data can also reveal the whole repertoire of other types of receptors, predicting functional cross-talk between glucocorticoids and any other type of signaling molecule.

2 | TARGET GENES

In relation to stress we assume that often MR and/or GR target genes fulfil a central role in establishing appropriate adaptive responses in cells, organs and eventually the whole organism. MR/GR target genes are also considered the mediators of the increased disease vulnerability during chronic glucocorticoid exposure, be it stress-induced or otherwise. All the transcriptional mechanisms that are linked to MR/GR activation result in cell-specific sets of target genes. Which of these are main drivers of changes in cellular (re-) activity, are there any "bystanders", and which target genes should be considered as therapeutic targets in stress-related disease? Such questions led to a substantial number of studies addressing the MR and GR target genes.

2.1 | MR/GR specificity

A first question to address is whether target genes are specific to either MR or GR. Classical target genes such as FKBP5, GILZ, SGK1, and PER1 can respond to both MR and GR activation, as is evident from responses to aldosterone (MR) and dexamethasone in different tissues,[74–77] and from gene regulation in both MR or GR knockout mice.[27] Receptor expression may simply be the major determinant for the regulation of such genes in a particular cell type. For example, in the hippocampus, microglia and oligodendrocytes do not express MR, and target genes that are specific to these cells will be GR-specific.[40] In cells where both receptor types are expressed, loci on the DNA can be specific to GR or MR (as discussed above), yet more than one GRE may be involved in the regulation of a particular target gene.[78] Therefore, GRE specificity does not necessarily translate into target gene specificity. Nevertheless, MR chromatin immunoprecipitation sequencing on whole tissue suggested that the mouse *Jdp2* gene is a selective MR target gene in the hippocampus.[27] Interestingly, although most characterized transrepression mechanisms apply to GR, we appear to lack knowledge of GRE-driven genes that are intrinsically responsive only to GR. The direct comparison of transcriptomes of MR and GR knockout mice at the single-cell level should help to answer such questions.

2.2 | The target gene or the ensemble?

In some systems, individual target genes may be central to a particular physiological response. For example, the induction of *Sgk-1* via MR in the kidney collecting duct appears to explain a major part of the aldosterone effects on salt retention, and the complete dependence of the *Pnmt* gene on the GR is crucial for adrenalin production in the adrenal medulla.[79] Similarly, induction of the extrahypothalamic *Crh* gene may be central to anxiogenic effects of glucocorticoids. Yet, any transcriptomics approach is bound to identify "long lists" of regulated genes. This may in part reflect multiple cell types that are present in "bulk" tissue RNAseq,[80] but we can speculate that single-cell approaches will lead to as many longlists as there are GR/MR expressing cell types.

Most likely, many glucocorticoid effects depend on ensembles of regulated genes, that belong to particular classes as revealed by gene ontology classes. After all, the concept of a *coordinated* transcriptional response is central to the effect of any transcription factor. An example can be found in muscle atrophy after glucocorticoid use, which depends on sets of induced "atrogenes" and repressed anabolic genes.[81] Therefore, we may aim to at least generate shortlists of regulated genes that are necessary and/or sufficient for glucocorticoid effects to occur. Indeed, the permissive nature of glucocorticoid signaling[82] perhaps requires that more genes get regulated than are necessary for a particular response. After all, there can be many causes for glucocorticoid secretion from the adrenal. Specific populations of cell types may be involved in the response to any challenge or stressor, and the requirements on the cell biology may be stressor specific.[83] Fine-tuning mechanisms did evolve, in which cross-talk with specific membrane associated signaling pathways limits the transcriptional response.[84] Nevertheless, probably not all target genes are essential for particular responses (be it cellular, physiological or behavioural). Therefore, the attempt of making shortlists from longlists may in fact be viable. We recently made two such attempts in relation to the well-established phenomenon of enhanced memory consolidation by glucocorticoids in rats and mice.

Corticosterone via GR activation may strengthen memory consolidation in a diversity of learning tasks, as established by GR antagonism, as well as by the administration of corticosterone.[85] The object location recognition paradigm can be set up in such a way that rats will not remember the localization of objects 24 h later. Under these conditions, corticosterone can act as a switch for memory consolidation: a post-training injection does lead to consolidation of the spatial information learned in the task.[86] Under the assumption that effects in the hippocampus are involved, we reasoned that the corticosterone-induced changes in the transcriptome under these conditions would contain the genes necessary for memory consolidation. Moreover, this gene regulation might occur in conjunction with other signaling pathways, namely those activated by the learning task itself. We therefore hoped to find genes that were exclusively regulated by corticosterone in the learning condition and not in animals that were not trained. However, the latter assumption was wrong: the (relatively mild) training procedure did not influence the hippocampal set of corticosterone-regulated genes. This may be either a true negative effect or the result of a context-specific gene regulation being present in a subset of neurons, which may be diluted out in bulk RNA sequencing. Therefore, this hypothesis also awaits single-cell approaches. Of note, based on the cell-type specific expression on

basal conditions, the corticosterone-induced target genes represented many changes in non-neuronal cell types, such as microglia.[80]

In a separate approach using fear conditioning of mice, we made use of the availability of four different ligands for GR: next to the agonist corticosterone and the antagonist RU486, we treated animals with two selective GR modulators (CORT108297 and CORT118335). CORT108297 in rats acted like corticosterone with respect to enhanced memory consolidation, whereas CORT118335 acted like the classical GR/PR antagonist RU486.[73,87] The SGRMs have unique sets of partially overlapping target genes. We reasoned that any transcriptional change that is potentially responsible for changes in memory consolidation strength should consistently vary with the behavioural effects of each ligand. This "pharmacological filter" helped to substantially reduce the number of hippocampal candidate target genes, to a short list of fewer than 15 genes. Of note, also this list contained many genes expressed in microglia. This may simply reflect the number of microglia cells and expression level of GR in these cells, or may point to microglia being part of the mechanism by which GR activation enhances memory consolidation.[88,89]

2.3 | Neurodegeneration and aquaporin 4

An exciting GR-regulated gene that, on its own, is potentially a major contributor to a particular glucocorticoid effect is related to neurodegenerative disease. A detrimental effect of chronically elevated glucocorticoids for neuronal viability has long been postulated.[4] Consequently, GR antagonists have been tested and were effective in a variety of animal models for neurodegeneration. These include a variety of Alzheimer's disease models and the wobbler mice, which models amyotrophic lateral sclerosis.[90–92]

Historically, GR activation has been linked to excitotoxicity and "neuro-endangerment".[93] In one study, however, beta amyloid content was substantially reduced only 24 h after 3 days of treatment with RU486.[94] Because amyloid accumulates slowly, this suggests a clearance mechanism that would be suppressed via GR activation. Indeed, we recently observed that the astrocyte-specific gene coding for aquaporin 4 (*Aqp4*) was strongly suppressed in the brains of the AdKO mouse model for Cushing's disease.[10] Aquaporin 4 is the limiting factor for the process of cleaning the brain via "glymphatic flow".[95] Moreover, chronic stress and dexamethasone were both shown to reduce glymphatic flow in the rat, in a GR-dependent manner.[96] These findings suggest that GR antagonists may have a generic attenuating effect on the consequences of neurodegeneration via an increased clearance of harmful factors from the brain. This hypothesis remains, for now, unproven and the mechanism by which GR stimulates aquaporin 4 expression is not known. Yet, it is an attractive "single target explanation" for a potentially clinically relevant effect of GR antagonism.

3 | CONCLUSIONS

Glucocorticoids can have many effects on brain function, for better and for worse. Both MR and GR have their respective roles, in an interplay that is incompletely understood. In this review, we have discussed transcriptional mechanisms and target genes that can underly their effects. Despite the complexities discussed, we have simplified things in at least two ways. First, we have implicitly assumed that effector genes are direct transcriptional targets, whereas glucocorticoids may regulate other transcription factors that may act as "master genes" for transcriptional effector programs. For example, chronic glucocorticoid exposure can induce androgen receptor expression in the liver.[97] Second, we have not explicitly covered cell-autonomous versus indirect effects: the transcriptome in a particular cell type need not be responsible for changes in (re-) activity of that cell type. As a speculative case in point, it may possible that glucocorticoid-dependent changes in synaptic strength require GR-mediated transcriptional effects in microglia cells.[88]

The non-cell autonomous effects are important to consider in relation to the use of reduced experimental systems, such as cell lines and organoids.

For the transcriptional effects of both receptor types, specific interactions with other transcription factors and coregulators are important, and more work on chromatin structure is needed to gain better insights. As with transcriptomics, it will be important to obtain much more detailed insight at the single-cell level. Linking the different levels of transcriptional regulation is one strategy to filter out relevant processes in what often appears to be a pleiotropic response. The first (multi-omics) studies addressing responses to stress at the single-cell type level have been published,[98–100] addressing responses at timescales from hours to months after stress exposure. Yet, these studies did not focus on glucocorticoid contribution to stress-induced changes in gene expression. The different levels that need attention include chromatin occupancy by receptors, local chromatin status and accessibility, and long-range chromatin interactions. Technological developments to help our understanding are many,[101] even if they often are challenging to perform in vivo and at the single-cell level. Yet, there is certainly progress, as exemplified by the recent approaches to identify estrogen receptor binding in the mouse brain.[102] Spatial transcriptomic approaches offer additional promise,[103] as well as for the study of post-mortem human brain samples.[104]

Often, however, multi-omics approaches are prohibitively expensive. We want to emphasize that also other strategies of identifying relevant (sets of) target genes for particular processes are possible, based on consistent correlation of (bulk transcriptomics) gene expression and functional outcome. Here, our "pharmacological filter" using the different GR ligands as discussed above may serve as an example, although many variations on the theme are conceivable.[88] In the end, definitive evidence for the functionality of gene regulation by glucocorticoids will have to come from removing the responsible regulatory genomic sequence (rather than the target gene as a whole). One example of a GRE-deletion was published based on a serendipitous finding,[16] but, with the advances of gene editing, the identification of other GREs that are necessary for particular functional effects of glucocorticoid may soon follow.

In parallel, glucocorticoid researchers may, and should, of course simply attempt to take good note of clinical data, as well as

neuroscientific and transcriptional mechanisms, and not forget the relevance of testing a well-defined hypothesis, rather than put all hopes on transcriptomics approaches.

AUTHOR CONTRIBUTIONS

Onno C. Meijer: Writing – original draft; writing – review and editing. **Jacobus C. Buurstede:** Conceptualization. **Eva Myriam Goussivi Viho:** Conceptualization; formal analysis; visualization. **Jorge Miguel Miguel Amaya:** Conceptualization. **Anne-Sophie Koning:** Conceptualization. **Merel van der Meulen:** Conceptualization. **Lisa T. C. M. van Weert:** Conceptualization. **Susana Paul:** Conceptualization. **Jan Kroon:** Conceptualization; writing – review and editing. **Lisa Koorneef:** Conceptualization; writing – review and editing.

CONFLICTS OF INTEREST

Onno C. Meijer receives funding from Corcept Therapeutics, who develop GR modulators for clinical use.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jne.13213.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this review because no new data were created or analyzed.

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How to cite this article: Meijer OC, Buurstede JC, Viho EMG, et al. Transcriptional glucocorticoid effects in the brain: Finding the relevant target genes. *J Neuroendocrinol*. 2023;35(2):e13213. doi:10.1111/jne.13213