Glucocorticoid-Dependent Hippocampal Transcriptome in Male Rats: Pathway-Specific Alterations With Aging

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Although glucocorticoids (GCs) are known to exert numerous effects in the hippocampus, their chronic regulatory functions remain poorly understood. Moreover, evidence is inconsistent regarding the long-standing hypothesis that chronic GC exposure promotes brain aging/Alzheimer disease. Here, we adrenalectomized male F344 rats at 15 months of age, maintained them for 3 months with implanted corticosterone (CORT) pellets producing low or intermediate (glucocorticoid receptor–activating) blood levels of CORT, and performed microarray/pathway analyses in hippocampal CA1. We defined the chronic GC-dependent transcriptome as 393 genes that exhibited differential expression between intermediate and low CORT groups. Short-term CORT (4 days) did not recapitulate this transcriptome. Functional processes/pathways overrepresented by chronic CORT–up-regulated genes included learning/plasticity, differentiation, glucose metabolism, and cholesterol biosynthesis, whereas processes overrepresented by CORT–down-regulated genes included inflammatory/immune/glial responses and extracellular structure. These profiles indicate that GCs chronically activate neuronal/metabolic processes while coordinately repressing a glial axis of reactivity/inflammation. We then compared the GC transcriptome with a previously defined hippocampal aging transcriptome, revealing a high proportion of common genes. Although CORT and aging moved expression of some common genes in the same direction, the majority were shifted in opposite directions by CORT and aging (eg, glial inflammatory genes down-regulated by CORT are up-regulated with aging). These results contradict the hypothesis that GCs simply promote brain aging and also suggest that the opposite direction shifts during aging reflect resistance to CORT regulation. Therefore, we propose a new model in which aging-related GC resistance develops in some target pathways, whereas GC overstimulation develops in others, together generating much of the brain aging phenotype. (Endocrinology 154: 2807–2820, 2013)

G lucocorticoids (GCs) are pleiotropic adrenal steroid hormones that affect nearly all tissues of the body and, in particular, regulate metabolic, developmental, and stress-related processes (1, 2). GCs also exert powerful anti-inflammatory actions, and synthetic analogs are the therapeutic agents of choice for many inflammatory/neuroinflammatory disorders (2–5). However, the long-term effectiveness of GCs is limited by severe metabolic side effects (3).

In the hippocampus, GCs exert a wide range of actions that depend on the duration and level of hormonal exposure (6–9). Although short-term effects are generally considered adaptive, long-term elevation of GCs (eg, as in chronic stress) has frequently been associated with cognitive deficits and/or neuropathological alterations (6–14), particularly at higher ranges of GCs (15). Moreover, a long-standing hypothesis links chronic GC exposure to
unhealthy hippocampal aging/Alzheimer disease (AD) (16–20). Nevertheless, given the brain’s continuous exposure to circulating adrenal steroids, it appears highly likely that, in addition to damaging effects, chronic exposure to GCs also mediates a number of biologically important brain functions.

Because of the complexity and extent of the GC signaling network (1, 7), however, elucidating chronic deleterious or adaptive processes has been difficult. GCs regulate hippocampal processes primarily via 2 nuclear receptors, the glucocorticoid receptor (GR), a ubiquitous transcription factor that mediates most GC-dependent transcription, and the mineralocorticoid receptor (MR), which has higher affinity for GCs and primarily mediates functions dependent on low corticosteroid concentrations (7, 21, 22). The GR has numerous isoforms and regulates transcription both via binding to specific DNA sequences (glucocorticoid response elements [GREs]) that positively (pGRE) or negatively (nGRE) modify transcription of adjacent target genes and via protein-protein interactions with other transcription factors, coactivators, and corepressors (1, 23–26). In addition, analogously to other steroids (27–29), GCs regulate various major physiological functions via nongenomic pathways (7, 8, 30–33). This diversity of isoforms and mechanisms enables GCs to regulate multiple pathways independently within the same tissue or cell.

One highly effective approach for dissecting complex systems such as hormonal target networks is gene expression profiling and associated pathway analysis (7, 34, 35). Microarrays or other expression profiling methods yield sets of differentially expressed genes that can be used to interrogate pathway databases and identify altered processes/pathways (36–40). Profiling techniques have been applied previously to analyze effects of stress or short-term exposure to GCs on gene expression in rodent hippocampus (41–47), but there have been no such studies on long-term exposure to normal-range levels of corticosterone (CORT), the naturally occurring GC in rodents. Consequently, neither the deleterious nor the adaptive processes mediated by chronic brain exposure to natural GCs are well understood.

Further, although it is clear that the effects of some steroids, including estrogen and progesterone, both change with aging and modify markers of brain aging (29, 48–51), evidence is inconsistent regarding the role of GCs in brain aging. Variations of the hypothesis that chronic exposure to GCs/stress promotes brain aging processes, especially in the hippocampus (16–19, 52), have garnered substantial support from studies showing that various biomarkers of brain aging can be accelerated or retarded by conditions that enhance or reduce GC impact, respectively (7, 10–12, 17, 18, 20, 53–56). Additional support has come from findings that GCs correlate with or accelerate markers of dysfunction in AD or AD models (57–61). However, other studies have found evidence that GCs do not accelerate brain aging and in fact can act in an apparent “antiaging” direction on some brain aging/AD markers (2, 13, 62–66). These contradictory results highlight the need for further tests of the general hypothesis, using a broader range of aging markers to control for anomalous indicators.

Here, we used microarray/pathway analyses to identify the chronic GC-dependent transcriptome and its associated functional processes in aging rat hippocampus. In addition, we analyzed the overlap of this GC transcriptome with a previously identified rat hippocampal aging transcriptome, to assess aging changes in the GC-dependent transcriptome and to test 2 implicit predictions of the hypothesis that GCs promote brain aging: (1) CORT-targeted genes should have a significantly higher probability of changing with aging than genes not targeted by CORT; and (2) most corticosterone- and aging-sensitive (CAS) genes should shift expression in the same direction with CORT and aging. The results reveal surprising effects of aging that indicate the need for new, more complex models of GC interactions with brain aging.

Materials and Methods

Animals and tissue preparation

All procedures were performed in accordance with federal animal care guidelines (Institutional Animal Care and Use Committee 00166M2000) at the University of Kentucky’s Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility.

Forty male Fischer 344 (F344) rats aged 14 to 15 months (late midage) were surgically prepared (sham operated, n = 10; adrenalectomized, n = 30) at Harlan, Inc (Indianapolis, Indiana) and shipped to the University of Kentucky 10 to 12 days after surgery. Upon arrival, adrenalectomized (ADX) subjects were maintained on isotonic saline (+1% sucrose) drinking fluid. Supplemental feed mash (standard chow mixed with water and warmed), apple chunks, and sc 1.25-mg corticosterone sulfate injections (Solu-Delta-Cortef; Pfizer, New York, New York) were provided. Pellet implant surgery was performed according to standard protocols. Sham animals received inert pellets. ADX animals were divided into 2 groups; low-dose animals (n = 20) received one 25-mg CORT pellet, whereas intermediate-dose animals (n = 10) received one 200-mg CORT pellet (slow release, catalog no. NG-111; Innovative Research of America, Sarasota, Florida). Incisions were closed with wound clips (removed after 10 days). Pellets were not presoaked, because the vendor’s instructions stated that pellets are designed for continuous and even CORT release for 90 days, beginning with initial subcutaneous tissue contact. No evidence of major pellet embedding/impaction was noted at study completion and terminal
plasma CORT levels confirmed that pellets were still releasing appropriate and anticipated amounts (Figure 1).

Animals were housed singly for 3 months after surgery and euthanized at ~18 months of age. Low-CORT animals received saline/sucrose drinking water. One ADX animal was removed for declining health. During week 12, just before study termination, 9 of the ADX low-CORT subjects were assigned to a short-term, high-dose group (low-CORT/ST) and given daily sc CORT injections (5 mg/d for 4 days; a relatively high dose) (67).

Final treatment groups (n = 9-10/group) consisted of the following: (1) sham (intact) control; (2) low-CORT (ADX); (3) intermediate-CORT (ADX); and (4) low-CORT/ST (ADX).

Animals were euthanized by deep CO2 anesthesia and decapitation between 8 and 10 AM on the last days of the study. CORT was measured from trunk blood using an RIA kit (ICN Biomedicals, Costa Mesa, California), according to the manufacturer’s directions. Duplicate readings were averaged and treated as single observations, and outlier values (>2 SD from the mean, with outliers included) were treated as missing values for statistical analysis.

Microarray procedures

Tissue preparation and quality control

Each animal’s hippocampal CA1 region was dissected and processed for microarray analysis (RGU34A; Affymetrix, Santa Clara, California) as described previously (37). Scanned microarray images were analyzed using GCOS (Affymetrix). Quality control measures, scaling factor (2.31 ± 0.06), RawQ (2.37 ± 0.06), percent present (46.1 ± 2.2), and GAPDH 3′:5′ (1.40 ± 0.11), were within normal ranges and did not differ significantly with treatment (P > .1; 1-way ANOVA). No visual microarray hybridization defects (residual signal analysis) (68) were found. This study has been uploaded to the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo/) under accession number GSE3761.

Prefiltering algorithm

A prefiltering algorithm was applied to reduce the number of multiple comparisons, as in prior studies (37, 69, 70). In brief, gene symbol–annotated, A grade probe sets (probe sets for which the manufacturer confirms most probes match the transcript perfectly) with sufficient signal intensity (>4 chips with ≥4.2 signal intensity) for reliable measurement were retained.

Statistical analysis

Filtered genes were analyzed by 1-way ANOVA (Figure 2), and significant results were evaluated with post hoc all-pairwise Fisher protected least significant difference testing. Multiple testing error (false-positive results) was estimated by the false discovery rate (FDR = expected/observed) (71) procedure, and median FDRs are reported. Identifying CAS genes

The GC-dependent transcriptome identified here was tested for overlap with an aging-dependent transcriptome constructed from 3 published microarray studies of aging male F344 rat hippocampus (Gene Expression Omnibus data deposition references): Ref. 37: GSE834, 3 months old (n = 9), 12 months old (n = 10), and 24 months old (n = 10); Ref. 72: GSE5666, 5 months old (n = 29) and 25 months old (n = 49); and Ref. 69: GSE9990, 3 months old (n = 9), 6 months old (n = 9), 9 months old (n = 9), 12 months old (n = 9), and 23 months old (n = 15). In total, this set comprised 158 individual microarray transcriptional profiles. The aging transcriptome was defined as all genes that differed significantly between young and aged animals in any 1 of the 3 studies. Common (overlapping) genes were defined as those genes present (identified) in both the aging- and the GC-dependent transcriptomes. Statistical comparison procedures (binomial tests) similar to those published previously (73–75) were used to find significant deviations from chance.

Biological process/pathway identification

Significantly overrepresented functional processes were identified using the Database for Annotation, Visualization and Integrated Discovery (DAVID) overrepresentation tool (76) on the Gene Ontology (GO) database (77). The table clustering option and streamlined GO-FAT subset of the GO were used for analysis. To reduce redundancy, a single process illustrative for each cluster (78), populated by between 3 and 50 genes, was identified at P < .05; k statistic/EASE score, is reported (see Results). In addition, the GO database is still in a work progress, and there is some underassignment of genes to relevant functional categories. Consequently, based on a literature review, some identified genes and functional processes that appeared appropriate to the lists obtained by DAVID/GO analysis are added (additions are underlined in figures and tables).

Real-time quantitative (q) PCR

Twelve CORT-dependent genes identified by microarray were validated using real-time qPCR on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, California) in RNA samples from the contralateral hippocampi of the same experimental animals. One-step RT-PCR was performed on 50 ng of cellular RNA in a 30-μL PCR mix containing mRNA-specific primers and Taq Man probes for each gene. For each target gene, RNA samples from all animals in the 4 experimental groups were analyzed in duplicate on the same 96-well PCR plate. The mRNA levels were quantified by plotting on a standard RNA serial dilution curve run in parallel. All results are normalized to Gapdh.

Immunohistochemistry

Immunohistochemical analysis was performed as in the prior work (69). The following primary antibodies and concentrations were used for overnight incubation: rabbit GR (1:1000, sc-1004; Santa Cruz Technology, Santa Cruz, California) and mouse glial fibrillary acidic protein (GFAP) (1:1000, clone G-A-5; Sigma-Aldrich, St Louis, Missouri). For double staining after the incubation in primary antibodies, the tissues were incubated in donkey anti-rabbit Cy3 and donkey anti-mouse fluorescein isothiocyanate secondary antibodies (1:500; both from Jackson ImmunoResearch Laboratories, Inc, West Grove, Pennsylvania). To obtain comparable results, all sections were stained simultaneously in the same staining tray.

Results

Plasma corticosterone and body weight

Blood was collected between 8 and 10 AM, near the trough of the rat CORT circadian cycle. However, CORT
concentrations in sham intact animals (~95 ng/mL) (Figure 1) were somewhat above the trough baseline generally reported for F344 male rats (79, 80). Therefore, the animals may have shown effects of mild stress or circadian disturbance from being transferred to the laboratory. Alternatively, the elevated values in sham rats may have reflected the aging-related increase in blood CORT reported in a number of rat studies (16, 56, 81–83). In ADX animals given low-CORT replacement, plasma CORT was severely reduced from normal levels (Figure 1). In contrast, ADX animals given intermediate-CORT replacement showed blood levels above those in sham controls (albeit nonsignificantly), in approximately the upper range of the normal circadian cycle (56, 79, 80, 82). This range activates GRs in hippocampus (21, 83).

Both low- and intermediate-CORT groups exhibited weight loss at the time of ADX surgery, and neither recovered to the level of sham animals (Table 1), consistent with previous long-term ADX studies (20, 84). However, these effects were not due to altered nutrient intake because both ADX groups showed food consumption similar to that of sham animals. In both ADX conditions, water intake remained elevated through the entire study, presumably related to a lack of adrenal mineralocorticoids. Unlike prior long-term ADX studies in which elevated CORT was administered in the drinking water (84) or by injection (15), the higher dose pellet–implanted animals in this study did not exhibit reduced food intake, additional weight loss, or general signs of physiological deterioration. The ADX-related modest weight loss apparently had little effect on gene expression regulation in the hippocampus, as indicated by the similar profiles of the ADX intermediate-CORT and the sham control groups. In addition, the major statistical comparisons were performed between the low- and intermediate-CORT groups (see below).

### Chronic CORT-dependent transcriptional profile

The algorithm used to identify the GC-dependent transcriptome (genes sensitive to long-term corticosterone exposure) is summarized in Figure 2A. First, total probe sets (8799) on the Affymetrix GeneChip array (RGU34A) were prestatistically filtered (see Materials and Methods) to select probe sets with a high-quality signal and an A grade gene symbol annotation (6685 genes). Of these, 2033 redundant probe sets were excluded, leaving 4652 gene probes for analysis. Each of the remaining 4652 gene probes was tested by 1-way ANOVA for significant difference (at $P \leq .05$) across the 4 treatment groups. As shown, 641 genes were found to differ significantly across the groups, generating a reasonably low FDR (median FDR = 0.19) (71) and indicating that the error of multiple testing contributed comparatively few false-positive results to the identified significant genes.

Specific groups between which genes differed were determined by all-pairwise testing (Fisher protected least significant difference, at $P \leq .05$) applied to ANOVA-significant genes. As expected, the sham vs intermediate-CORT pairwise comparison generated the smallest number of significantly different genes (135), whereas sham vs low-CORT (446) and intermediate-CORT vs low-CORT (393) comparisons yielded considerably more genes with differential expression. To exclude confounds from the

### Table 1. Body Weight, Food Intake, and Water Consumption

|          | 3 Wk          | 12 Wk         |
|----------|---------------|---------------|
|          | Sham | Low   | Intermediate | Sham | Low | Intermediate |
| Weight, g* | 403 ± 9 | 350 ± 8 | 325 ± 12 | 399 ± 4 | 338 ± 9 | 331 ± 11 |
| Food, g/2 days | 37 ± 6 | 32 ± 1 | 40 ± 2 | 34 ± 1 | 31 ± 1 | 36 ± 2 |
| Water, mL/2 days* | 45 ± 2 | 92 ± 6 | 146 ± 15 | 34 ± 2 | 89 ± 4 | 76 ± 17 |

Measurements obtained early (~3 weeks after implant surgery) or late (final week) in the study, are shown for body weight, food intake, and water consumption. Data are means ± SEM.

* Significant weight and water consumption differences: $P < .01$, 1-way ANOVA, for both time points.
nonspecific effects of ADX that might be present in comparisons between sham vs low-CORT (ADX) animals, we defined the CORT-dependent transcriptome as the 393 genes for which hippocampal expression differed significantly between the intermediate-CORT (ADX) and low-CORT (ADX) groups (47). These 393 genes were separated for DAVID pathway analysis according to whether expression was activated (n = 156; Figure 2B, white) or repressed (n = 237; Figure 2B, black) by chronic CORT (up-regulated or down-regulated, respectively, in the intermediate-CORT group relative to the low-CORT group). (All 393 genes that differed significantly between the intermediate- and low-CORT groups are listed in Supplementary Table 1 published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org.)

Validation by qPCR

We validated accuracy of the microarray findings by performing qPCR on 12 genes identified as CORT-dependent in the microarray analysis (6 genes activated and 6 genes repressed). Of these 12, 11 were changed by CORT in the same direction (up or down) in both analyses (P = .0034, χ² test). Moreover, a Spearman rank correlation test between the microarray and qPCR analyses of the relative magnitude of the CORT effect for each of the 12 genes (ranked from most repressed to most activated) was highly significant (Figure 3), indicating strong agreement in the relative degree of change between the 2 measures of expression.

Figure 2. Microarray and pathway analysis. A, Filtering and statistical testing algorithm. Total probe sets were filtered to retain probe sets with at least 5 presence calls, and an A grade unique gene symbol level annotation. The retained 52% of probe sets were each tested by 1-way ANOVA (P ≤ .05) across the 4 treatment groups. The FDR for statistically significant genes is shown. B, Post hoc pairwise analysis (Fisher protected least significant difference, P ≤ .05) identified 393 genes that differed between low- and intermediate-CORT conditions (separated into up- and down-regulated categories), defining the GC-dependent transcriptome. C, Functional process analysis (DAVID; see Materials and Methods) revealed processes/pathways overrepresented by GC-dependent genes. For each overrepresented GO process, the number of GC-dependent genes identified for that process (#), and the overrepresentation P value (Overrep p-value) are shown. Underlined processes were added by the authors.

Figure 3. Correlation between microarray and RT-qPCR measures. Twelve genes identified by microarray analysis as chronic CORT dependent (6 up- and 6 down-regulated) were also assessed by RT-qPCR in extracted RNA from the same subjects (see Materials and Methods). Genes are ranked from the most significant decrease (1) to the most significant increase (12) for both microarray (y-axis) and PCR (x-axis) data. A highly significant Spearman rank correlation (P = .009) was found between datasets obtained with the 2 technologies, indicating strong validation of both direction and relative magnitude of expression change.
**Functional pathways/processes overrepresented in the GC-dependent transcriptome**

DAVID functional analysis revealed that genes whose expression was activated by CORT overrepresented several processes associated with neuronal plasticity and energy metabolism, including regulation of synaptic plasticity, learning, and memory (including immediate early genes [IEGs]), cholesterol biosynthesis, the glucose metabolic process, and response to steroid hormone and cofactor binding (Figure 2C).

In contrast, genes repressed by CORT overrepresented processes associated with immune/inflammatory responses, cell damage, oxidative systems, apoptosis, and extracellular matrix organization (Figure 2C). Many inflammatory/immune genes are expressed primarily in microglia (85, 86), but CORT also repressed at least 12 additional glial marker genes, including *Gfap* and others linked to reactive astrogliosis (87). Although not identified by DAVID analysis, these genes were assigned by the authors to a glial activation/structure process in Figure 2C (underlined).

**Localization and density of immunoreactive GR**

Immunohistochemical analysis was used to assess the distribution and relative concentration of GRs among cell types in the hippocampal field CA1 and adjacent corpus callosum of intact F344 rats (Figure 4). Figure 4, A and B, illustrate that pyramidal neurons exhibited intense GR immunostaining compared with that of glial cells scattered throughout other layers and white matter, as reported previously for rats (88). Double-label immunohistochemical analysis of GR and GFAP showed that nuclei with low GR immunoreactivity were frequently localized adjacent to astrogial cytoplasmic GFAP structures (Figure 4D), identifying them as likely astrocyte nuclei or were not clearly associated with GFAP but instead were aligned in parallel to white matter fibers, identifying them as likely oligodendrocytes (Figure 4C). These findings are consistent with the interpretation that glial cell nuclei contain low concentrations of GR relative to those in neurons (81, 88).

**Insensitivity of chronic CORT-dependent genes to short-term CORT treatment**

To determine whether long-term CORT exposure was in fact required for the manifestation of CORT dependence in the identified chronic transcriptome, we tested one of the low-CORT groups for sensitivity to a short-term high-dose CORT regimen (low-CORT/ST, 5 mg/rat injection for 4 days). Of the 393 genes found to differ between low-CORT and intermediate-CORT in the long-term study (Figure 2), only 124 (~32%) also differed significantly between the low-CORT and the low-CORT/ST, 122 of which were shifted in the same direction by long-term and short-term CORT. Therefore, expression of most chronic CORT-dependent genes was not altered by short-term CORT exposure. The 122 genes that also exhibited short-term sensitivity may constitute a more responsive subset (short-term CORT sensitivity of chronic CORT-dependent genes is reported by yes or no designations in Supplemental Table 1).

**Aging changes in CORT-dependent gene expression**

We assessed overlap of the CORT-dependent transcriptome with an aging-dependent transcriptome generated by combining 3 prior microarray studies of rat hip-
However, of these 217 CAS genes, a substantial majority (98) were down-regulated by CORT but up-regulated by aging and 47 were up-regulated by CORT but down-regulated by aging (“opposite direction genes”), whereas 31 were up-regulated by both CORT and aging and 41 were down-regulated by both CORT and aging (“same direction genes”) (all dual-sensitive genes are listed by a combination of directional change in Supplemental Table 1). The 217 CAS genes were grouped according to the 4 combinations of CORT and aging directional change above, and each grouping was assessed separately by DAVID analysis to identify overrepresented functional pathways/processes. Results are shown in Table 2 and are considered in the Discussion.

**Discussion**

In this study, we defined a chronic glucocorticoid-dependent transcriptome comprising 393 genes in aging rat hippocampus and interrogated the GO database to identify the biological processes and pathways overrepresented by this transcriptome. Our results revealed that chronic GC exposure activated genes encoding multiple neuronal and metabolic processes, while concomitantly repressing genes encoding an apparent “glial axis” of reactivity (including inflammation/immune response and astrogliosis). In addition, to assess how the GC-dependent transcriptome changes with brain aging, we determined its overlap (genes in common) with a previously identified hippocampal aging transcriptome. These analyses were used to test 2 implicit quantitative predictions of the hypothesis that chronic GCs advance brain aging, namely, 1) that genes comprising the GC transcriptome should be more likely than non-GC targets to change with aging and 2) that GCs and aging should regulate expression of common target genes in the same direction (19). Our findings supported the first prediction. However, they clearly contradicted the second, because a substantial majority (~67%) of genes common to both transcriptomes changed expression in opposite directions with CORT and aging. Thus, rather than promoting aging, the effect of CORT opposed the aging change for many of its targets and can be viewed as “antiaging.”

**GC-dependent transcriptome**

The GC-dependent transcriptome was defined by differential expression between the intermediate- and low-CORT groups. Because the GR is bound by ligand only at the higher plasma CORT levels present in the intermediate-CORT group (Figure 1), it is likely that most differentially expressing genes comprising this transcriptome were regulated primarily by the GR (47, 83). This con-
The 217 genes regulated by both long-term CORT exposure and aging (Figure 5) were sorted by combination of CORT- and aging-regulated directions and subjected to DAVID/GO functional process/pathway analysis. Significantly overrepresented functional processes are listed for each combination of regulated directions, according to the P value (DAVID score). No. represents the number of identified genes. Genes and processes added based on a literature review are underlined, and genes consolidated from multiple categories are listed in parentheses.

The conclusion is consistent with findings that hippocampal gene expression induced at higher concentrations of CORT is blocked selectively by a GR antagonist (89). Nevertheless, these observations do not preclude the possibility that some identified genes were regulated via nongenomic GC signaling pathways (8, 90) or secondarily by primary targets (83).

**Functional processes/pathways overrepresented by genes of the GC-dependent transcriptome**

Processes identified by genes activated by chronic CORT included synaptic plasticity, learning (IEGs), cholesterol biosynthesis, glucose metabolism, neurite outgrowth, and differentiation (Figure 2 and Supplemental Table 1). Although it is well established that acute CORT treatment or stressful conditions associated with learning paradigms can activate expression of some IEGs, notably Arc and Egr1 (64, 72, 91, 92), the present results show that chronic GC exposure activates a much broader genomic network important for learning/plasticity than recognized previously. The cholesterol biosynthetic pathway also is important for learning/plasticity because of its role in generating membrane for neurite outgrowth and because it provides isoprenoids that modulate long-term potentiation (90, 93). It should be noted, however, that some synaptic and plasticity genes also respond to shorter GC exposures (46, 94, 95) (also see Short-Term CORT Sensitivity column in Supplemental Table 1).

Conversely, processes identified by genes repressed by chronic CORT included inflammatory/immune and reactive glial responses, proteasomal/lysosomal proteolysis, apoptosis, proliferation, protein kinase cascades, extracellular matrix (ECM), and cytoskeletal structure. Approximately 50% more genes were down-regulated than up-regulated by chronic CORT, an effect related primarily to the large number of repressed inflammatory/immune genes (Figure 2). Based on prior studies of cell-specific expression (69, 85, 87, 96, 97), most target genes repressed by CORT are expressed primarily in glial cells.
Moreover, many of these repressed genes play important roles in reactive responses of different glial cell types to injury/pathology, including inflammatory/immune genes in microglia, Gfap and other genes (Pla2g4a, S100a4, Vcam, and Vim) in astrocytes, and Plip in remyelinating oligodendrocytes (Table 2D and Supplemental Table 1). Taken together, these data suggest that a major function of GCs is to coordinate suppress a “glial axis of reactivity.”

However, CORT also repressed some genes in neurons and activated others expressed primarily in glia. For example, glycolytic pathway genes were activated by CORT (Figure 2), and increasing evidence suggests that glycolysis in the brain may occur largely within astrocytes (98, 99). By virtue of the diversity of its isoforms and regulatory mechanisms, the GR can activate and repress different sets of genes within the same cell (1, 78). Ligand-activated GRs regulate target genes by direct binding of GR homodimers to positive (activating) or negative (repressing) GREs (the “direct” mode or cis-regulation) and by tethering to other transcription factors at non-GRE binding sites (the “indirect” mode or trans-regulation), as well as by recruiting chromatin-modifying coactivators or corepressors (23, 100–103). Moreover, GR-mediated transcriptional regulation can be modulated by postranslational modifications or by concurrent expression of dominant negative splice variants and other isoforms. In particular, GRβ, a major splice variant of the primary human gene (GRα), forms heterodimers with GRα and acts as a dominant negative inhibitor of GRα function (1, 104). Therefore, the ratio of GRα to GRβ, which varies substantially among cell types and conditions, can modulate the GC sensitivity of specific gene targets and cell types. In addition, the GR can form heterodimers with the MR (105) and the androgen receptor (105) that inhibit GR function.

Furthermore, GR-mediated indirect-mode repression via protein-protein interactions may occur at lower GC concentrations than activation/repression via direct-mode GR dimer binding to GREs (1, 3, 23, 100, 101). Considering that GR immunoreactivity is lower in glia than in CA1 neurons (Figure 4 and Ref. 88) and that inflammatory gene expression is predominantly localized in glia, our results raise the interesting possibility that GC repression of inflammatory/immune genes (Figure 2) is mediated by indirect-mode GR mechanisms in glia. Similar indirect mechanisms are used by GCs in repressing peripheral inflammatory responses (1, 3).

Thus, the present results indicate that a major action of GCs in the hippocampus is the broad coordination of differential activation between neuronal and glial compartments. The functional role of this neuronal-glial imbalance is not fully clear, but it could reflect processes required for adequate allocation of bioenergetic resources to energy-expensive neuronal plasticity or, alternatively, for maintaining neuronal systems in states of biochemical readiness, enabling rapid responses to incoming stimuli.

**Aging-related expression shifts of GC-dependent genes**

As shown in Figure 5, of the 393 genes identified as the chronic CORT-dependent transcriptome, 217 also shifted expression with aging (Supplemental Table 1) and are referred to here as CAS genes. One likely interpretation of an aging shift in expression of a CORT-sensitive gene appears to be a change in the efficacy of CORT signaling. Specifically, if CORT signaling were strengthened during aging, the gene’s expression should shift in the same direction promoted by CORT. Conversely, if CORT signaling were weakened during aging, the gene’s expression should shift in the direction opposite that promoted by CORT. Of the 217 CAS genes, the majority (67%) shifted expression during aging in the direction opposite that promoted by CORT, consistent with aging-related weakening of or resistance to the regulatory actions of CORT. The remaining 33% of CAS genes shifted expression during aging in the same direction promoted by CORT, consistent with strengthening of the regulatory actions of CORT in aging.

Although additional studies will, of course, be needed to confirm these interpretations, development of GC target resistance is a well-recognized pathogenic mechanism in a number of clinical disorders. In particular, resistance to GC-mediated inflammatory gene repression, possibly resulting from diminished availability of corepressors or elevated expression of the dominant negative isoform, GRβ, is implicated in major inflammatory diseases such as severe asthma and chronic obstructive pulmonary disease (2, 3, 104, 107, 108). Conceivably, similar mechanisms might underlie aging-related alterations in GC signaling in selective hippocampal genomic networks. Regardless of the mechanisms, however, the effects of CORT opposed, rather than promoted, aging changes for most CAS genes. Therefore, these data clearly contradict the long-standing basic hypothesis that GCs primarily promote brain aging changes (see Figure 2 in Ref. 19).

**Functional consequences of aging changes in GC-dependent pathways**

With aging, plasticity gene expression declines (Table 2A) and inflammatory gene expression increases (Table 2D). Both of these shifts are in the direction opposite that promoted by CORT, suggesting potential age-related weakening of CORT actions (eg, possible GC resistance). Moreover, these opposite direction aging changes reflect a...
relative reversal during aging of the maintenance of neuronal over glial activation by chronic CORT (Figure 2). Interestingly, the impaired activation of plasticity and metabolic processes (37, 49, 72, 91, 94, 95, 109) and excessive activation of glial reactivity/inflammation (16, 69, 85, 110, 116) represent canonical biomarkers of the aging brain, suggesting that alterations in GC signaling may have an important contribution to the brain aging phenotype.

Similarly, a number of processes and CAS genes that shift expression during aging in the same direction promoted by CORT also seem likely to induce established components of the brain aging phenotype. These include metabolic dysregulation, apoptosis, and aberrant growth (Table 2B) and ECM degradation, protein catabolism, and neuronal atrophy (Table 2C). Furthermore, same direction up-regulation of CORT-activated metabolic genes during aging (eg, Table 2B) might play a role in an aging-related shift in bioenergetic gene and protein expression that is seen consistently in the brains of female (49, 109) and male (69, 72) animal models of aging and in female models of Alzheimer disease (117). Therefore, these results suggest the striking conclusion that multiple phenotypic hallmarks of brain aging are generated by aging-related modifications (weakening or strengthening) of selective GC signaling pathways.

New model of GC interactions with brain aging: alterations in GC signaling (Figure 6)

As noted, the basic hypothesis that chronic GCs primarily promote brain aging (19) no longer appears tenable. Accordingly, we propose a new, more complex working model of GC-brain aging interactions. In this view, rather than GCs promoting brain aging, aging modifies GC signaling, and the resulting altered functions of GC regulatory pathways generate much of the brain aging phenotype (Figure 6).

We suggest that aging processes modify GC regulation of expression in selective pathways in 2 general ways: (1) strengthening of GR direct-mode signaling and (2) GC resistance to indirect-mode signaling. Strengthening of the direct-mode results from increased binding of GR dimers to pGREs and nGREs. Several aging-related changes that could lead to such increased ligand-activated GR binding to GREs have been described, including an increase in circulating GCs, found in many, but not all, rodent and human studies (16, 56, 79, 82, 118), or increased expression of GRs and/or epigenetic cofactors (53, 54, 74, 81, 121, 122). (The genes encoding the MR [Nr3C2] and the GR [Nr3C1] were repressed by CORT, but only Nr3C2 was changed [down-regulated] with age as shown in Supplemental Table 1.)
Concurrently, GC resistance develops to the indirect GC regulatory mode, possibly resulting from aging-dependent decreased availability of coregulators (3, 102, 103) or increased expression of dominant negative isoforms that can form heterodimers with GRα (83, 104). Given the diversity of GC signaling pathways, it appears highly feasible that parallel strengthening and weakening of different gene regulatory pathways could develop within the same region or cell type, even in an environment of elevated GCs/GRs. Thus, in this model, dissimilar and largely independent aging changes in direct and indirect GC regulatory pathways combine to generate much of the canonical brain aging phenotype. Clearly, alternative models are possible. Nevertheless, the present model incorporates newly uncovered complexity of hippocampal aging-GC interactions and, therefore, may inform future studies aimed to identify novel therapeutic targets in unhealthy brain aging/AD.

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