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Glucocorticoid Regulation of Gene Expression in Hippocampal CA3 and Dentate Gyrus

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Glucocorticoids (GCs) alter metabolism, synaptogenesis, apoptosis, neurogenesis, and dendritic morphology in the hippocampus. To better understand how glucocorticoids regulate these aspects of hippocampal biology, we studied gene expression patterns in the CA3 (Hippocampal pyramidal cell field CA3) and dentate gyrus (DG). Litter-matched Lewis inbred rats treated for 20 days with either 9.5 mg per day sustained-release corticosterone or placebo pellets were compared with high-density oligonucleotide microarray analysis (Rat Neurobiology U34 Arrays, Affymetrix). In placebo-treated rats, 32 genes were expressed at greater levels in CA3 than DG, whereas 3 genes were expressed at great levels in DG than CA3. Regional differences were also apparent in corticosterone-induced changes in the hippocampal transcriptome. Six genes in CA3 and 41 genes in DG were differentially regulated by corticosterone. As per the glucocorticoid effects on gene transcription in the brain, forty three of these genes were upregulated, and 4 genes were downregulated. Genes differentially expressed in hippocampus included those for 13 neurotransmitter proteins, 5 ion channel related proteins, 4 transcription factors, 3 neurotrophic factors, 1 cytokine, 1 apoptosis related protein, and 5 genes involved in synaptogenesis. Interestingly, GCs can have suppressive effects on brain BDNF mRNA transcription, one of the neurotrophic factors. These results indicate the diversity of targets affected by chronic exposure to corticosterone and highlight important regional differences in hippocampal neurobiology.

Key words – Corticosterone, transcripts, oligonucleotide microarray, CA3, DG, rat, BDNF

Introduction

The hippocampus is a brain structure that plays a crucial role in learning and memory in human and animals [1,8]. The hippocampus consists of a set of anatomically distinct subregions, Dentate Gyrus (DG), CA3, CA2 CA1 and CA4. Among these subregions, the DG consists mainly of granule neurons projecting largely to CA3 by means of the mossy fiber, whereas the CA region consists mainly of pyramidal neurons projecting to CA1 by means of the Schaeffer collaterals [4,17].

Extensive studies of the hippocampus as a target of stress and stress hormones have revealed a considerable degree of structural plasticity in the adult brain [7,9,10]. This structural plasticity of hippocampus is involved in vulnerability to damage from seizures, ischemia, and head trauma [5,15,16]. Repeated stress causes shortening and debranching of dendrites in the CA3 region of the hippocampus and suppresses neurogenesis of DG granule neurons [2,12]. Despite of the significance of the stress in hippocampus plasticity, our understanding of the genetic change by glucocrticoids underlying hippocampus plasticity is quite limited. To begin to analyze this genomic events, we were used Affymetrix oligonucleotide microarray to profile comprehensive molecular and genetic change by stress hormone, corticosterone.

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Materials and Methods

Animals

A total of eight adult male Lewis purchased from Harlan (Indianapolis, IN), were housed in groups of 2-3 rats per cage under 12/12 hr light/dark cycles in standard laboratory conditions. Rats were acclimated to these conditions for 7 days prior to the treatment conditions. Rat chow and

water were provided ad libitum. Cage bedding was changed weekly with the last change occurring 1-week before the brain tissue collection. Litter-matched Lewis inbred rats received subcutaneous injections of either sustained-release 200 mg corticosterone or placebo pellets and housed each two rats in a cage for 20 days. After 20 days housed, each eight corticosterone rats and control rats were decapitated, measured weight and blood samples were collected for corticosterone analyses. For isolation of DG and CA3 in hippocampus, thick sections (500~600 μm) were sliced with a vibratome, and the structure were dissected from these sections on the cold plate under a dissecting scope, following delineations from the rat brain atlas. This study was approved by the Administrative Panel on Laboratory Animal Care (A-PLAC) of Stanford University.

RNA Preparation

Tissue samples were homogenised with a motorised pellet pestle, and total RNA from each sample was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions and previous work [6]. Quantification was carried out by spectrophotometric analysis, and gel electrophoresis was used to verify the integrity of each RNA sample. The 260/280 ratios of total RNAs were all above 1.77 and at least 20 μg total RNA (CA3) and 10 μg total RNA (DG) were purified from two-left hemisphere of rats.

Microarray Analysis

Oligonucleotide microarray analysis was performed as described in Technical Manual (Affymetrix, Santa Clara, CA). Briefly, cRNA was prepared from 6 μg of total RNA, hybridized to Rat Neurobiology U34 oligonucleotide arrays comprising 1,200 rat genes, scanned, and analyzed. The data were analyzed by Genechip analysis suite 5.0 (Affymetrix). Comparassion and clustering analysis was performed by using dChip 1.1 (Harvard School, Boston, MA). The results described in the text tables were derived from perfect match (PM) probe intensities by Li and Wong [7].

Results and Discussion

DG and CA3 samples of the brain tissues were collected 20-days after the sustained-release 200mg corticosterone or placebo pellets from eight rats. Four of the eight rats were treated to corticosterone pellets and matched four rats served as placebo controls. Five total RNAs of the CA3 sample including two placebo sample and three corticosterone treated samples were recovered with high quality and six total RNAs of the DG samples including two placebo sample and four corticosterone treated sample were prepared with high quality, too. Due to poor extraction of RNA sample such as low 260/280 ratio and concentration, five RNA samples (three CA3 region and two RNA samples of the DG region) were discarded in this study.

These studies investigate the effects of GCs on the generalized transcriptional rates in two brain regions known to be effected by GC, the hippocampal DG and CA3 region. A total of 1176 probe sets target known genes on the rat NeuroArray (Affymetrix). Of this number, 726 had a Call Rate > 20% in our sample of 11 genechips. Hierarchical clustering of samples from the eight rats was performed with the remaining 87 genes to identify similarities and differences across the two hippocampal regions of interest. The resulting dendrogram (Figure 1) clustered all samples

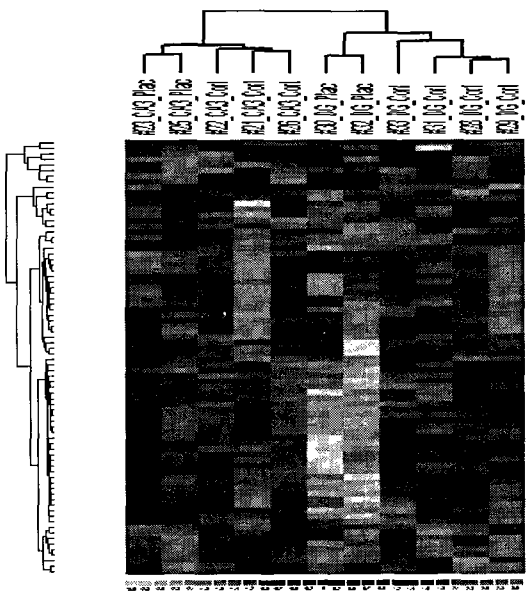

Fig. 1. Hierarchical clustering depicts the relationships between CA3 and DG samples in hippocampal region. Differential Gene expression in CA3 and DG. Hierarchical cluster analysis of 87 genes (selected by filtering by 0.30 ≤ standard deviation / mean) in each pair samples. Dendrogram shows the overall similarity in gene expression of CA3 and DG samples. Cluster of genes with overexpression in DG or in CA3 are displayed with averaged gene profile patterns. Columns represent the gene expression levels (Li-Wong estimates, corrected for matching) in individual samples; rows represent individual genes. Red and green indicate transcript levels above and below the median for each gene across all samples respectively [7].

from CA3 separately from samples of DG. The dendrogram topography was highly robust, and did not differ appreciably with either RMA or dChip microarray data (Data not shown). To identify specific individual genes enriched in CA3 compared to DG regions for rats from placibo group, t-tests were performed for each of the genes detected as present on one or more of the CA3 microarrays. Of the 590 genes detected as present in CA3, 32 were expressed at significantly ($P<0.05$) higher levels in CA3 compared to DG as discerned in dChip microarray data.

For the 32 genes differentially expressed in the data set, expression levels were, on average, 116% higher in CA3 compared to DG. Among the genes enriched in CA3, many are known to be associated with snaptogenesis and, neurotransmitters e.g., synaptojanin II, Synapsin I, SNAP-25B, synaptotagmin associated 35 kDa protein, Prostaglandin F receptor, GABA-A receptor alpha-5 subunit, Glutamate dehydrogenase (Table 1). Of 504 genes called present on one or more microarrays from DG, three were expressed at significantly ($P<0.05$) higher levels in DG compared to CA3

Table 1. Genes expressed at significantly higher levels ($P<0.05$) in CA3 relative to DG regions or vice versa as discerned in dChip microarray data

Probe Set	Signal in DG	Signal in CA3	FC	t-test	Gene Name
High expression in the CA3					
M22254	3959.12	6321.79	1.60	0.04	Sodium channel, voltage-gated, type II, alpha
L16532	4748.20	7679.61	1.62	0.03	2',3'- Cyclic nucleotide 3'-phosphodiesterase
D38380	6851.75	11198.25	1.63	0.03	Transferrin
M27812	4830.38	7905.06	1.64	0.00	Synapsin I
M22357	3374.28	5537.90	1.64	0.02	Myelin-associated glycoprotein
D84450	6253.47	10394.70	1.66	0.04	ATPase, Na+K+ transporting, beta polypeptide 3
D12519	1934.47	3221.45	1.67	0.03	Synaptotagmin associated 35kDa protein
AI170268	3771.65	6584.23	1.75	0.01	Prostaglandin F receptor
AI072770	7911.97	13852.15	1.75	0.03	Proteolipid protein
A03913	1932.28	3394.50	1.76	0.02	Glia-derived neurite-promoting factor (GdNPF)
U90312	1752.80	3114.64	1.78	0.00	Synaptojanin II
M13707	5753.00	10364.65	1.80	0.01	Protein kinase C, type I (gamma type)
X55812	4127.33	7448.89	1.80	0.01	Cannabinoid receptor 1
AB004267	1409.86	2556.45	1.81	0.02	Calcium/calmodulin-dependent protein kinase 1, beta
AI179613	4633.50	8426.43	1.82	0.04	Glutamate dehydrogenase
M17526	4797.64	8794.83	1.83	0.02	GTP-binding protein
AJ006855	4940.56	9137.13	1.85	0.01	Synaptojanin
AB003992	856.04	1624.68	1.90	0.01	SNAP-25B
AF016296	1742.51	3474.23	1.99	0.02	Neuropilin
L21192	4329.02	8687.78	2.01	0.02	GAP-43
D12524	383.32	794.91	2.07	0.04	c-kit receptor tyrosine kinase
X63744	423.18	882.64	2.09	0.03	Solute carrier family 1, member 3
U31866	1438.93	3049.14	2.12	0.04	Nclone10
M25890	7717.17	16455.15	2.13	0.03	Somatostatin
X62839	1823.66	3911.17	2.14	0.00	RRPCP3120
X07287	2559.93	5720.67	2.23	0.04	Protein kinase C gamma
M28648	2589.58	6790.17	2.62	0.01	Na,K-ATPase alpha-2 subunit
U08290	7247.00	20302.85	2.80	0.02	Neuronatin alpha
AA818677	1050.49	3058.01	2.91	0.02	Neurofilament, heavy polypeptide
L08494	335.06	1101.93	3.29	0.03	GABA-A receptor alpha-5 subunit
X06889	2421.80	8039.41	3.32	0.03	Ras-related small GTP binding protein 3A
U56261	463.32	1930.13	4.17	0.00	SNAP-25a
High expression in the DG					
E03082	1513.46	852.78	1.77	0.01	Nerve growth factor
M35162	3730.07	2075.96	1.80	0.01	Gamma-aminobutyric acid (GABA-A) receptor, delta
AA818604	446.35	229.18	1.95	0.03	EST

FC; Fold Change, FG; Functional Group

according to dChip. Taken together, a total of 35 unique genes were enriched in either CA3 or DG as discerned in dChip analysis.

In DG, the number of genes differentially expressed in rats exposed to corticosterone pellets compared to control rats was 41 according to dChip analysis (Table 2). Most of the affected genes in DG were up-regulated to a modest extent. Gene expression fold-differences that exceeded 50% were discerned for 23 of 41 genes in DG that were affected by corticosterone according to dChip microarray data.

Table 2. Genes identified in dChip data as being differentially expressed (P<0.05) in DG for rats exposed to corticosterone pellets compared to placebo control rats

Probe Set	FC	LBFC	UBFC	Gene Name
Down Regulated				
AF037199	-2.80	-1.22	-2.56	Zinc finger transcription factor REST
U53450	-2.36	-1.30	-4.97	Jun dimerization protein 1
Up Regulated				
L04739	1.32	1.21	1.42	Plasma membrane calcium ATPase isoform 1
D30666	1.33	1.21	1.45	Brain acyl-CoA synthetase II
X06554	1.38	1.24	1.53	Myelin-associated glycoprotein (S-MAG)
L08228	1.38	1.20	1.56	N-methyl-D-aspartate receptor (NMDAR1)
X15466	1.43	1.26	1.63	GABA(A) receptor beta-1 subunit
X53455	1.43	1.26	1.62	Microtubule-associated protein 2
AI179613	1.43	1.24	1.67	Glutamate dehydrogenase
AF007758	1.44	1.20	1.78	Synuclein, alpha
AI145044	1.44	1.28	1.62	Glycine receptor alpha 2 subunit
AA860030	1.44	1.24	1.71	EST
M96375	1.46	1.25	1.69	Non-processed neurexin I-beta
U39549	1.46	1.22	1.77	Synaptogyrin 1
AI101255	1.47	1.26	1.70	EST
AI233216	1.48	1.34	1.63	Glutamate dehydrogenase
M22254	1.49	1.27	1.75	Sodium channel, voltage-gated, type II, alpha polypeptide
Z38067	1.49	1.31	1.72	c-myc
U71089	1.50	1.34	1.69	Interleukin 8 receptor, alpha
L08490	1.50	1.21	1.92	GABA-A receptor alpha-1 subunit
AF022819	1.51	1.35	1.69	Putative potassium channel TWIK
U56261	1.52	1.21	1.89	SNAP-25a
D84450	1.54	1.22	2.06	ATPase, Na+K+ transporting, beta polypeptide 3
AI227608	1.55	1.29	1.82	Microtubule-associated protein tau
S61973	1.55	1.25	2.01	NMDA receptor glutamate-binding subunit
X60769	1.56	1.37	1.76	Liver activating protein (LAP, NF-IL6)
AF021923	1.57	1.21	2.20	Potassium-dependent sodium-calcium exchanger (NCKX2)
U87971	1.58	1.21	2.12	Syntaxin 5
AF060173	1.61	1.41	1.86	SV2 related protein (SVOP)
AI170613	1.62	1.20	2.37	Heat shock 10 kD protein 1 (chaperonin 10)
AI145494	1.67	1.27	2.33	Synapsin II
M10088	1.69	1.29	2.24	Prodynorphin
AF016296	1.69	1.36	2.03	Neuropilin
AI170268	1.70	1.40	2.08	Prostaglandin F receptor
E05646	1.75	1.57	1.97	Hippocampal cholinergic neurostimulating peptide,HCNP
X04139	1.75	1.23	2.94	Protein kinase C beta
S54008	1.77	1.32	2.64	FGF receptor-1
X06889	1.82	1.25	2.86	Ras-related small GTP binding protein 3A
AF030091	1.87	1.28	3.15	Activity and neurotransmitter-induced early gene 6 (ania-6)
M60654	1.95	1.57	2.43	Adrenergic receptor, alpha 1d
AF037067	5.20	1.72	10.00	OX40 ligand (Ox40l)

FC; Fold Change, LBFC; Lower Bound of Fold Change, UBFC; Upper Bound of Fold Change

Table 3. Genes identified in dChip data as being differentially expressed (P<0.05) in CA3 for rats exposed to corticosterone pellets compared to placebo control rats

Probe set	FC	LBFC	UBFC	Gene Name
Down Regulated				
D49395	-1.60	-1.25	-2.05	Serotonin 5-HT3 receptor
X67108	-1.65	-1.29	-2.23	Brain derived neurothrophic factor
Up Regulated				
AF065431	1.49	1.26	1.78	Bcl-2 related apoptotic gene product BimL
AF030089	1.55	1.22	1.97	Activity and neurotransmitter-induced early gene protein 4 (ania-4)
AI236828	1.91	1.38	2.70	EST
X16003	8.44	1.22	10.00	Putative potassium channel subunit protein (RCK5)

FC; Fold Change, LBFC; Lower Bound of Fold Change, UBFC; Upper Bound of Fold Change

Expression levels for all but 2 of the 41 genes were diminished in DG after exposure to corticosterone pellets as 2 of 6 transcripts were down regulated by corticosterone pellets in CA3 region (Table 3).

In an effort to understand the molecular and genetic changes by glucocorticoids in anatomically distinct substructures in hippocampus, we have used olignucleotide microarray analysis. By using Rat Neurobiology U34 Genechips, we identified 32 genes expressed at greater levels in CA3 than DG, whereas 3 genes expressed at great levels in DG than CA3 in placebo-treated rats. Regional differences were also apparent in corticosterone-induced changes in the hippocampal transcripts. Six genes in CA3 and 41 genes in DG were differentially regulated by corticosterone. Forty-three of these genes were upregulated, and 4 genes were downregulated. Genes differentially expressed in hippocampus included those for 13 neurotransmitter proteins, 5 ion channel related proteins, 4 transcription factors, 3 neurotrophic factors, 1 cytokine, 1 apoptosis related protein, and 5 genes involved in synaptogenesis, etc.

Neurotrophic factors are a heterogeneous group of peptides known to play important roles in neuronal plasticity. Stress-induced plasma GCs may therefore bring about effects on the hippocampus by changing transcription rates of neurotrophins within hippocampal cells. Brain derived neurothrophic factor (BDNF) is one of these trophic factors involved in processes for the proliferation, differentiation, and maintenance of neurons. It is distributed in the cortex and particularly in the hippocampus. The present study found that corticosterone treatment decreases -1.65 fold change of the BDNF expression in the CA3 (Table 3). Our findings are in agreement with other evidence that dexamethasone or corticosterone administration also suppresses BDNF concentrations in ADX rats [13]. Further, acute models of immobilization (restraint) suppress BDNF mRNA expression throughout the hippocampus [14]. The effects of stress on hippocampal gene transcription seems to be primarily mediated by the GC component of the stress response, as supported by evidence that removal of the adrenals elevates BDNF levels in the hippocampus [3]. Neurotrophins like BDNF bind and activate tyrosine receptor kinase signal transduction receptors (trks) to bring about trophic effects, and both the trkB receptor subtype (the subtype that is activated by BDNF), and trkC are distributed in adult hippocampi. Although previous studies provide evidence that both stress and GCs can have suppressive effects on brain BDNF mRNA transcription, 2 h immobilization stress has no effect on mRNA levels of trkB or trkC receptors (Data not shown). No effect of immobilization stress was observed on the p75 molecule (LNGFR), which binds NGF, BDNF, NT-3, and NT4/5 [18]. Expression of catalytic trkB, is increased in the rat hippocampus following repeated immobilization stress (45 min/day, 10 days) but not acute immobilization (1 day). These results were reproducible in a varied stress paradigm of cage rotation, swim stress, cold isolation, and other stresses [14].

In conclusion, these results demonstrate first, a clear association between gene expression patterns and the neuroanatomy of the hippocampus, second, the diversity of targets affected by chronic exposure to corticosterone, with evidence for anatomically structural differences underlying functional differences in hippocampal neurobiology.

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초록 : 글루코코티코이드 호르몬에 의한 뇌해마의 CA와 Dentate Gyrus 부분의 유전자 발현 변화

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글루코코르티코이드는 해마 조직에서 대사, 스냅신 형성, apoptosis, 신경세포 생성과 세포에 있어서 수지상의 형태에 영향을 준다. 글루코코르티코이드 호르몬에 의한 해마조직의 생리학적 조절을 이해하기 위하여, CA3와 DG (dentate gyrus)에서 유전자 발현에 대하여 조사하였다. Lewis 쥐에 9.5mg의 코르티코스테론 알약 또는 플라시보 알약을 20일 동안 처리한 후에 올리고머 유전자 칩을 이용하여 유전자 발현을 조사 하였다 (Rat Neurobiology U34 Arrays, Affymetrix). 플라시보 알약을 처리한 쥐에서 32 유전자들이 DG보다 CA3에서 발현이 높았으며, 3개 유전자는 CA3보다 DG에서 높은 발현을 보였다. 코르티코스테론 호르몬 처리에 의한 해마조직의 유전자 발현 형태는 해부학적 구조의 차이를 보였다. 특히, CA3에서 6개의 유전자와 DG에서 41 개의 유전자가 호르몬에 의하여 조절 받았으며, 이중 43개의 유전자가 상승 발현하였으며, 4개의 유전자가 하강 발현 하였다. 이들 유전자를 기능에 의해 분류하면, 13개의 신경전달물질관련 유전자, 5개의 이온채널, 4개의 전사인자, 3개의 neurotrophic인자, 1개의 각 사이토카인과 apoptosis관련 유전자, 그리고 5개의 스냅신형성관련 유전자가 해마조직에서 발현의 변화를 보였다. 특히, 스트레스 호르몬에 의하여 CA3에서 BDNF의 감소를 볼 수 있었다. 이러한 결과는 호르몬에 의하여 해마구조의 생리학적인 다양성을 내포 하고 있다.