Early-Life Forebrain Glucocorticoid Receptor Overexpression Increases Anxiety Behavior and Cocaine Sensitization

Supplement 1

Supplemental Methods and Materials

Generation of Inducible Glucocorticoid Receptor Overexpression in Forebrain (GRov) Mice

The codons encoding the influenza hemagglutinin (HA) epitope were added to 5'-end of the full length mouse glucocorticoid receptor (GR) complementary DNA (cDNA) with primer pair 5'-GCG CGG ATC CAC CAT GTA CCC ATA CGA CGT CCC AGA CTA CGC TGA CTC CAA AGA ATC CTT AGC-3' and 5'-AAT TAA CCC TCA CTA AAG GG-3'. The transactivation properties of HA-GR were verified by glucocorticoid response element-chloramphenicol acetyltransferase assay (supporting information in ref. 1). The HA-GR cDNA was cloned into the response plasmid pTRE (tetracycline-response element) of the Tet-Off inducible system (Clontech, Mountain View, CA). The transgene fragment containing TRE-HA-GR was microinjected into F_2 zygotes from C57BL/6J $\times$ SJL parents to generate transgenic mice (the Transgenic Animal Core of the University of Michigan). Genomic DNA prepared from tail biopsies was screened for the presence of the transgene by polymerase chain reaction (PCR) assay using primer 5'-GGG TCG AGT AGG CGT GTA CGG TGG G-3' from the pTRE plasmid and 5'-CAG GTG TTG AAC TCT TGG GAT TCT CTG G-3' from the mouse GR cDNA. A PCR assay for β–globin was used as a positive control for the quality of the genomic DNA sample. The β–globin primers are 5'-CCA ATC TGC TCA CAC AGG ATA GAG AGG GCA GG-3' and 5'-CCT TGA GGC TGT CCA AGT GAT TCA GGC CAT CG-3'. All transgenic mice are maintained as hemizygotes. Following identification, founder TRE-HA-GR mice were crossed with CaMKIIα-tTA mice (2) (The Jackson Laboratory; Bar Harbor, ME) to produce bigenic mice. The primer pair 5'-GCG AGC TGT GAG CAG CCA CAG TGC-3' and 5'-GAG CAG GCC CTC GAT GGT AGA C-3' was used to detect the CaMKIIα-tTA mice. The bigenic mice express the tet-transactivator (tTA) protein only in the forebrain under the control of the CaMKIIα promoter, and tTA binds to the TRE sequence to

induce transgene GR transcription in the forebrain in the absence of doxycycline (Dox), a tetracycline analog. In the presence of Dox (200 mg/kg chow, Bio-Serv, Frenchtown, NJ), tTA does not bind the TRE and transgene GR messenger RNA (mRNA) is not transcribed. We utilized the following conditions: 1) induction of GR throughout the lifetime of the animal (Lifetime GRov); 2) induction of GR only during the first 3 weeks of life (Early Life GRov); 3) induction of GR after weaning and into adulthood (Adult GRov); 4) no induction of GR in the forebrain (No-GRov). All experiments were performed with male bigenic mice. Mice were maintained on a 14:10 light/dark schedule with lights on between 5:00 and 19:00. Food and water were available ad libitum. At the time of testing, the bigenic mice were between 3 and 6 months old. Mice were housed individually for one week before the study. All experiments were conducted in accordance with the principles and procedures outlined in the National Institutes of Health Guidelines for the Care and Use of Animals and approved by the University of Michigan Committee on the Use and Care of Animals.

In Situ Hybridization

Sections (12 μm) were fixed in 4% paraformaldehyde for 1 hr, followed by three washes in 2 × SSC (1 × SSC is 150 mM sodium chloride, 15 mM sodium citrate). The sections were then placed in a solution containing acetic anhydride (.25%) in triethanolamine (.1 M), pH 8.0, for 10 min at room temperature, rinsed in distilled water, and dehydrated through graded alcohols (50, 75, 85, 95, and 100%). After drying, the sections were hybridized with a ^{35}S-labeled complementary RNA (cRNA) probe. The transgene-specific probe was a 230-bp nucleotide fragment directed against the 3' untranslated region of TRE-HA-GR mRNA, located just upstream of SV40 polyadenylation signal. The GR probe was a 597-bp fragment directed against the mouse GR mRNA coding sequence. The mineralocorticoid receptor probe was a 300-bp fragment directed against the mouse mineralocorticoid receptor mRNA. The probes were labeled in a reaction mixture consisting of 1 μg of linearized plasmid, 1 × transcription buffer, 125 μCi of [^{35}S]UTP, 125 μCi of [^{35}S]CTP, 150 μM ATP and GTP, 12.5 mM dithiothreitol, 20 U of RNase inhibitor, and 6 U of RNA polymerase. The radiolabelled cRNA hybridization probes were separated from unincorporated nucleotides using a Sephadex G50-50

column. The cRNA probes were diluted in hybridization buffer (50% formamide, 10% dextran sulfate, 3 × SSC, 50 mM sodium phosphate buffer, pH 7.4, 1 × Denhardt's solution, .1 mg/ml yeast transfer RNA, and 10 mM dithiothreitol) to yield 10^6 dpm/70 μl. After hybridization overnight at 55°C, the sections were rinsed twice in 2 × SSC for 5 min each and then incubated for 1 hr in RNase (200 μg/ml in 10 mM Tris buffer containing .5 M NaCl, pH 8.0) at 37°C. The sections were washed in increasingly stringent solutions of SSC (2 ×, 1 ×, and .5 ×) for 5 min each, followed by incubation for 1 hr in .1 × SSC at 65°C. After rinsing in distilled water, the sections were dehydrated and exposed to Kodak XAR film for a few days. The specificity of hybridization was confirmed by control experiments using sense probes. The autoradiograms were digitized using a Sony XC-ST70 camera connected to a computer running MCID software (Imaging Research, Ontario) or using a flat bed scanner (Microtek ScanMaker 9800XL, Carson, CA). Signal pixels of a region of interest were defined as being 3.5 standard deviations above the mean of a fiber-tract area. The regional boundaries of nuclei were determined according to the mouse brain atlas. For all probes, a series of sections spaced 96 μm apart for each region of interest were measured, and then averaged to produce one data point for each brain region for each animal. The intensities of the radioactive signal are dependent on density of labeled cells and X-ray film exposure time. Therefore, they are not comparable across probes or studies. However, it should be noted that each study included its own control, and all comparisons were made within each study.

Protein Analysis

Hippocampal tissue was rapidly dissected and homogenized in TEDGM buffer (20 mM sodium molybdate, 20 mM Tris-HCl, 1 mM EDTA, 5 mM dithiothreitol, 10% glycerol, .2 mM phenylmethylsulfonyl fluoride, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, 10 μg/ml aprotinin, pH 7.6 at 4°C) and centrifuged at 112,000 × g to collect the supernatant. Proteins were electrophoresed in 7% SDS-polyacrylamide gel and then transferred onto Immobilon-P membranes (Millipore Corp, Billerica, MA). Proteins were detected with either an anti-HA antibody (F-7, Santa Cruz Biotechnology, Santa Cruz,

CA) or an anti-GR antibody (BuGR2, Affinity Bioreagents, Rockford, IL), and immunoreactive bands were visualized by chemiluminescence (Amersham ECL kit, Piscataway, NJ).

Blood Collection and RIA Analysis

For determination of basal plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT) concentrations, blood samples were collected by decapitation less than 20 sec after opening the cage at 2 hr after lights on. Plasma ACTH was determined using an ACTH RIA kit (MP Biomedicals, Orangeburg, NY). Plasma CORT was assayed using a highly specific antibody developed in our laboratory.

Elevated Plus Maze Test

The elevated plus maze consisted of four arms (27 × 6 cm) arranged in a plus form, and elevated 50 cm above the floor. Two opposing arms were surrounded with 15-cm-high clear Plexiglas walls (closed arms) while the other arms were devoid of walls (open arms). The light intensity measured in the open arms was 275 Lux. Mice were placed in the center of the apparatus, facing an open arm to begin the 5 min test period. An entry was defined as having 4 paws in an arm of the plus maze.

Light-Dark Box Test

The light-dark box was 46 × 27 cm (L × W). It was designed so that 2/3 of the length consisted of a white Plexiglas box and 1/3 of its length was black Plexiglas box. A black partition with an open doorway (7 × 7 cm) at the bottom separated the two compartments. A black removable top fit on top of the black compartment. The light intensity measured in the light box was 300 Lux. Mice were placed on the dark side to begin the 5 min test period. A transition was defined as all 4 paws moving from one side to the next.

Defensive Withdrawal Test

This test was conducted in a brightly lit polycarbonate cage (36 × 36 cm) containing a small opaque chamber 12 cm deep and 8 cm in diameter. The chamber was situated in

the middle of the cage with the open end facing the corner. The light intensity measured in the cage was 200 Lux. Mice were introduced into the chamber to begin the 5 min test period. Latency to first exit the chamber (all four paws in the open field) was scored.

Open Field Test

Locomotor activity was measured in a 42 × 42 cm cage using the Digiscan Activity Monitor (Accuscan Instruments, Inc., Columbus, OH). General locomotor activity was measured for 30 min for 3 consecutive days. We measured horizontal activity and distance traveled in 5 min bins. For cocaine-induced behavioral sensitization, mice were first habituated to the apparatus for 15 min, then given cocaine HCl (17.5 mg/kg; intraperitoneal) and immediately placed back into the apparatus. After daily injections of cocaine for 5 consecutive days, mice were undisturbed for 8 days, and then given a challenge with the same dose of cocaine on Day 14.

Laser Capture Microdissection (LCM)

LCM was performed as described previously (3, 4). Briefly, brains were collected in the morning under undisturbed condition. Brains were cryostat-sectioned (-20°C) in the coronal plane at 12 μm and stored at -80°C. LCM was performed on unstained sections adjacent to those processed for histochemical staining. Slides were thawed and dehydrated prior to LCM. LCM was performed with an AutoPix instrument (Molecular Devices, Sunnyvale, CA); laser settings ranged from 50-75 mW (power), 1,500-3,500 ms (duration) and 200-250 mV (intensity). Position of anatomical landmarks from histochemically-stained images was used for LCM. Regions in unstained sections that corresponded to boundaries of the hippocampal dentate gyrus (DG) and the nucleus accumbens (NAcc) were then microdissected under a 4x objective using CapSure macrocaps (Molecular Devices). Each cap contained 2 DG or 4 NAcc microdissected areas from serial sections for each animal.

RNA Isolation and Amplification

RNA extraction and isolation of LCM samples was performed using the PicoPure RNA Isolation kit (Molecular Devices) according to manufacturer's instructions including

DNase treatment. RNA quality and concentration were analyzed and determined using an Agilent bioanalyzer (Palo Alto, CA). RNA samples were then subjected to two rounds of amplification (RiboAmp OA RNA kit, Molecular Devices) and subsequent biotin labeling (PerkinElmer, Waltham, MA). After the first round of amplification, a portion of amplified double-stranded cDNA was saved for quantitative real-time PCR (qRT-PCR). Following the second round of amplification, 15 µg of biotinylated amplified RNA from each nucleus was hybridized to Affymetrix Mouse Genome 430_2.0 GeneChips (Affymetrix, Santa Clara, CA) per manufacturer's instructions. Arrays were hybridized for 18 h at 45°C, washed, and stained on an Affymetrix fluidics station using the standard EukGEWS2v4_450 protocol and scanned.

Microarray Data Analysis

GeneChip signal intensity data were interpreted by use of a custom chip description file (filename MM430_2_REFSEQ_v13). Design of the custom chip description file was described in detail previously (5). This file is available at http://brainarray.mbni.med.umich.edu. CEL intensity files from the Affymetrix GeneChip arrays were normalized by the robust multi-array average (RMA) algorithm available at www.bioconductor.org. To determine the number of altered transcripts, gene was considered to be significantly changed if: 1) the *p*-value was < .05; 2) the $\log_2$ intensity value was ≥ 5 according to RMA algorithm; and 3) the change in the mRNA expression was either ≤ -1.10-fold or ≥ 1.10-fold.

Quantitative Real-Time PCR

qRT-PCR employing SYBR Green chemistry was used to confirm microarray results. Amplification reactions and fluorescence quantification were performed in real time using a Bio-Rad iCycler (BioRad, Hercules, CA) in combination with a SYBR-488 detection protocol (3). Amplification reactions were carried out in 96 well PCR plates (Bio-Rad). Each well contained 5 µl of amplified double-stranded cDNA (aDNA; 35 pg/µl) that was obtained following the first round RNA amplification. aDNA was quantified using Quant-iT PicoGreen dsDNA kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Each qPCR well also contained 5 µl of forward and reverse

strand primers (final concentration: 500 nM) and 10 µl of iQ SYBR Green Supermix (Bio-Rad). Primers were designed to generate amplicons of 70-150 bp in length at the 3' of the target mRNA. Each primer pair was tested using a 5-fold dilution series of pooled cDNA template to ensure that the efficiency of amplifications was linear and that the primers specifically produced a single amplified product. The cycle threshold was calculated with iCycler software.

Supplemental Results

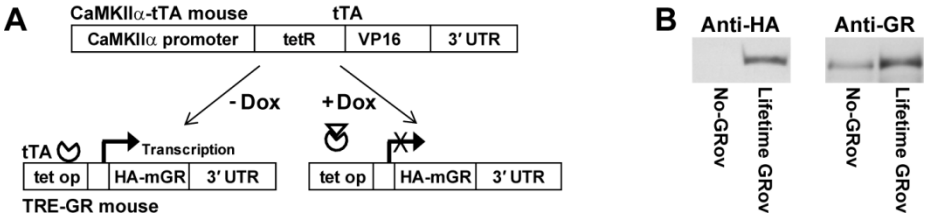

Figure S1. Inducible glucocorticoid receptor overexpression in forebrain (GRov) mice. **(A)** Schematic representation of the inducible CaMKIIα-tTA Tet-Off expression system. **(B)** Western blot analysis showed that the influenza hemagglutinin (HA) tagged glucocorticoid receptor (GR) protein was expressed in the hippocampus of Lifetime GRov mice (anti-HA, *left panel*) and that total GR protein was significantly increased in the hippocampus of Lifetime GRov mice compared to No-GRov mice (anti-GR, *right panel*). Dox, doxycycline; mGR, mouse glucocorticoid receptor; tet op, tetracycline operator sequences; TRE, tetracycline-response element; tTA, tet-transactivator; UTR, untranslated region.

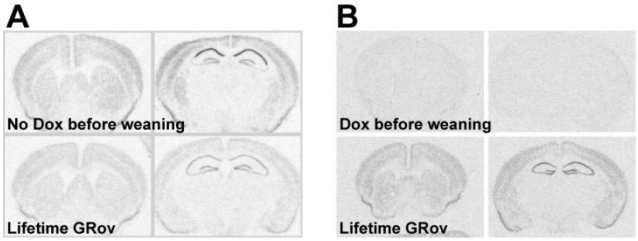

Figure S2. (A) *In situ* Hybridization (ISH) experiments using a probe specific to the transgene showed that the bigenic glucocorticoid receptor overexpression in forebrain (GRov) mice had comparable distribution patterns of the transgene-specific glucocorticoid receptor (GR) messenger RNA (mRNA) at postnatal day 21 (weaning) in the absence of doxycycline (Dox) compared to the Lifetime GRov mice in adult. **(B)** By contrast, there was no detected transgene-specific GR mRNA at postnatal day 21 in

bigenic mice that were treated with Dox before weaning (*top row*). Representative images in **(A)** and **(B)** are from independent ISH experiments. *n* = 2-4 mice per group.

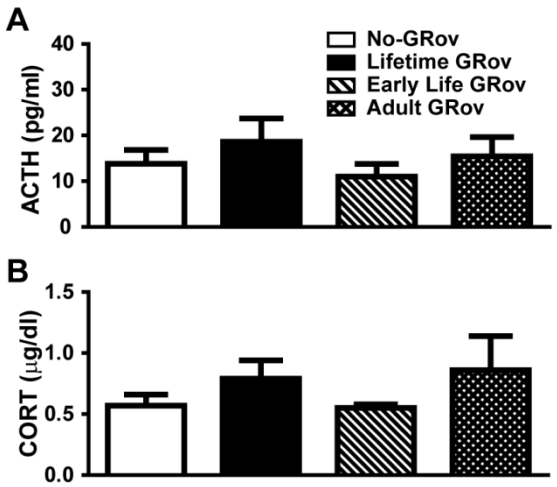

Figure S3. Normal basal stress hormones secretion in inducible mice. There were no significant differences in basal circulating adrenocorticotropic hormone (ACTH) **(A)** and corticosterone (CORT) **(B)** levels in adult mice following the glucocorticoid receptor overexpression in forebrain (GRov) during different periods in development. *n* = 5-7 mice per group.

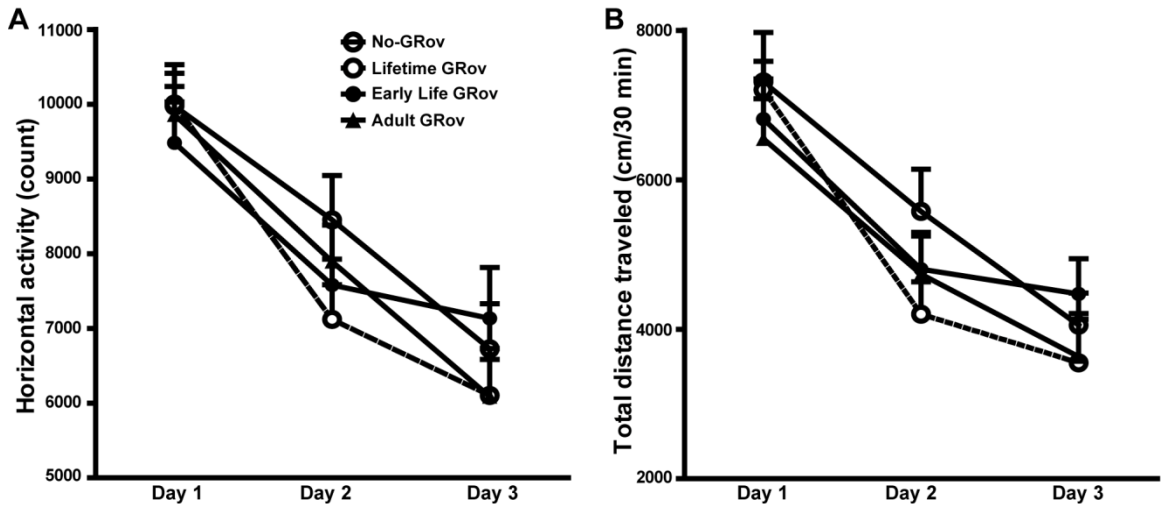

Figure S4. General locomotor activity in inducible glucocorticoid receptor overexpression in forebrain (GRov) mice. General locomotor activity was measured in adult mice for 30 min in the open field with repeated measures for 3 consecutive days. The results did not reveal any group differences in horizontal activity **(A)** or total travel distance **(B)**, indicating there is no significant effect of genotype or doxycycline (Dox) treatment on this behavior. These groups of bigenic mice showed habituation to the novel environment, as demonstrated by decreasing horizontal activity ($p < .001$) or total travel distance ($p < .001$). There was no interaction of group x day in these measures. n = 7-8 mice per group.

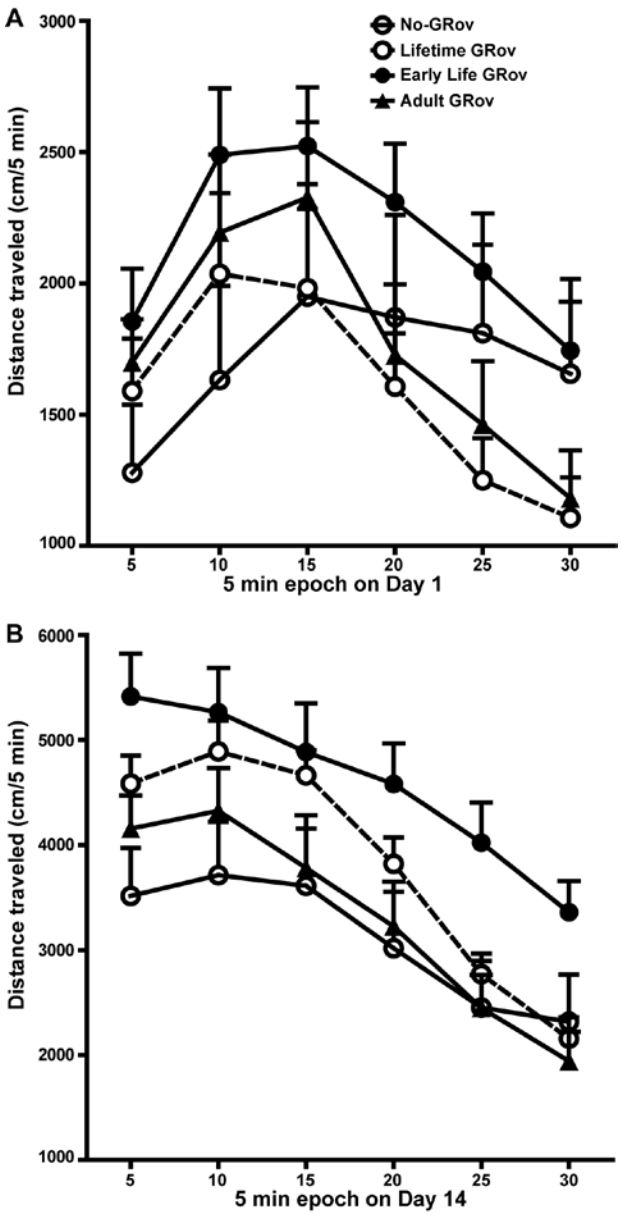

Figure S5. Distance traveled in response to cocaine in 5 min bins. **(A)** Acute locomotor response to cocaine on Day 1. ANOVA with repeated measures revealed no significant group difference on Day 1. However, there was a significant group × time interaction ($p < .05$). Bonferroni post hoc comparisons revealed that Early Life GRov mice significantly differed from No-GRov and Lifetime GRov mice ($p < .01$ and $p < .001$, respectively). **(B)** Locomotor activity in response to the cocaine challenge on Day 14. ANOVA with repeated measures yielded a significant group difference ($p < .05$) and a significant group × time interaction on Day 14 ($p < .05$). Bonferroni post hoc comparisons revealed

that Early Life GRov mice significantly differed from all other groups ($p < .001$). Bonferroni post hoc comparisons also revealed that Lifetime GRov mice significantly differed from No-GRov mice ($p < .01$). GRov, glucocorticoid receptor overexpression in forebrain. n = 9-16 mice per group.

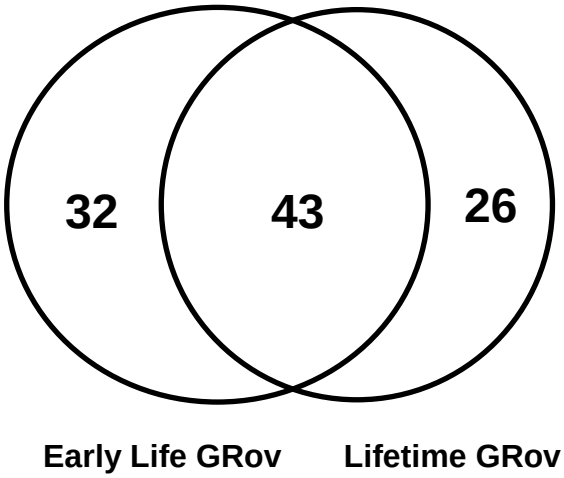

Figure S6. Venn diagram showed the number of genes changed in glucocorticoid receptor (GR) signaling pathway in the hippocampal dentate gyrus in Early Life or Lifetime GRov when compared to No-GRov controls. Forty-three transcripts involved in GR signaling were altered and shared by Early Life and Lifetime GRov mice (see Table 1). There were 32 genes involved in GR signaling uniquely altered in Early Life GRov mice compared to No-GRov controls, including *CD247, CEBPB* (up-regulated)*, CHUK, CREB1, CREBBP, DUSP1, ESR1, GTF2A1, GTF2F1, GTF2H2, HSP90B1, IL4, KRT36, MAP2K2, MAP3K14, NFKBIB, NFKBIE, PCK1, PIK3CD, PIK3R1, PIK3R6, POLR2C, SCGB1A1, SMAD4, SMARCD2, STAT3, TAF1, TAF4B, TAF5L, TAF6L, TAT,* and *TSC22D3*. There were 26 genes involved in GR signaling uniquely changed in Lifetime GRov compared to No-GRov mice, including *CDK7, CEBPB* (down-regulated)*, FCGR1A, FGG, FKBP4, HSPA1A, ICAM1, IL13, IL1B, IL1R2, KRT32, MAPK10, MMP1, NFATC2, NFATC3, NR3C1, POLR2B, POLR2F, PPP3CB, PRKACA, RAC1, RAF1, TAF12, TGFB2, TGFB3,* and *TGFBR1.* GRov, glucocorticoid receptor overexpression in forebrain. n = 6 mice per group.

Table S1. Intensity of the Radioactive Signal for GR messenger RNA in Mouse Brain

	No-GRov	Lifetime GRov	Early Life GRov	Adult GRov
PFC	.077 ± .003	.289 ± .029**	.071 ± .003	.164 ± .017**,††
Striatum	.051 ± .002	.252 ± .021**	.049 ± .003	.245 ± .047**
NAcc	.058 ± .003	.245 ± .022**	.059 ± .003	.229 ± .012**
Septum	.047 ± .002	.189 ± .013**	.048 ± .002	.186 ± .013**
PVN	.146 ± .007	.172 ± .005*	.144 ± .009	.157 ± .012
Amygdala	.053 ± .004	.130 ± .006**	.055 ± .004	.103 ± .009**,†
HC-CA1	.174 ± .007	.794 ± .044**	.194 ± .012	.803 ± .029**
HC-CA3	.073 ± .003	.422 ± .029**	.081 ± .006	.395 ± .019**
HC-DG	.200 ± .009	.853 ± .035**	.192 ± .013	.878 ± .035**

The levels of glucocorticoid receptor (GR) messenger RNA were measured in adult bigenic mice. GRov, glucocorticoid receptor overexpression in forebrain; PFC, prefrontal cortex; NAcc, nucleus accumbens; PVN, paraventricular nucleus of the hypothalamus; HC-CA, hippocampal cornu ammonis; HC-DG, hippocampal dentate gyrus. *$p < .05$; **$p < .01$ versus No-GRov mice. †$p < .05$; ††$p < .01$ Adult GRov versus Lifetime GRov mice. $n = 5–7$ mice per group.

Table S2. Intensity of the Radioactive Signal for MR messenger RNA in the Hippocampus

	No-GRov	Lifetime GRov	Early Life GRov	Adult GRov
HC-CA1	.314 ± .010	.323 ± .018	.319 ± .010	.311 ± .007
HC-DG	.344 ± .017	.349 ± .023	.330 ± .021	.336 ± .012

The levels of MR messenger RNA were measured in adult bigenic mice. MR, mineralocorticoid receptor; GRov, glucocorticoid receptor overexpression in forebrain; HC-CA, hippocampal cornu ammonis; HC-DG, hippocampal dentate gyrus. $n = 5–7$ mice per group.

Table S3. qRT-PCR Results for Genes Involved in GR Signaling in DG

		Early Life vs No-GRov		Lifetime vs No-GRov	
Gene	**RefSeq ID**	**FC**	***p***	**FC**	***p***
GTF2A2	NM_001039519	7.05	.113	NS	
GTF2H1	NM_008186	-9.44	.062	-2.37	.056
GTF2H5	NM_181392	NS		NS	
HSP90AA1	NM_010480	6.38	.055	6.70	.025
HSP90AB1	NM_008302	8.34	.026	6.22	.019
HSPA2	NM_001002012	7.07	.038	6.90	.022
JAK1	NM_146145	4.60	.029	5.46	.050
JAK3	NM_010589	-6.15	.090	NS	
JUN	NM_010591	6.23	.033	4.62	.020
MAP2K1	NM_008927	7.03	.047	7.20	.022
MAP3K7IP1	NM_025609	-12.18	.020	-3.14	.065
MAPK1	NM_001038663	6.29	.045	6.24	.025
MAPK11	NM_011161	NS		NS	
NCOR1	NM_011308	NS		NS	
NFKBIA	NM_010907	NS		NS	
NRIP1	NM_173440	NS		5.53	.097
POLR2A	NM_009089	-10.99	.061	-7.98	.036
RRAS	NM_009101	-4.51	.099	-4.51	.058
SMACA2	NM_011416	NS		NS	
TAF10	NM_020024	-9.85	.058	-2.23	.089
TGFB1	NM_011577	-5.21	.020	-5.62	.033
TRAF2	NM_009422	-8.17	.097	-7.05	.062

Fold changes (FC) of gene expression represent either Lifetime or Early Life GRov mice compared with No-GRov animals by qRT-PCR. GR, glucocorticoid receptor; GRov, glucocorticoid receptor overexpression in forebrain; DG, dentate gyrus; NS, not significant; qRT-PCR, quantitative real-time polymerase chain reaction. *n* = 6 mice per group.

Table S4. Alterations in Axonal Guidance Signaling Pathway in DG

		Early Life vs No-GRov		Lifetime vs No-GRov	
Gene	**RefSeq ID**	**FC**	***p***	**FC**	***p***
ABLIM1	NM_001103177	1.24	.023	1.19	.036
ABLIM3	NM_001164491	1.76	.003	1.56	.014
ACTR3	NM_023735	1.49	.013	1.38	.010
ADAM15	NM_009614	-1.24	.001	-1.23	.002
ADAM19	NM_009616	-1.15	.020	-1.17	.007
ADAM20	NM_010086	-1.16	.003	-1.16	.010
ADAM22	NM_001007221	-1.20	.04	-1.21	.024
ARHGEF12	NM_027144	1.26	.003	1.22	.005
BMP1	NM_009755	1.16	.006	1.24	.002
BMP7	NM_007557	-1.17	.007	-1.21	.001
BMP15	NM_009757	-1.18	.002	-1.22	.001
BMP8B	NM_007559	-1.13	.032	-1.17	.009
CRK	NM_133656	1.46	.005	1.30	.010
EIF4E	XM_001004193	-1.32	.004	-1.33	.017
EPHA1	NM_023580	-1.23	.007	-1.21	.010
EPHA7	NM_010141	1.52	.012	1.30	.047
EPHA8	NM_007939	-1.27	.016	-1.23	.008
EPHB2	NM_010142	-1.13	.050	-1.38	.001
EPHB4	NM_001159571	-1.10	.040	-1.14	.035
ERBB2	NM_001003817	-1.25	.014	-1.21	.031
FES	NM_010194	-1.10	.046	-1.15	.018
FZD8	NM_008058	-1.17	.004	-1.14	.035
FZD9	NM_010246	-1.18	.015	-1.16	.019
GNA12	NM_010302	-1.15	.044	-1.15	.038
GNAO1	NM_001113384	-1.13	.025	-1.13	.018
GNAQ	NM_008139	1.14	.036	1.17	.004
GNAT1	NM_008140	-1.25	.037	-1.24	.049
GNB5	NM_138719	-1.14	.037	-1.29	.001
GNG2	NM_001038637	1.38	.009	1.42	.008
GNG12	NM_001177556	-1.23	.020	-1.21	.008
GNG13	NM_022422	-1.88	.001	-1.76	.003

ITGA3	NM_013565	-1.16	.002	-1.15	.002
ITGA5	NM_010577	-1.28	.001	-1.24	.001
KALRN	NM_177357	1.15	.035	1.20	.012
KLK2	NM_010642	-1.31	.001	-1.28	.001
NGFR	NM_033217	-1.20	.002	-1.13	.029
NRAS	NM_010937	1.11	.045	1.10	.040
NRP1	NM_008737	1.44	.001	1.43	.004
NRP2	NM_001077403	1.45	.001	1.83	.001
NTF3	NM_001164034	1.43	.007	1.48	.009
PAK7	NM_172858	-1.17	.008	-1.20	.019
PIK3C2G	NM_011084	-1.14	.014	-1.12	.004
PIK3R4	NM_001081309	1.38	.002	1.32	.001
PLCB3	NM_008874	-1.18	.013	-1.13	.047
PLXNA1	NM_008881	-1.17	.006	-1.14	.048
PLXNB3	NM_019587	-1.24	.030	-1.34	.003
PRKCE	NM_011104	1.29	.006	1.29	.005
PRKCQ	NM_008859	-1.23	.004	-1.27	.002
RGS3	NM_001081650	-1.13	.021	-1.18	.023
ROBO1	NM_019413	1.50	.047	1.96	.001
ROCK1	NM_009071	1.35	.002	1.15	.049
RRAS	NM_009101	-1.14	.043	-1.18	.005
RTN4R	NM_022982	-1.21	.043	-1.21	.003
SEMA3B	NM_001042779	-1.13	.027	-1.15	.014
SEMA3F	NM_011349	-1.15	.024	-1.15	.014
SEMA4B	NM_013659	-1.18	.015	-1.17	.011
SEMA4C	NM_001126047	1.75	.002	2.16	.001
SEMA5A	NM_009154	1.60	.008	1.61	.008
SEMA6A	NM_018744	-1.12	.025	-1.18	.001
SEMA6B	NM_001130456	1.24	.021	1.29	.006
SUFU	NM_001025391	-1.14	.026	-1.17	.032
UNC5B	NM_029770	-1.28	.001	-1.28	.001
WNT1	NM_021279	-1.27	.003	-1.24	.013
WNT2	NM_023653	-1.36	.009	-1.41	.011
WNT3A	NM_009522	-1.21	.034	-1.20	.045

WNT5B	NM_009525	-1.22	.032	-1.24	.019
WNT9B	NM_011719	-1.15	.025	-1.13	.028

This list includes genes that are altered in the dentate gyrus and shared by Lifetime and Early Life GRov mice when compared to No-GRov mice. Fold changes (FC) of gene expression represent either Lifetime or Early Life GRov mice compared with No-GRov animals. GRov, glucocorticoid receptor overexpression in forebrain; DG, dentate gyrus. *n* = 6 mice per group.

Table S5. Alterations in GR Signaling Pathway in NAcc of the Lifetime GRov Mice

Gene	RefSeq ID	FC	*p*
ANXA1	NM_010730	-1.21	.047
CD3D	NM_013487	-1.13	.029
CD3G	NM_009850	-1.15	.023
HSP90AA1	NM_010480	1.27	.035
HSPA1L	NM_013558	-1.10	.022
IL13	NM_008355	-1.13	.039
MAP2K4	NM_009157	1.14	.039
NCOA3	NM_008679	-1.14	.022
NCOR1	NM_011308	1.21	.003
NR3C1	NM_008173	1.27	.010
NRIP1	NM_173440	1.27	.030
PIK3CA	NM_008839	1.16	.043
PIK3R1	NM_001024955	1.11	.018
PIK3R5	NM_177320	-1.20	.009
RRAS2	NM_025846	1.14	.003
SMAD4	NM_008540	1.14	.022
SMARCA4	NM_001174078	1.15	.028
STAT5B	NM_001113563	-1.11	.038
TAF1	NM_001081008	1.12	.005
TAF6	NM_009315	-1.10	.020
TAF12	NM_025579	-1.13	.027

Fold changes (FC) of gene expression represent Lifetime GRov mice compared with No-GRov controls. GR, glucocorticoid receptor; GRov, glucocorticoid receptor overexpression in forebrain; NAcc, nucleus accumbens. *n* = 6-7 mice per group.

Table S6. qRT-PCR Results for Genes Involved in Dopamine Receptor Signaling in NAcc

		Early Life vs No-GRov		Lifetime vs No-GRov	
Gene	**RefSeq ID**	**FC**	***p***	**FC**	***p***
ADCY1	NM_009622	2.93	.097	-3.92	.157
ADCY7	NM_001037724	4.27	.101	NC	
MAOA	NM_173740	NS		NC	
PPP1CA	NM_031868	-3.80	.008	NC	
PPP1R1B	NM_144828	-6.28	.007	NS	
PPP2R5C	NM_001081458	-9.05	.032	NC	
PPP2R5D	NM_009358	NS		NC	
PRKAG1	NM_016781	-3.16	.015	NC	
AADC	NM_016672	NC		4.69	.015
PPP1CB	NM_172707	NC		2.93	.021
PPP1R12A	NM_027892	NC		3.10	.047
PPP1R14C	NM_133485	NC		5.49	.024
PPP1R3C	NM_016854	NC		2.03	.033
SMOX	NM_001177836	NC		NS	

Fold changes (FC) of gene expression represent either Lifetime or Early Life GRov mice compared with No-GRov controls. GRov, glucocorticoid receptor overexpression in forebrain; NAcc, nucleus accumbens; NC, no change; NS, not significant; qRT-PCR, quantitative real-time polymerase chain reaction. n = 6–7 mice per group.

Table S7. Alterations in Axonal Guidance Signaling Pathway in NAcc

		Early Life vs No-GRov		Lifetime vs No-GRov	
Gene	**RefSeq ID**	**FC**	***p***	**FC**	***p***
ADAM28	NM_001048175	-1.15	.009	-1.13	.050
ARHGEF15	NM_177566	-1.16	.035	-1.10	.019
CFL1	NM_007687	1.17	.045	NC	
DPYSL2	NM_009955	1.12	.006	NC	
GNB5	NM_138719	-1.32	.034	NC	
GNG2	NM_001038637	1.20	.003	NC	
GRB2	NM_008163	-1.11	.006	NC	
IGF1	NM_001111274	1.12	.045	NC	
NRP2	NM_001077406	1.45	.009	1.35	.013
PAK7	NM_172858	1.29	.010	NC	
PLXNC1	NM_018797	1.15	.015	NC	
PRKAG1	NM_016781	-1.23	.024	NC	
PRKCH	NM_008856	-1.14	.034	NC	
PRKCZ	NM_001039079	-1.23	.023	NC	
RASSSF5	NM_018750	1.14	.014	NC	
RRAS2	NM_025846	1.15	.050	1.14	.023
SEMA4F	NM_011350	1.36	.033	1.15	.047
SEMA5A	NM_009154	1.13	.024	1.14	.012
UNC5B	NM_029770	1.10	.032	NC	
ABL1	NM_001112703	NC		1.10	.015
ABLIM1	NM_001103177	NC		1.21	.014
ABLIM2	NM_001177696	NC		-1.13	.038
ABLIM3	NM_001164491	NC		1.16	.040
BAIAP2	NM_001037754	NC		-1.13	.018
BMP2	NM_007553	NC		1.30	.038
GNG13	NM_022422	NC		-1.32	.017
KLK2	NM_008457	NC		-1.16	.002
MYL3	NM_010859	NC		-1.15	.005
NTNG1	NM_001163348	NC		1.17	.002
PAK4	NM_027470	NC		-1.14	.049
PIK3CA	NM_008839	NC		1.16	.044

PIK3R1	NM_001024955	NC	1.11	.019
PIK3R5	NM_177320	NC	-1.20	.009
PLXNA2	NM_008882	NC	1.15	.030
RASA1	NM_145452	NC	1.18	.043
ROBO1	NM_019413	NC	1.53	.023
RTN4	NM_194051	NC	1.15	.025
SDC2	NM_008304	NC	1.16	.020
SEMA3C	NM_013657	NC	1.21	.008
SEMA4A	NM_001163489	NC	1.11	.042
SEMA4C	NM_001126047	NC	1.30	.010
SEMA7A	NM_011352	NC	-1.10	.005
UNC5D	NM_153135	NC	1.51	.016

Fold changes (FC) of gene expression represent either Lifetime or Early Life GRov mice compared with No-GRov controls. GRov, glucocorticoid receptor overexpression in forebrain; NAcc, nucleus accumbens; NC, no change. *n* = 6–7 mice per group.

Supplemental References

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