Maternal care and Gene - Environment Interactions Defining Development

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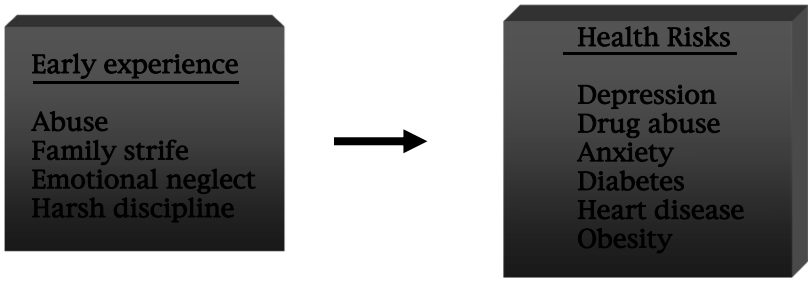
The development of an individual is an active process of *adaptation that occurs within a social and economic context*:

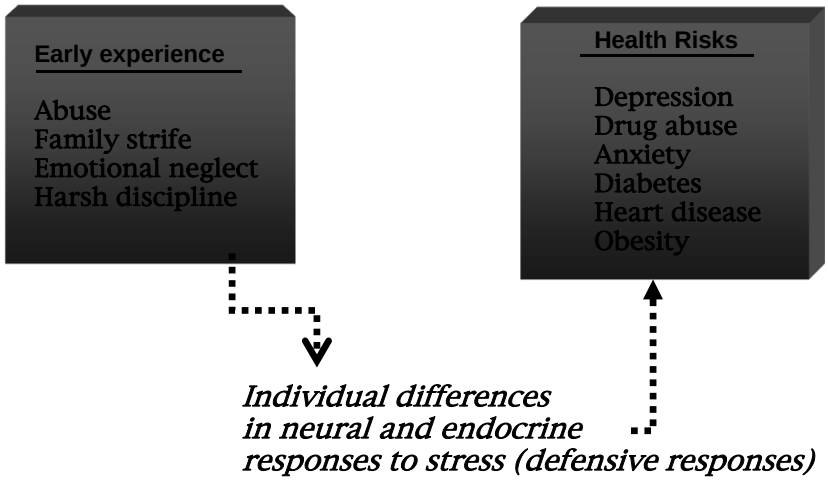
- To resource (food, shelter, safety) availability.
- To social interactions.
- To independence from the parent.

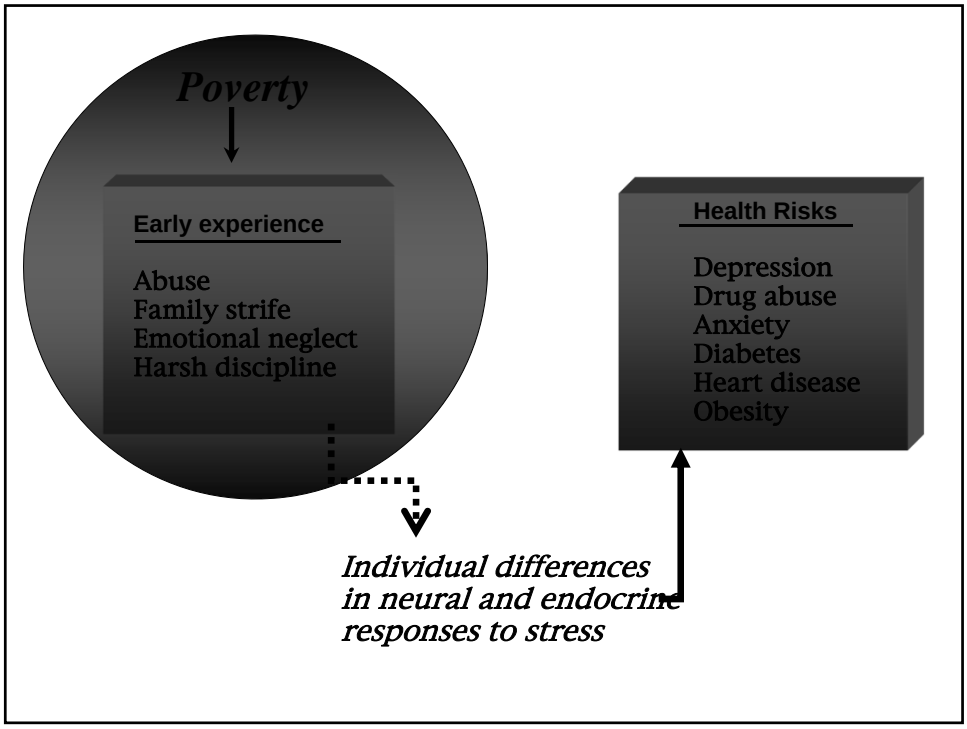
Developmental Origins of Adult Disease

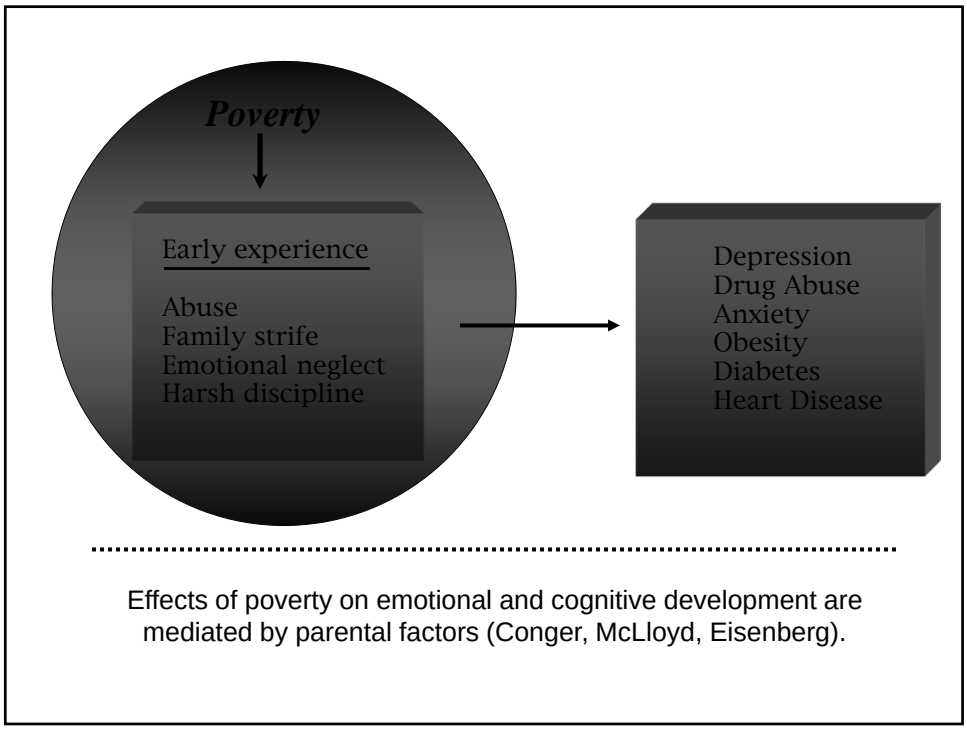


Mechanism?

Stress Diathesis Models




Poverty
Early experience
Abuse
Family strife
Emotional neglect
Harsh discipline
Health Risks
Depression
Drug abuse
Anxiety
Diabetes
Heart disease
Obesity
Individual differences
in neural and endocrine
responses to stress


Poverty
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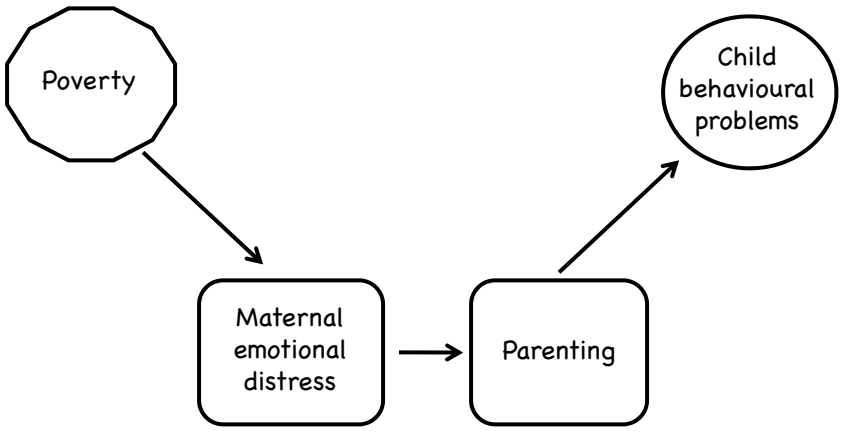
Diabetes
Heart Disease
Effects of poverty on emotional and cognitive development are mediated by parental factors (Conger, McLloyd, Eisenberg).



Linver, Brooks-Gunn & Kohen Dev Psychol 2002; same model predicts child cortisol levels (Lupien, McEwen, Meaney Biol Psychiatry 2000)

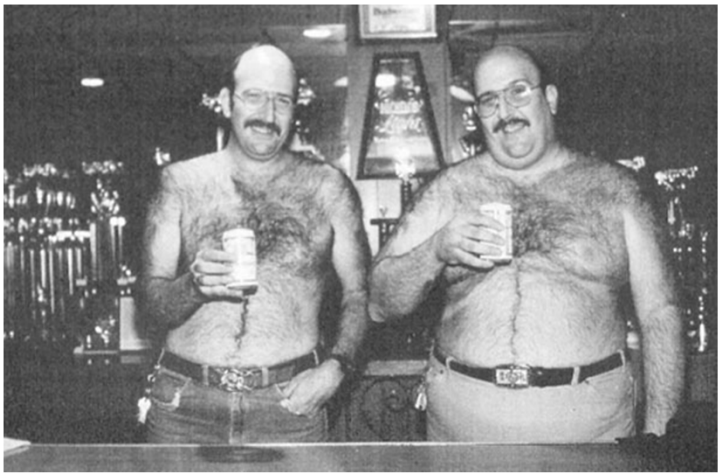
How might the parent-child relations that define early family life influence the development of individual differences in brain development and function?

Summary

- Parental care affects the activity of genes in the brain that regulate stress responses, neural development and reproduction.
- This parental effect involves a form a "plasticity" at the level of the DNA.

Epigenetics: Any functional change in the genome that does not involve an alteration of DNA sequence.

Multiple phenotypes from a common genotype



Every cell in your body has the same nuclear genes, but…?

If they ask you anything you don't know, just say its due to epigenetics.

Environmental epigenetics hypothesis: Environmental events activate intracellular signals that remodel the epigenome, leading to sustained alterations in the structure and function of the genome, and thus stable effects on gene transcription.

Warning!!!

Incomprehensible scientific jargon
will follow…

Genetic code is defined by the sequence of four nucleotides that produce proteins and other molecules that serve cell function.

C T A C G T A C T C G G A A T C T C G

↓

RNAs, Protein

Epigenetic effects refer to modifications of the chemistry of the DNA, but <u>not</u> to a change of sequence. Epigenetics alters the activity of the gene, but not its function.

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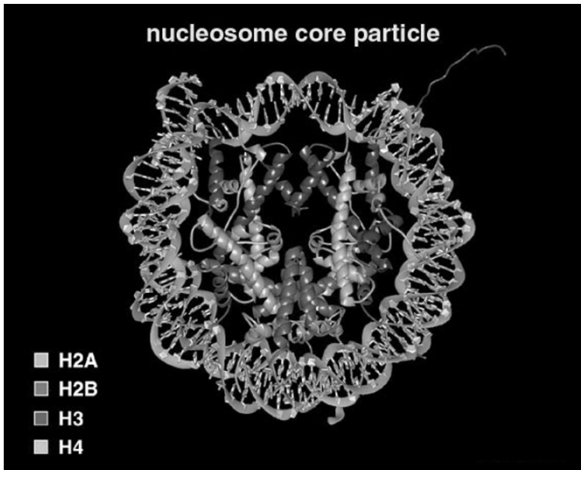
- Epigenetics alters the activity of the gene, but not its function.

- DNA methylation: The addition of a methyl group onto a cytosine.
- DNA methylation is chemically stable (potentially lasting for the life of the organism).
- DNA methylation silences gene expression.

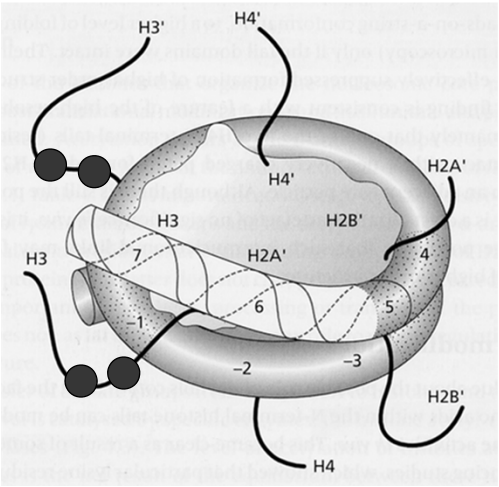


Prevents TF binding to DNA

TF binding involves alteration of chromatin structure

Nucleosome core particle: ribbon traces for the 146-bp DNA phosphodiester backbones (brown and turquoise) and eight histone protein chains (Luger et al. Nature 1997).

Acetyl group



B. Turner. Chromatin structure and gene regulation. 2001.

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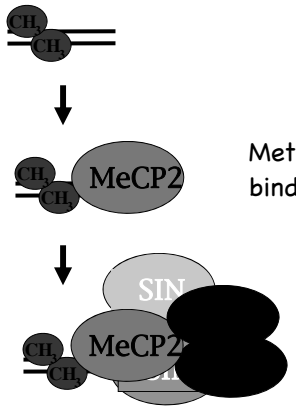
CH_3 CH_3 CH_3

C T A C G T A C T C G G A A T C T C G

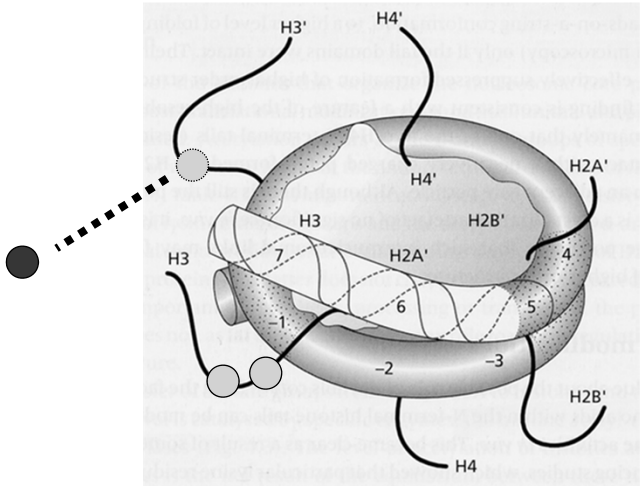
DNA Methylation can inhibit gene expression by blocking transcription factors binding



Methylated DNA binding protein

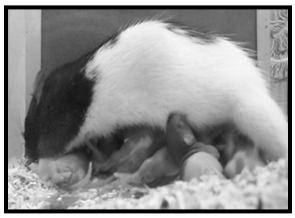
HDAC: Histone deacetylase

HDAC



B. Turner. Chromatin structure and gene regulation. 2001.

Parental care → Epigenetic mark → Gene expression → Phenotype



Naturally-occurring variations in maternal care

Expression of specific genes in brain regions

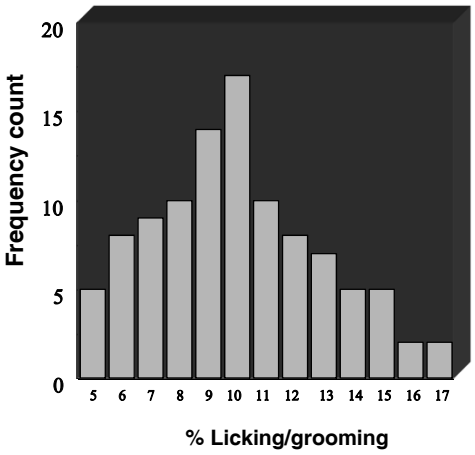
HPA function

Stable individual differences in stress reactivity

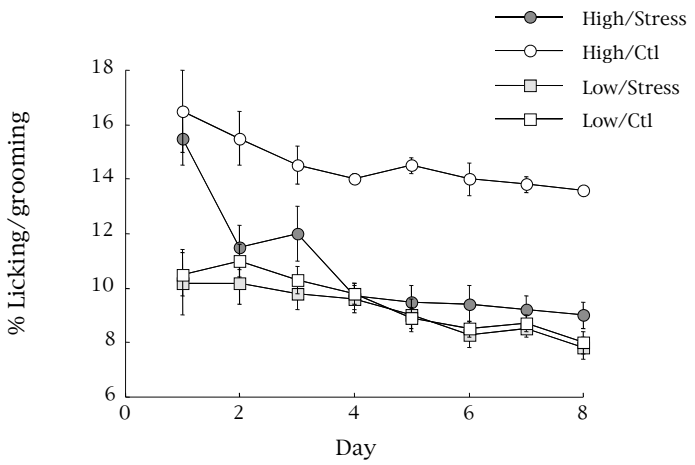
Maternal licking/grooming

Source of tactile stimulation/nurturance: Enhances activity of endocrine systems (e.g., GH/IGF) that promote somatic growth, suppresses those (glucocorticoids) that inhibit growth

Variations in maternal care



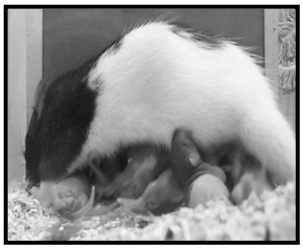
Gestational stress/maternal behaviour



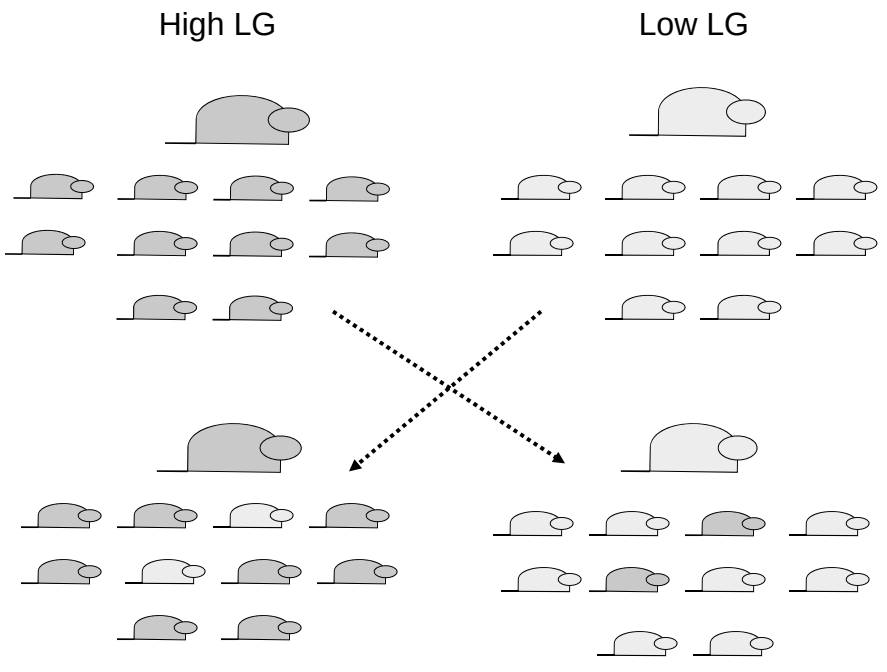
Comparable effects in humans

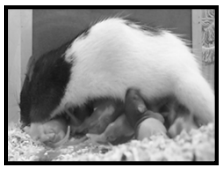
Popeski et al: Pictures of 'own' infant increase activity in ventral striatum (nucleus accumbens – dopamine target) by comparison to other infant or neutral stimuli in fMRI studies – this effect is abolished by chronic stress.

Broad range of parental effects



- Stress responses
- Neural development
- Learning & memory
- Metabolism
- Reproduction (females)



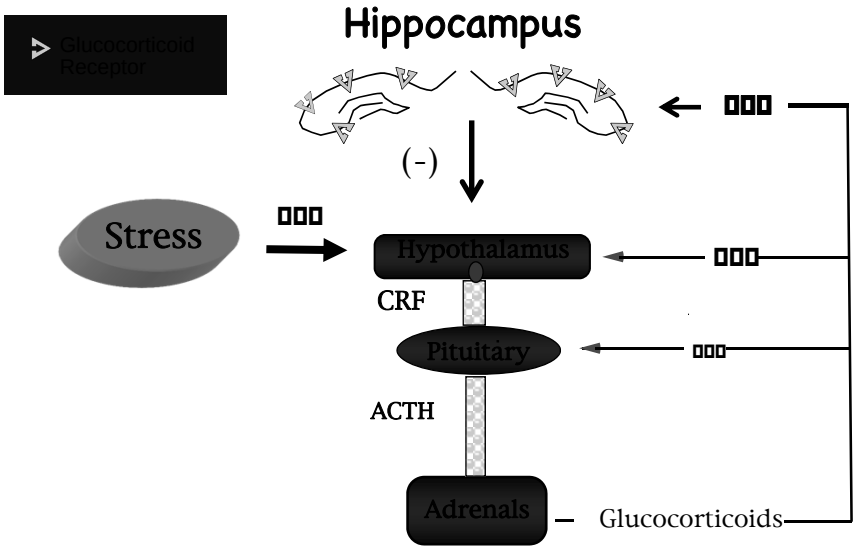


Defensive Responses

Endocrine stress response

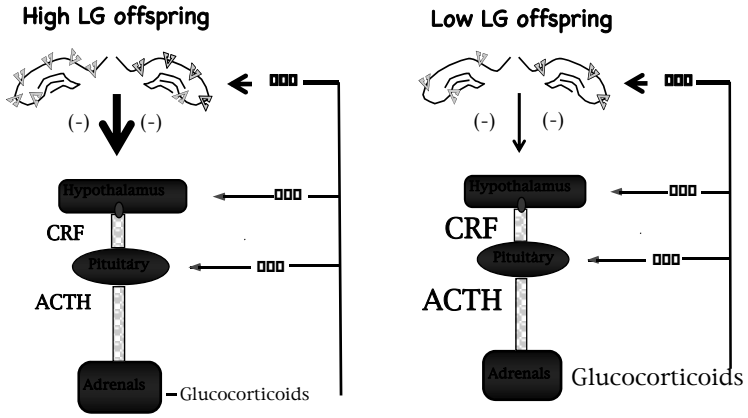
Fearfulness

Immune responses

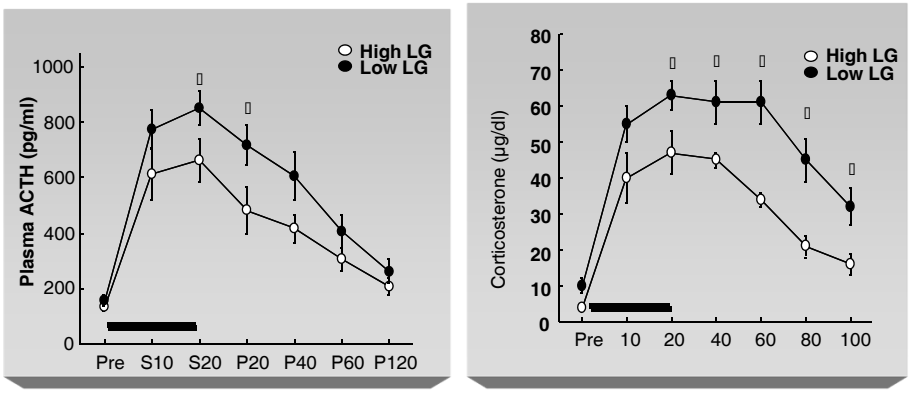


CRF: corticotropin releasing factor. ACTH: adrenocorticotropin

Individual differences in glucocorticoid receptor levels lead to altered pituitary-adrenal responses to stress

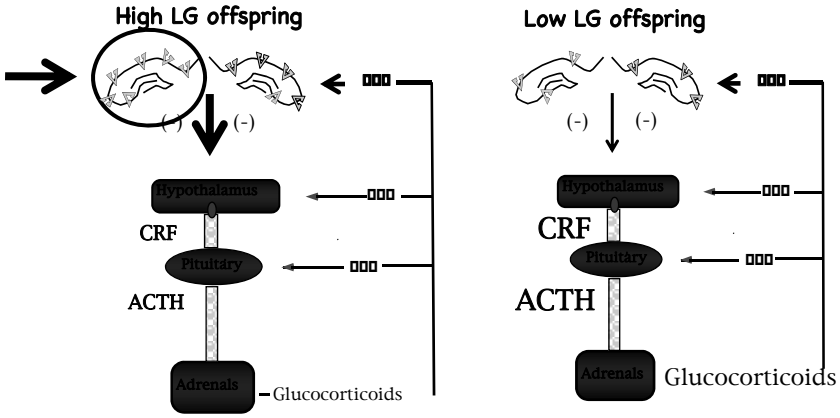


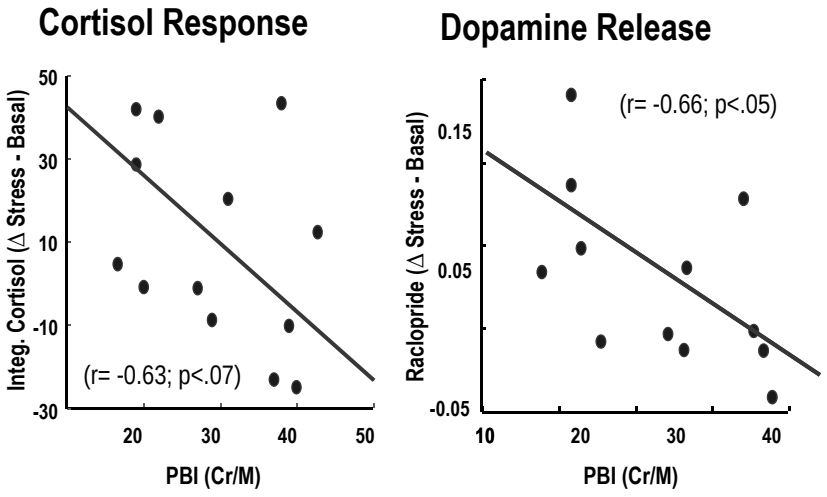
Adult offspring of High LG mothers show more modest HPA responses to stress



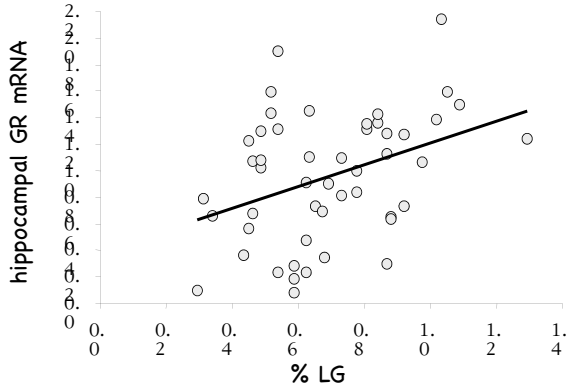
Intra-hippocampal infusion of a GR antagonist completely eliminates the maternal effect on HPA responses to stress

Individual differences in glucocorticoid receptor levels lead to altered pituitary-adrenal responses to stress

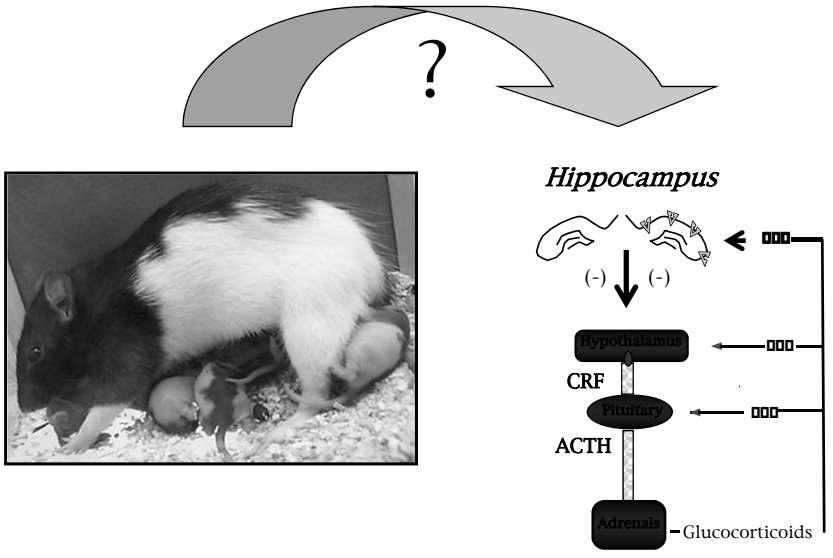




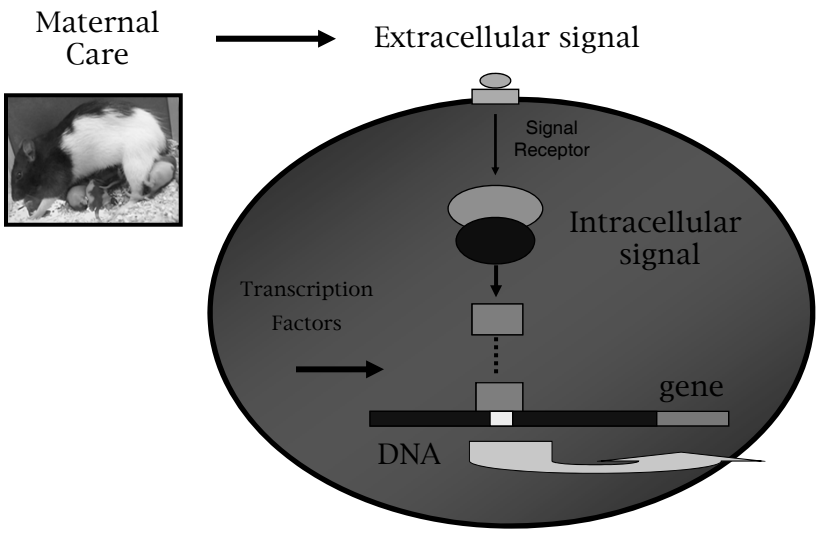
Within-litter variation in pup LG frequency predicts hippocampal glucocorticoid receptor gene expression



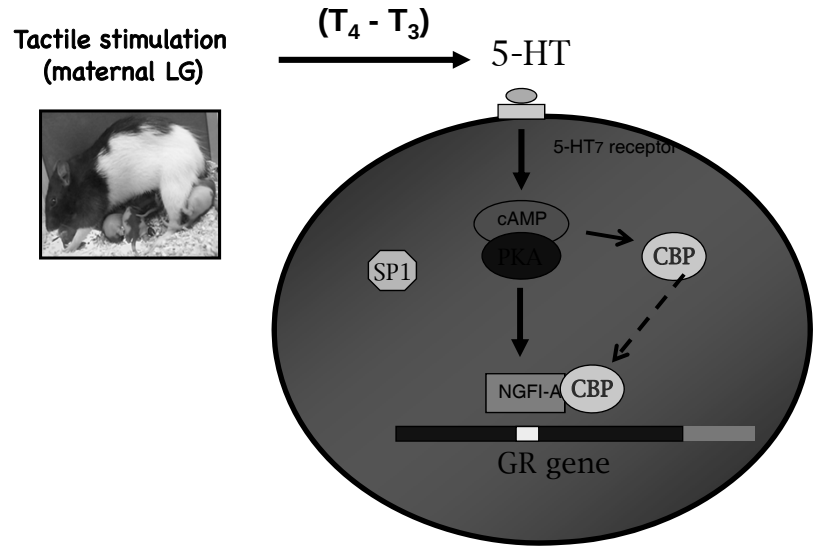
r= 0.375, p= 0.008



Relevant gene - environment interaction

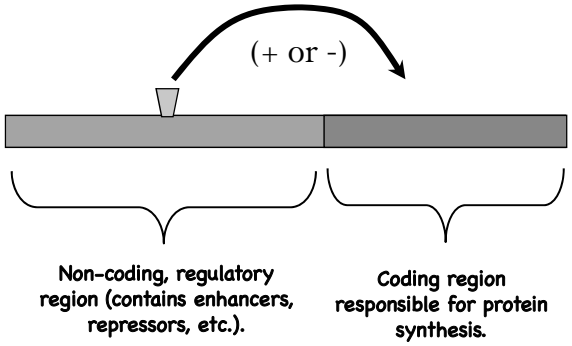


Summary of *in vivo* and *in vitro* studies

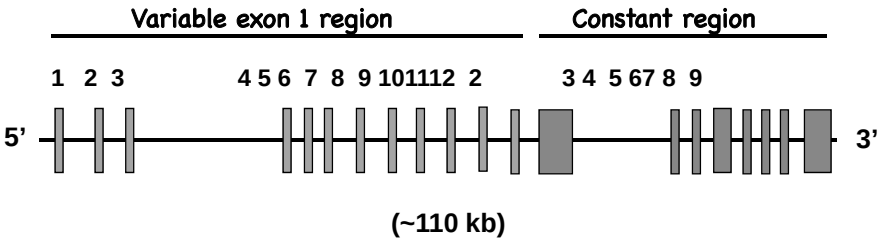


NGFI-A (egr-1, zif-268, krox-24)

Gene organization



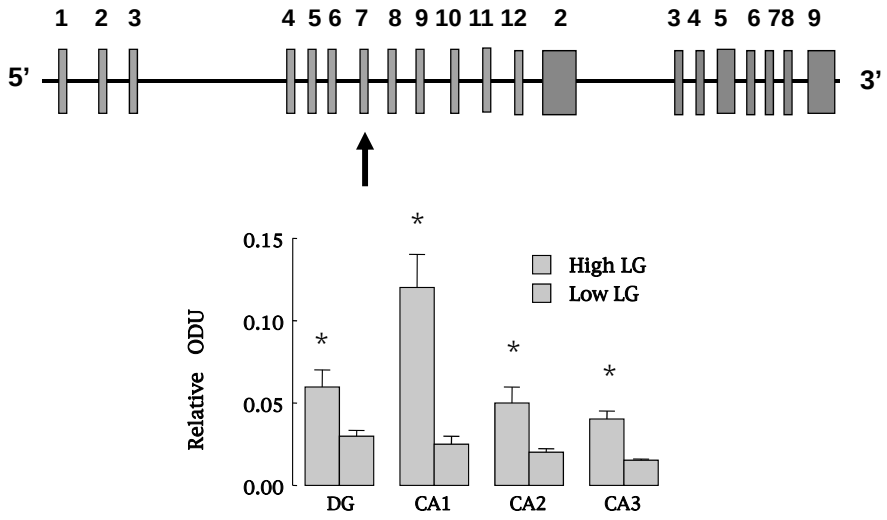
Glucocorticoid receptor gene



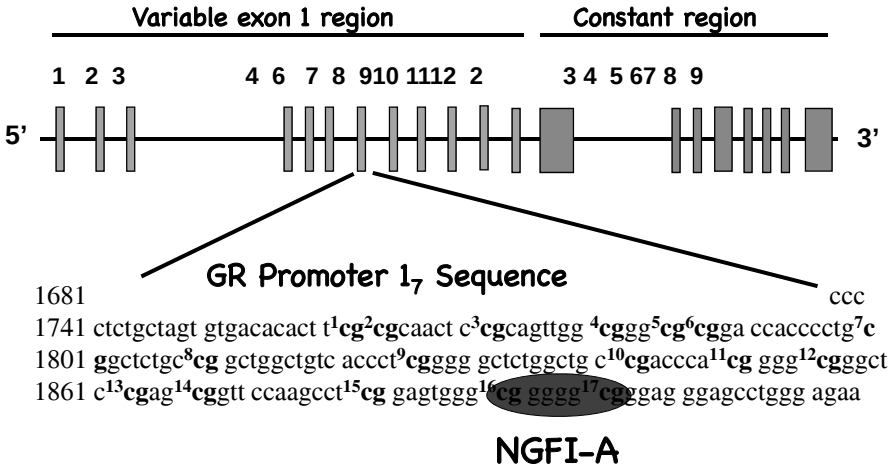
Clone the 5' untranslated region of the rat hippocampal glucocorticoid receptor gene

Transfection studies with promoter-reporter constructs reveal exon 1_7 sequence has considerable transactivational capacity.

Hippocampal GR(1_7) mRNA



DNA sites that regulate glucocorticoid receptor gene



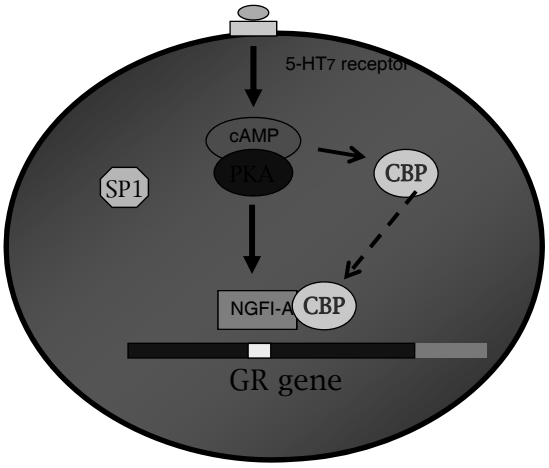
1681 ccc
1741 ctctgctagt gtgacacact t[1]**cg**[2]**cg**caact c[3]**cg**cagttgg [4]**cg**gg[5]**cg**[6]**cg**ga ccacccctg[7]**c**
1801 **g**gctctgc[8]**cg** gctggctgtc accct[9]**cg**ggg gctctggctg c[10]**cg**accca[11]**cg** ggg[12]**cg**ggct
1861 c[13]**cg**ag[14]**cg**gtt ccaagcct[15]**cg** gagtggg[16]**cg** gggg[17]**cg**ggag ggagcctggg agaa

NGFI-A

Summary of *in vivo* and *in vitro* studies

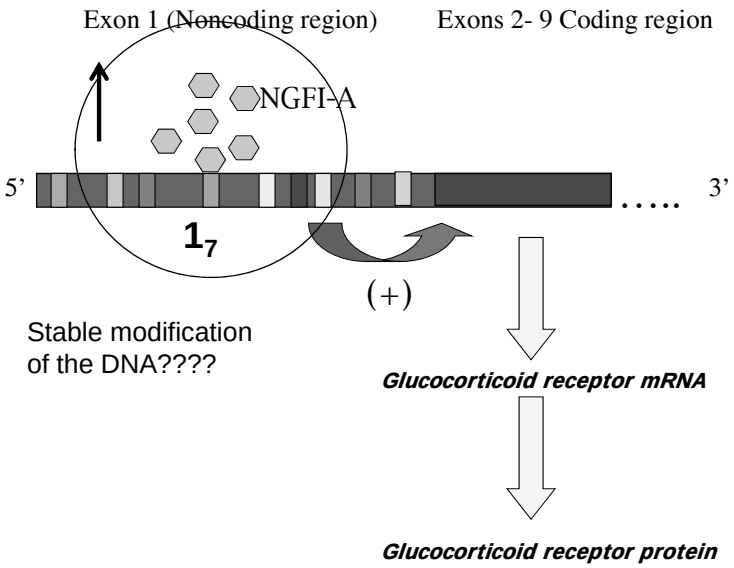
Tactile stimulation (maternal LG)

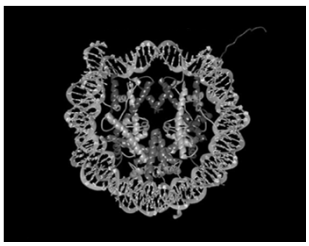
$(T_4 - T_3)$ → 5-HT

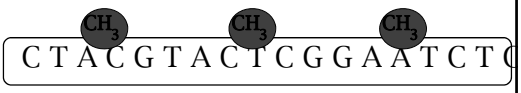


NGFI-A (egr-1, zif-268, krox-24)

Offspring of High LG mothers

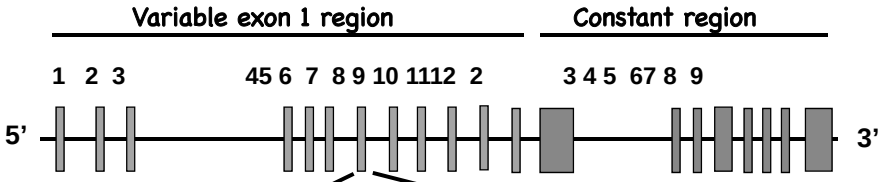






GR expression & HPA function

DNA sites that regulate glucocorticoid receptor gene



GR Promoter 1_7 Sequence

1681 ccc
1741 ctctgctagt gtgacacact t[1]**cg**[2]**cg**caact c[3]**cg**cagttgg [4]**cg**gg[5]**cg**[6]**cg**ga ccacccctg[7]**c**
1801 **g**gctctgc[8]**cg** gctggctgtc accct[9]**cg**ggg gctctggctg c[10]**cg**accca[11]**cg** ggg[12]**cg**ggct
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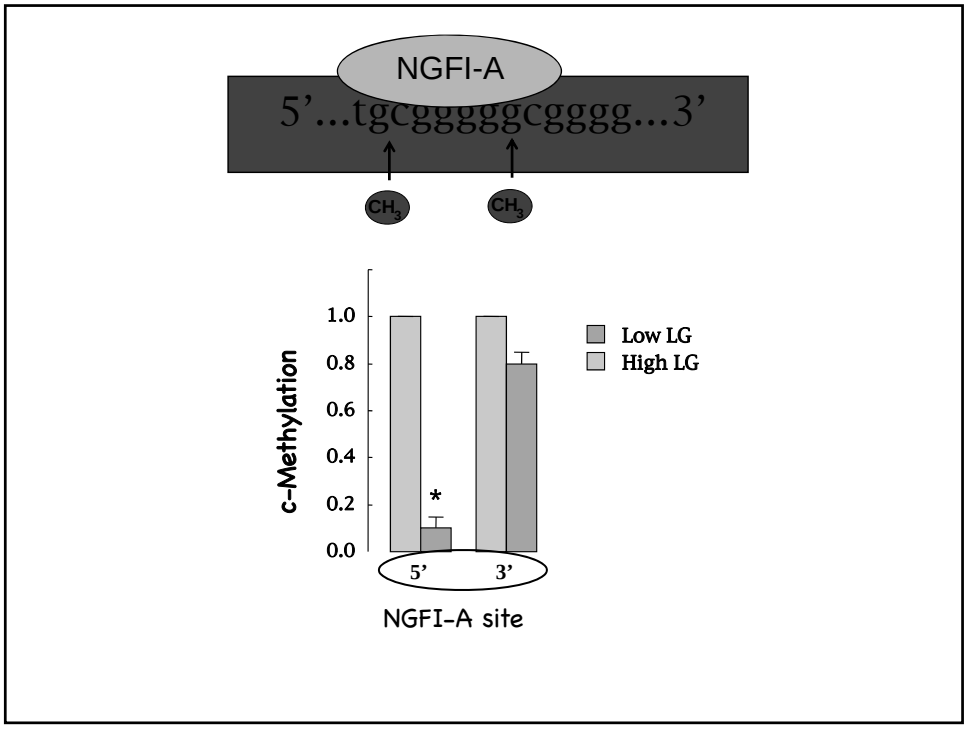
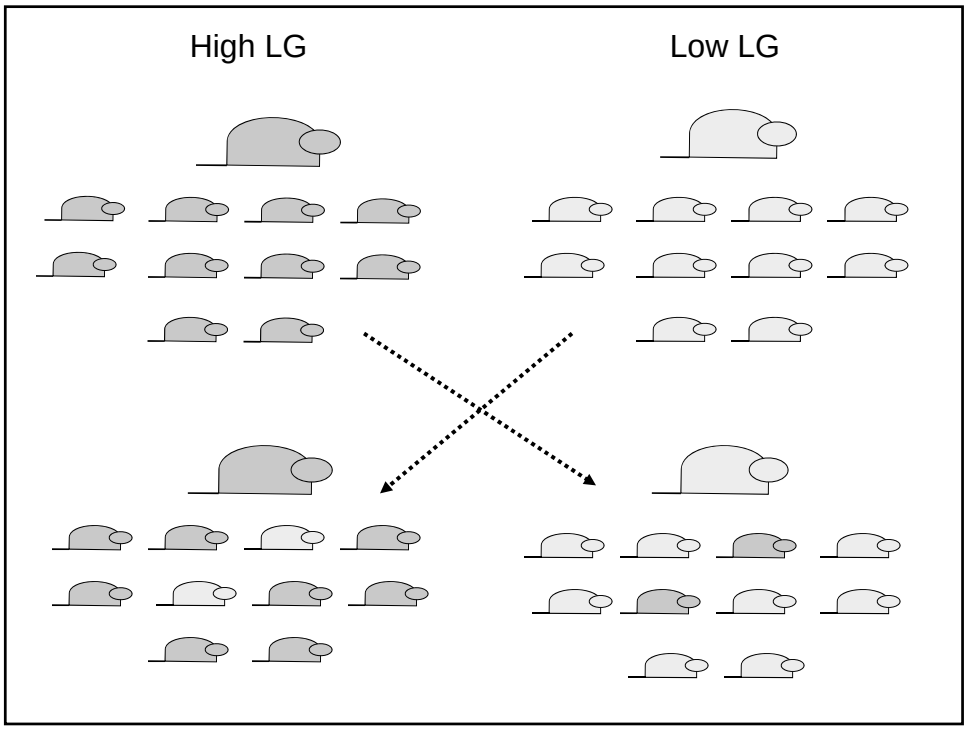
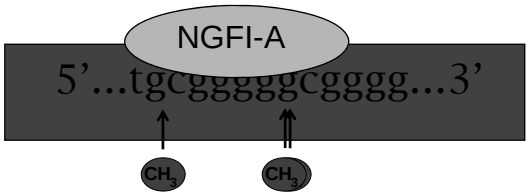
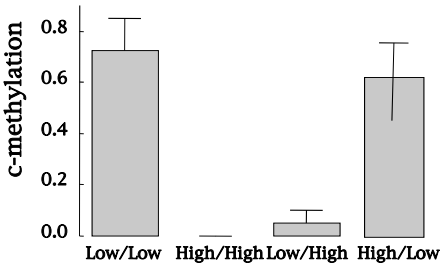
NGFI-A


NGFI-A
5'...tgcgggggcgggg...3'
CH_3
CH_3
c-Methylation
1.0
0.8
0.6
0.4
0.2
0.0
*
5'
3'
NGFI-A site
Low LG
High LG

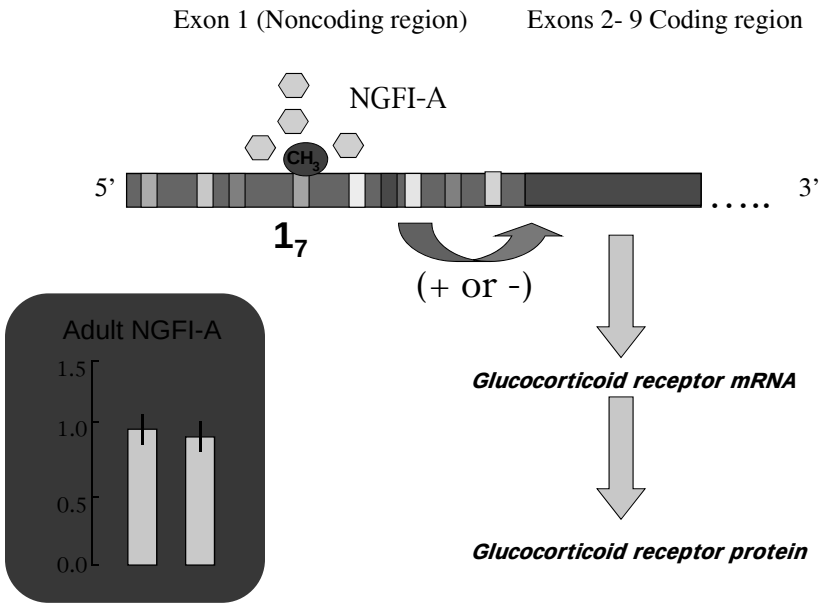

High LG
Low LG



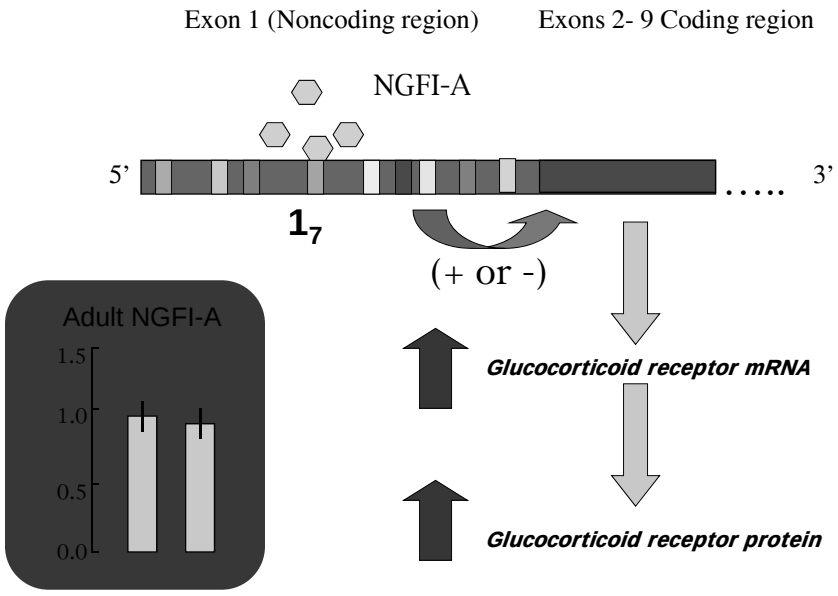
5' CpG region of NGFI-A/RE



Offspring of Low LG mothers



Offspring of High LG mothers



Offspring of Low LG mothers

GGAGCCTGGG AGAA CCAAGCCT**CG** GAGTGGG**CG** GGGG**CG**GGAG

Lower levels of GR in hippocampus -
Increased stress response

Offspring of High LG mothers

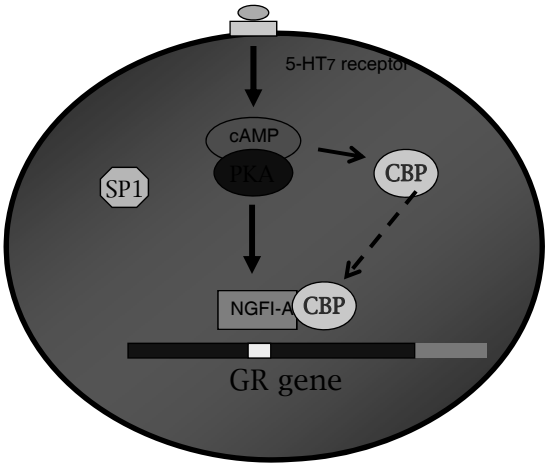
GGAGCCTGGG AGAA CCAAGCCT**CG** GAGTGGG**CG** GGGG**CG**GGAG

High levels of GR in hippocampus -
Modest stress response

Summary of *in vivo* and *in vitro* studies

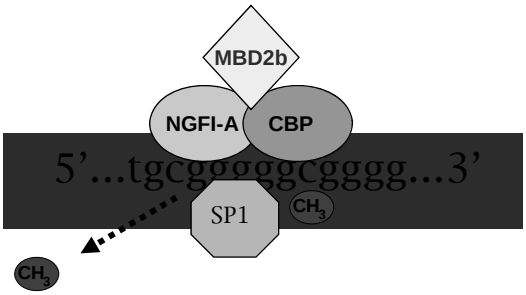
Tactile stimulation (maternal LG)

$(T_4 - T_3)$ → 5-HT

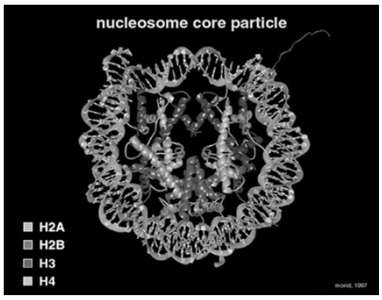


NGFI-A (egr-1, zif-268, krox-24)

High LG → high NGFI-A levels

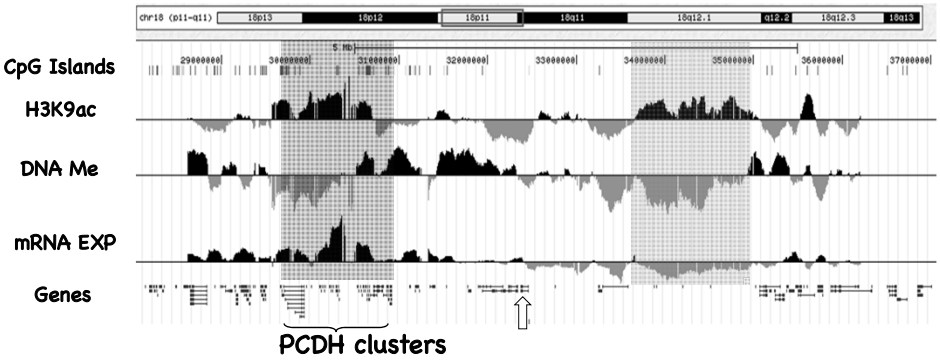


DNA methylation serves to imprint social factors,
such as maternal behavior, upon the offspring's genome.

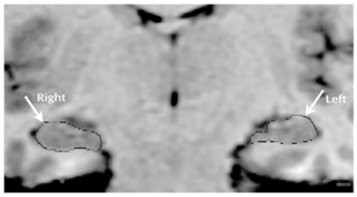


DNA methylation serves as an interface between
the dynamic environment and the fixed genome

NR3C1 (GR) gene locus (Chromosome 18)

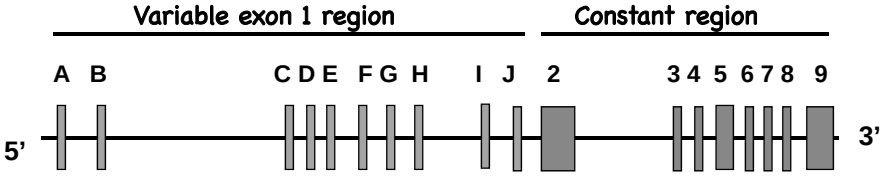


Do comparable processes occur in humans?

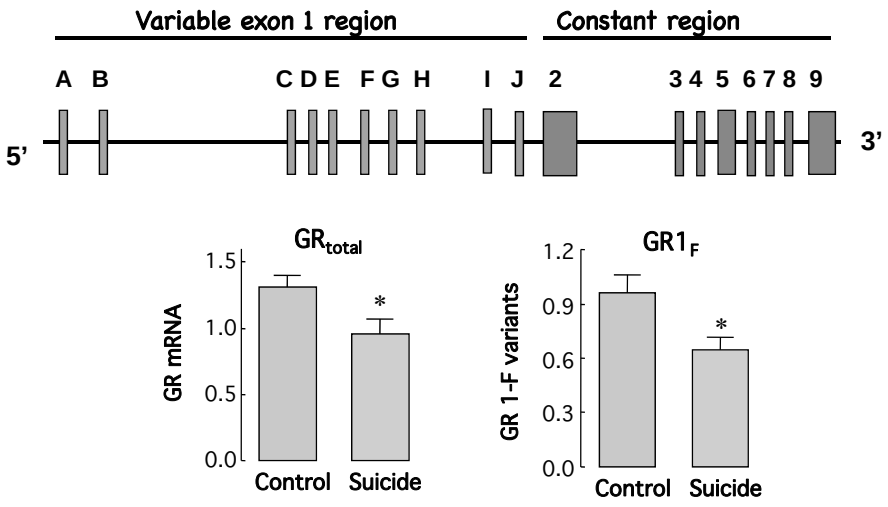


- **Post-mortem studies of hippocampus.**
- **Samples from suicide victims/controls.**
- **QSBB (Gustavo Turecki) – forensic phenotyping.**
- **Human exon 1F promoter (Turner & Muller, 2005)**

Human glucocorticoid receptor gene



Human glucocorticoid receptor gene

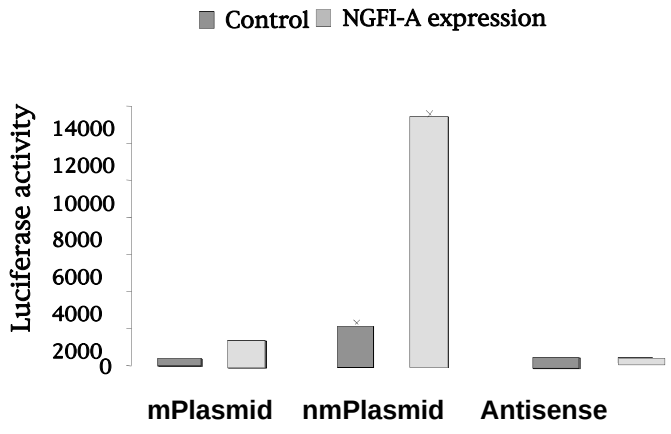


Suicide vs abuse - GR expression

Suicide vs abuse - CpG methylation

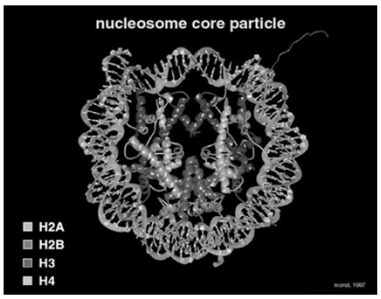
McGowan et al. PlosOne 2008, Nature Neuroscience 2009

Co-transfection studies (NGFI-A vector w/ human GR exon 1F-luciferase construct)

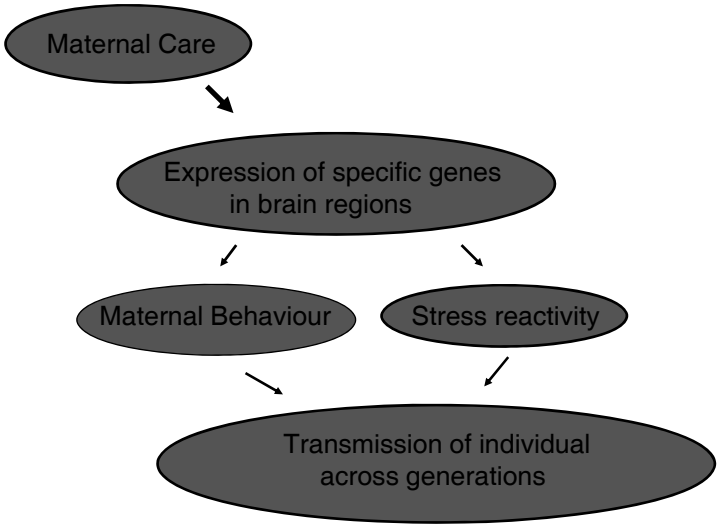


McGowan et al. PlosOne 2008, Nature Neuroscience 2009

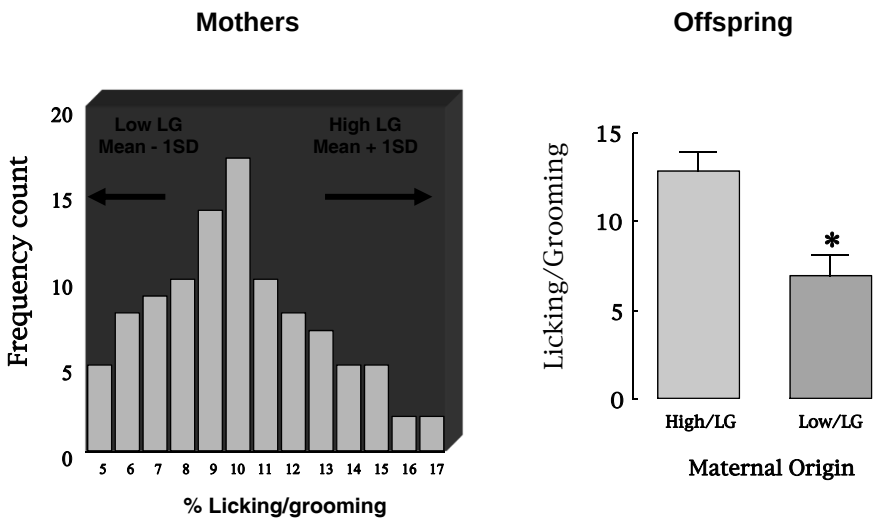
DNA methylation serves to imprint social factors,
such as maternal behavior, upon the offspring's genome.

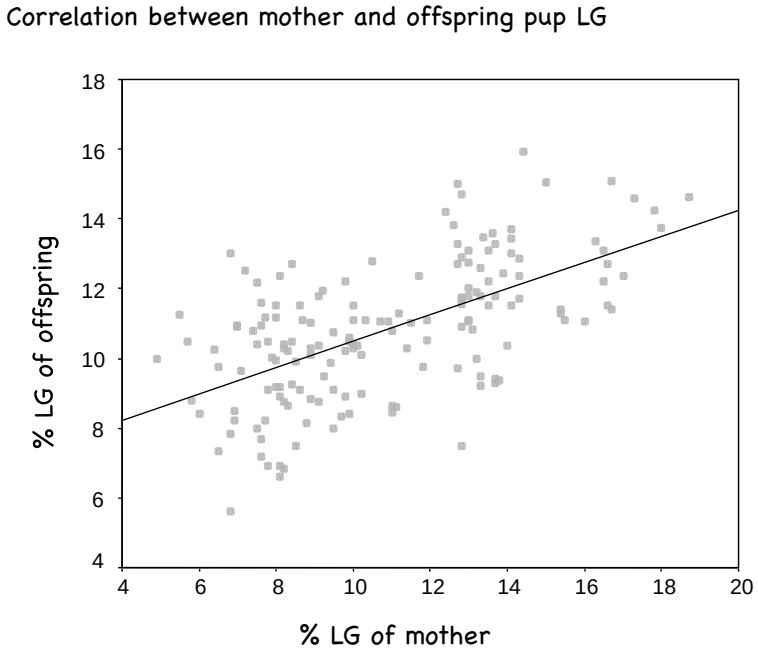


DNA methylation serves as an interface between
the dynamic environment and the fixed genome

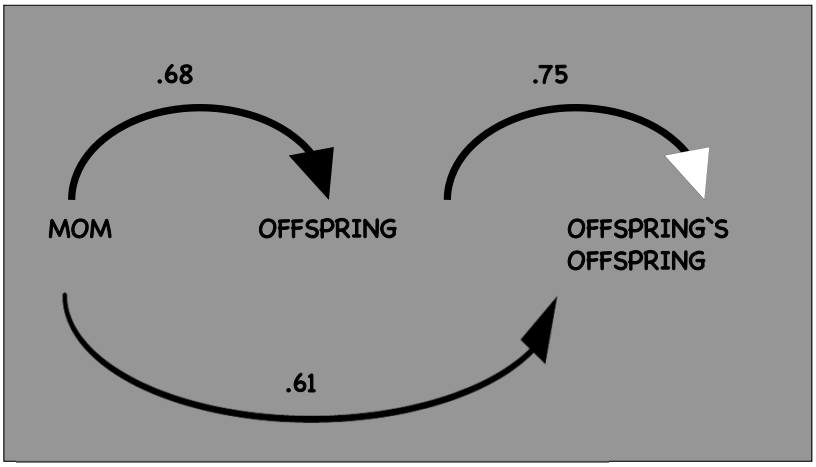


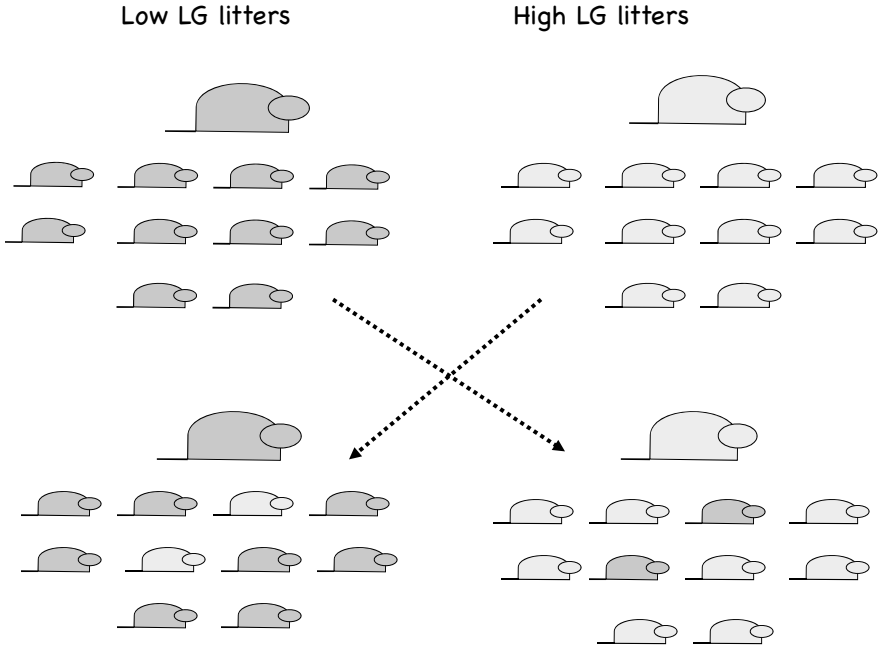
Transmission of individual differences in maternal care



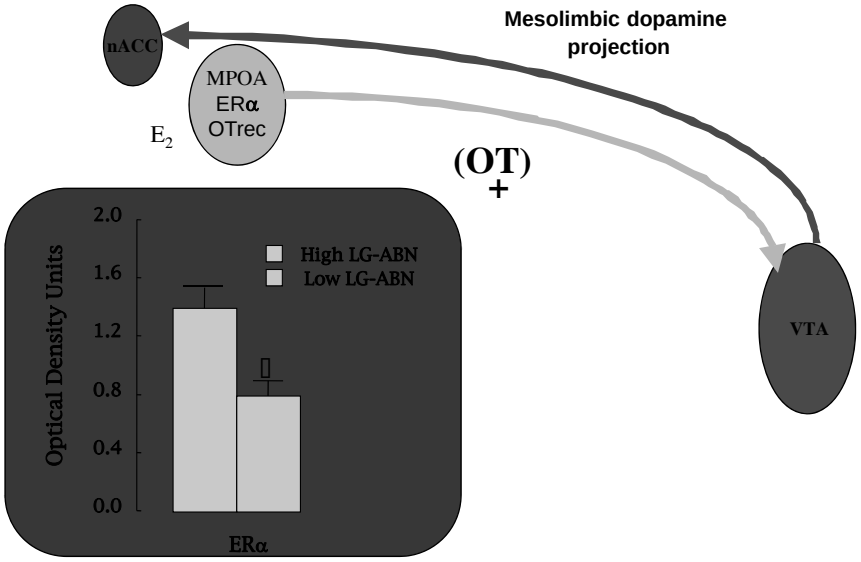


Transmission of maternal behaviour over generations under stable environmental conditions



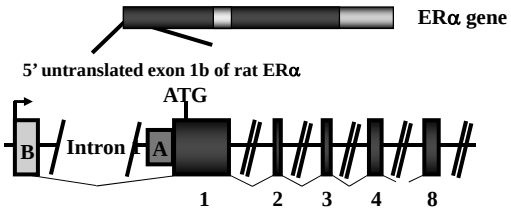


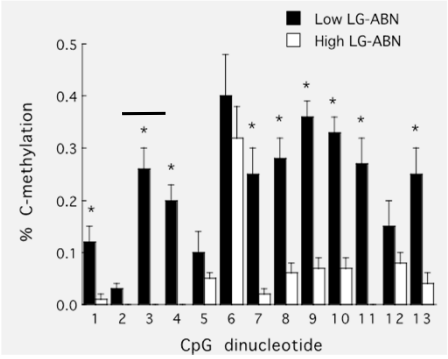
Relevant neural systems



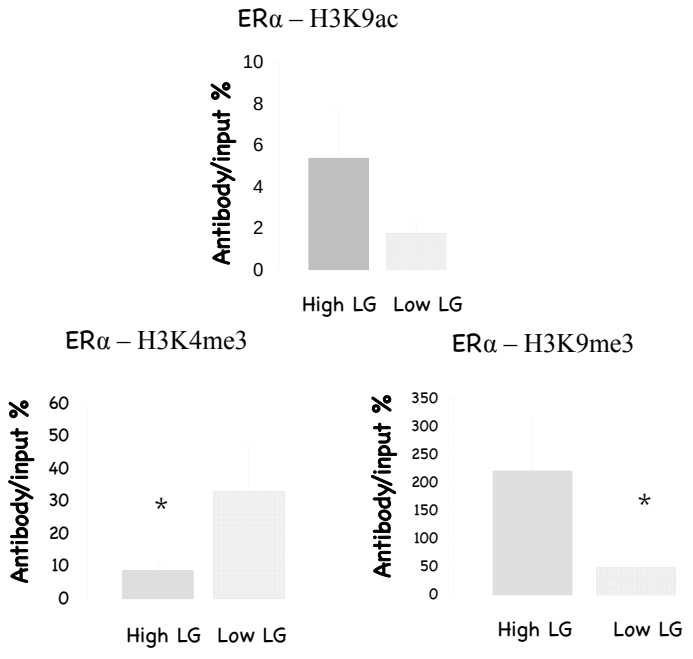
- Cross-fostering reverses the differences in both ER expression and maternal behaviour.
- The ER promoter is differentially methylated as a function of maternal care.

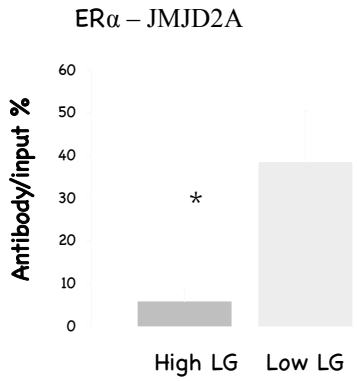
Promoter sites of the ERα gene





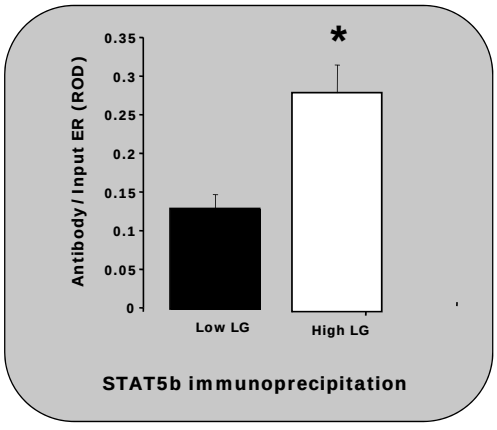
- Site 3 contained within a Stat5 binding site.
- Stat5 regulates ERα expression.





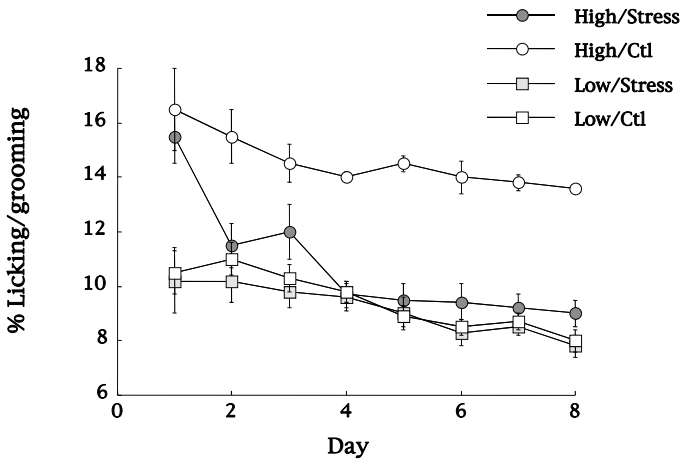
JMJD2A is a lysine demethylase that targets H3K9me3 and is present in co-repressor complexes N-CoR (nuclear hormone co-repressor complex). Through its tandem tudor domains, JMJD2A associates with H3K4me3 and demethylates H3K9 (Huang Y et al. *Science* 2006).

Stat5b binding to the ERα exon 1b promoter in adults

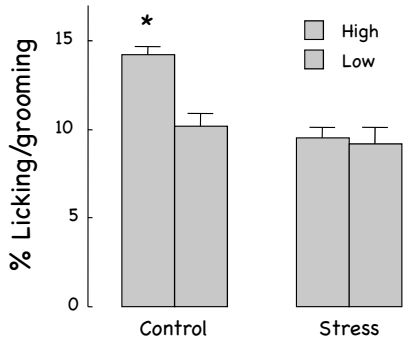


1st litter	Characterize High and Low Mothers
2nd litter	One-half of each group are exposed to stress during gestation.
Prediction:	If the quality of the environment regulates maternal care, then High mothers/Gestational stress should resemble Low mothers.

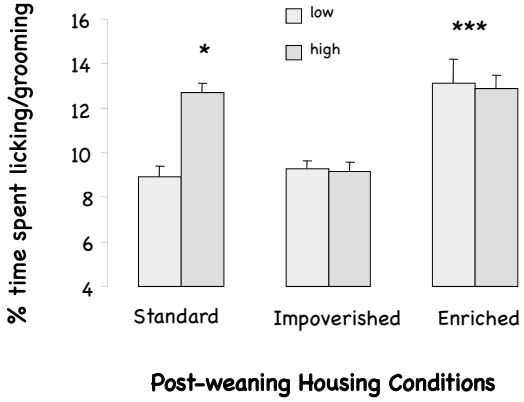
Gestational stress/maternal behaviour

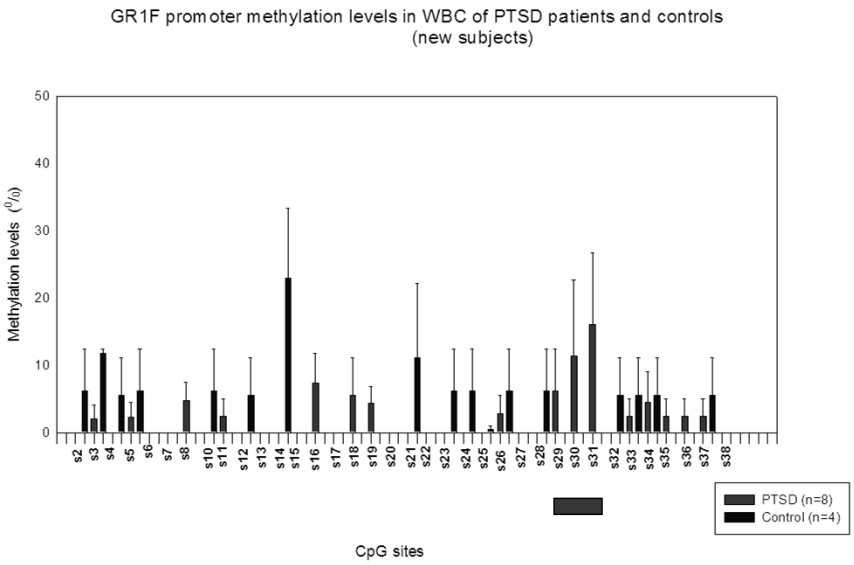


Gestational stress: Maternal behaviour in the offspring

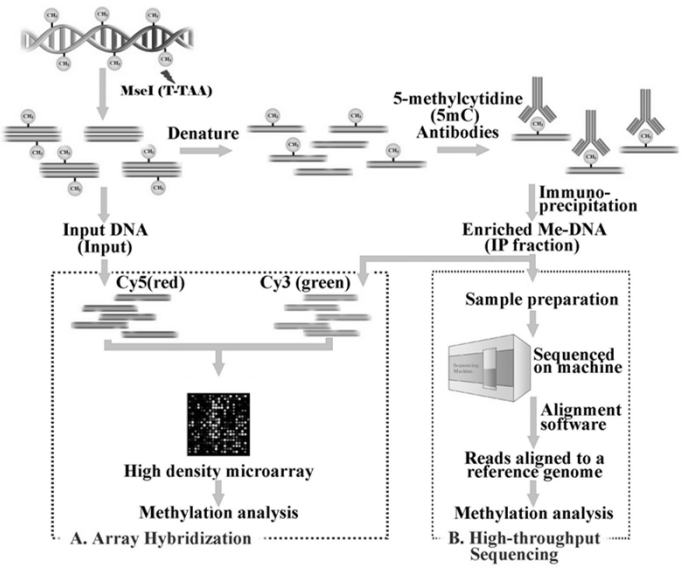


Post-weaning influences on maternal care





MeDIP



http://en.wikipedia.org/wiki/File:Medip-final-3.jpg

2.1 M Deluxe Promoter Array

- Single array design which covers ~10kb of all annotated promoters
- This array tiles through all annotated CpG islands
- Includes positive, negative and non-CpG control regions for quality control

Input data for this analysis

- Scaled $\log_2$-ratio values

$$\log_2 \frac{Intensity\ of\ enriched\ MeDNA}{Intensity\ of\ total\ DNA}$$

Data =

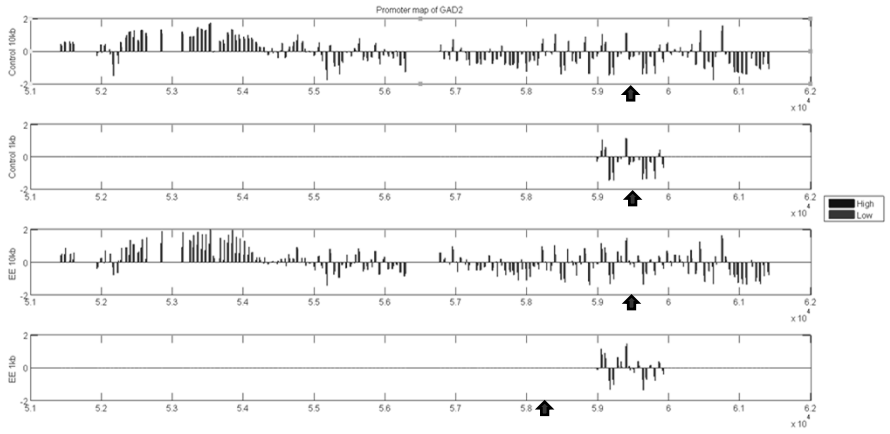
- 7 samples (4 Highs and 3 Lows) for each rearing condition

  (Control vs. Environmental Enriched)

Range of observation (CpG sites)

10kb (8kb before and 2kb after TSS)

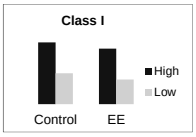
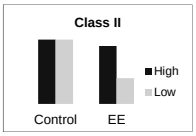
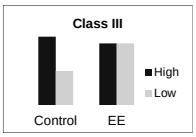
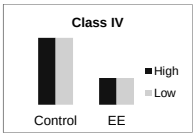
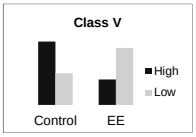
1kb (500bp before and 500bp after TSS)



⬆ - Transcription starting site (TSS)

Preliminary filtering

- Student's *t*-test between Highs and Lows
- Filter sites that are differentially methylated in Highs and Lows via p-value calculated from t-test ($p<0.05$)

	7 CONTROL	7 EE	14 TOTAL
	X	X	X
	O	X	X/O
	X	O	X/O
	O	O	X
	X	X	O

Number of genes with differential methylated CpG sites in Highs vs. Lows

	Control	EE	TOTAL
1kb	• 7834	• 12816	• 5474
10kb	• 17599	• 17872	• 16603

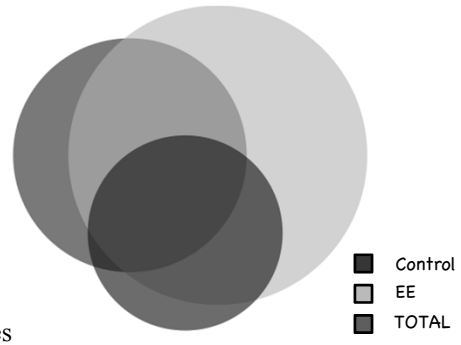
VENN diagram on 1kb data:

MC ∩ EE = 5952 genes

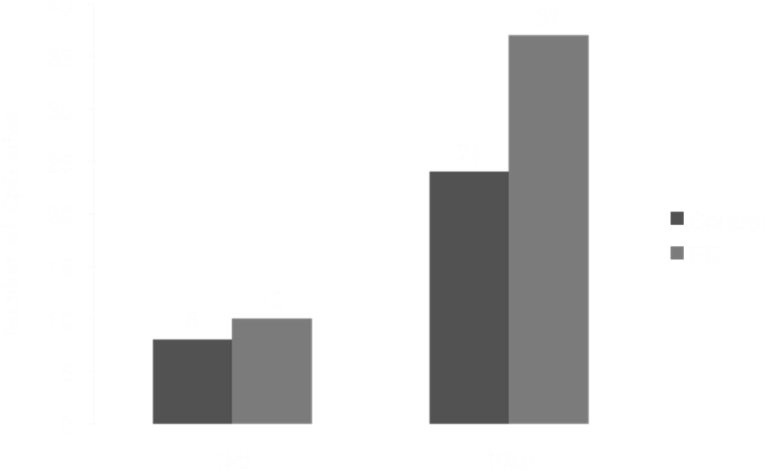
TOTAL ∩ MC = 3120 genes

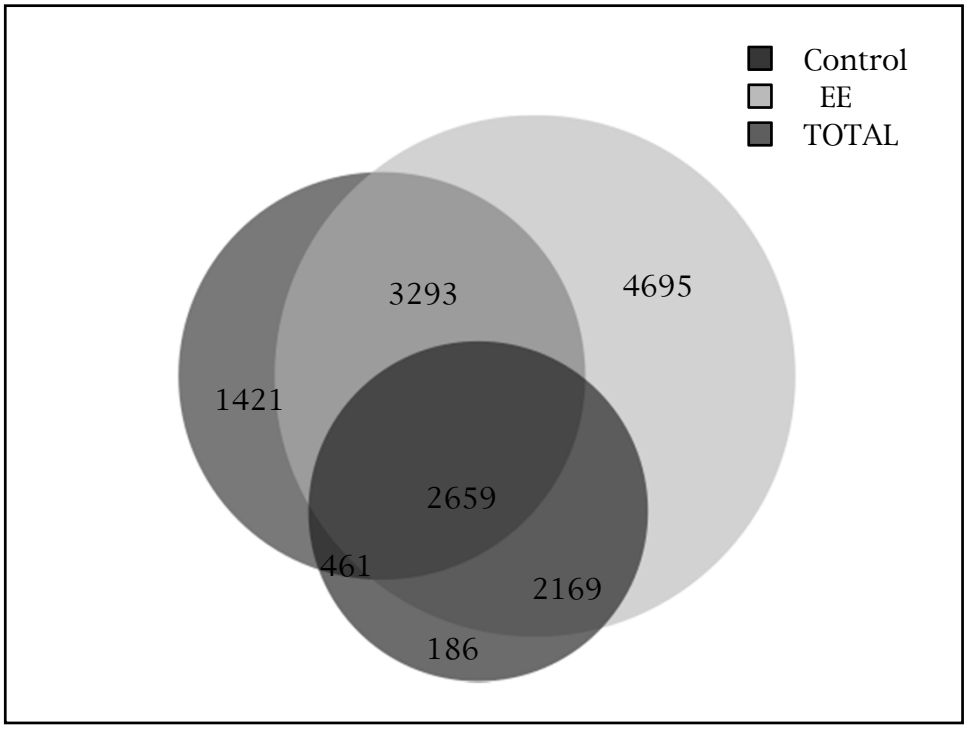
TOTAL ∩ EE = 4828 genes

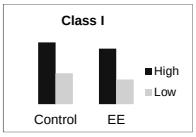
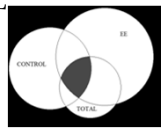
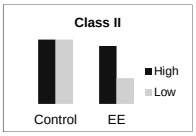
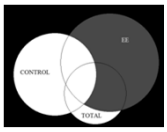
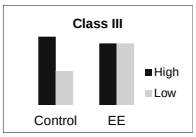
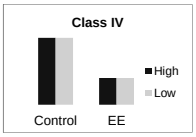
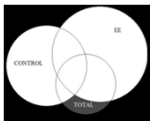
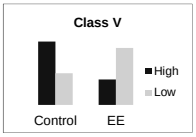
TOTAL ∩ CONTROL ∩ EE = 2659 genes



Maximum number of CpG sites differentially methylated in Highs and Lows of one gene





	7 CONTROL	7 EE	14 TOTAL	
	X	X	X	
	O	X	X/O	
	X	O	X/O	
	O	O	X	
	X	X	O	

Example – Grid2ip

- Glutamate receptor, ionotropic, delta 2 (Grid2) interacting protein 1
- From class V – show DNA **methylation variance in both CONTROL and EE, but NOT in TOTAL**