

From biomarkers to drug targets: genetic animal models of stress and psychiatric disorders

Eva E. Redei, Ph.D.

David Lawrence Stein Professor

Department of Psychiatry and Behavioral Sciences

Northwestern University Feinberg School of Medicine

GENE or ENVIRONMENT?

- Complex, but even single-gene diseases have genetic and environmental contribution to individual variation
- Complex diseases affect the most people
- Environment is everything that is not heritable, but what about epigenetics?!?!?

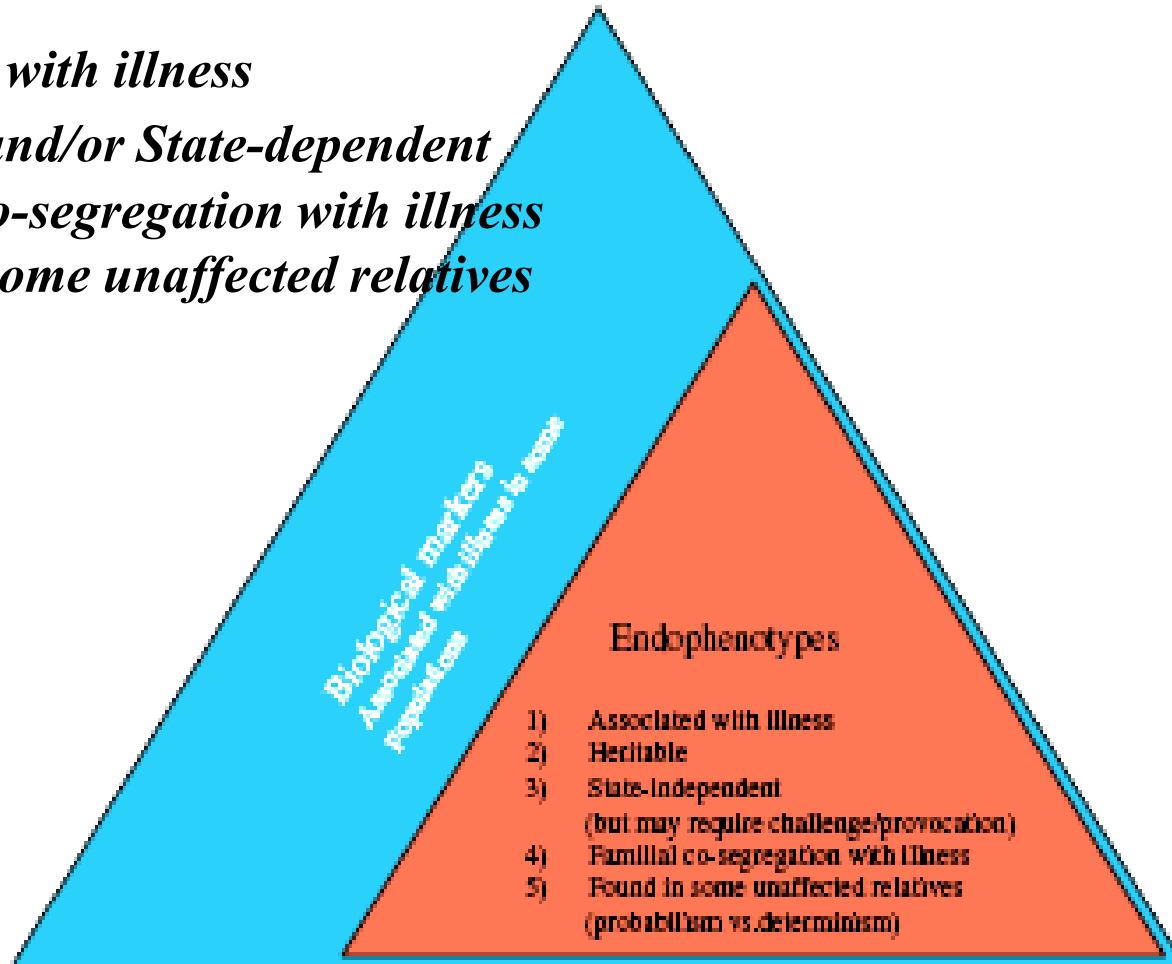
Animal Models to Study Human Diseases

No single animal model can fully replicate any human disorder. Each model, however, can help to clarify certain aspects of the disorder (the concept of endophenotypes).

Biological markers: associated with illness in some population

Endophenotypes are specific manifestation of an illness:

- *Associated with illness*
- *Heritable and/or State-dependent*
- *Familial co-segregation with illness*
- *Found in some unaffected relatives*



Genome-wide association studies, and why animal models are still useful

- **GWAS** can
- successfully localize positive signal to a few tens of kilobases, usually the span of a single gene, therefore,
- identify the genetic determinants of human complex disease directly
- But,
- In most diseases, genetic variation segregating at these genes explains only a small fraction of cases that could be accounted for by genetic causes
- Environment cannot be controlled in humans
- Function of the gene(s) can be elucidated in animal models

Outline

- ❖ Genetic contribution to individual coping with stress
 - Quantitative Trait Loci analysis
 - X-congenic strains
 - Genetic and transcriptomics: a combined analysis
- ❖ Genetic contribution to depressive behavior
 - Quantitative Trait Loci analysis
 - Genetic and transcriptomics: a combined analysis
 - Biomarkers for depression: a translational approach

What is Coping?

- Definition 1: The cognitive, behavioral and physiological processes aimed at diminishing or terminating **stress**.
- Definition 2: General **adaptive** patterns of responses to environmental challenges

Coping Styles are:

- Stable trait characteristics: active and passive coping strategies
- Genetic contribution: higher concordance in identical twins; heritable in animals

Implications for health and disease:

- **Passive coping increases vulnerability to**
 - Heart disease
 - Hypertension
 - Diabetes
 - Rheumatoid arthritis pain (perception)
 - Anxiety disorders
 - Depressive disorders, etc.
- **Active coping increases resilience in the face of adversity**

How do we measure coping strategies?

Any behavioral test with a choice for active or passive response to a threat, without punishment!

DB (Defensive Burying) a test with ethological validity

- The animal can avoid the threat by actively burying the prod or passively avoiding it.
- Approaching the prod is a form of stereotypy

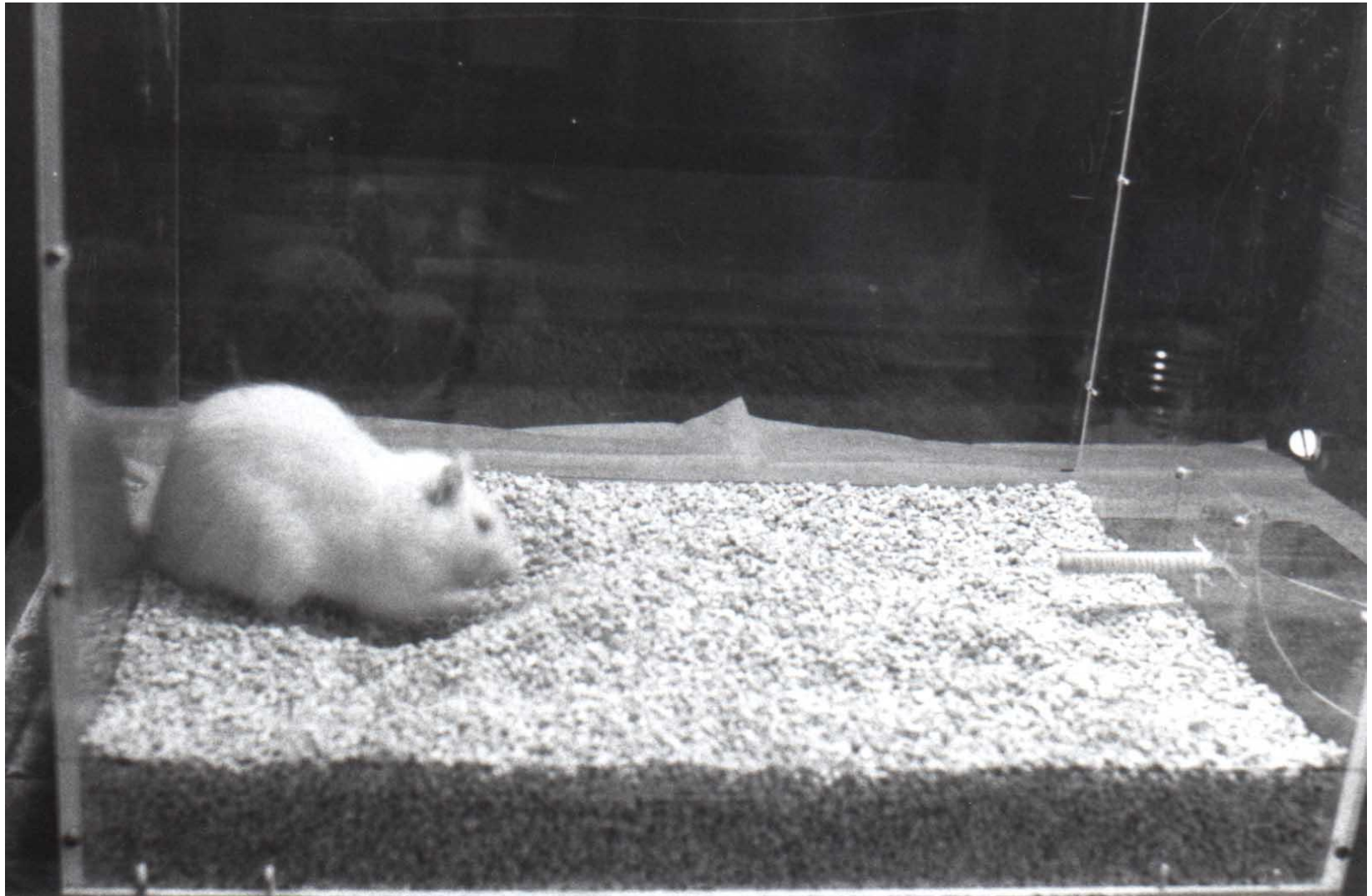


Neuroendocrinology Symposium,
Istanbul, 2009



QuickTime™ and a
DV/DVCPRO - NTSC decompressor
are needed to see this picture.

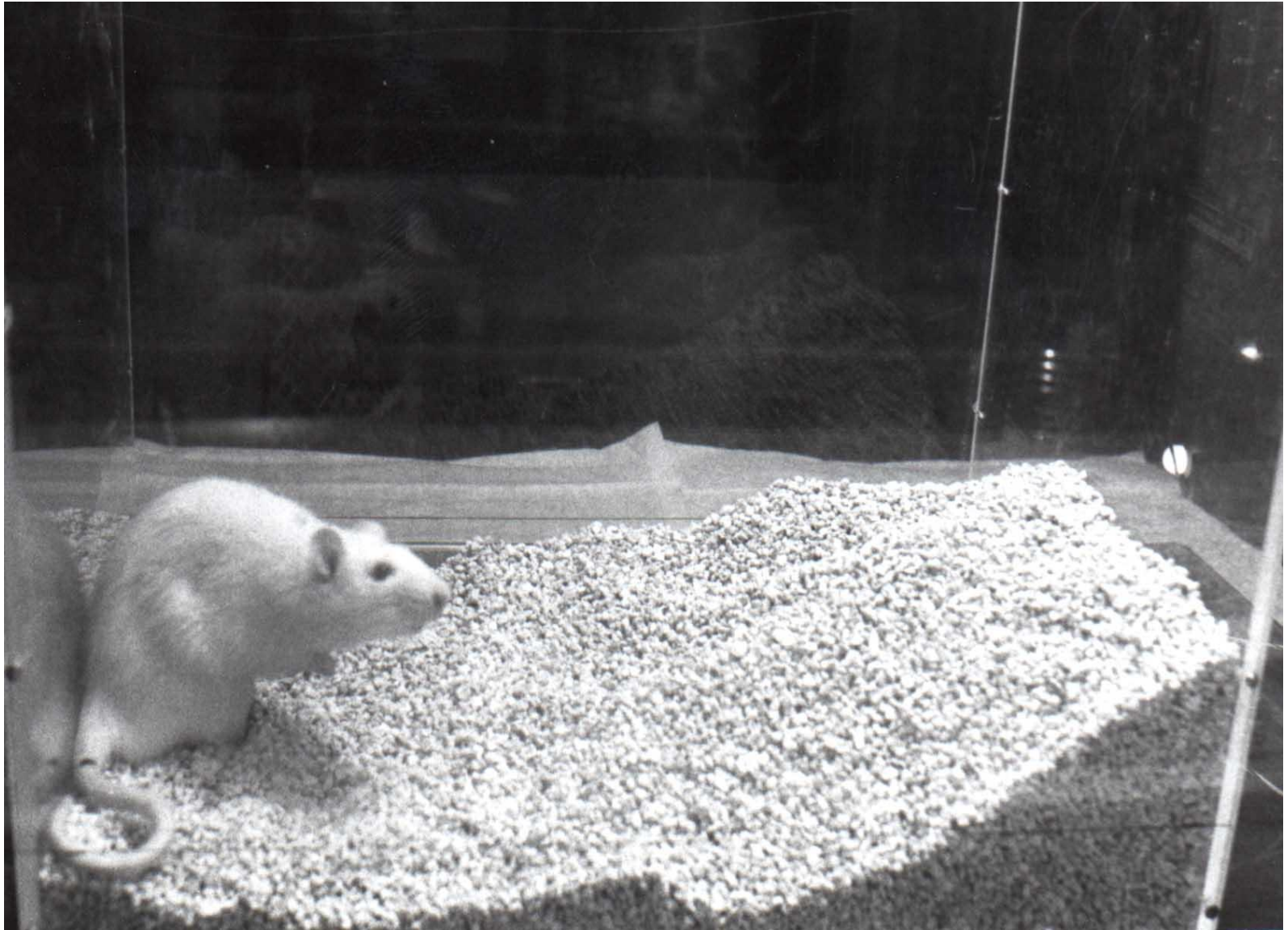
NOV 29 1999
3:15:49 PM



Neuroendocrinology Symposium,
Istanbul, 2009



Neuroendocrinology Symposium,
Istanbul, 2009



Neuroendocrinology Symposium,
Istanbul, 2009



Neuroendocrinology Symposium,
Istanbul, 2009

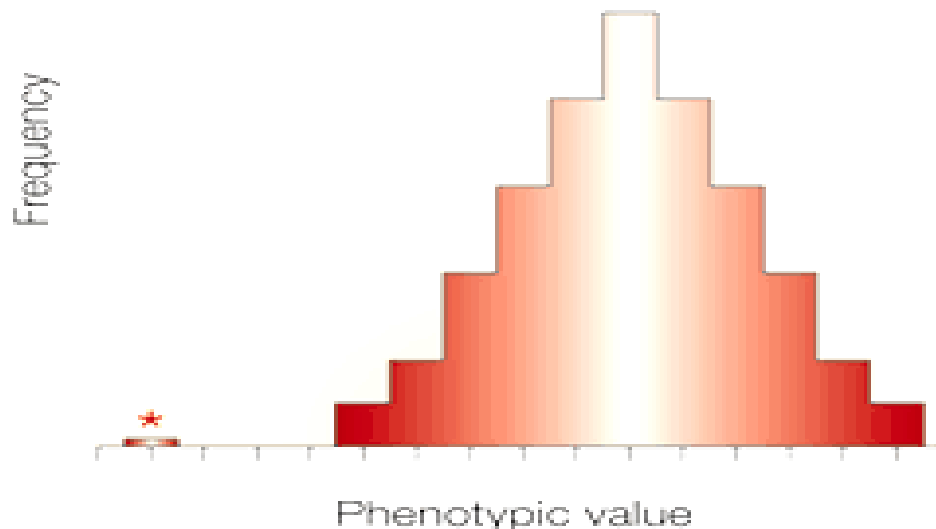
Strategy to identify Quantitative Trait Genes

- QTL analyses identify chromosomal location
- Congenic strains are generated
- Congenic strains are characterized behaviorally
- SNP analysis of parental strains compared to reference strain BN narrow regions
- Microarray analyses of relevant brain regions identify differentially expressed genes within these narrow regions
- These genes are candidate QTGs waiting functional confirmation

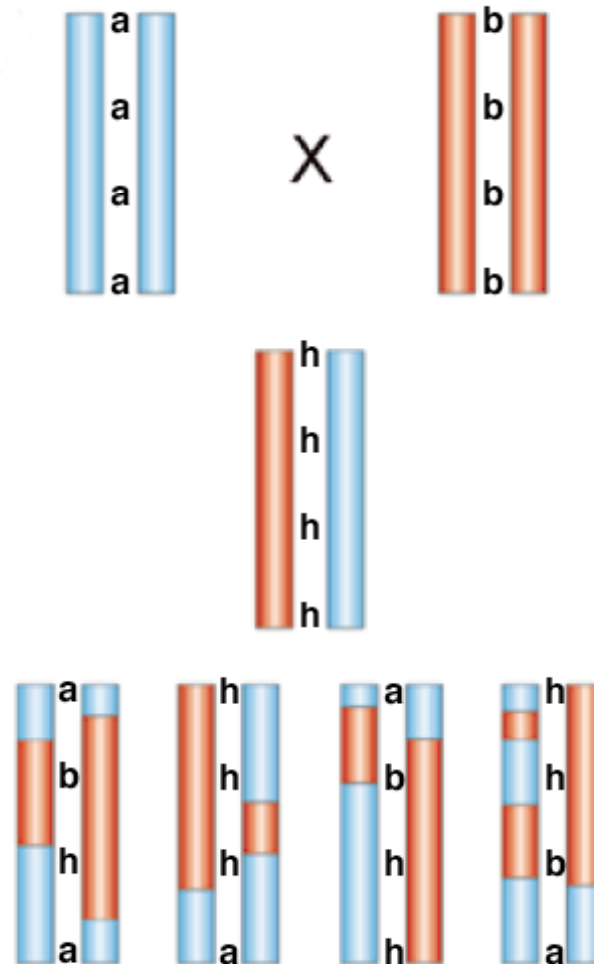
Quantitative Trait Loci Analysis (QTL)

Quantitative Trait – a heritable character that is transmitted as continuous variation

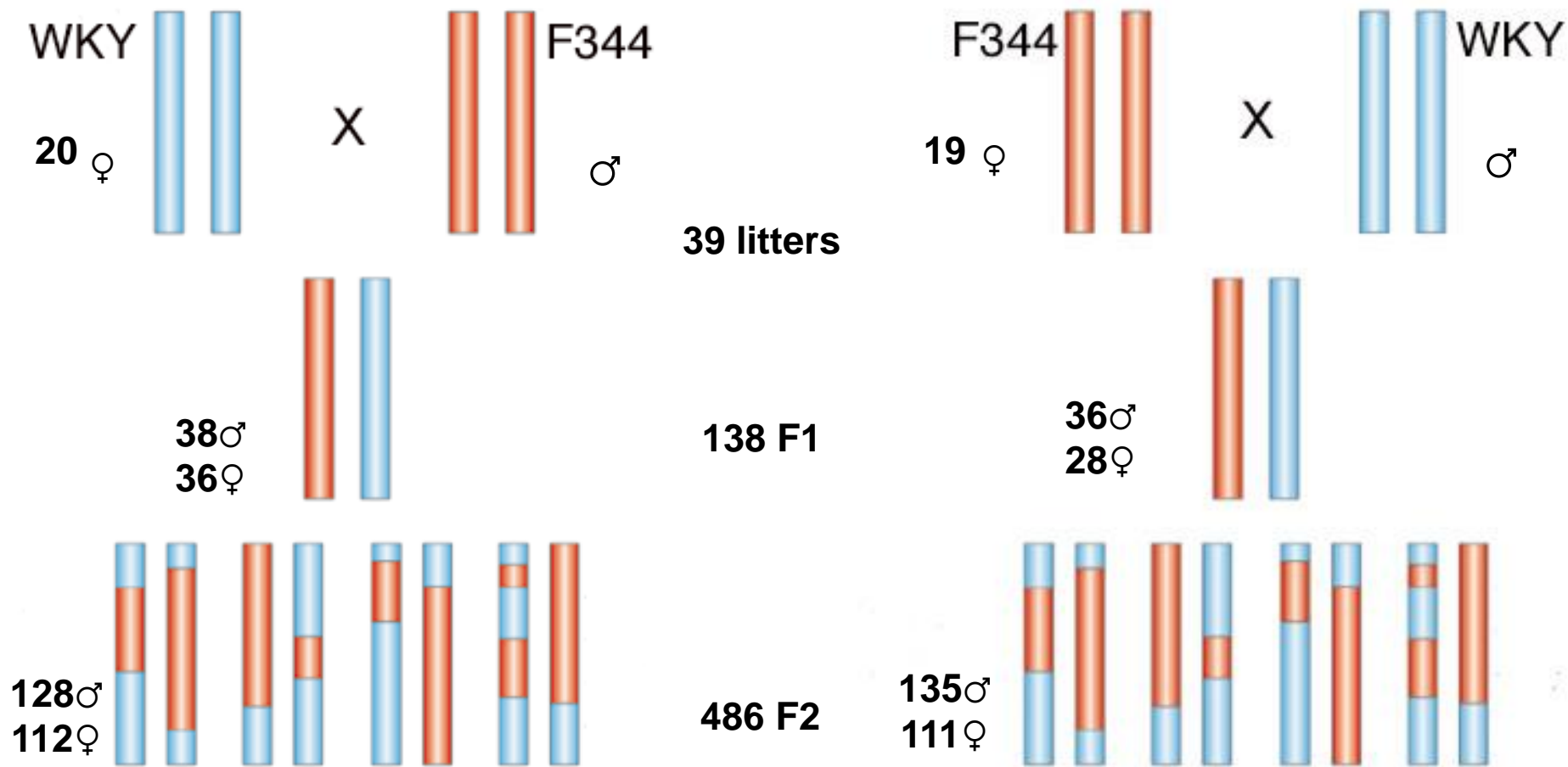
Loci - positions that genes occupy on a chromosome



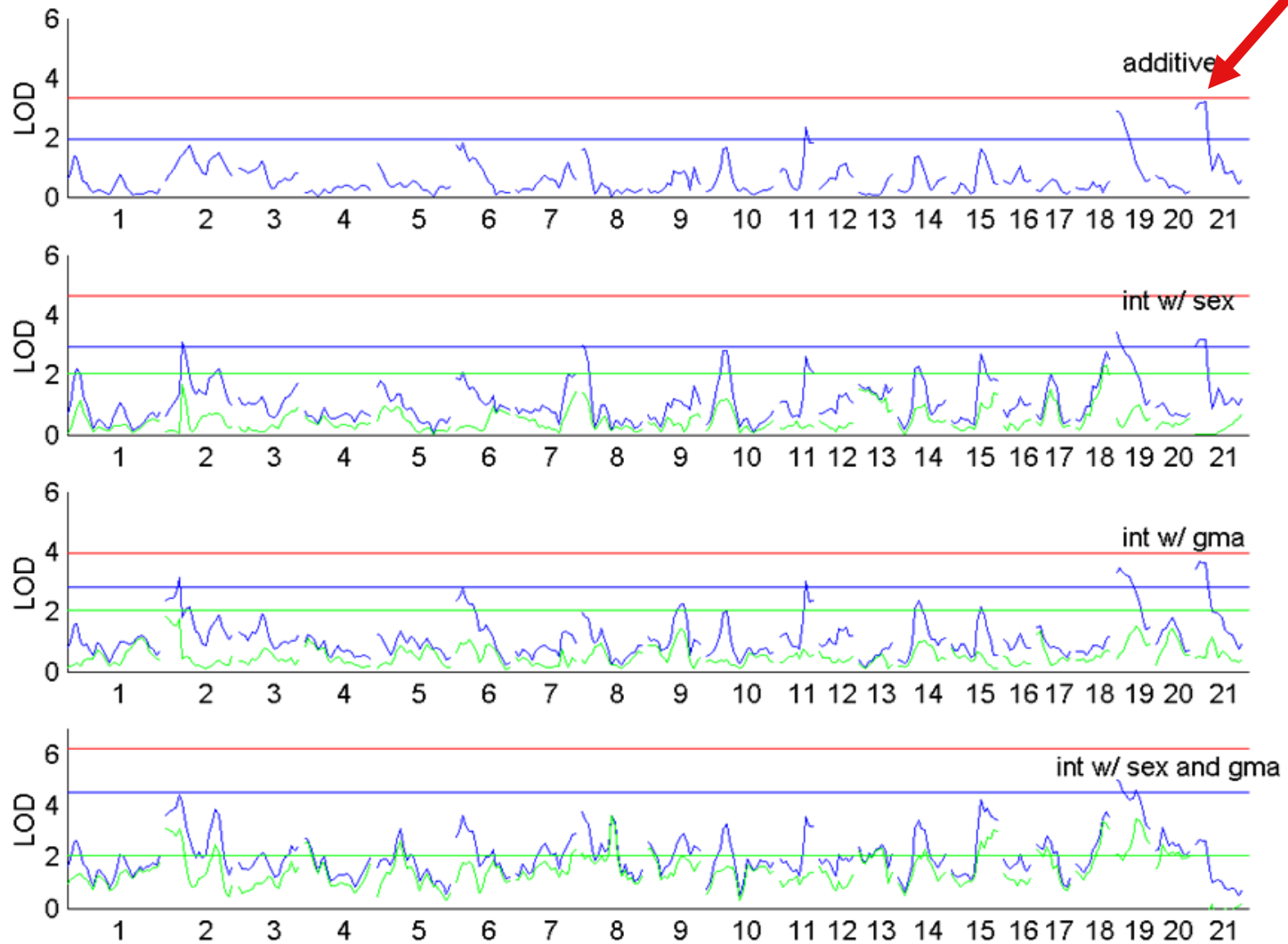
Quantitative Trait Locus Analysis

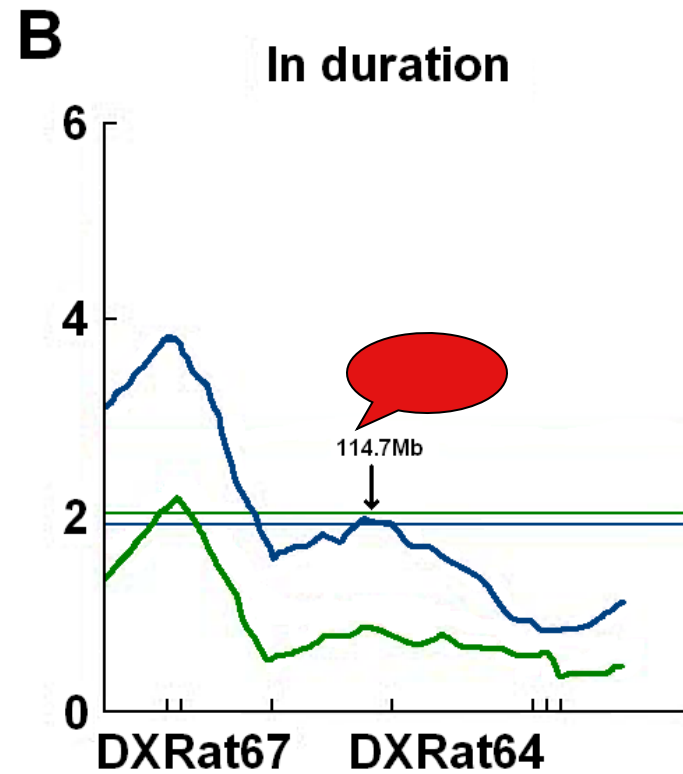
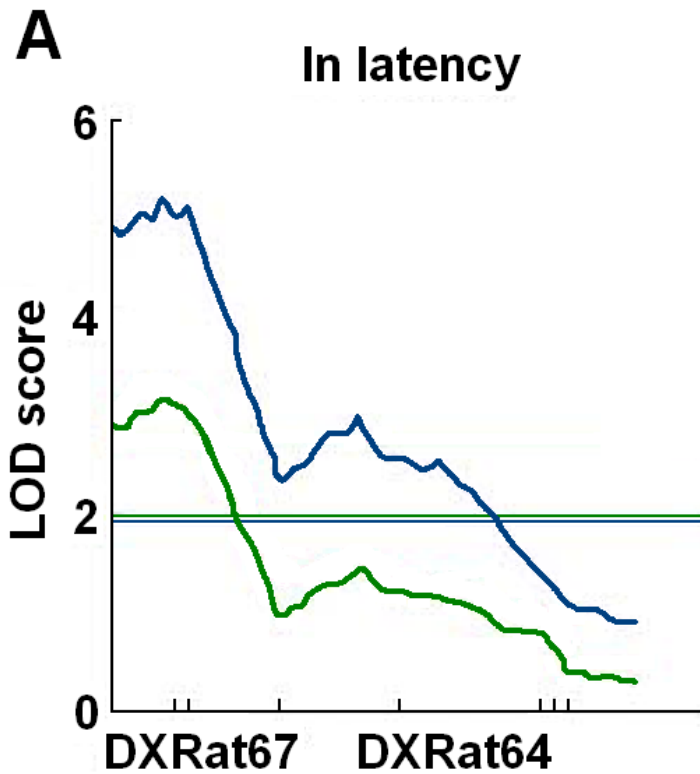


Quantitative Trait Locus Analysis



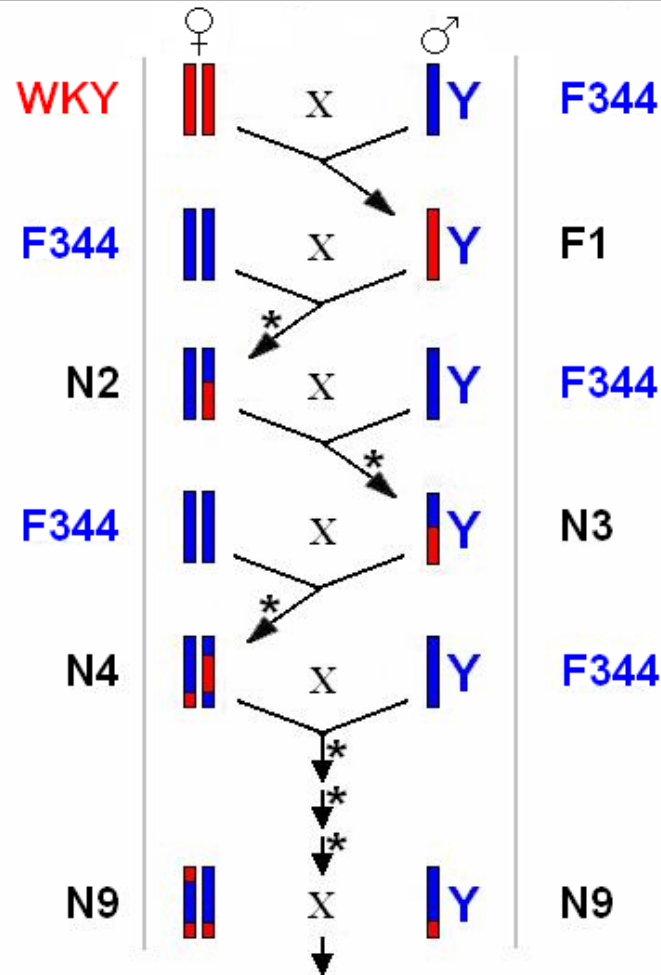
Genome scan for duration of burying





Blue: unadjusted, no covariates	Green: sex and lineage as additive covariates
(1) $y = b_0 + b_1q$	(3) $y = b_0 + b_1x + b_2q$
(2) $y = b_0$	(4) $y = b_0 + b_1x$

X-Congenetic Strain Construction

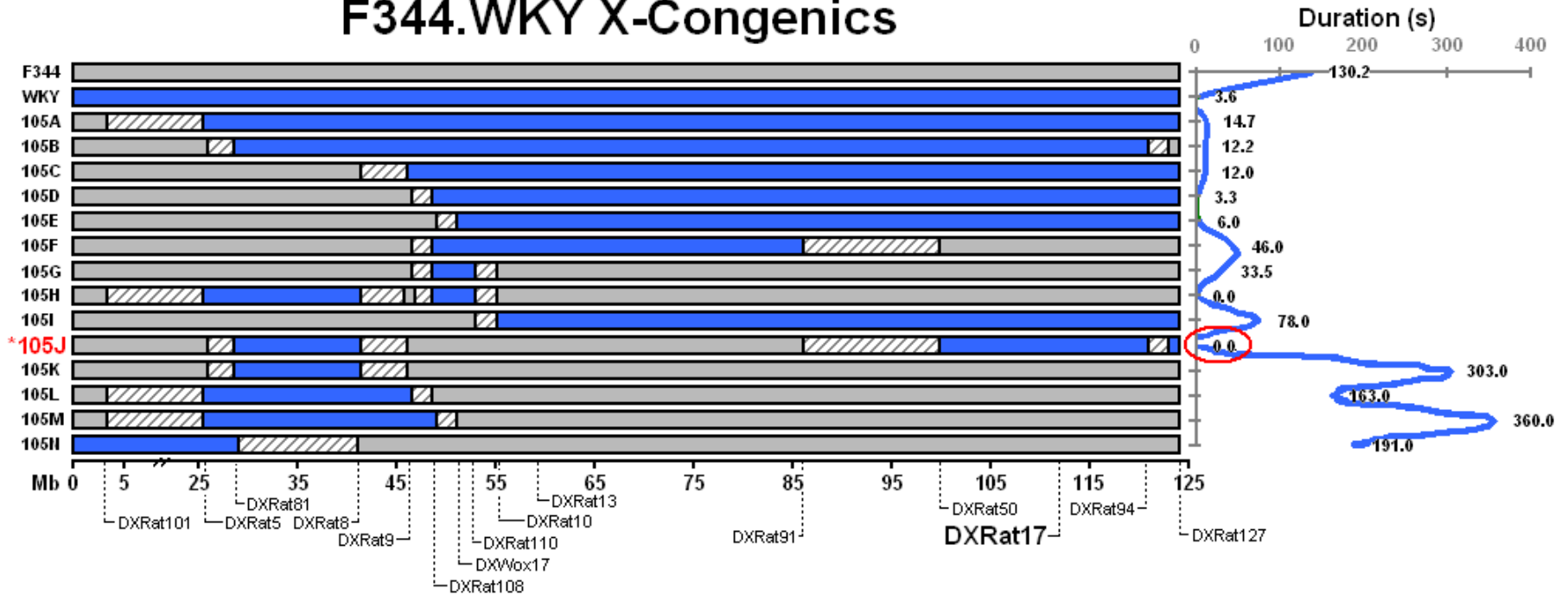


F344.WKY (DXWox30-D1Got217)Eer

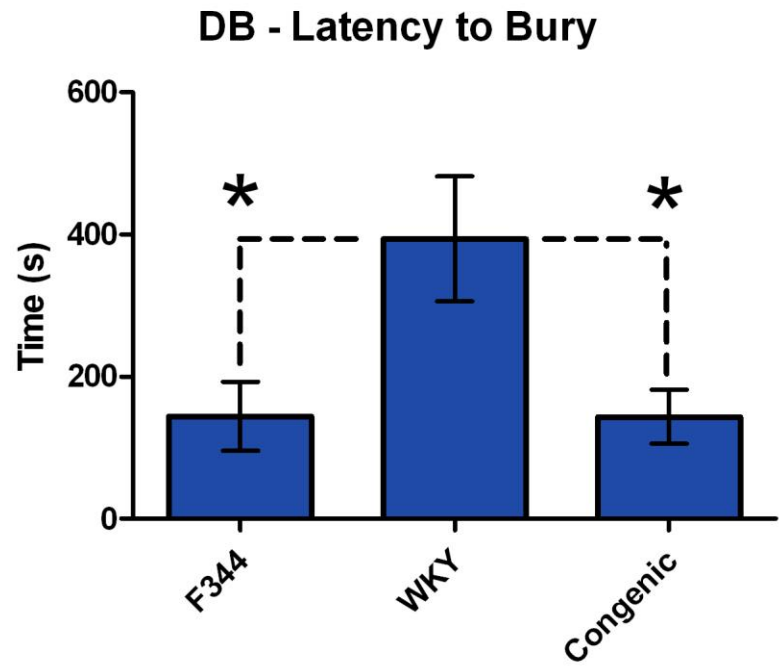
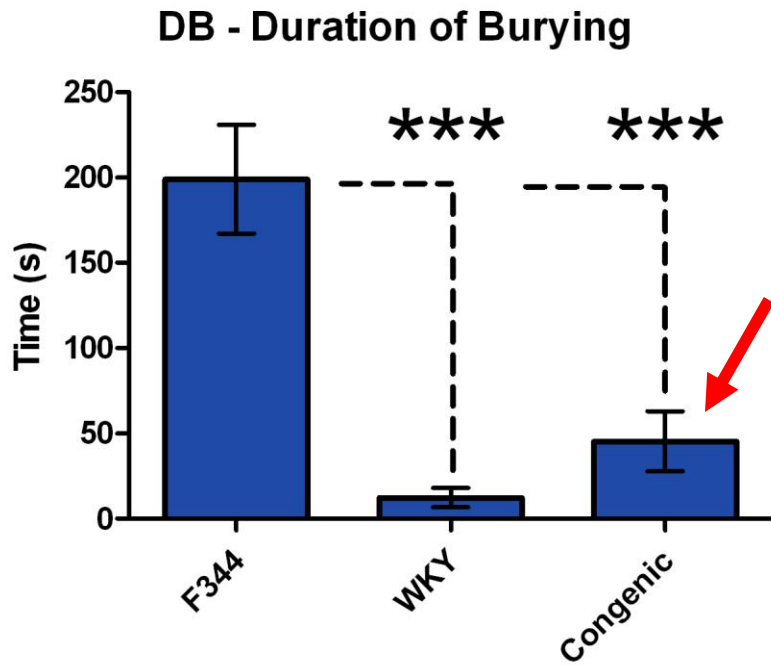
* Selected for WKY *Stresp3*-locus and F344 genotypes on autosomes

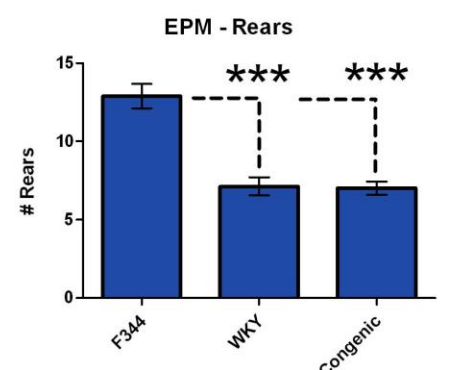
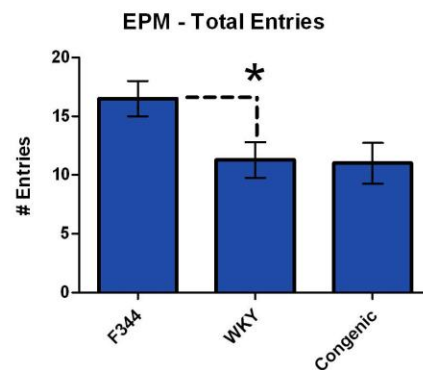
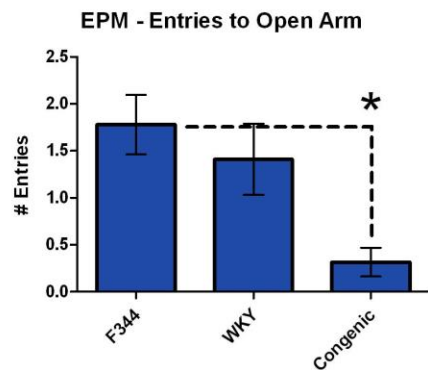
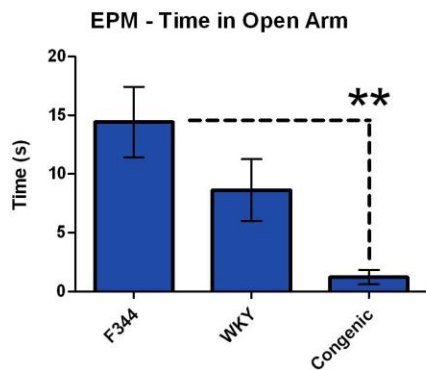
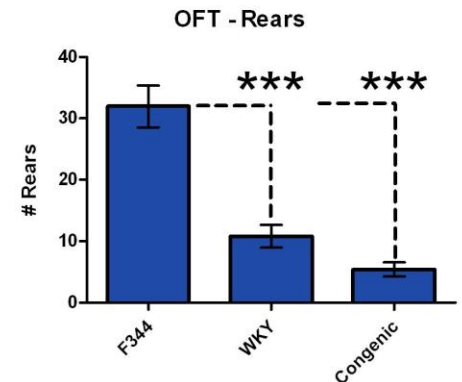
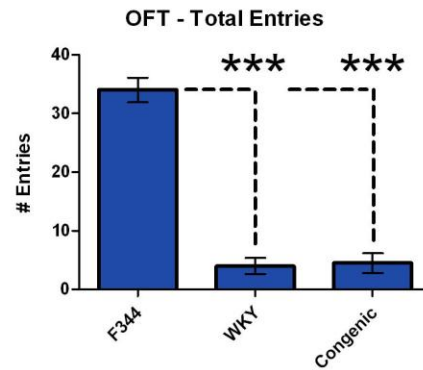
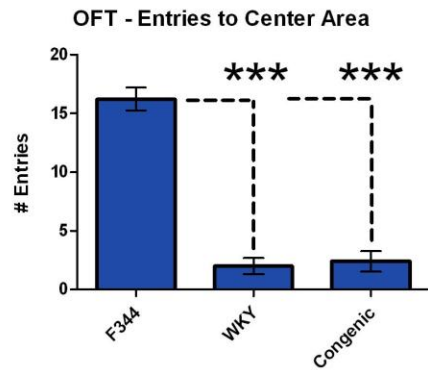
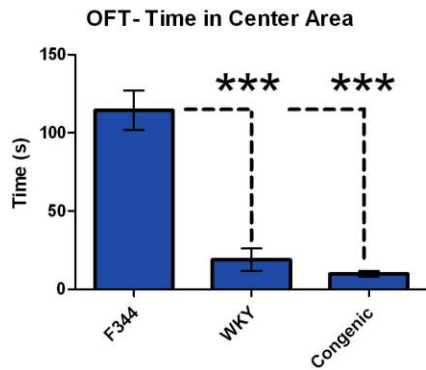
Neuroendocrinology Symposium,
Istanbul, 2009

F344.WKY X-Congenic

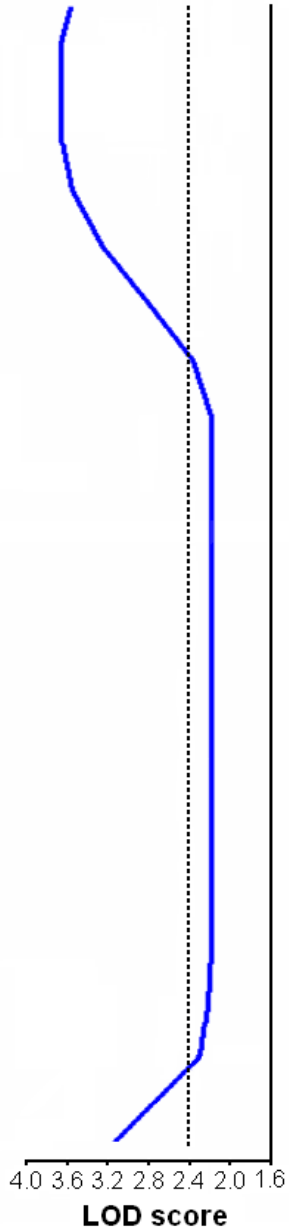


*F344.WKY-(DXRat50-DXRat96)/Eer - preliminary congenic





— Duration LOD Score
 Significance Threshold



Genotype	Marker or SNP	Location (Mb)
F344		88,514,169
?		
?		
WKY	G/T	95,074,160
WKY	A/G	95,487,735
WKY	C/T	96,012,286
WKY	C/T	96,457,403
WKY	C/T	96,722,166
WKY	A/T	98,285,593
WKY	A/C	98,998,455
WKY	DxRat50	99,773,357
WKY	A/C	100,781,233
WKY	G/T	103,022,500
WKY	C/T	104,799,115
WKY	A/T	106,125,462
WKY	A/G	106,474,065
WKY	C/T	107,012,927
WKY	C/T	107,227,193
WKY	A/G	107,956,175
WKY	C/T	108,613,039
WKY	DxRat71	109,514,212
WKY	A/G	110,819,544
WKY	G/T	111,531,390
WKY	A/G	112,109,283
WKY	DxRat17	112,204,504
WKY	C/T	112,483,874
WKY	A/G	114,036,787
WKY	A/G	115,067,688
WKY	A/G	115,467,035
WKY	G/T	116,110,562
WKY	A/T	116,944,293
WKY	DxRat94	120,688,640
WKY	DxRat96	122,069,838
?		
F344		124,590,225

Candidate Locations

← 1 - 4

← 5

← 6

← 7

← 8

← 9 - 13

← 14

Candidate	Brain Region	Fold Change
1.	Amygdala	-1.53
	Frontal Cortex	-1.42
	Hippocampus	-1.51
2.	Amygdala	-1.46
	Hippocampus	-1.56
3.	Amygdala	-1.20
	Hippocampus	-1.24
4.	Frontal Cortex	-1.19
	Hippocampus	-1.23
5.	Amygdala	-1.36
6.	Hippocampus	1.34
7.	Amygdala	-1.32
8.	Hippocampus	1.15
9.	Hippocampus	-1.22
10.	Hippocampus	-1.21
11.	Frontal Cortex	-1.16
12.	Frontal Cortex	1.11
13.	Frontal Cortex	1.12
14.	Amygdala	-1.34
	Hippocampus	-1.51

■ F344 Genotype
 □ Marker/SNP = BN ≠ F344
 ■ Marker/SNP ≠ BN ≠ F344
 ■ Region with no Markers/SNP

Conclusions:

- **QTL analysis of the reciprocal F2 intercross of WKY and F344 rat strains identified three loci on the X chromosome contributing to the variance in coping behaviors: *Stresp1*, *Stresp2* and *Stresp3*.**
- **A novel locus, at the proximity of *Stresp2*, emerged for duration of burying that is confirmed by generation of a congenic strain (X:88,514,169-124,590,225 Mb).**
- **Since hypoactivity is consistent with the decreased duration of burying phenotype, this congenic strain presents a consistent profile of inhibited or passive behavioral responses to environmental challenges.**

Conclusions (cont):

- **SNP analysis identifies regions/haplotypes that likely harbor genes responsible for these behaviors, as these regions differ from those of F344s and BN; both of which show active behaviors in these tests.**
- **Microarray results combined with the QTL analysis of the congenic strains and SNP-based haplotype analysis identified two major regions: at X:88.5-99.0Mb and at X:120.8-123.0Mb.**
- **Within these regions there are five potential QTGs that show significant expression differences between WKYs and F344s in more than one brain area.**

Outline

- ❖ Genetic contribution to individual coping strategies
 - Quantitative Trait Loci analysis
 - X-congenic strains
 - Genetic and transcriptomics: a combined analysis
- ❖ Genetic contribution to depressive behavior
 - Quantitative Trait Loci analysis
 - Genetic and transcriptomics: a combined analysis
 - Biomarkers for depression: a translational approach

Evaluation of Putative Endophenotypes for Major Depression

Table 1 Evaluation of Putative Endophenotypes for Major Depression^a

	Specificity	State-independence	Heritability	Plausibility	Total
<i>Psychopathological endophenotypes</i>					
Depressed mood (mood bias)	+	+	0	+++	6
Anhedonia (impaired reward function)	+	+	+	+++	9
Impaired learning and memory	±	+	0	+	2
Direction of appetite change	-	±	+	++	4
Diurnal variation	++	+	+	++	6
Exec. cogn. function (response speed)	+	+	++	+	5
Psychomotor change	-	-	0	+	1
Increased stress sensitivity	-	+	++	+++	9
<i>Biological endophenotypes</i>					
REM sleep abnormalities	±	+	+	++	7
Increased amygdala activity	++	+	0	+++	6
Decreased subgenual PFC activity	+	+	0	+++	5
Left ACC volume reduction	+	++	0	++	7
Hippocampal volume reduction	-	++	+	++	6
Reduced 5-HT _{1A} receptor BP	+	++	+	+++	7
Tryptophan depletion	+++	+++	++	+++	14
Catecholamine depletion	+	+++	0	+++	7
dexCRH test	±	++	++	+++	11
CRH dysfunction	+	+	+	+++	6

Animal Models of Depression

Stress Models

- Stress induced
 - Chronic Mild Stress
 - Social Defeat
 - Prenatal Stress
 - Maternal Separation
- Tests for despair/depressive behavior
 - Forced Swim Test
 - Tail Suspension
 - Learned Helplessness

Genetic Models

- Selectively Bred
 - Maudsley reactive
 - Roman high and low avoidance
 - Flinders Sensitive Line
 - High swim/Low swim
 - **Wistar Kyoto More/Less Immobile**
- Transgenic models
 - Single candidate gene-background!!
- Inherent behavior
 - **Wistar Kyoto (WKY)**

Criteria for a Major Depressive Episode:

(at least 5 symptoms present in a 2-week period)

- Depressed mood
- Diminished interest or pleasure in activities
- Significant weight loss or gain
- Insomnia or hypersomnia
- Psychomotor agitation or retardation
- Fatigue or loss of energy/despair
- Diminished ability to concentrate
- Recurrent thoughts of death

Modeled
in animals

Modeled
in WKY

No

Yes

Yes

Yes

Yes

Yes

Yes

No

Yes

Yes

Yes

Yes

Yes

?

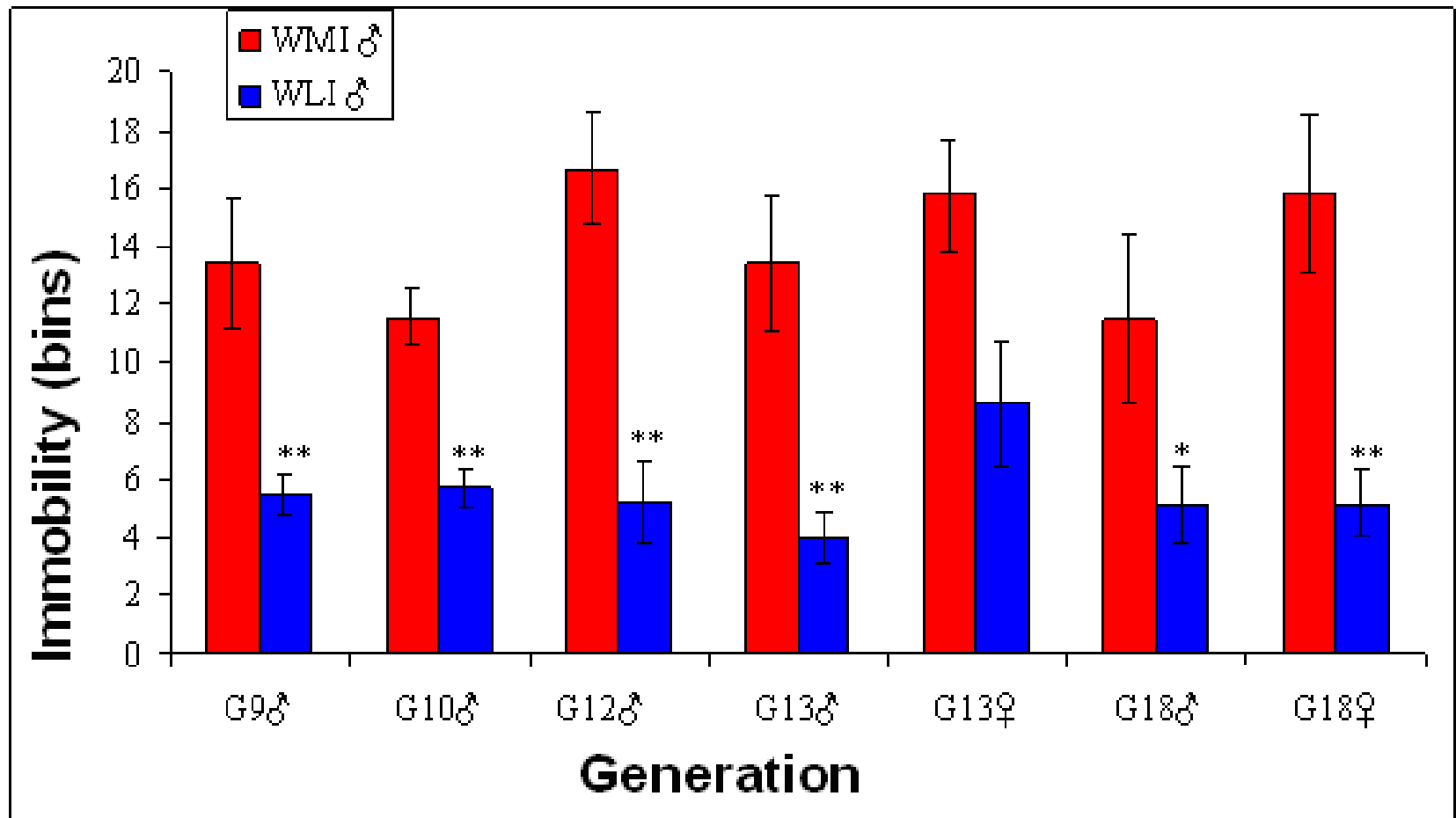
WKY as an animal model of depression

- Exhibits behaviors analogous to those seen in depressed patients
- Depressive-like behavior is reversed after treatment with antidepressants (TCAs and MAOIs)
- Exhibits neuroendocrine and sleep changes similar to those seen in depressed patients

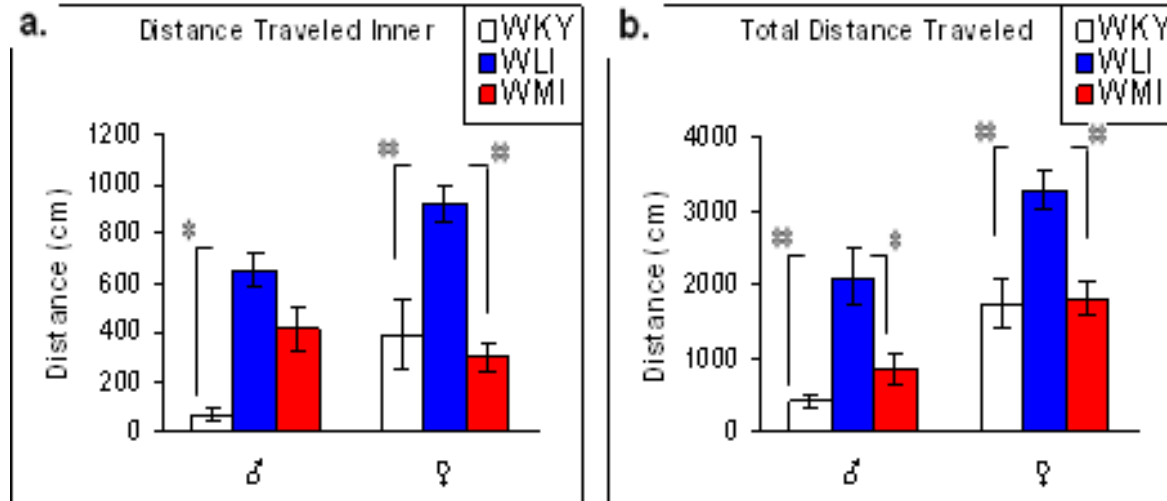
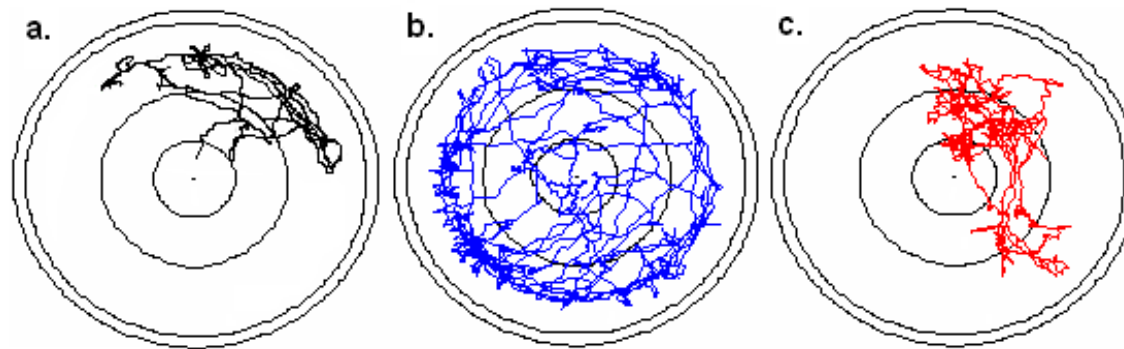
Affimetrix microarray studies comparing gene expressions in:

1. amygdala, hippocampus, frontal cortex and striatum of **male and female WKY and Fisher 344**, WKY more immobile (**WMI**) and WKY less immobile (**WLI**), chronically treated with vehicle or desipramine. (192 arrays)
2. in peripheral blood of the same animals

Selectively bred Wistar Kyoto substrains



Behavior of WMI and WLI substrains in the open field test



Strategy to identify biological markers for depression: a translational approach

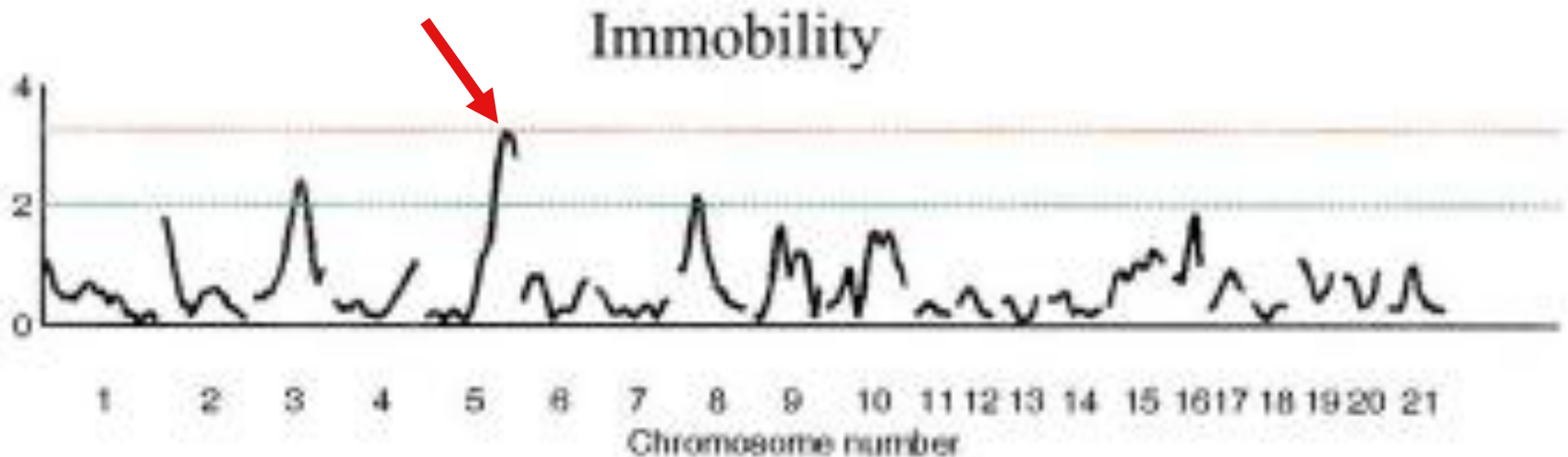
Genetic Markers

1. QTL of F2 WKY x F344 analyses identify locus
2. SNP analysis of parental strains compared to reference strain BN narrow regions
3. Selectively bred sub-strains
4. Microarray analyses of relevant brain regions between QTL strains and selectively-bred strains identify differentially expressed genes within these narrow regions
5. Differentially expressed genes mapped to syntenic region with human loci identified by GWA studies are candidates

Expression Markers

1. Selectively bred sub-strains
2. Microarray analyses of relevant brain regions AND BLOOD between selectively-bred strains identify genes differentially expressed in both BRAIN AND BLOOD
3. Validate candidate biomarkers in blood samples of depressed patients and controls

***Despr3* locus syntenic to human depression and neuroticism locus at Chr 1:24,207,596 -42,694,514bp**



Identification of potential QTGs

SNP analysis of *Despr3* locus

	Chr	bp	WKY	F344
G/T	5	1407384 59	0	2
C/T	5	1411734 87	2	0
A/G	5	1425019 22	2	0
C/G	5	1425592 51	0	2
C/T	5	1432281 63	0	2
A/C	5	1434718 54	0	2
C/T	5	1436335 65	0	2
C/T	5	1449941 77	2	0
A/G	5	1450319 80	0	2
A/G	5	1475696 68	0	2
C/T	5	1477185 86	2	0
A/G	5	1501987 28	0	2
C/T	5	1503522 36	2	0
C/T	5	1505112 34	2	0
A/T	5	1509849 43	0	2
C/T	5	1517123 42	0	2
A/G	5	1528404 41	0	2

0=same as BN 2=differs from F344 and BN

Candidate genetic biomarkers at Depr3 locus between WMI and WLI

Gene Title	Gene Symbol	Rat Chr5		Human Chr 1	
		Start (p)	Stop (bp)	Start (bp)	Stop (bp)
Stromal membrane-associated protein 1-like	Smap11	14154485	14159088	40612315	40661581
Mitochondrial ribosomal protein S15	Mrps15	14539772	14540885	36702556	36693960
Similar to RIKEN cDNA 1810007P10 Organic solute transporter protein 1	RGD1306596/OS1	14541086	14544142	36656095	3668869
Similar to 2610027C13ik protein	RGD1308876/ Fam176b	14550283	14550424	36562342	3656029
Collagen alpha-2(VIII) chain precursor	Col8a2	14567981	14568285	36333433	3633843
serine incorporator 2	Serinc2	14927936	14930185	31658550	3168012
pumilio 1 (Drosophila)***	Pum1	14951267	14953080	31311151	3117690
Hypothetical protein LOC688757 transmembrane protein 200B	LOC688757/ Tmem200b	15077798	15078038	29318526	2932308

*** all three brain regions highlighted genes are primary candidates

Candidate genes at Despr3 locus between WKY and F344

Gene Title	Gene Symbol	Rat Chr 5		Human Chr 1		
		Start (bp)	Stop (bp)	Start (bp)	Stop (bp)	
Similar to schlafen-like 1	LOC500540/ Slfn1	1411292	981411337	69	41176358	41620940
similar to RIKEN cDNA 3110037I16/ chromosome 1 open reading frame 176	RGD1309802/ C1orf176	1415076	831415091	54	40747000	40754799
stromal membrane-associated protein 1-like	Smap11	141544545	141590968		40612315	40661581
CAP, adenylate cyclase-associated protein 1 (yeast)	Cap1	1421744	411422011	20	40278842	40310908
hippocalcin-like 4	Hpcal4	1424904	621424983	59	39929676	39917232
RGD1559909/ chromosome 1 open reading frame 122	RGD1559909/C1orf122	1441808	891441834	89	38046556	38047713
mitochondrial ribosomal protein S15	Mrips15	14539777	145408365		36702556	36693960
Collagen alpha-2(VIII) chain precursor	Col8a2	145679681	145682325		36333433	36338437
ADP-ribosylhydrolase like 2	Adprhl2	1456854	951456911	73	36327073	36332120
tyrosyl-tRNA synthetase	Yars	1483503	631483789	89	33005620	33013427
serine incorporator 2	Serinc2	149279136	149301805		31658550	31680112
syndecan 3	Sdc3	1494879	381495189	17	31154067	31118567
Hypothetical protein LOC688757/transmembrane protein 200B	LOC688757/Tmem200b	150777398	150780378		29318526	29323008
YTH domain family 2	Ythdf2	1510805	991510893	93	28935723	28968874

Strategy to identify biological markers for depression: a translational approach

Genetic Markers

1. QTL of F2 WKY x F344 analyses identify locus
2. SNP analysis of parental strains compared to reference strain BN narrow regions
3. Selectively bred sub-strains
4. Microarray analyses of relevant brain regions between QTL strains and selectively-bred strains identify differentially expressed genes within these narrow regions
5. Differentially expressed genes mapped to syntenic region with human loci identified by GWA studies are candidates

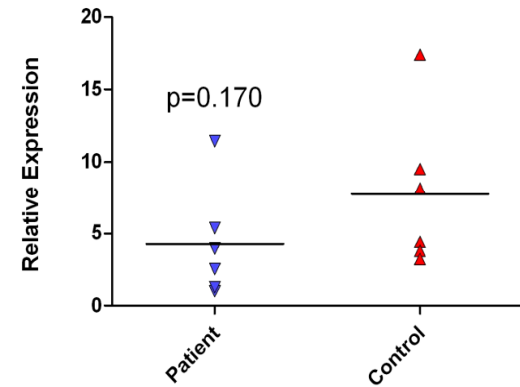
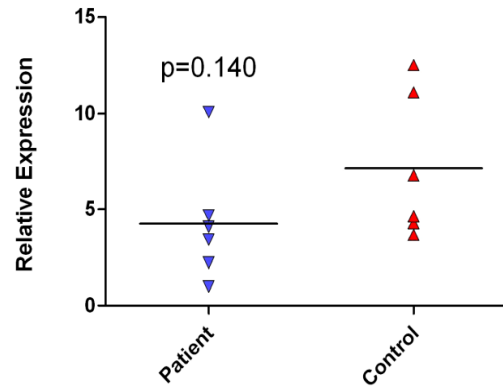
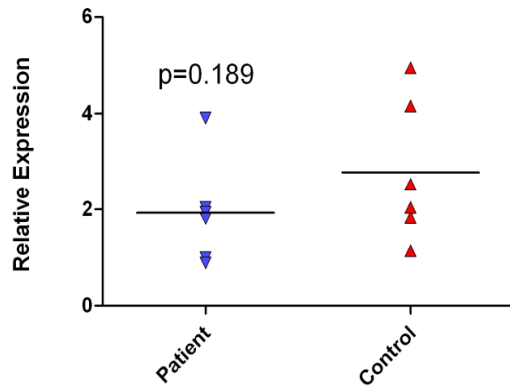
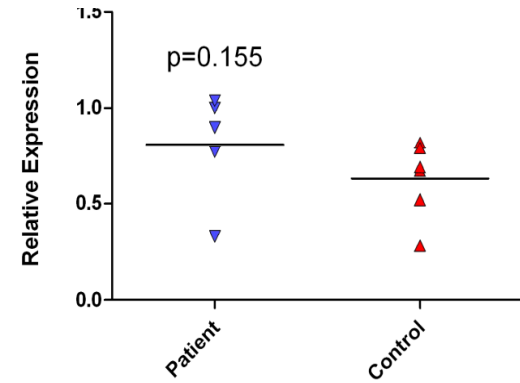
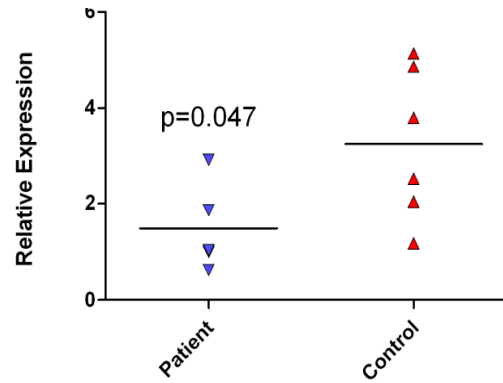
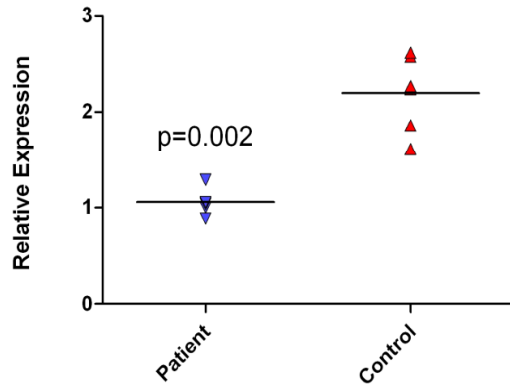
Expression Markers

1. Selectively bred sub-strains
2. Microarray analyses of relevant brain regions AND BLOOD between selectively-bred strains identify genes differentially expressed in both BRAIN AND BLOOD
3. Validate candidate biomarkers in blood samples of depressed patients and controls

Candidate genes from expression profiling

- **155** genes whose expression was greater in WLI blood compared to WMI blood
- **61** genes whose expression was greater in WMI blood compared to WLI blood.
- **101** genes differed in both brain and blood between the two sub-strains.
- **25** genes showed the greatest difference in both brain regions and blood: these genes are our primary candidates for biomarkers

Biomarker candidates for human depression



Summary

- Identified novel genetic animal models of passive coping and depression
- Passive coping may underlie many mood disorders
- The combination of genetic and transcriptomic analyses provide candidate genes that contribute to these endophenotypes
- Preliminary evidence indicate that blood biological markers can be identified based on the animal model data.

Collaborators

- **Northwestern**

(past and present lab members)

Claire Will

Nasim Ahmadiyeh

Leah Solberg Wood

Amber Baum

Kelsey Budd

Fraser Aird

Kristen Debus

Brian Andrus

Daniel Schaffer

Laura Sittig

Pradeep K. Shukla

Joe Takahashi

Kazuhiro Shimomura

David Mohr

Joyce Ho

- **Jackson Lab**

Gary Churchill

Peter Vedell

Renhua Li

Liam O'Brian

Qian Li

- **Free University
Berlin**

Kathryn Staar