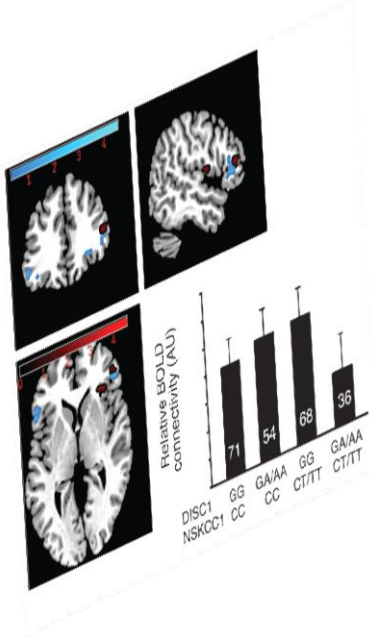
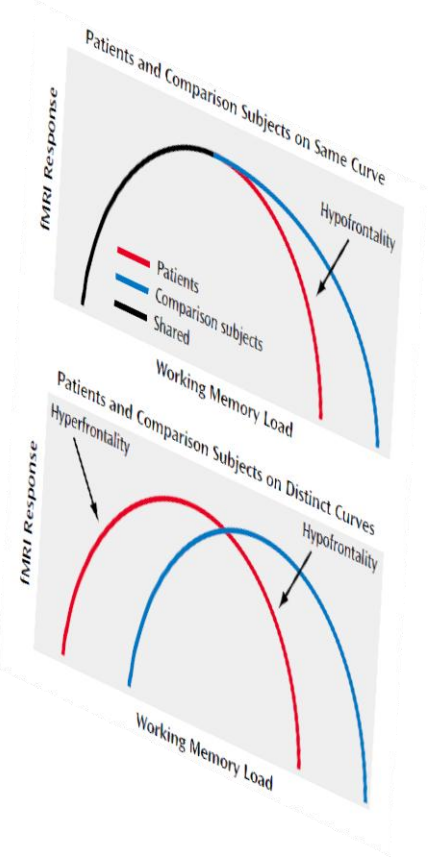


fMRI and Genetics

Joseph Callicott, MD
CBDB, NIMH
August 2, 2013
Summer FMRI Course at the NIH

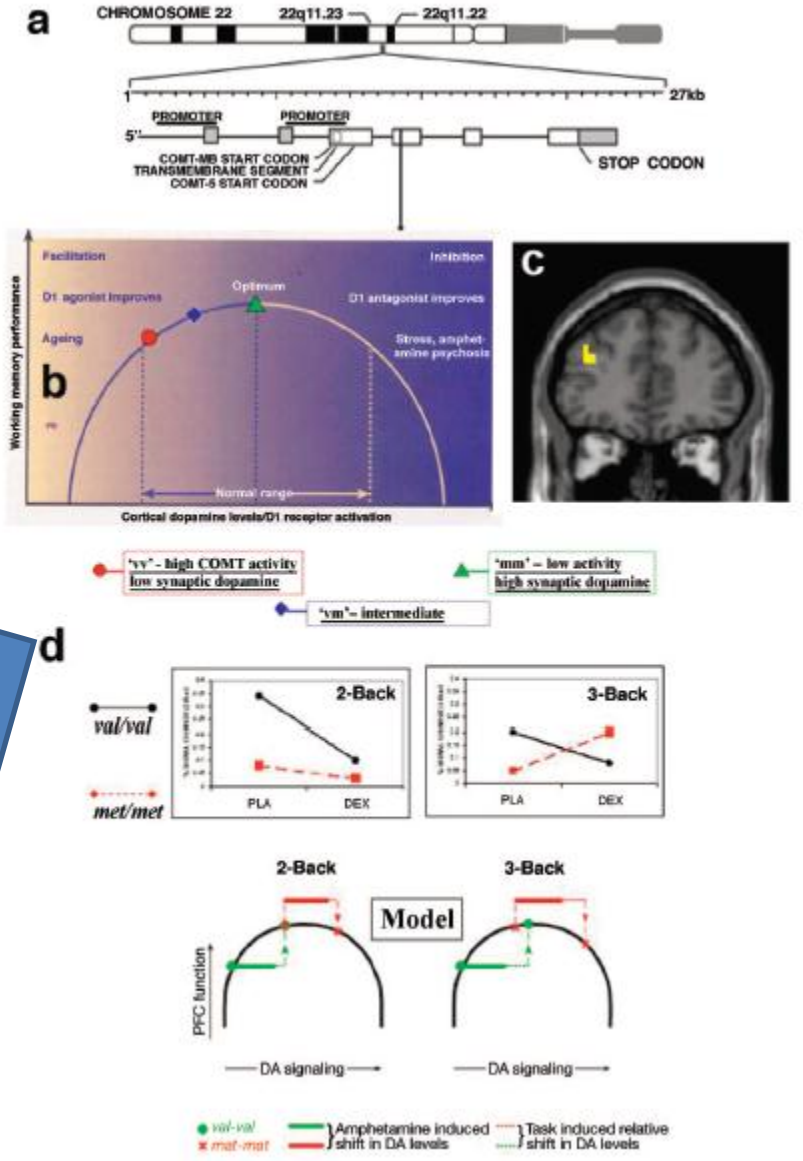


Simple plan, high impact

- Have an fMRI task in a relatively large sample?
  - Healthy controls preferable
  - N > 40
- Draw blood or swab cheek
- Genotyping at most resolutions fast & cheap*
- In SPM, ANCOVA or regression

* Collaborations with our lab already have whole genome genotypes > 1000 local controls

COMT led from primate to human imaging, novel cognitive enhancement



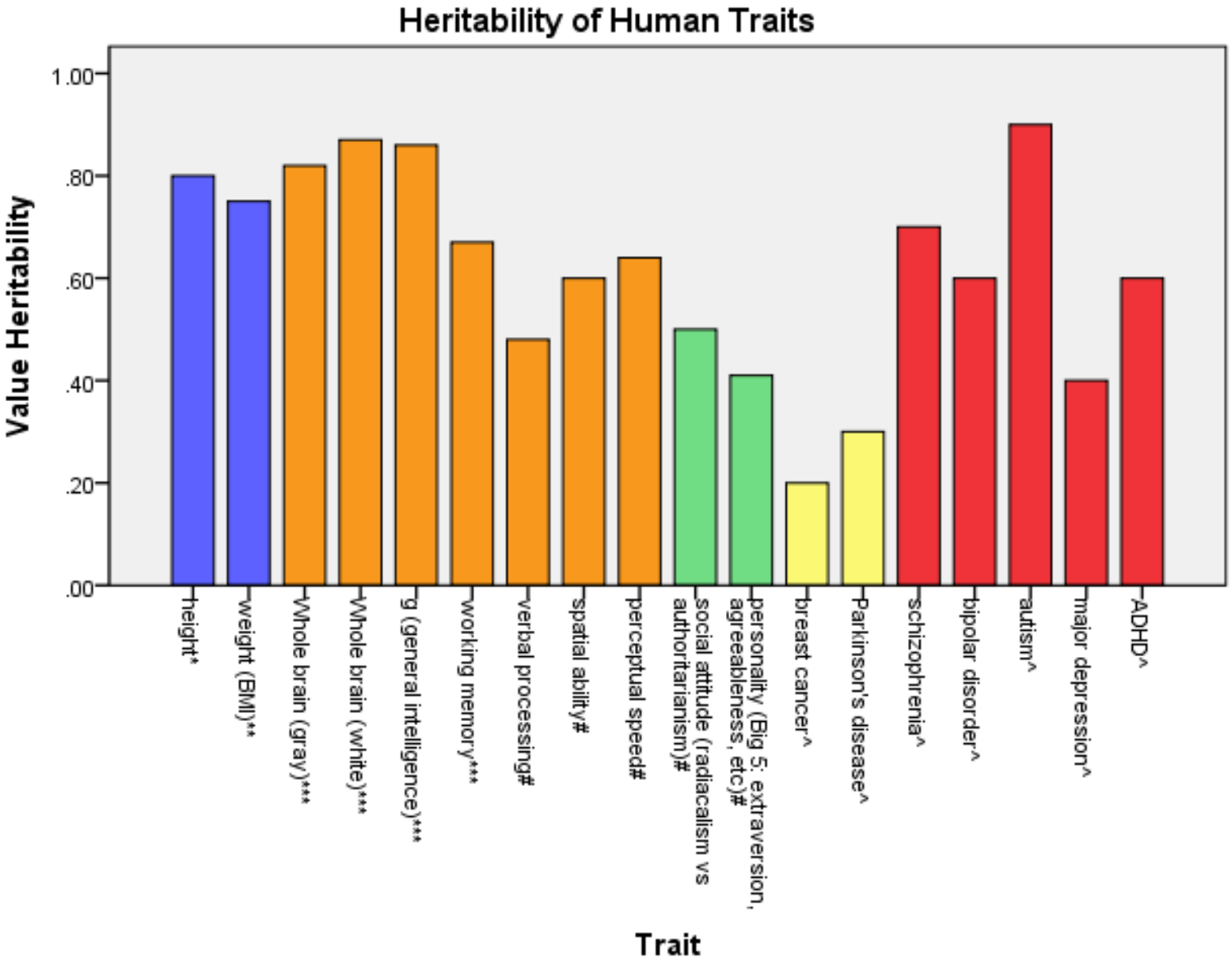
Why Imaging Genetics?

- Few routes to neural mechanism using in vivo human data
  - Animal model
  - Drug study
  - BOLD fMRI (MRSI, MEG, EEG)
- Genes do not code for mental illness, per se
  - Genes code for heritable aspects of brain function, intermediate- or endo-phenotypes
  - Genetic risk for illnesses like schizophrenia is polygenic, heterogeneous
  - Gene interact with each other and the environment

- BOLD fMRI, as an alternate metric of specific or general cognitive systems, offers 'real world validation'
  - Putative genetic mutations (including private mutations (CNVs)
    - In spite of growing sample sizes, association studies risk false positives
  - RDoC domains and constructs
    - If these do not correspond to brain systems we can map, then may be as doomed as DSM

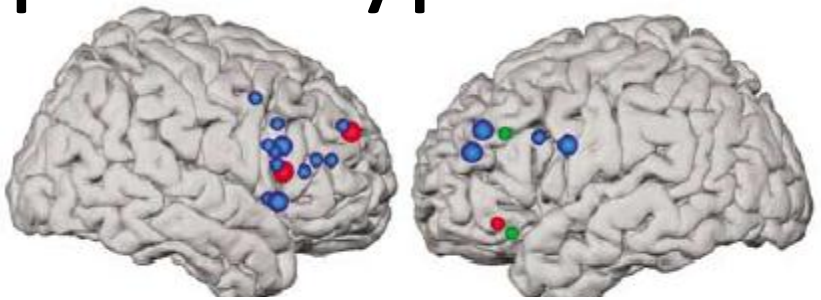
Imagers make the best geneticists

- Both methods based on recent technologies
  - High field magnets vs. sequencing chips
- Both highly computerized and utilize open-source code
  - SPM (fMRI) vs. PLINK (genetic association)
  - Active user communities
- Statistical issues similar
  - Sampling size
    - Whole brain vs. whole genome
  - ‘Resolution’
    - Voxel size vs. tag SNPs
  - ‘Smoothing’
    - FWHM vs. LD
  - Multiple comparisons problem
    - T test vs. Chi square
    - Correction (both based on modified Bonferroni)
      - Whole brain vs. GWAS ($p < 1x10^{-8}$)
      - ROI vs. candidate gene
      - FWE/FDR both



(*Visscher et al., 2010; Nan et al. 2012**; Postuma et al. 2002***; McGue & Bouchard, 1998#;^Burmeister et al., 2008)

BOLD: powerful phenotypes



Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bertolino; 2006a	0,922952	0,286766	0,082235	0,360901	1,485002	3,218487	0,001289
Bertolino; 2006b	0,886313	0,403607	0,162899	0,095257	1,677369	2,195979	0,028093
Bertolino; Retrieval; 2006c	1,570141	0,519027	0,269389	0,552666	2,587416	3,025161	0,002485
Bertolino; Encoding; 2006c	1,372262	0,495105	0,245129	0,401874	2,342651	2,771657	0,005577
Bertolino; NBack, 2008	0,756444	0,257418	0,066264	0,251914	1,260973	2,938584	0,003297
Bertolino; Encoding; 2008	0,437292	0,230333	0,053053	-0,014153	0,888737	1,898518	0,057628
Bishop; 2006	4,279867	1,493896	2,231726	1,351884	7,207849	2,864902	0,004171
Caldu; 2007	0,619117	0,246737	0,060879	0,135521	1,102713	2,509216	0,012100
Domschke; 2008	1,020774	0,495812	0,245830	0,049000	1,992548	2,058791	0,039514
Drabant; 2006	-0,992655	0,320724	0,102864	-1,621263	-0,364048	-3,095044	0,001968
Egan; 2001	2,389708	0,827971	0,685535	0,766916	4,012501	2,886224	0,003899
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Gothelf; 2007	0,129659	0,556929	0,310170	-0,961903	1,221220	0,232810	0,815909
Mattay; 2003	2,905350	0,747761	0,559146	1,439766	4,370935	3,885400	0,000102
Meyer-Lindenberg; 2006	0,796669	0,194114	0,037680	0,416212	1,177126	4,104126	0,000041
Schott; Retrieval; 2006	2,028647	0,435653	0,189793	1,174783	2,882511	4,656567	0,000003
Schott; Encoding; 2006	1,545247	0,403484	0,162799	0,754433	2,336062	3,829761	0,000128
Smolka; 2005;	-1,649081	0,458239	0,209983	-2,547214	-0,750948	-3,598732	0,000320
Tan; Retrieval; 2007	0,345094	0,442885	0,196147	-0,522945	1,213132	0,779195	0,435865
Tan; Encoding; 2007	1,494534	0,544830	0,296840	0,426687	2,562380	2,743120	0,006086
Tan; 2007	1,377671	0,414906	0,172147	0,564471	2,190872	3,320444	0,000899
Williams-Gray; 2007	0,735051	0,371324	0,137881	0,007270	1,462831	1,979542	0,047755
Williams-Gray; 2008	1,222767	0,406450	0,165202	0,426140	2,019395	3,008407	0,002626
Yacubian; 2007	-1,640767	0,345251	0,119198	-2,317446	-0,964088	-4,752392	0,000002
	0,734802	0,208750	0,043577	0,325659	1,143944	3,520007	0,000432

Statistics for each study

Std diff in means and 95% CI

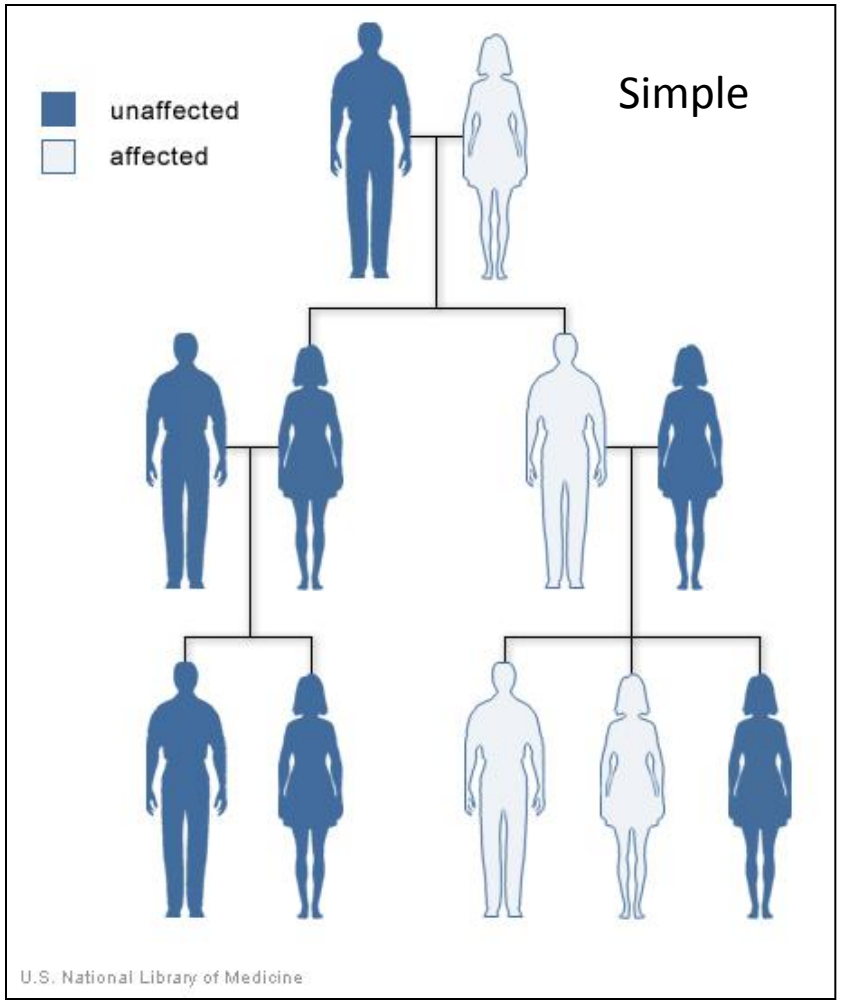
-4,00 0,00 4,00 8,00

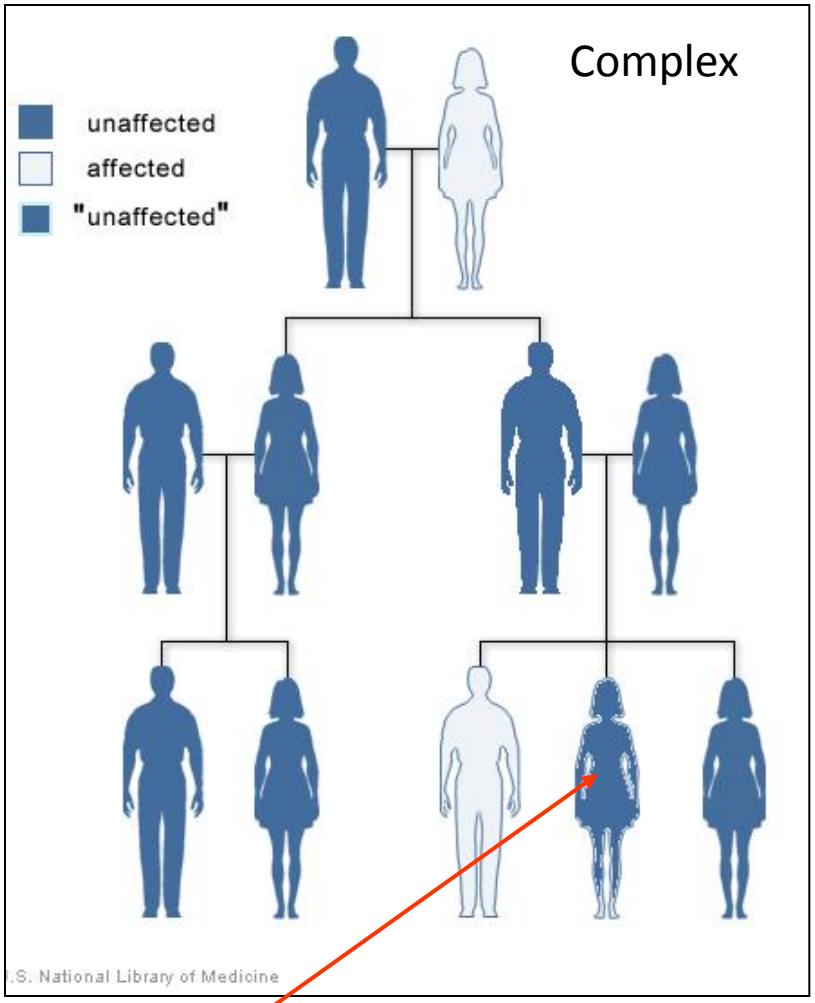
Met > Val Val > Met

Meta Analysis

Figure 2 Meta-analysis (random effects) of the functional imaging studies examining the association of the COMT polymorphism with prefrontal activation. Central box reflects the effect size of the study; box size is proportional to the sample size, and bars indicate the 95% CI. In the last row, the mean effect size of all studies is shown.

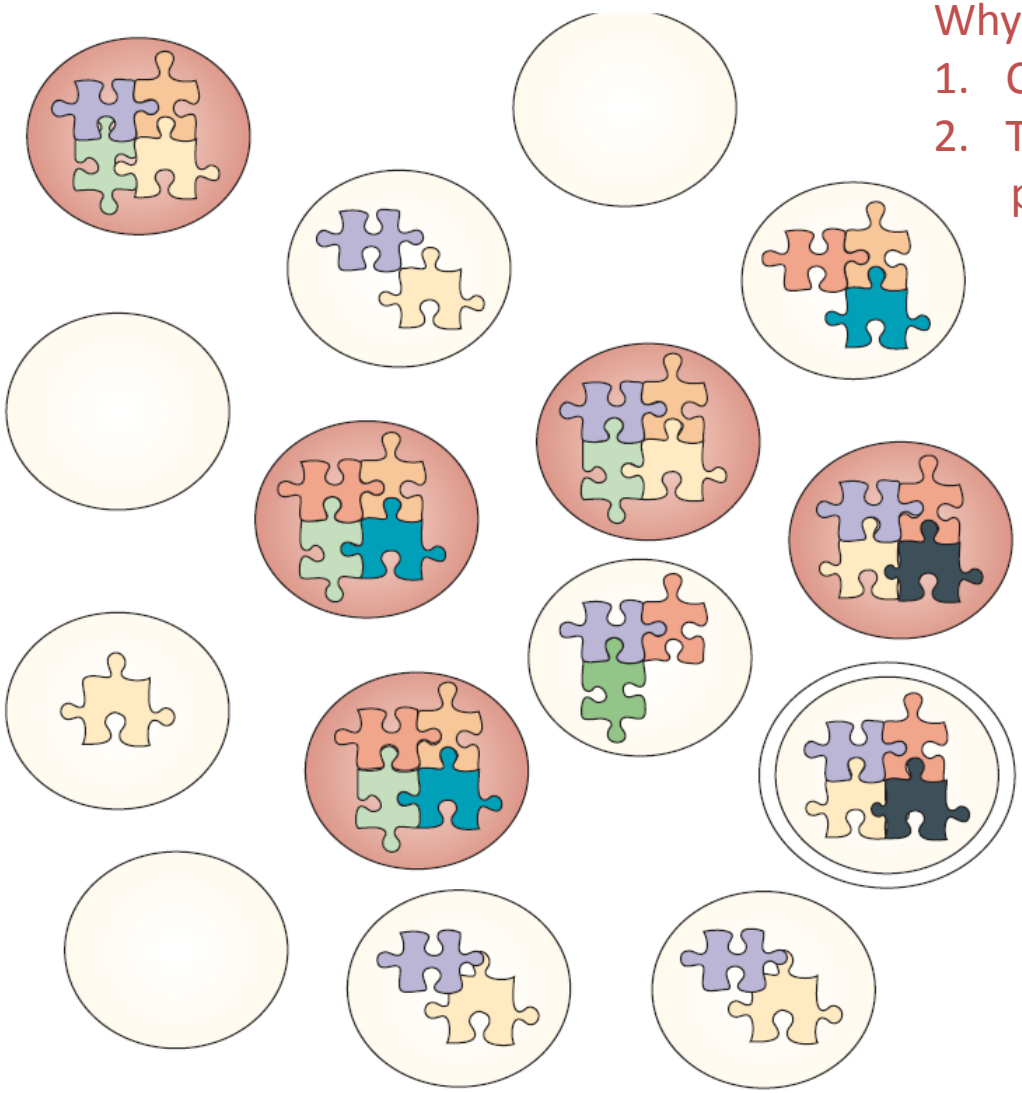
Genetics = the study of how human diseases are passed by genes through families





Clinically unaffected, but shares ~ ½ risk genes

Finding these genes is difficult for most inherited diseases….



Why?

1. Caused by many (100-1000s) of genes
2. The effects of a mutation vary between people

affected person

Has all the genes
(note this doesn't mean the exact same set)

unaffected

May still carry some genes
(like a parent of a sick person)

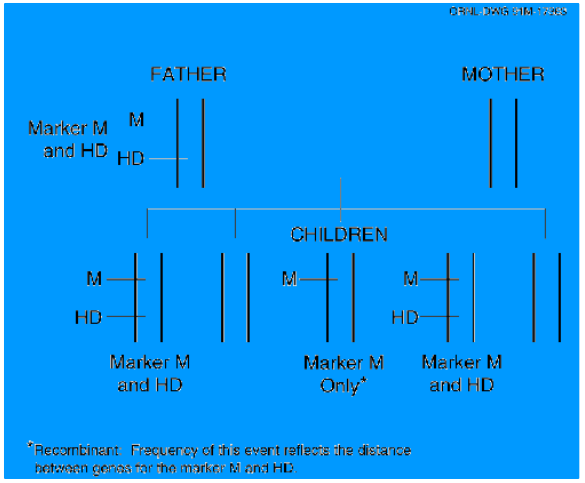
"nonpenetrant"

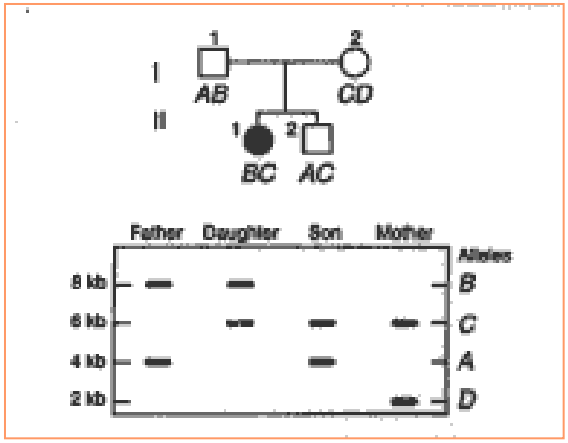
Has all of the genes but is NOT sick for reasons we can't explain

(Goldman et al. Nat Rev Genet 2006)

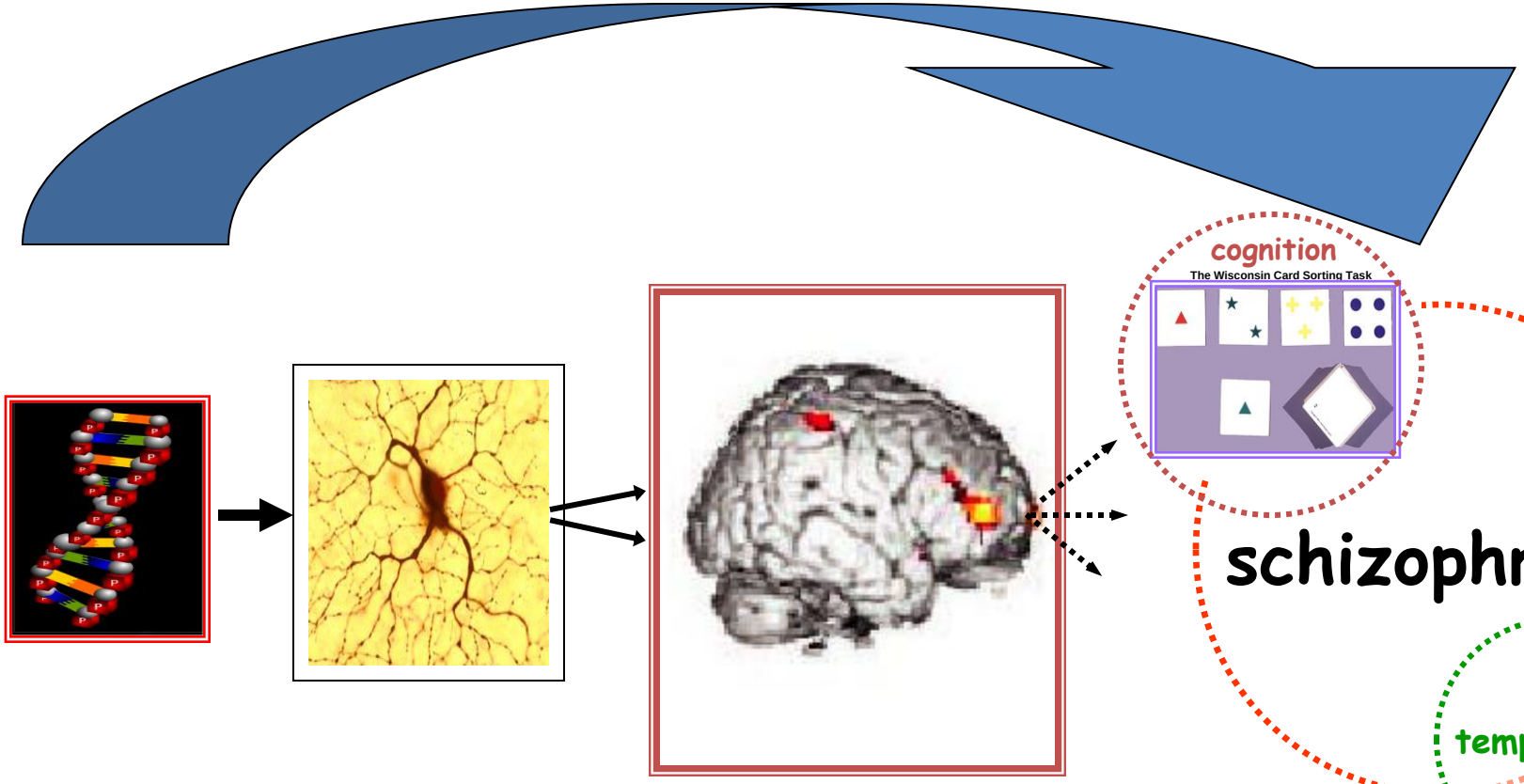
Techniques for finding genes

- Linkage
  - In large families, searching for markers (signposts) transmitted to sick children
- Association
  - In families, searching for links between mutations in suspected genes and sick children
- Intermediate phenotypes
  - short cut





The intermediate phenotype (half way there)



Genes:

multiple susceptibility alleles each of small effect

Cells:

subtle molecular abnormalities

Systems:

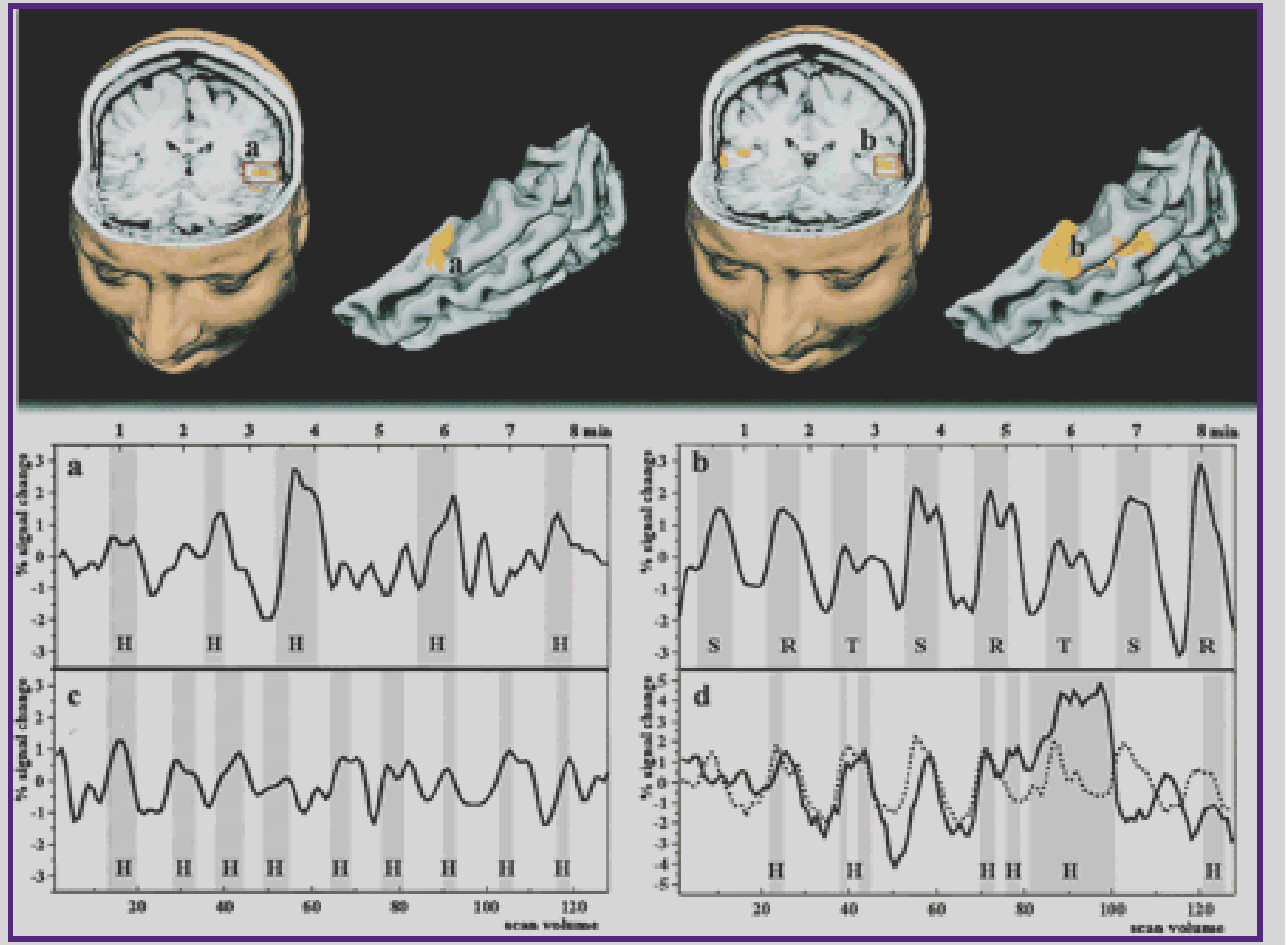
abnormal information processing

Behavior:

complex functional interactions and emergent phenomena

(After: Callicott and Weinberger, Mol Methods Med 2003)

Imaging genetics would be easy if we could take a picture of the disease, and not just the intermediates



fMRI study that showed parts of the brain active when someone was hearing voices

(Dierks et al., Neuron, 1999)

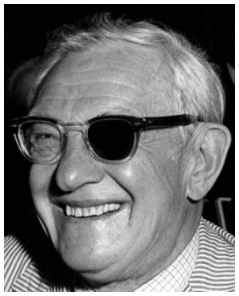
Catechol O-methyltransferase (COMT): NIMH Intramural Success Story

Enzymatic O-Methylation of Epinephrine and Other Catechols*

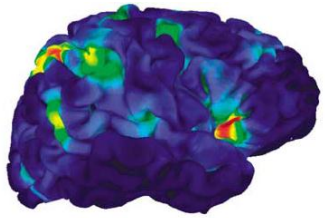
JULIUS AXELROD AND ROBERT TOMCHICK

From the National Institute of Mental Health, United States Public Health Service, Bethesda, Maryland

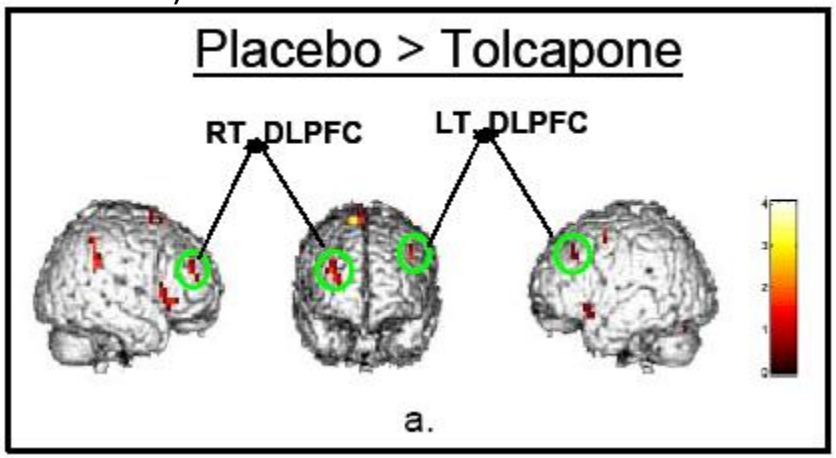
(Received for publication, February 24, 1958)



(Egan et al. 2001)

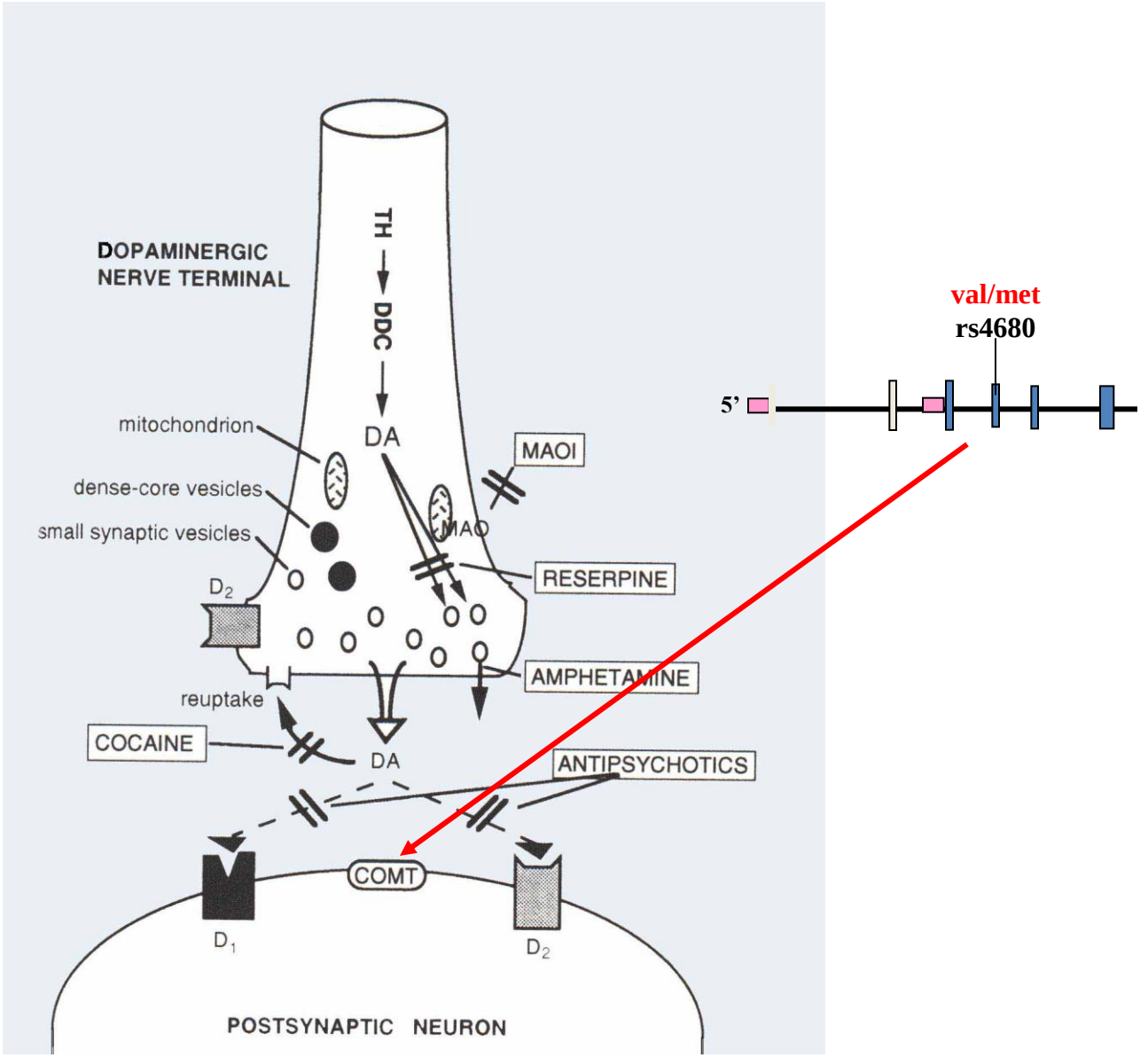


(Meyer-Lindenberg et al. 2006)



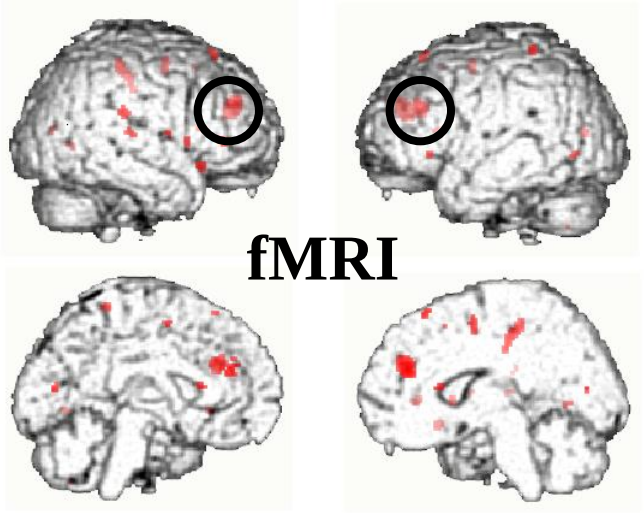
(Apud et al. 2006)

Dopaminergic Nerve Terminal



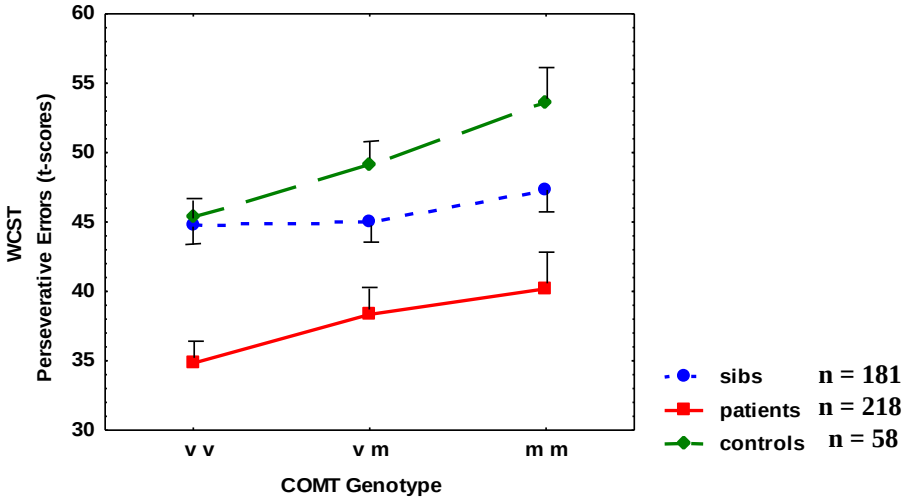
Effect of COMT genotype on frontal lobe function

Physiological efficiency



***vv>vm>mm*, SPM 99, p<.005**

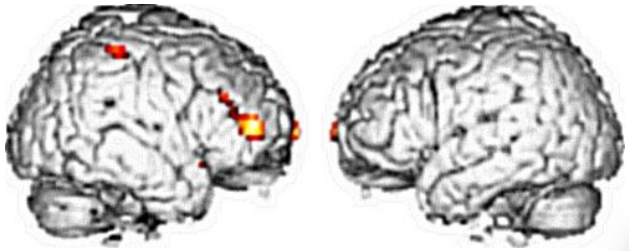
Executive cognition



genotype effect F=5.41, df= 2, 449; p<.005.

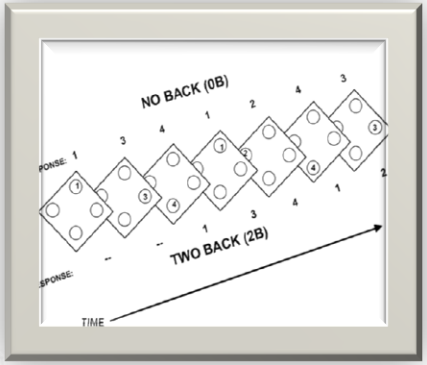
Egan et al *PNAS* 2001

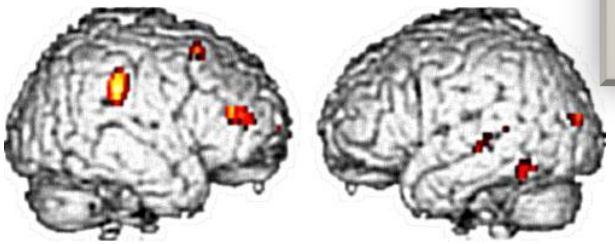
Interest in imaging genetics predicated on heritability of phenotypes…



Patients > Controls
(N=13) (N=18)

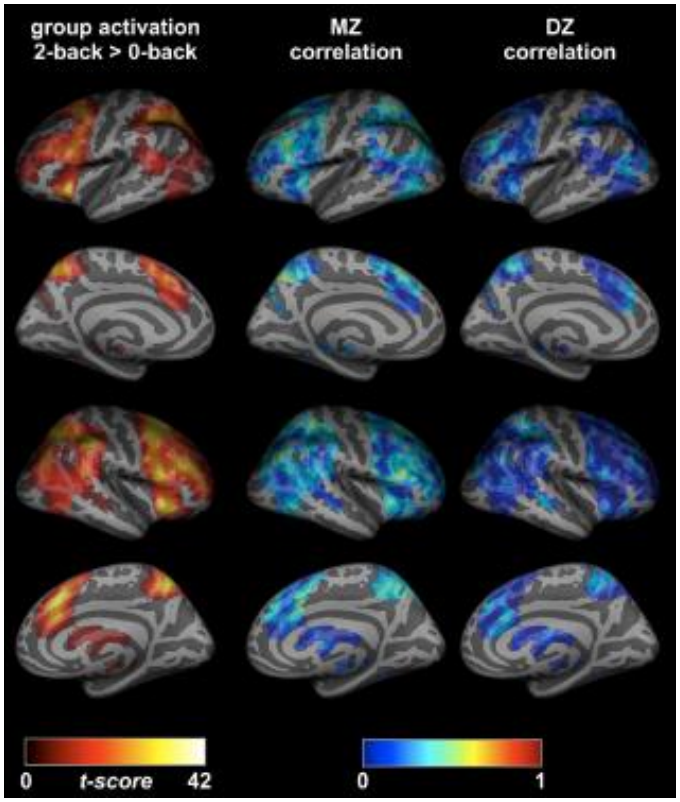
Callicott *et al. Cereb Cortex* 2000





Healthy Siblings > Controls
(N=48) (N=33)

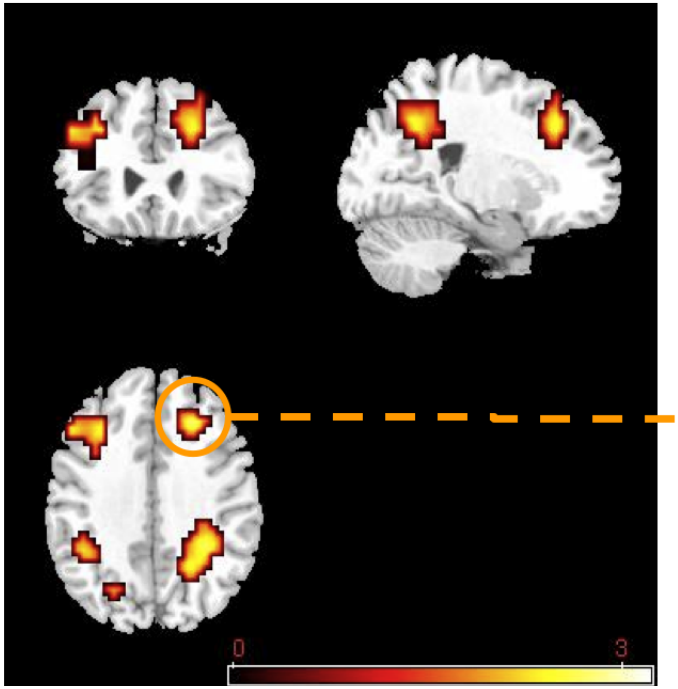
Callicott *et al. Am J Psychiatry* 2003



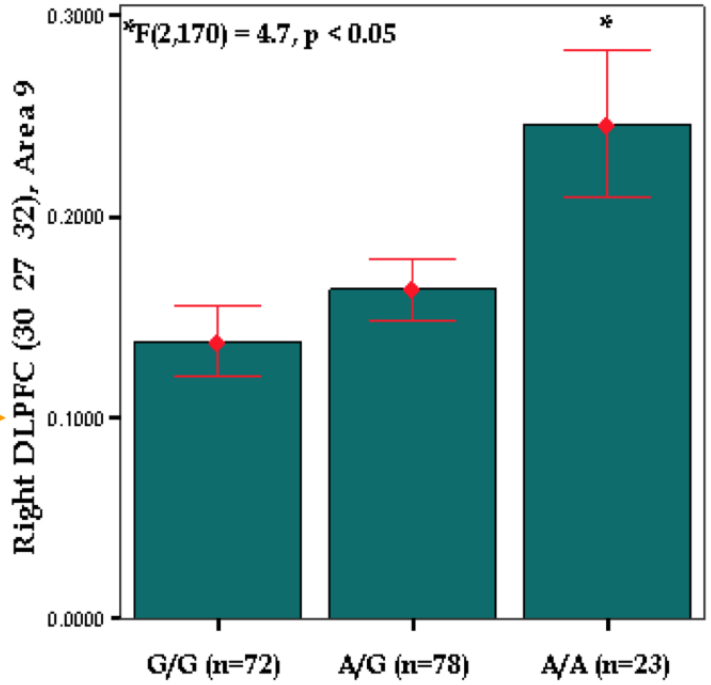
PFC BOLD during our Nback h2 = 0.4-0.5

Blokland e*t al. Biol Psych* 2009, *J Neurosci* 2011; Koten et al. *Science* 2009

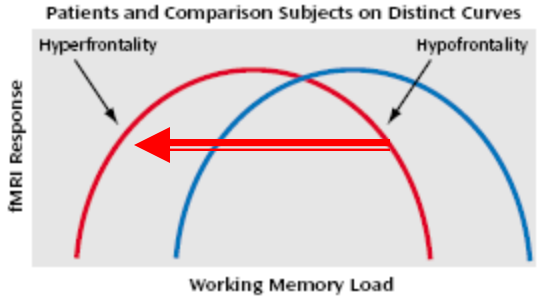
Putting brain imaging and genetics together



(SPM5, display at p < 0.001, uncorrected; PFC p < 0.05 SVC)



Huffaker et al. 2009



Mutations that increase risk for schizophrenia are disadvantageous for all of us

Imaging genetics v 1.0

- Candidate genes
  - KIBRA impaired memory & expressed in hippocampus (Papassotiropoulos et al., Science 2006)
  - Replication in 3 independent populations in behavioral memory measures
  - In 30 healthy subjects, KIBRA associated with reduced hippo activation

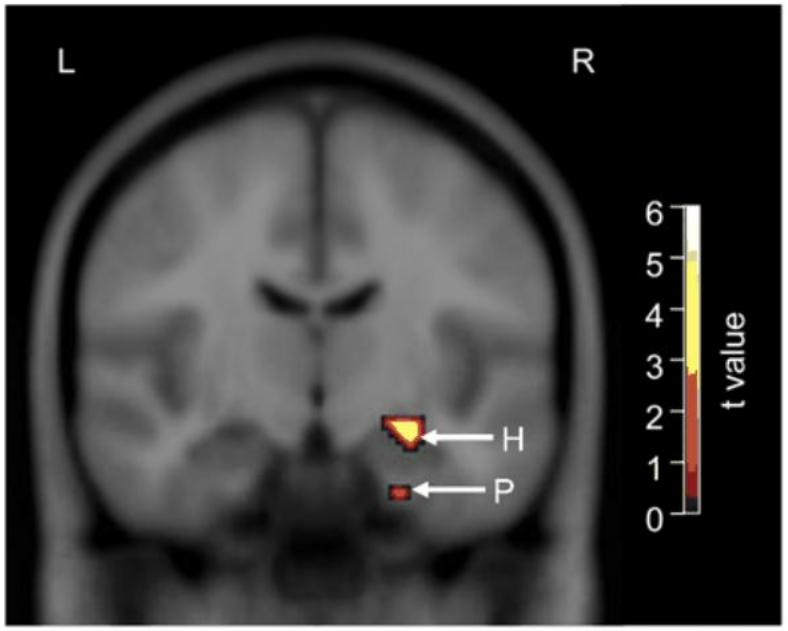
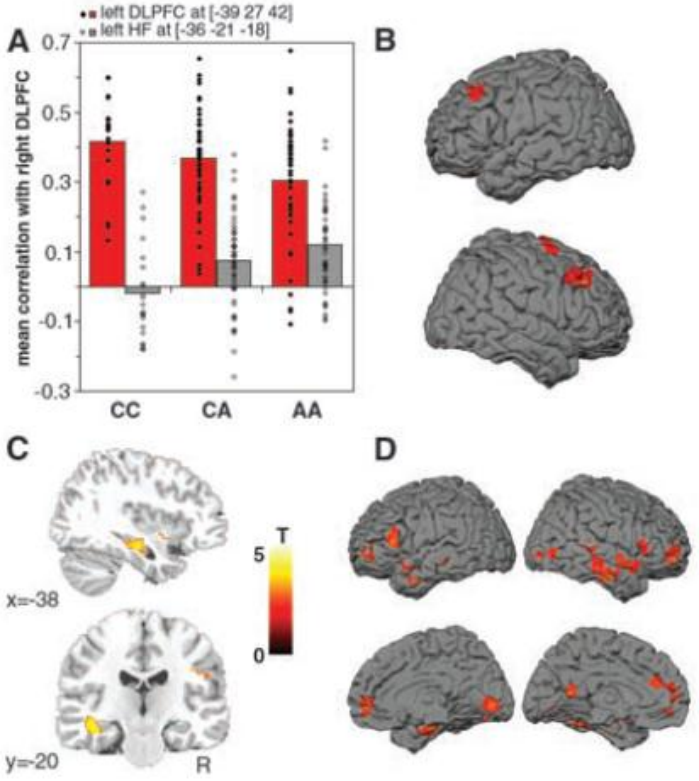


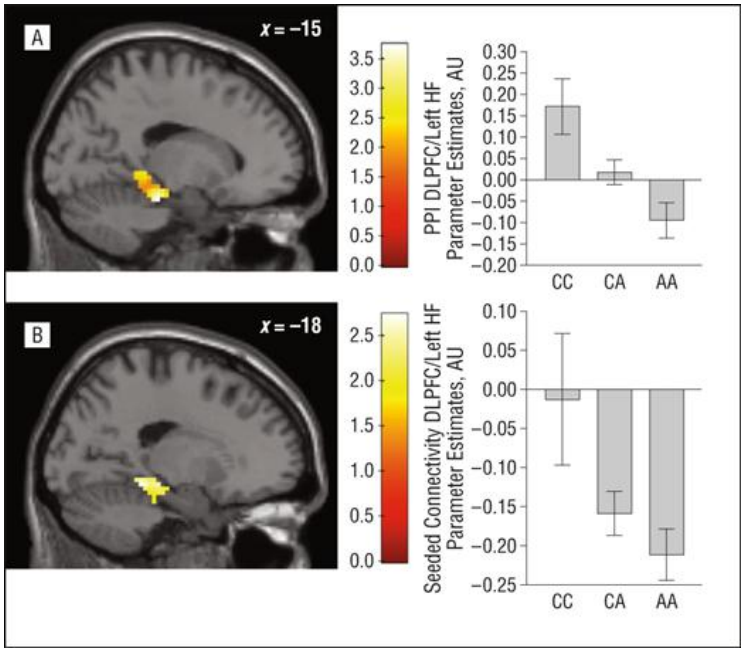
Fig. 3. .

Significant *KIBRA* allele–dependent differences in hippocampal activation as measured with fMRI. Activations are significantly increased in the hippocampus in noncarriers (n = 15) of the T allele of SNP rs17070145 than in the hippocampus of T allele carriers (n = 15). Activations from all 30 individuals were overlaid on a coronal section of a T1-weighted magnetic resonance image of SPM2 and displayed in color-coded t values. Threshold: $P < 0.001$. H, hippocampus; P, parahippocampal gyrus; L, left side of the brain; R, right side of the brain.

Imaging genetics v 2.0

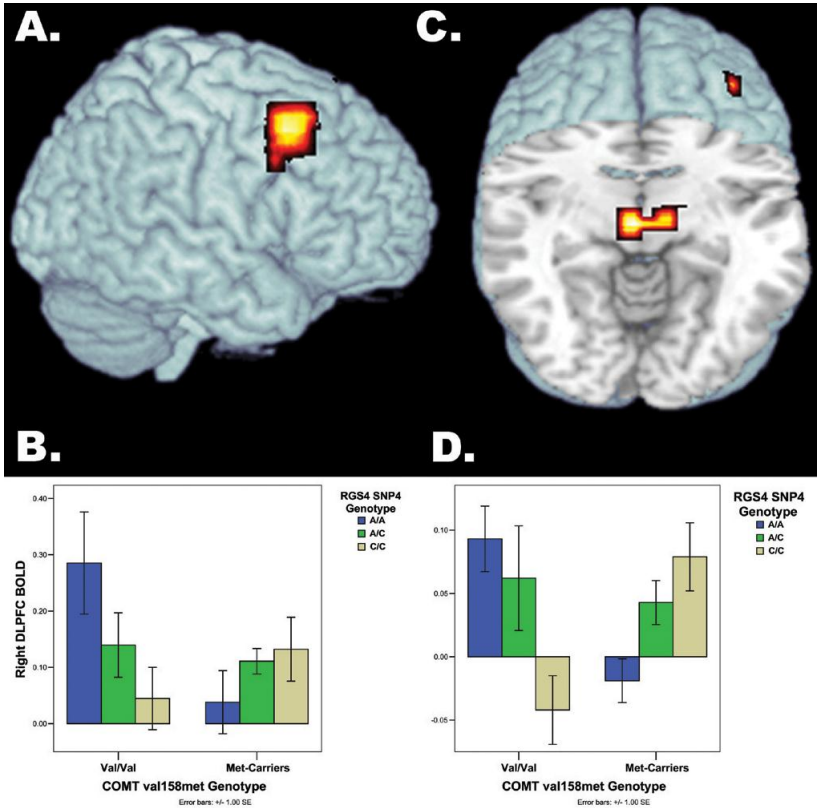
- Genetic mutations modeled in cell culture or animals
- Association based on disease GWAS (ZNF804A)
  - Esslinger et al. 2009 (Science)
  - Rasetti et al. (Arch Gen Psychiatry)

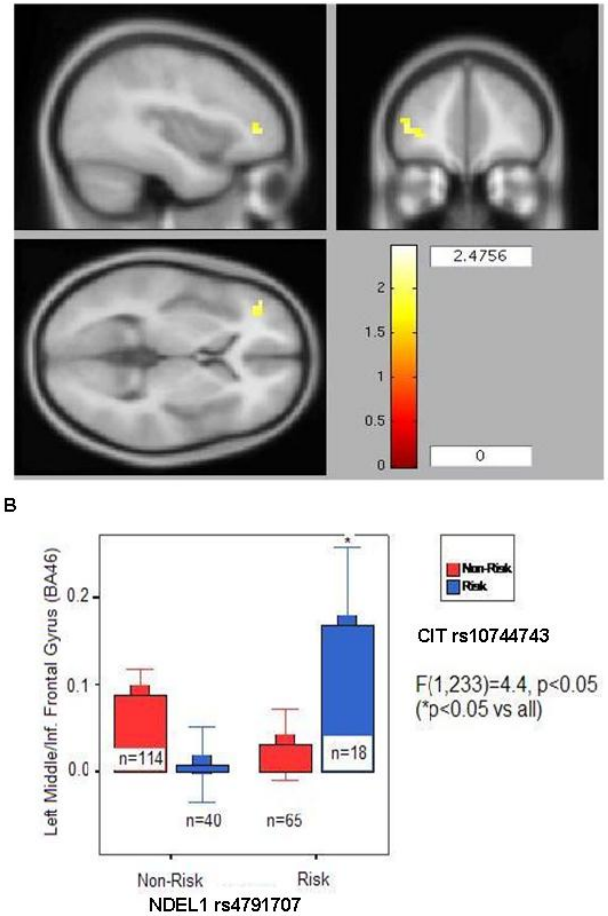




Imaging genetics v 2.1

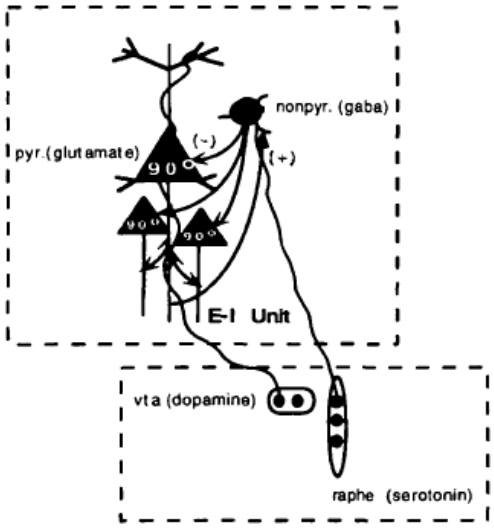
- Epistasis (gene-gene interaction)
  - Initially based on candidate-by-candidate
    - Buckholtz et al., Mol Psychiatry 2007
  - Data-driven (machine learning)
    - Nicodemus et al., Hum Genet 2010
  - Now predicated on detailed cellular or animal modeling
    - Papaleo et al., in press; Callicott et al. JCI 2013





PFC neuronal function: 'optimized' by dopamine & GABA interactions

Figure 3. A basic excitatory-inhibitory (E-I) functional unit in the cerebral cortex



(Goldman-Rakic & Selemon 1997)

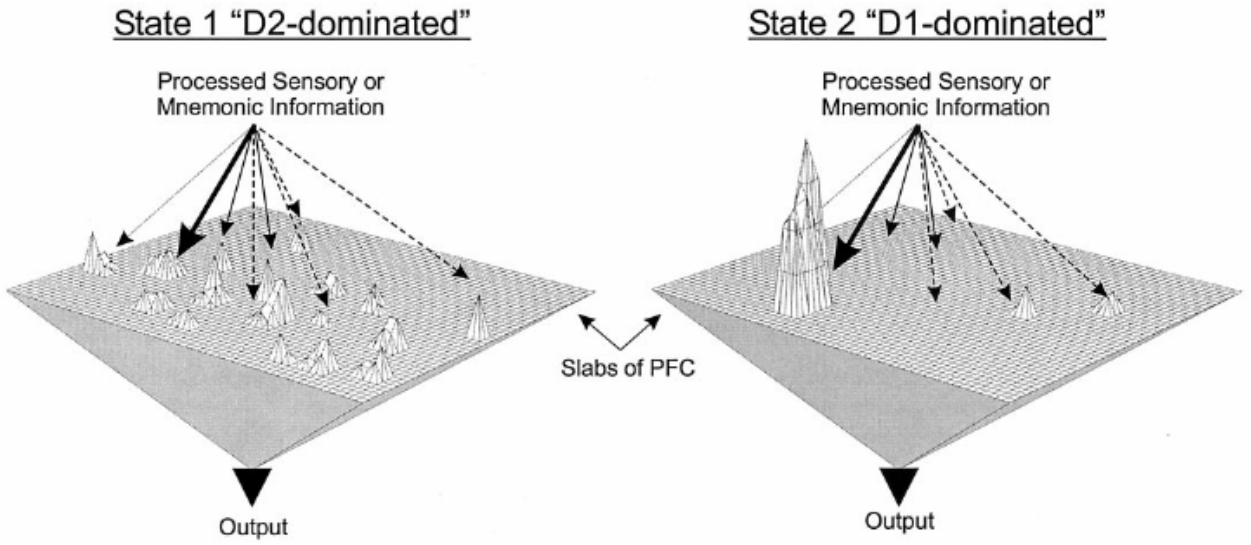


Figure 7. Theoretical implications of a bidirectional change in inhibition in the PFC. *Left*, In state 1, the D2 modulation predominates, and there is a net reduction in inhibition. As a result, multiple inputs impinging on the PFC have access to the working memory buffers, allowing multiple representations (i.e., sustained activity driven by recurrent excitation that encodes working memory information) to be held in PFC networks nearly simultaneously. *Right*, In state 2, the D1 modulation predominates, and there is a net increase in inhibition. As a result, inputs have difficulty accessing PFC networks. However, particularly strong inputs, which can overcome the effects of heightened inhibition, benefit from the simultaneous D1-mediated increases in long-lasting inward currents (i.e., persistent Na^+ and NMDA currents), which produce very active and stable network representations, even after the offset of the initiating stimulus (Yang and Seamans, 1996; Durstewitz et al., 2000; Seamans et al., 2001). In this way, dopamine may first allow an exploration of the input space (state 1), entertaining multiple network representations nearly simultaneously. Subsequent transition into state 2 shuts off the influence of weak inputs on PFC networks and strongly stabilizes one or a limited set of representations, which would then have complete control of PFC output.

(Seamans et al., 2001)

ORIGINAL ARTICLE

Allelic variation in GAD1 (GAD_{67}) is associated with schizophrenia and influences cortical function and gene expression

RE Straub, BK Lipska, MF Egan, TE Goldberg, JH Callicott, MB Mayhew, RK Vakkalanka, BS Kolachana, JE Kleinman and DR Weinberger

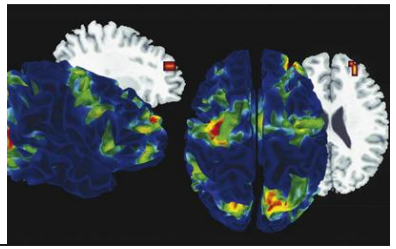


Table 1: GAD1-COMT Statistical Epistasis

Table Results of GAD1–COMT interaction analysis

GAD1 M#	*GAD1 genotype*	*COMT val158met[a] (rs4680) genotype*	*Genotype interaction*		*Odds ratio*	*95% CI lower*	*95% CI upper*	*Case* N	*Control* N
			P-value	*Type*					
M05	1/2 (A/G)	1/1 (G/G; Val/Val)	0.001	Risk	6.57	2.076	20.79	36	19
M06	1/2 (C/G)	1/1 (G/G; Val/Val)	0.008	Risk	5.00	1.521	16.434	36	18
M10	1/2 (C/G)	1/1 (G/G; Val/Val)	0.003	Risk	4.30	1.623	11.388	45	30

From Straub et al., 2007

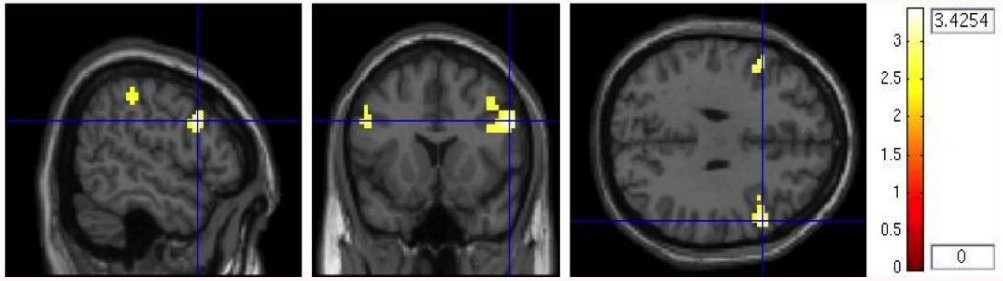
Table 2: Representative demographics

GAD1 (rs2356236; M5) x COMT ($Val^{158}Met$)

	GAD1 (rs2356236; M5) x COMT ($Val^{158}Met$) interacti on Demographics (n = 134)							
	A/G + Val/Val (n = 18)		A/G + Met Carriers (n = 40)		A/A + Val/Val (n = 20)		A/A + Met Carriers (n = 56)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	32.2	10.5	31.8	10.2	32.2	9.8	29.6	8.0
Gender (M:F)	5:13		19:21		9:11		26:30	
Educati on (years)	17.1	2.1	17.1	3.0	17.2	2.7	16.6	2.6
WAIS IQ	108.2	9.2	109.6	9.7	110.0	11.8	105.9	8.2
2-back Accuracy	0.83	0.13	0.84	0.17	0.76	0.20	0.82	0.16
2-back Reacti on Time (sec)	0.581	0.238	0.461	0.195	0.536	0.231	0.472	0.220

PFC efficiency associated with dopamine and GABA epistatic interaction

Figure 3: GAD1 (rs2356236; M5) x COMT Val^{158}Met (rs4680) Interaction (n=134)



DLPFC Region (53, 16, 27) (BA 9, Middle and Inferior Frontal Gyri)

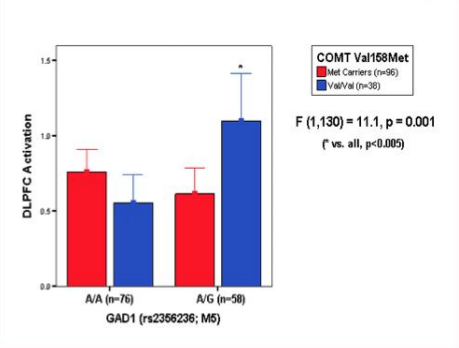
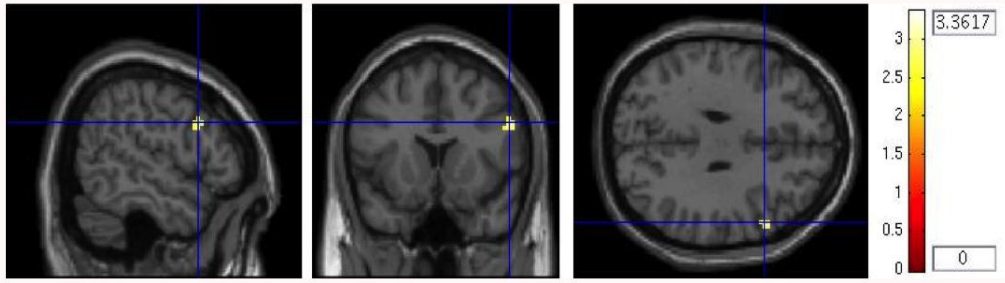
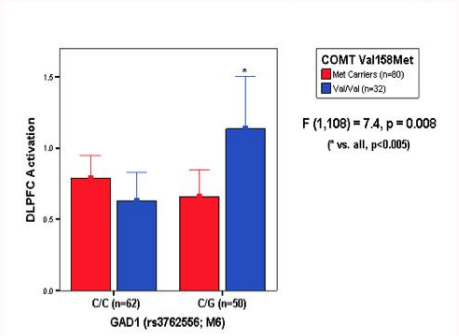


Figure 4: GAD1 (rs3762556; M6) x COMT Val^{158}Met (rs4680) Interaction (n=112)



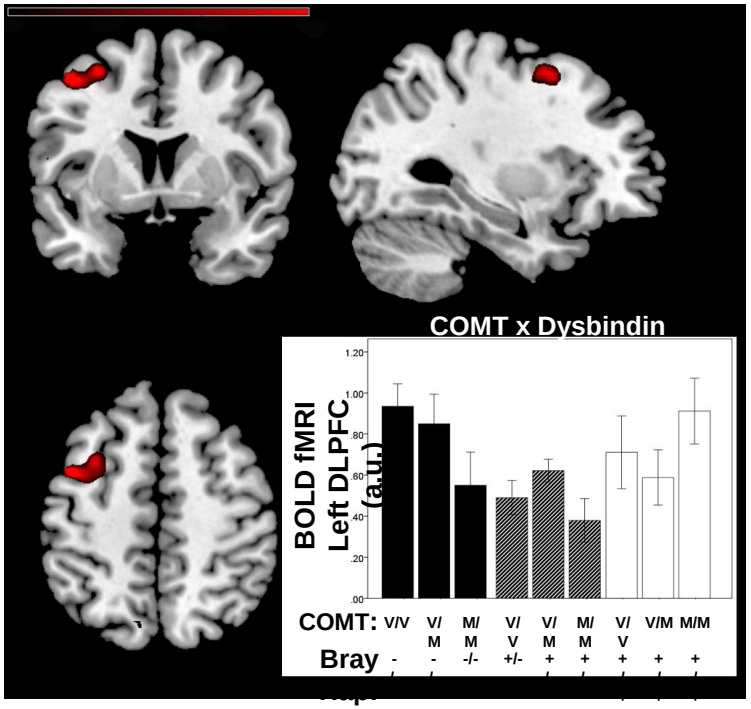
DLPFC Region (53, 16, 27) (BA 9, Inferior and Middle Frontal Gyri)

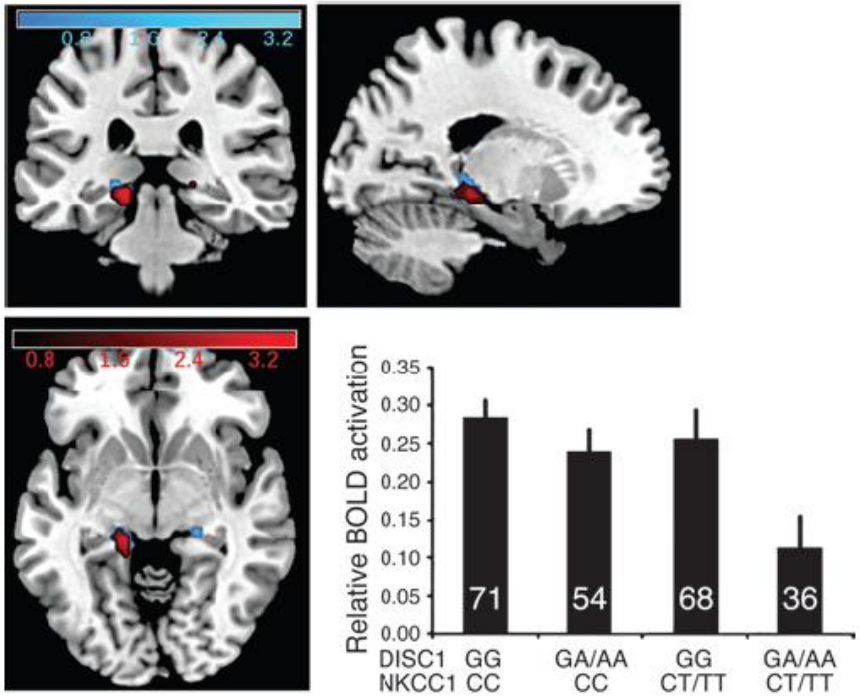


($p < 0.05$ SVC, display at $p < 0.01$ uncorrected; error bars represent 1 standard error of the mean)

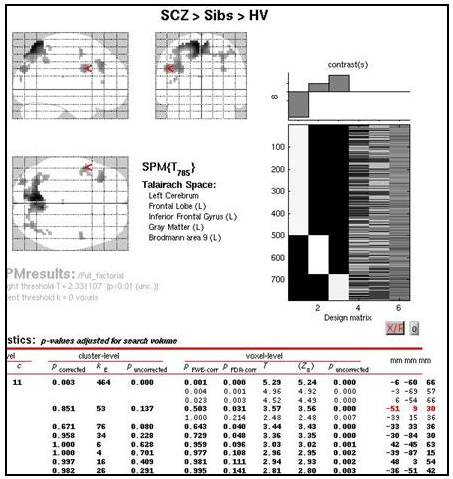
Imaging genetics v 2.1

- Epistasis (gene-gene interaction)
  - Initially based on candidate-by-candidate
    - Buckholtz et al., Mol Psychiatry 2007
  - Data-driven (machine learning)
    - Nicodemus et al., Hum Genet 2010
  - Now predicated on detailed cellular or animal modeling
    - Papaleo et al., in press; Callicott et al. JCI 2013

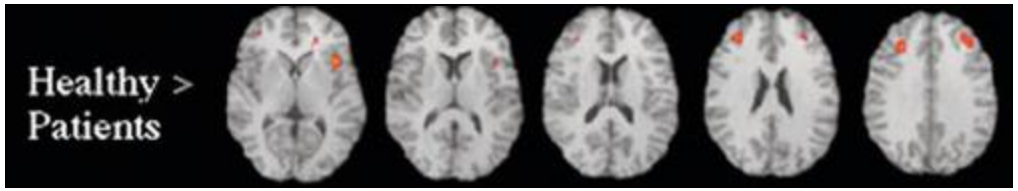




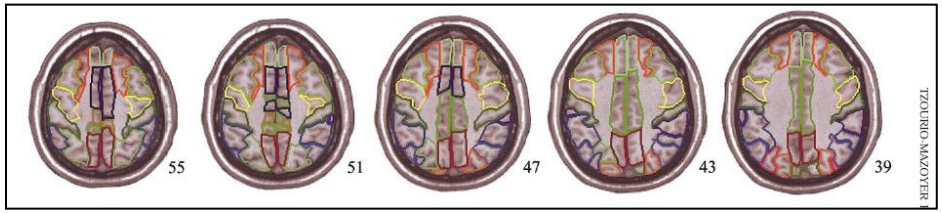
Imaging genetics v 2.2



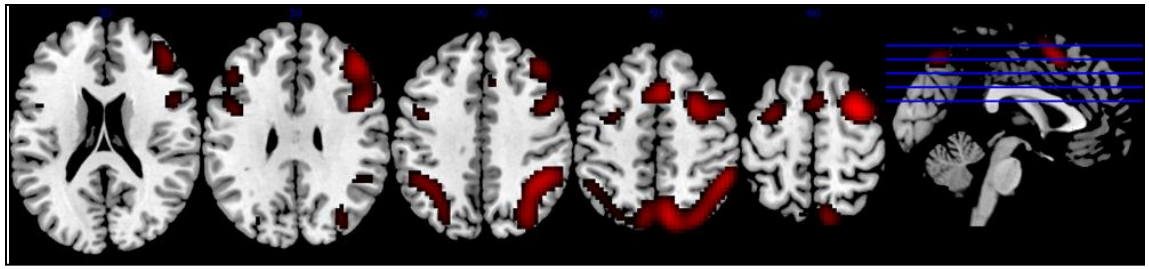
PFC inefficiency defined by intermediate phene



Meta-analysis driven ROIs



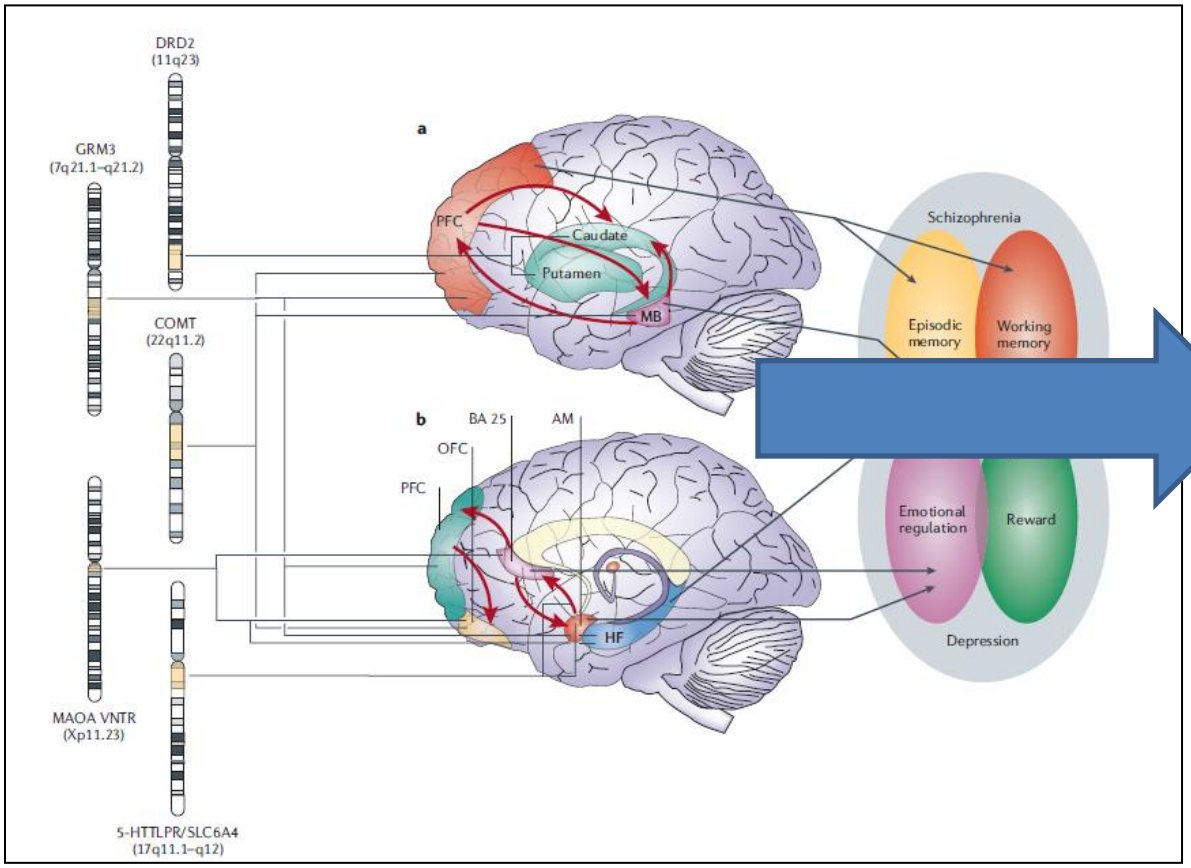
AAL: Extraction across whole brain

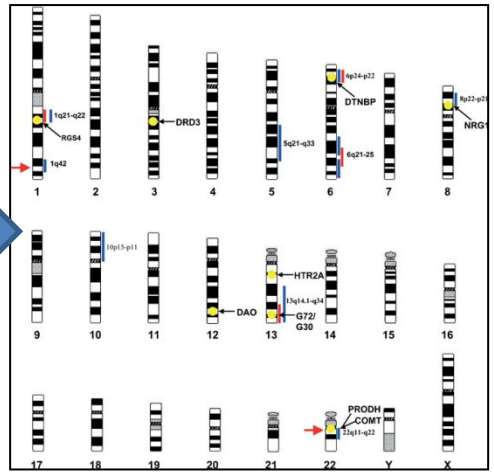


CPCA: networks

Imaging genetics v 3.0

- BOLD fMRI intermediate phenotypes, like PFC efficiency, seem particularly useful in elucidating potential physiological manifestations of risk genes for schizophrenia.
- Establishing potential heritability was an essential part of this argument (e.g., Weinberger et al. 1992, Berman et al. 1992).
  - If DLPFC BOLD signal is a heritable quantitative trait, then we should expect some associations between genome-wide datasets and estimates of BOLD activation:
    - BOLD fMRI PFC inefficiency in 2 separate cohorts of unafffected siblings (Callicott et al. 2003)
    - Blokland et al. 2008: Nback in 60 twin pairs (29 MZ vs 31 DZ)
    - Koten et al. 2009: SIRP in 10 MZ twin pairs + additional brother
- Will BOLD fMRI GWAS identify risk variants that have been 'invisible' to case-control GWAS due to the diagnostic heterogeneity, phenotypic complexity, and other poorly-understood aspects of the clinical diagnosis of schizophrenia?
  - Caveat: BOLD fMRI may always be better poised to generate novel phenotypes for candidate gene studies
  - Likely to generate false positives or at least many findings unrelated to risk per se



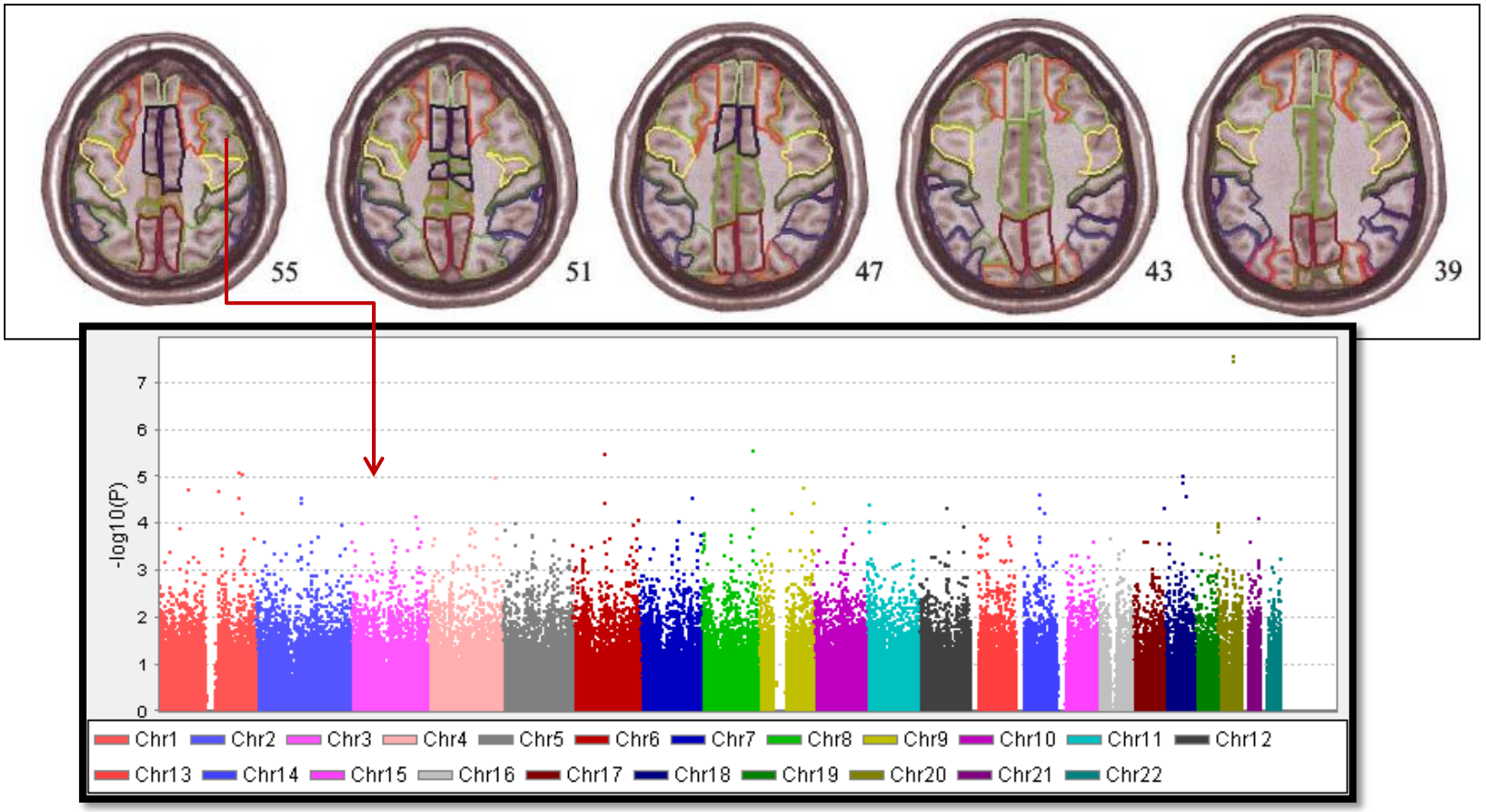


(Owens et al. 2004)

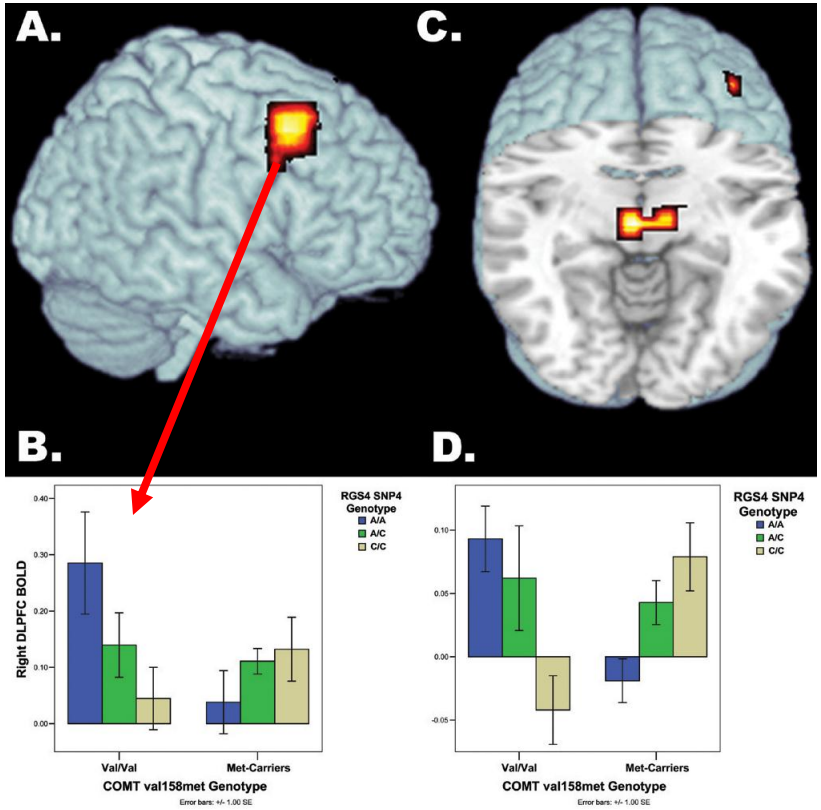
(Meyer-Lindenberg & Weinberger 2006)

Imaging genetics v 3.0

- BOLD fMRI GWAS
- Nback (n = 364)
- Illumina 650K chip genotyping
- Automated extraction of AAL ROIs
- First GWAS + using BOLD fMRI (Callicott, Spencer, et al., in prep)



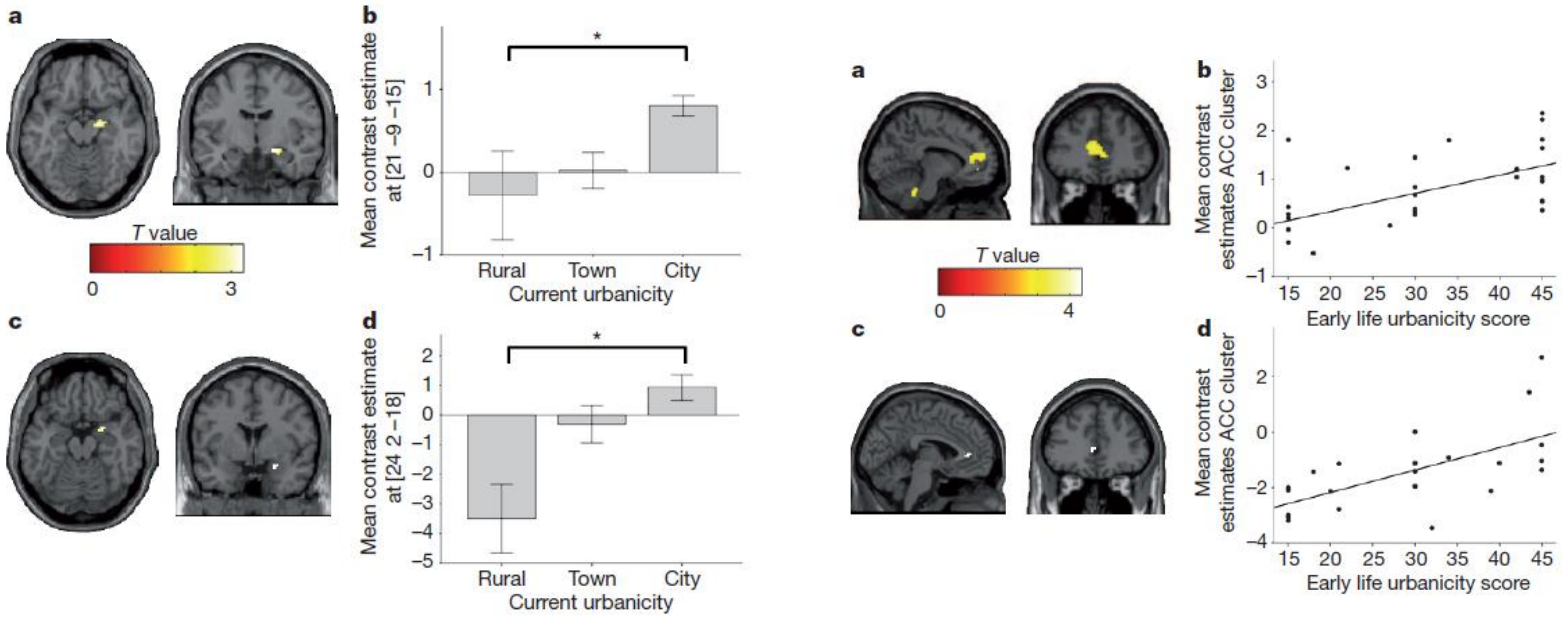
We’re looking for lots of genes acting together, so we’ve started looking for signs of these “interactions”



(Buckholtz et al. Mol Psychiatry; 2007)

As a heritable trait, BOLD fMRI phenotypes show other sensitivities…..

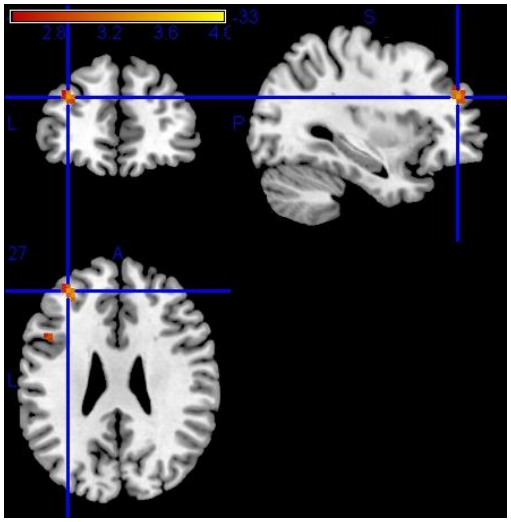
- fMRI during social stress task influenced by environment
  - Urban upbringing or urbanicity

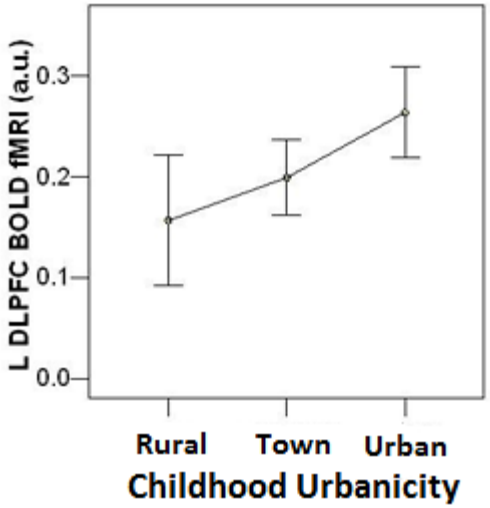


Lederbogen et al., Nature, 2012

As a heritable trait, BOLD fMRI phenotypes show other sensitivities…..

- fMRI during WM (3 cohorts (USA1 = 124; USA2 =92; Italy1=226)
  - Same sensitivity to environment (Urbanicity)
  - Gene-by-environment interaction (COMT x Urbanicity)





Ihne et al., in preparation

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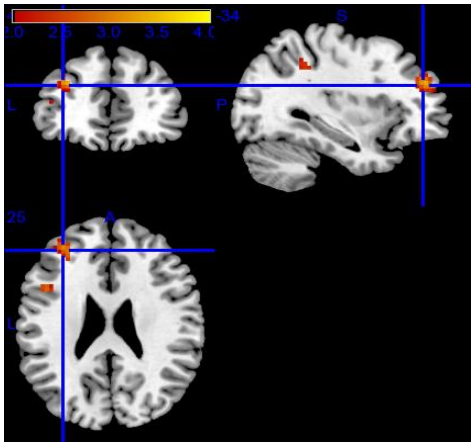
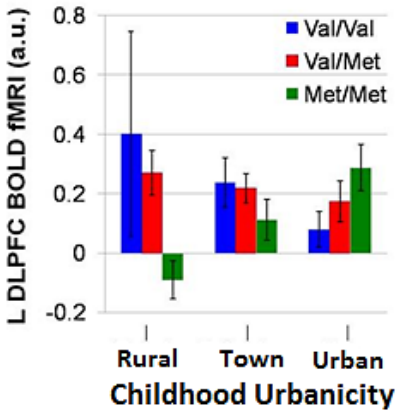
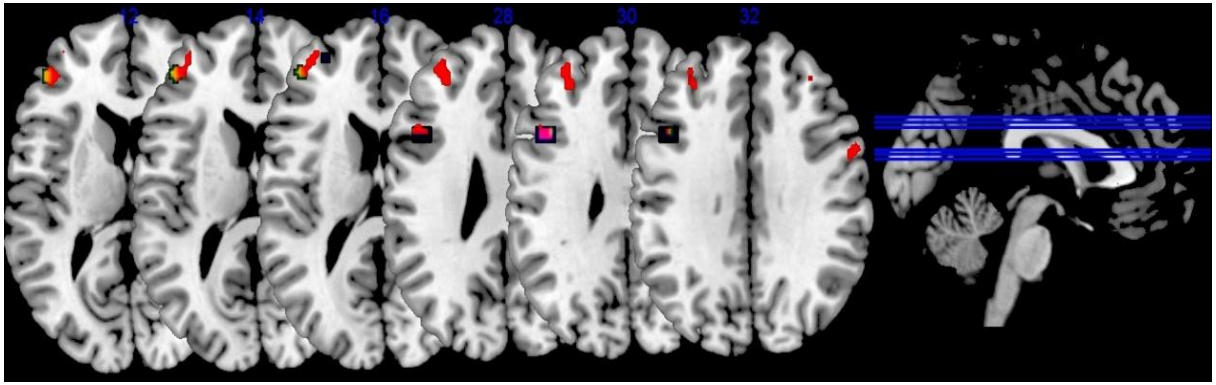
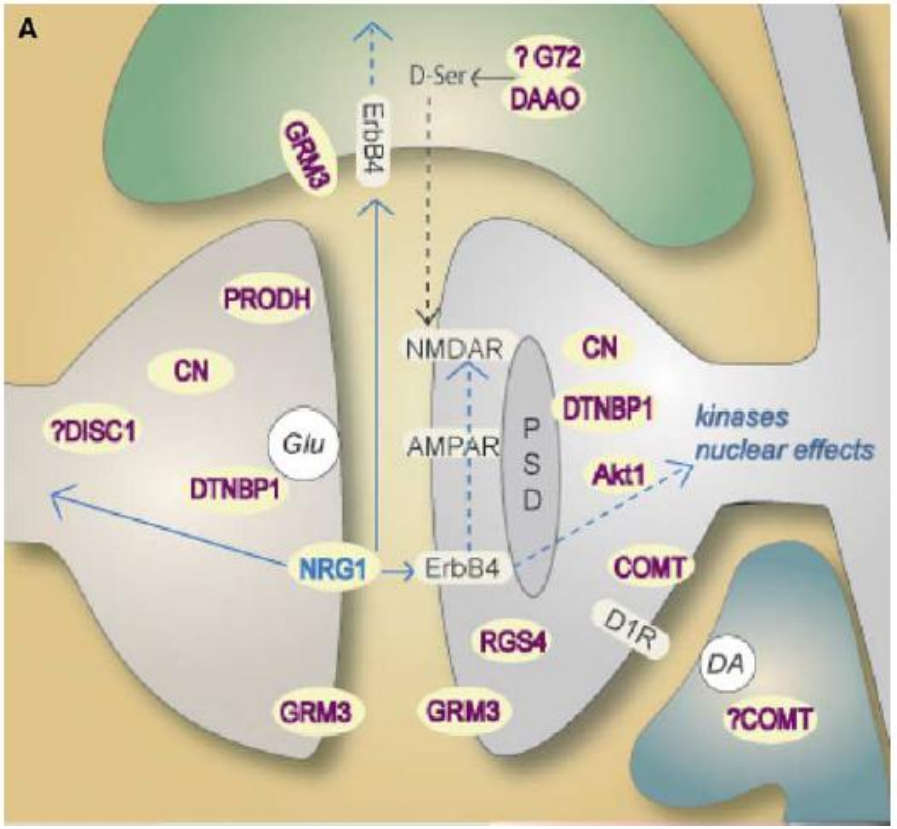






Until we understand healthy brain cell function, fMRI lets us check our work

If a mutation is important to brain function, then mapping these effects (taking a picture) lets us feel more confident we are on the right track.



(Harrison and Weinberger Mol Psychiatry 2005)

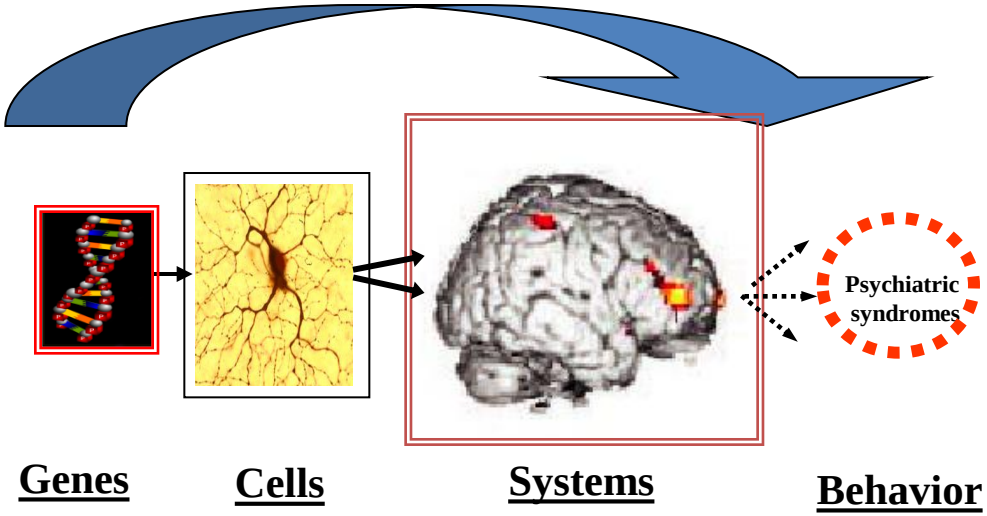
“The most incomprehensible thing about the world is that it is comprehensible.”
A. Einstein (1936)

- Many features that appear state-independent are poor candidates for intermediate phenotypes:
  - Put another way, not all biomarkers are intermediate (‘endo’) phenotypes for a given disease OR may be heritable for all humans, but that that heritability not particularly altered by disease
    - Characterization of siblings, both twins and full sibs, in parallel to probands remains the only way to determine familial co-segregation and heritability (Gottesman & Gould 2003)
    - Thus, our primary intermed phene, PFC inefficiency (Callicott et al. 2003, Karlsgodt et al. 2007), may have had so much success b/c PFC activation is heritable (Blokland et al. 2008; Koten et al. 2009, Karlsgodt et al. 2010, Blokland 2011)
    - One the other hand, amygdala activation, also linked to several genetic polymorphisms (Hariri et al. 2002), appears not to be heritable in schizophrenia (Rasetti et al. 2009)
- Outstanding issues
  - How dependent are these associations on task characteristic (i.e., are genetic effects specific or generalized)?
  - Gene x gene (epistatic) analyses are thought critical in complex heritability. Will such analyses in imaging space hold when the genes are no longer candidate A x candidate B, but the kind of unpredicted A x novel B, as from GWAS or empirical statistical analyses?
  - Effect size appears larger for imaging vs. other phenes. Will this mean that smaller sample sizes can yield GWAS significant findings using intermediate phenes?

Conclusions

- ✓The next generation of studies
  - ✓Task characteristics:
    - ✓Genetic effects easy to search for
      - ✓Finding heritable phenotypes (GCTA)
    - ✓Candidate gene strategy replaced by GWAS, model, data-driven
  - ✓Epistasis:
    - ✓Usual suspects: GAD1 x COMT
    - ✓Novel approaches: DISC1 interactome, GWAS +, cell or animal model
  - ✓PFC efficiency is a valid phene with its own set of GWAS findings
    - ✓These genes will be relevant far beyond scz; any neurological disorder with PFC implicated pathology
    - ✓Unique avenues to cognitive remediation
- ✓Building the better mousetrap: rethinking the next generation of tasks and potential phenes:
  - ✓Processing speed suggests its second order processes that are most disrupted, not the highest executive levels we've studied (Dickinson et al. 2008)
  - ✓Are there lacunae of intact function (aspects of fluid intelligence) that survive illness (just as most of crystallized intelligence does)?
    - ✓More targets for remediation

Summary



1. **Genes don't give you a mental illness, they build a brain that is vulnerable.**

2. **Short-cut: Find the genes that cause a brain to have trouble with short term memory and with some luck, some of those may be responsible for the vulnerable brain.**

3. **The vast majority of diseases that cause pain and suffering in humans are inherited in this 'many genes' fashion – this could work for lots of other diseases besides psychiatry.**