MONASH University

Medicine, Nursing and Health Sciences

Neurobiology of Attention Deficit Hyperactivity Disorder (ADHD)

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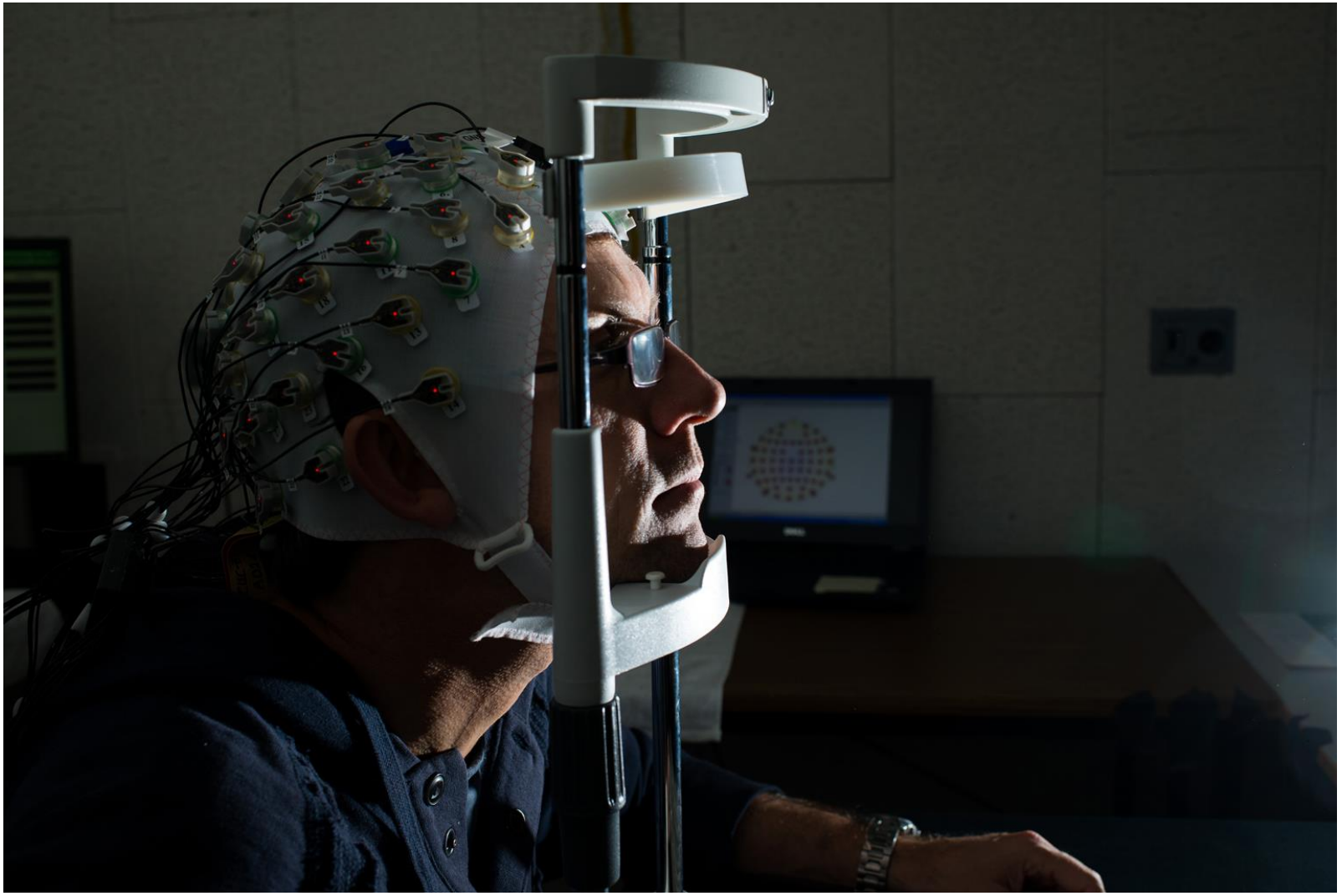
Bellgrove Lab

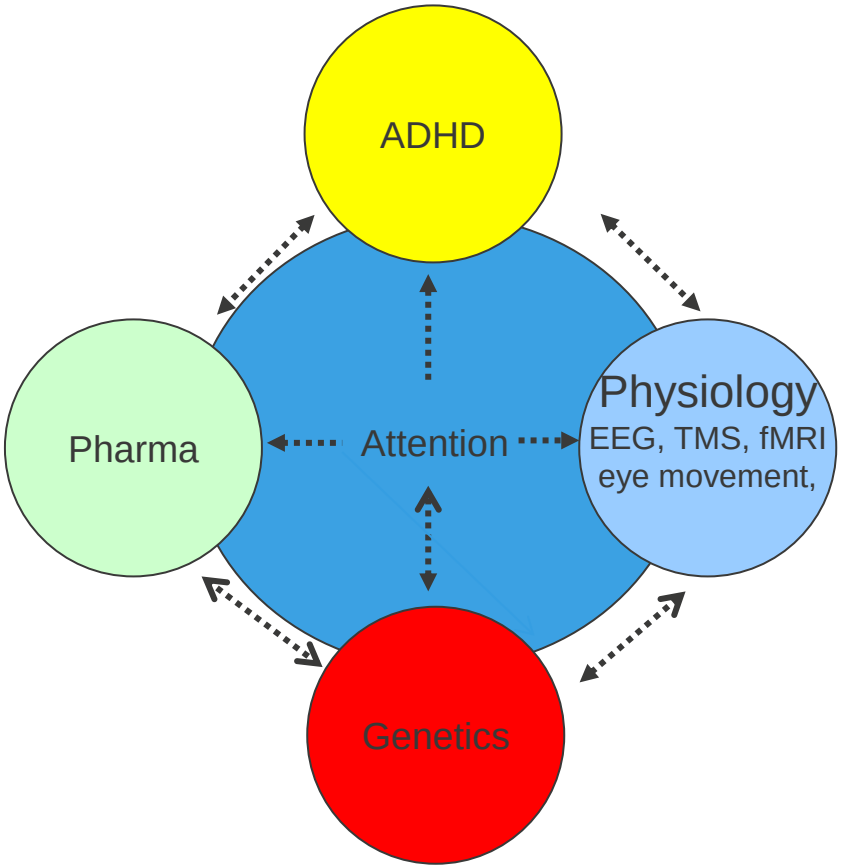
Functional Genomics Laboratory

Bellgrove Lab



Cognitive Neuroscience Laboratories

Our Paradigm for Discovery



Identify the molecular mechanisms of individual differences in attention and map susceptibility pathways for psychiatry

ADHD- not just a modern disorder

- Alexander Crichton (1798): Mental Restlessness.

"nervous problem which may be born with the person or be the effect of accidental disease… when born with the person it becomes evident at a very early period of life, and has a very bad effect, in as much as it renders him incapable of attending with constancy to any one object of attention. But it is seldom so great a degree as to totally impede all instruction; and what is very fortunate it generally diminishes with age"

"every impression seems to agitate the person, and gives him an unnatural degree of mental restlessness. A slight noise, too much light, too little light all destroy constant attention in so much as it is easily excited by every impression"

Overview

- Pharmacology of ADHD
  - Mode of action of psychostimulants
- Genetics of ADHD
  - Focus on catecholamine signalling pathways
- Neuropsychology and Brain Imaging in ADHD
  - Executive function
    - Response Inhibition
    - Spatial Attention

Pharmacology of ADHD

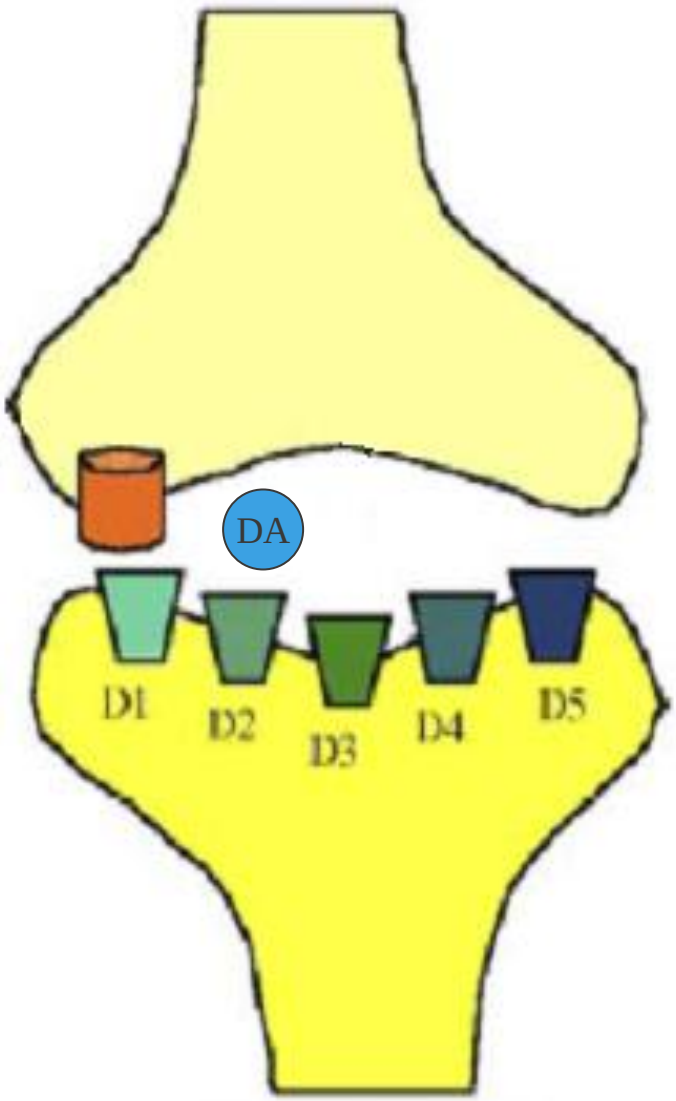
- Methylphendiate or Ritalin
- Atomoxetine

Action of Methylphenidate or Ritalin

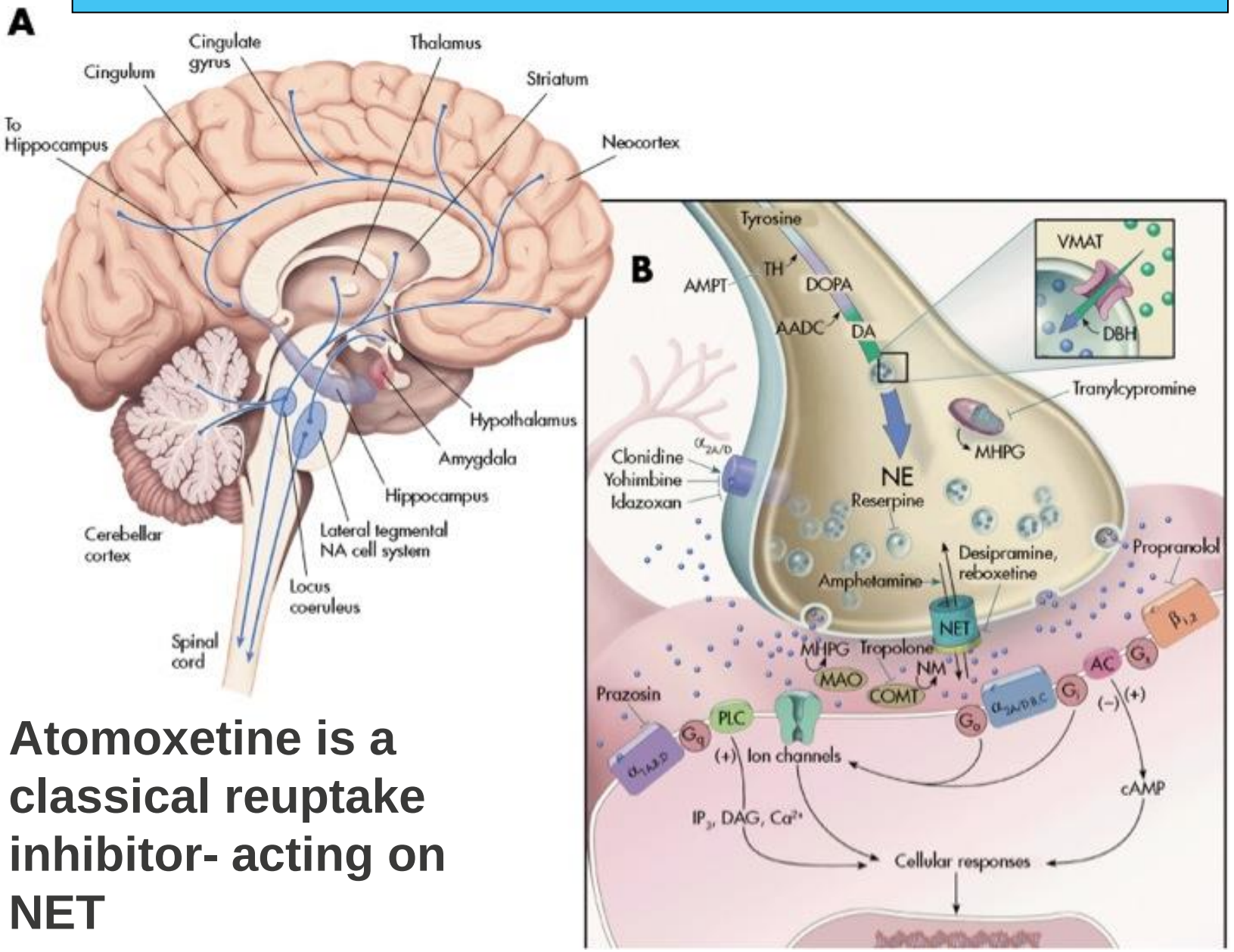
Dopamine Transporter (DAT)

Methylphenidate or Ritalin

- Inhibits reuptake via DAT
- Increases synaptic DA in striatum
- In PFC DAT is sparse and reuptake occurs via NET
- MPH modifies alpha 2a and D1 signalling in PFC



Action of Atomoxetine



Atomoxetine is a classical reuptake inhibitor- acting on NET

Pharmacological Treatment

Childhood

- Stimulants
  - Methylphenidate (10-40mg/day)
  - Dexamphetamine (10-30mg/day)
- Non-stimulants
  - Atomoxetine (1.2mg/kg/day)
- Effect sizes:
  - Stimulants>non-stimulants

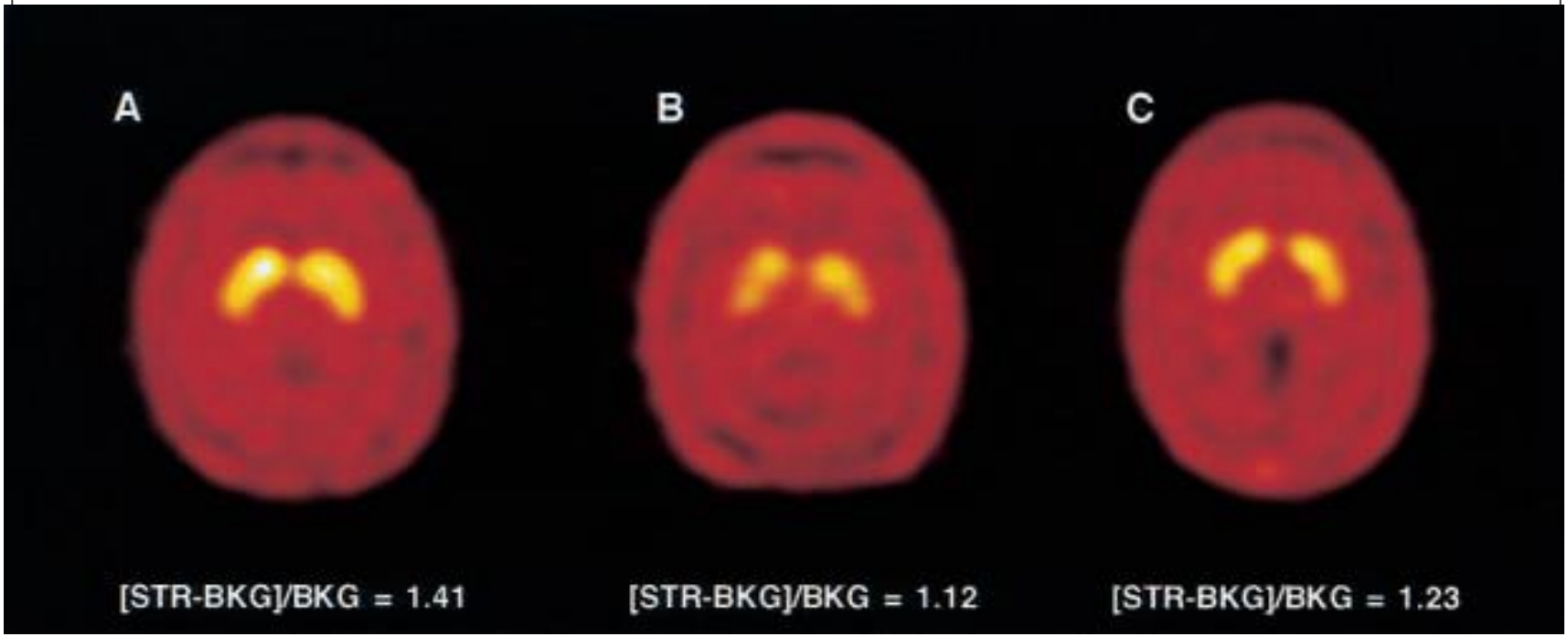
Adulthood

- Stimulants
  - Methylphenidate (20-100mg/day)
  - Dexamphetamine (10-60mg/day)
- Non-stimulants
  - Atomoxetine (40-150mg/day)
- Effect sizes:
  - Stimulants > non-stimulants

Catecholamine hypothesis of ADHD

- Increased activity of the dopamine transporter (DAT), particularly within the striatum, reduces availability of synaptic dopamine for subsequent signal transduction
- Treatment with methylphenidate inhibits the reuptake of dopamine, leaving more synaptic dopamine available.
- DAT is sparse in prefrontal cortex, so reuptake of methylphenidate occurs via the noradrenaline transporter (NET), with receptor level effects occurring at D1 and alpha2a receptors

Upregulated DAT in ADHD



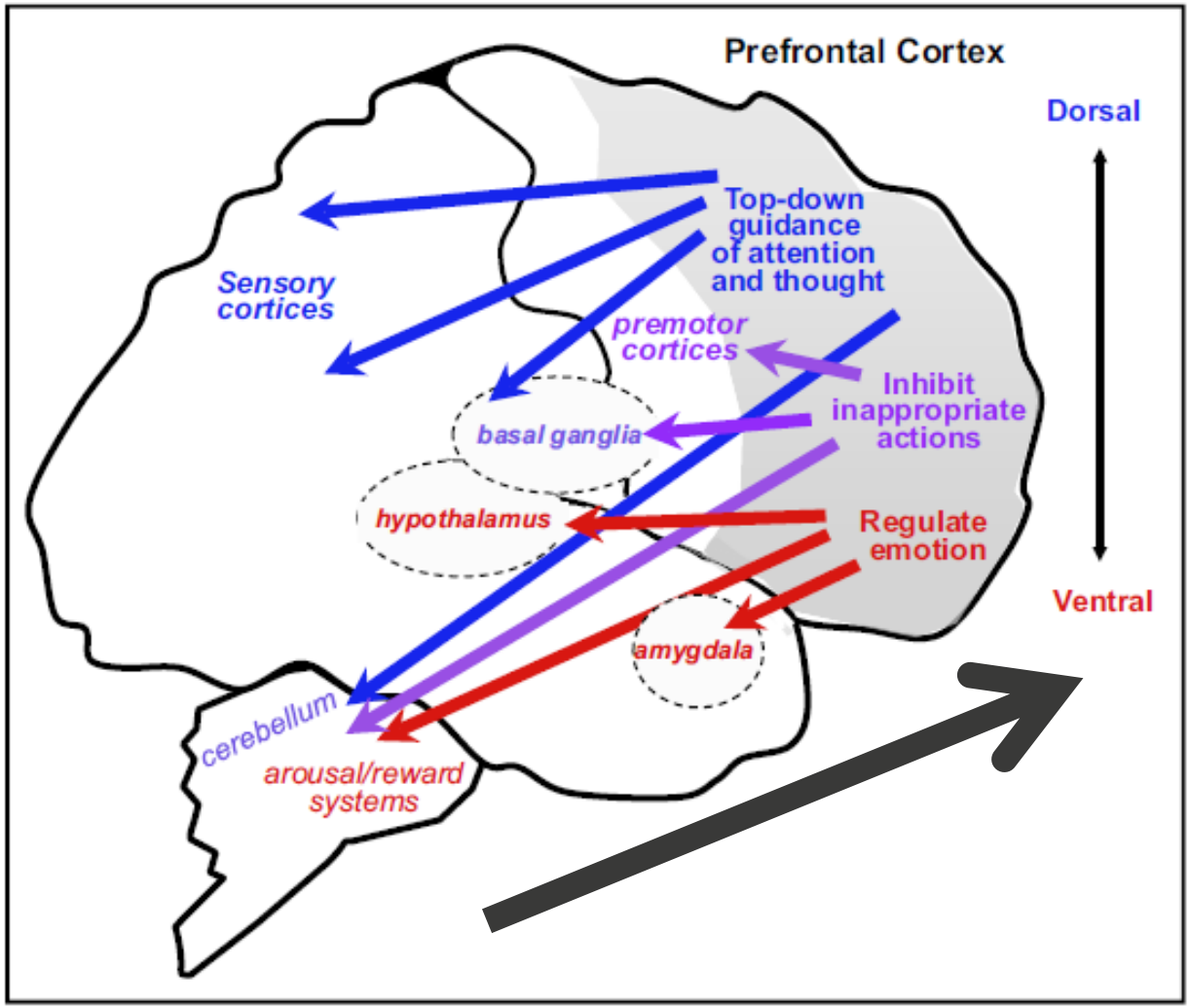
Dresel et al (2000)

(A) ADHD patient displays increased uptake of radiolabeled ligand in striatum which is diminished with methylphenidate (B)

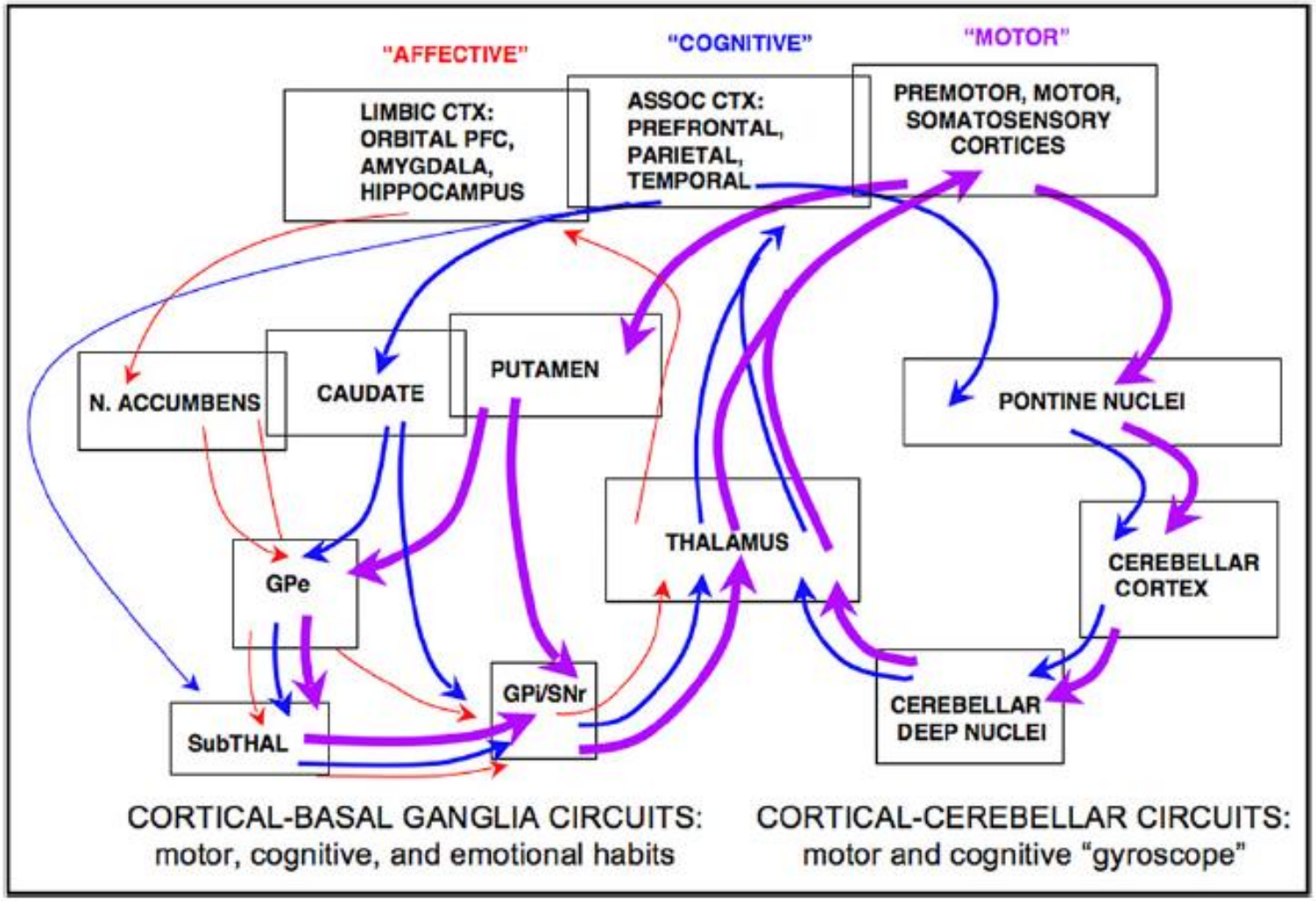
Spencer et al, 2007: elevated DAT binding in the right striatum

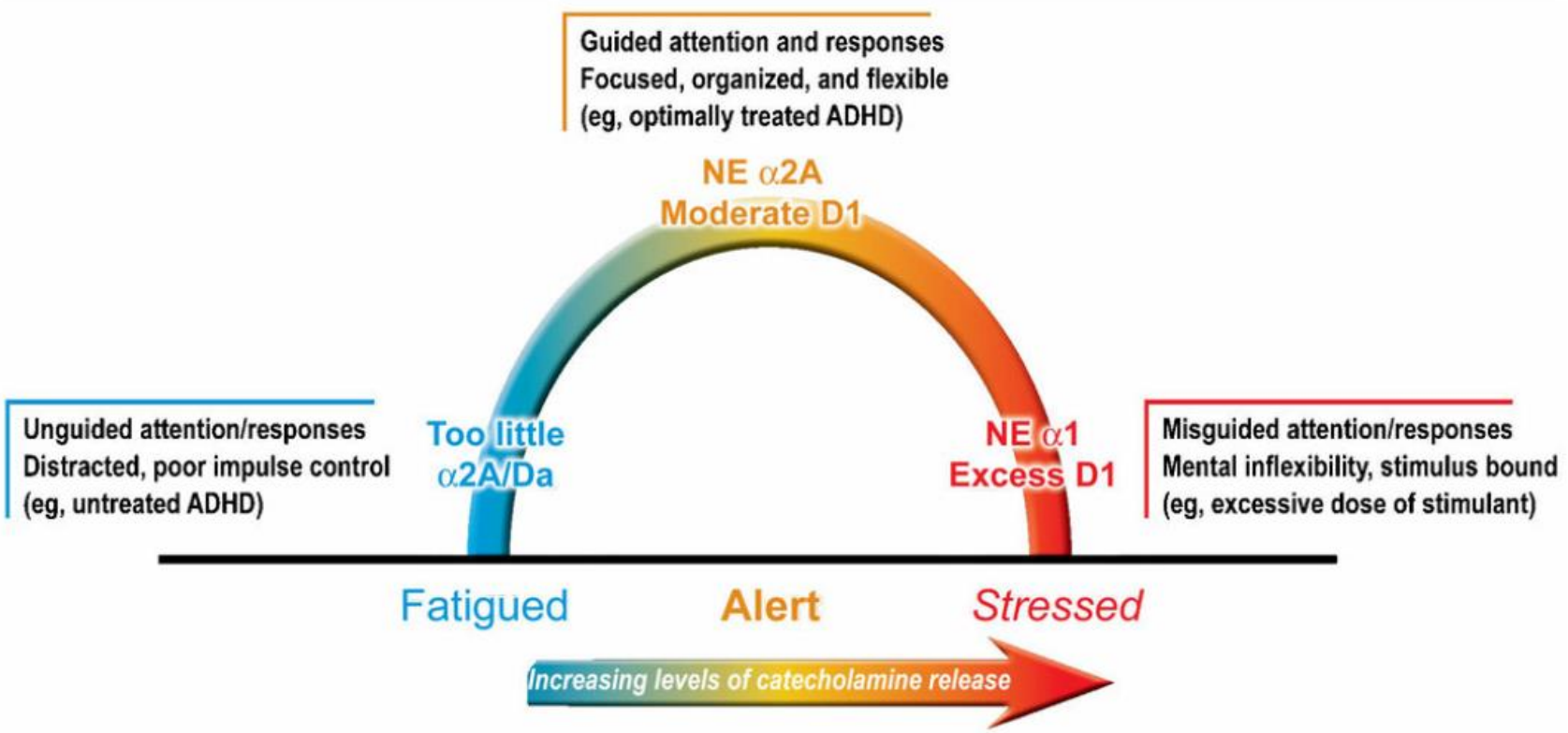
Effects of treatment on DAT binding results are possible: Fusar-Poli et al, 2012, AJP

Top down and bottom up control of cognition



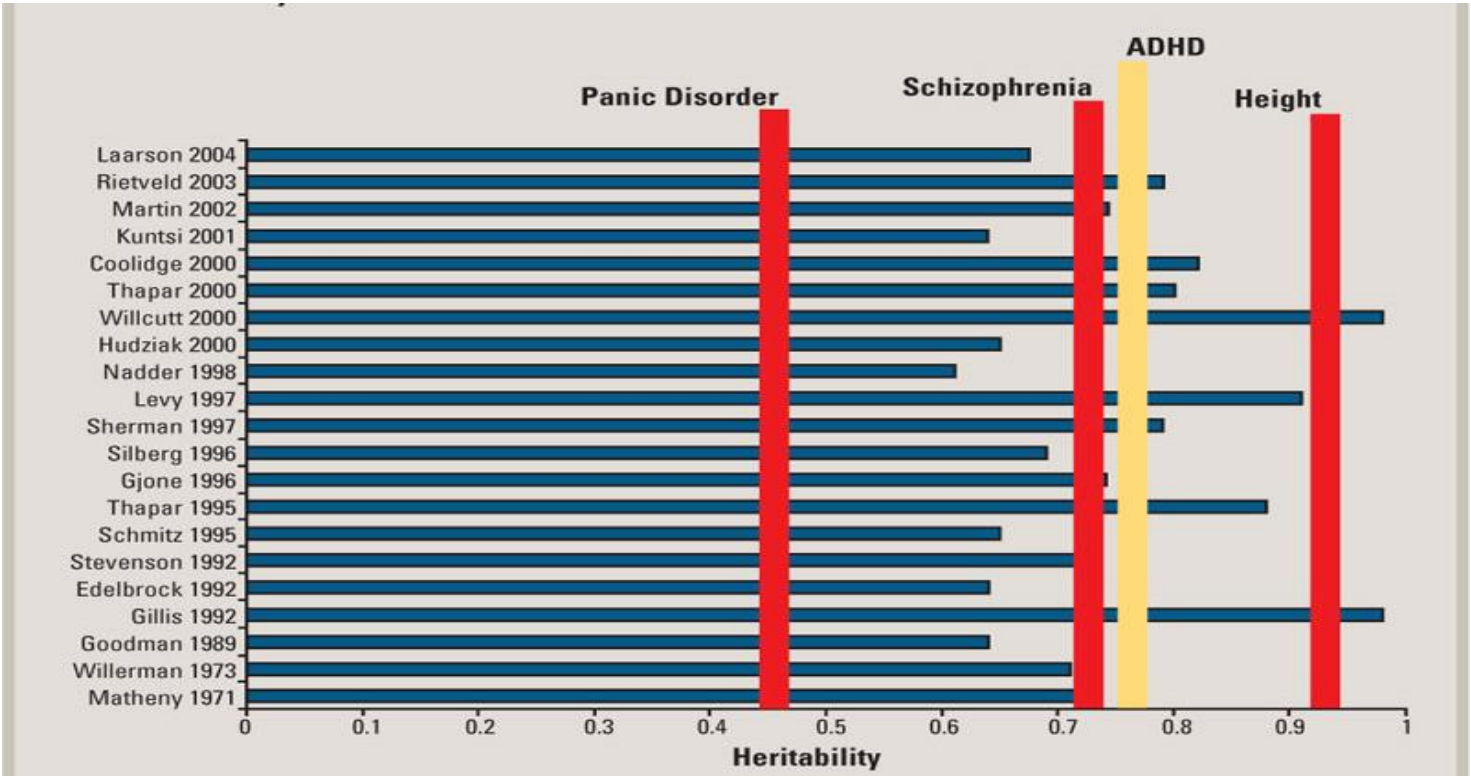
Distinct brain circuits for Affect, Cognition and Motor Function





Arnsten and Pliska 2011

Genetics of ADHD



Mean heritability of ADHD = .75. ADHD=attention-deficit/hyperactivity disorder.

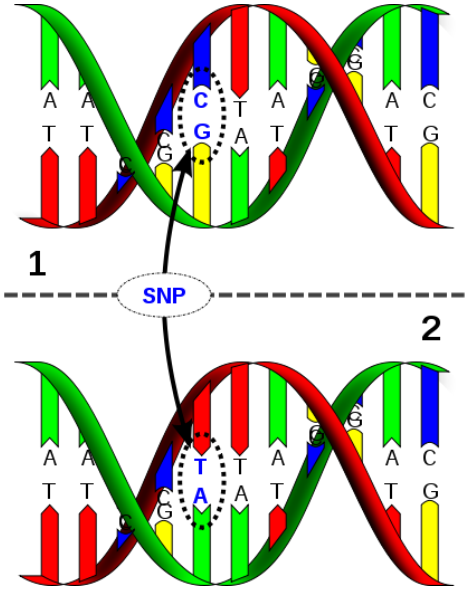
How do we study the genetics of ADHD?

- One approach is called the CANDIDATE GENE APPROACH
- This approach selects genes of interest based upon knowledge of the disorder
- In the case of ADHD we know that stimulants like Ritalin are effective in treating ADHD
  - We look for genes that are involved in the therapeutic action of stimulants
  - DOPAMINE
  - NORADRENALINE

How do we study the genetics of ADHD?

- By comparing the frequency of mutations in a gene in a sample of children with ADHD compared to controls, we can determine whether a gene is “ASSOCIATED” with ADHD.

Single nucleotide polymorphisms (SNPs)



AAGC**C**TA

C- Allele

Individual 1 differs from 2 at a single Base-pair location

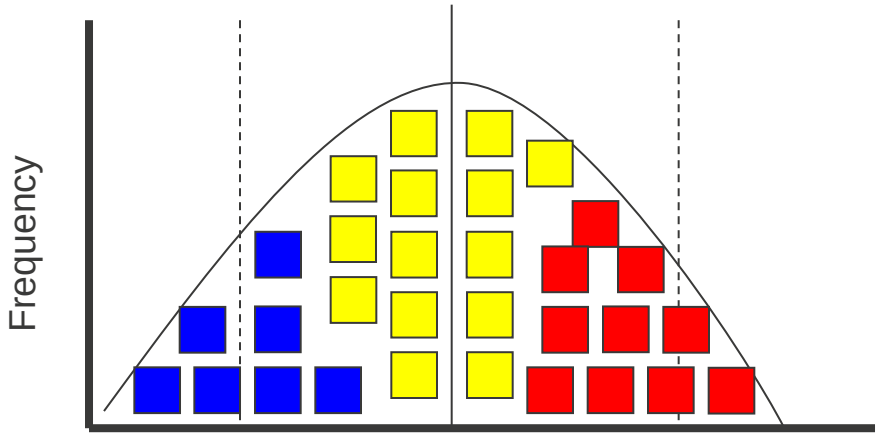
C / T SNP.

AAGC**T**TA

T-Allele

Within a Population, you have:

- C/C genotypes
- C/T genotypes
- TT genotypes



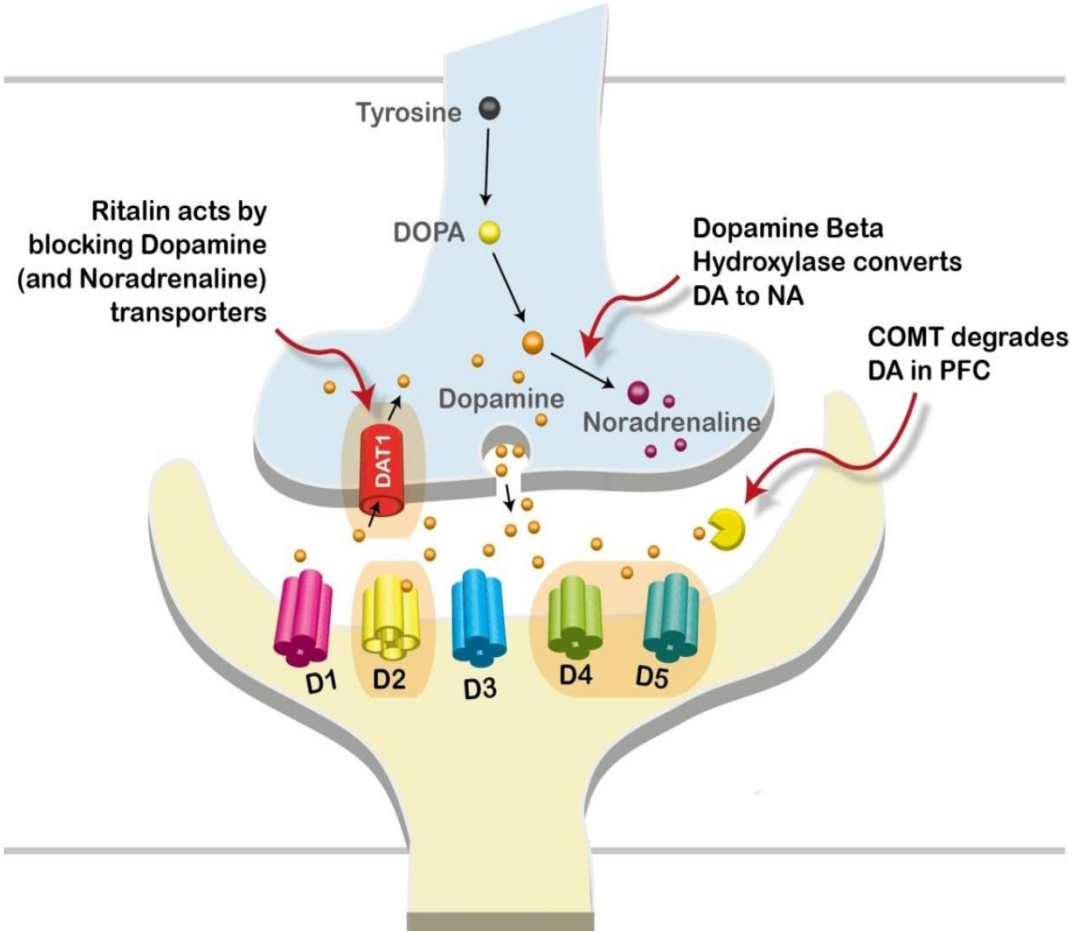
Non-ADHD — Inattention — **ADHD**

Candidate Gene Studies of ADHD: clues from pharmacology

Susceptibility Genes

- DAT1
- DRD4
- DRD5
- SNAP-25
- 5HTT
- HTR1B

- Small effect sizes



DAT1 gene variants influence ADHD symptoms in 517 non-clinical adults

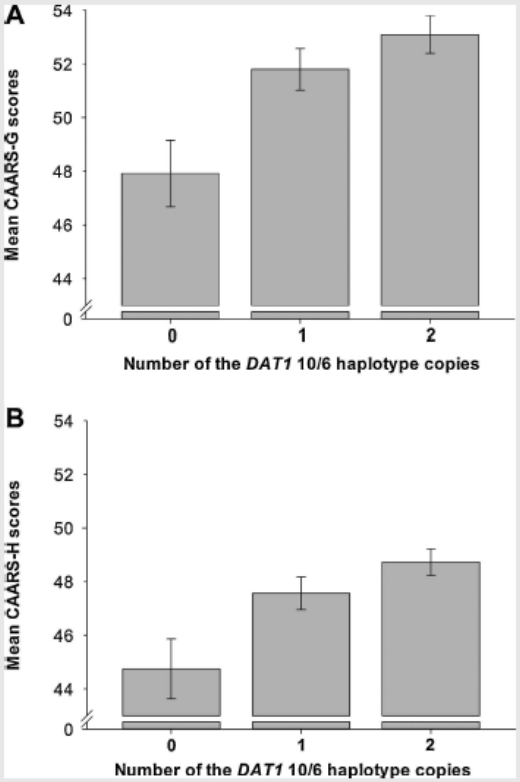


Fig. 1. Additive DAT1 10/6 haplotype effect on CAARS-G and CAARS-H in healthy adults. A and B showed increased DAT1 haplotype effect of the ADHD-risk associated 10-repeat of VNTR at 3' UTR and 6-repeat of VNTR at intron 8 of DAT1 correlates on CAARS-G and CAARS-H, respectively. Error bars denote the standard error of the mean.

Tong et al, AJMG: Neuropsychiatric Genetics, 2015

Additive increases in self-report ratings of ADHD like symptoms as a function of DAT1 gene variants

Leading candidate genes in ADHD- Hawi et al 2015.

Table 1. Candidate genes showing replicated evidence of association with ADHD

Gene	*Associated variant*	*Location*	*Biological function*	*References*
SLC6A3	40 bp VNTR	3′ UTR	Regulator of extracellular dopamine and mediates the reuptake of dopamine from the synapse.	Cook *et al.*[91a]; Gizer *et al.*[92b]
DRD4	48 bp VNTR	Exon	GPCR activated by the neurotransmitter dopamine.	La Hoste *et al.*[93a]; Gizer *et al.*[92b]
DRD5	148 bp dinucleotide repeats	5′ flanking	Transduces extracellular signals in the form of dopamine into several intracellular responses, including effects on adenylate cyclase, Ca^{2+} levels and K^{+} conductance.	Daly *et al.*[94a]; Gizer *et al.*[92b]
SLC6A4	40 bp indel	5′ flanking	A member of a transporter family that is Na^{+} and Cl dependent. Mediates the reuptake of serotonin from synapses.	Manor *et al.*[95a]; Gizer *et al.*[92b]
HTR1B	rs6296	Exon1	GPCR for serotonin. A prime target for antidepressant drugs and psychoactive substances	Hawi *et al.*[96a]; Gizer *et al.*[92b]
SNAP25	rs3746544	3′ UTR	Plasma membrane protein essential for synaptic vesicle fusion and neurotransmitter release	Brophy *et al.*[97a]; Gizer *et al.*[92b]
SLC9A9	Inversion breakpoints	Region 3p14—q21	A member of large solute carrier family 9. Acts in electroneutral exchange of hydrogen/sodium ions across membranes.	de Silva *et al.*[98a]; Lasky-Su *et al.*[21c]; Mick *et al.*[23c]
LPHN3	Haplotype encompassing exons	Exon 4–19	Encodes a member of the latrophilin subfamily of GPCR. May act in signal transduction and cell adhesion.	Arcos-Burgos *et al.*[99a]; Ribases *et al.*[100d]
GIT1	rs550818	Intron	GPCR kinase. Thought to be involved in vesicle trafficking, cell adhesion and increasing the speed of cell migration. Overexpression of GIT1 is known to regulate the beta2-adrenergic receptor.	Won *et al.*[101a]
NOS1	180–210 bp CA repeat	Exon	Mediates several biological processes including neurotransmission and is reported to associate with neurodegenerative conditions.	Reif *et al.*[102a]; Franke *et al.*[103c]

Abbreviations: ADHD, attention deficit hyperactivity disorder; GPCR, G-protein-coupled receptors; GWAS, genome wide association studies; UTR, untranslated region; VNTR, variable number tandem repeat. [a]First reported by. [b]Meta-analysis article. [c]GWAS finding. [d]Association in large sample or validation using animal model.

Genome Wide Association Designs vs. Candidate Gene

- The Human Genome Project aimed to identify sources of genetic variation between individuals that could be used to map disease and quantitative traits
- As a result we are now able to interrogate the whole genome for association with traits, such as cognitive ability.
- GWAS is a discovery platform and is hypothesis free, meaning that no *a priori* knowledge about a gene is needed for it to be linked to a trait
- High throughput genotyping platforms can now type literally 100,000s of SNPs with analyses testing variation in each SNP (0.vs.1vs. 2 copies of an allele) against the phenotype, across the whole genome.
- **Problem?**

Genome Wide Association Designs vs. Candidate Gene

- The vast number of statistical tests performed between the SNPs across 30,000 genes and the trait measure means that the potential for Type I error is vastly inflated
- In order to keep the experiment error at α=0.05, a significance value of 10e-0.08 is required
  - 0.00000010

GWAS in ADHD

- 7 GWAS in childhood ADHD (4 family based; 2 case-control; 1 quantitative trait)
- No SNP association at GWAS significance ($p \leq 10–8$).
- Reasonable evidence for a SNP in Cadherin 13
- Numerous hits in the $p \leq 10–5$ range which may informative in larger samples

Contribution of common variation to the heritability of ADHD

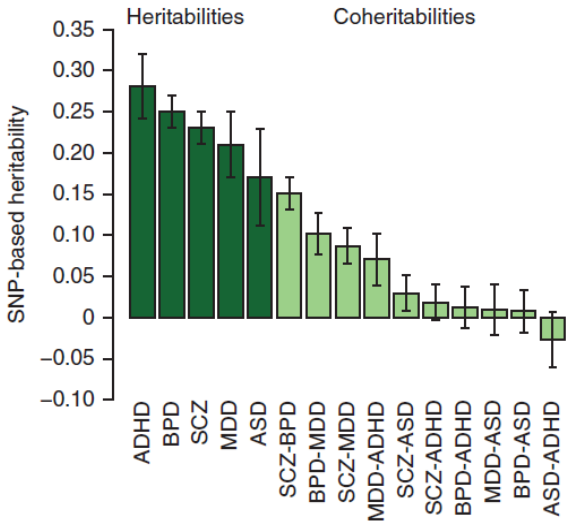
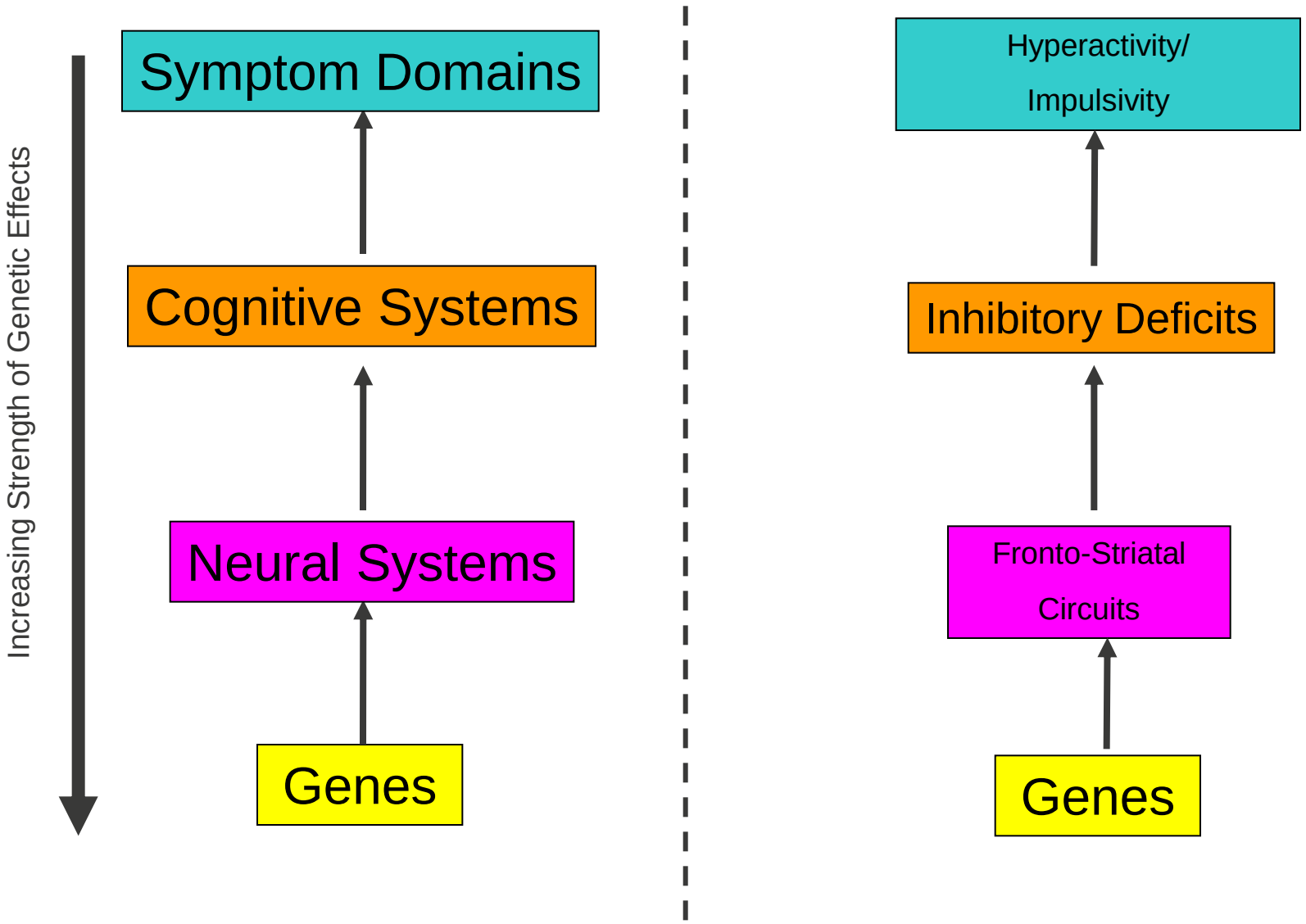


Figure 1 Evidence for genome-wide pleiotropy between psychiatric disorders. Proportion of variance in liability (SNP-based heritability) and proportion of covariance in liability between disorder (SNP-based coheritability) for five major psychiatric disorders. The 95% error bars represent the estimates ± 1.96 s.e. SCZ, schizophrenia; MDD, major depressive disorder; BPD, bipolar disorder.

- Strong contribution of common variation to heritability of ADHD (SNP-based heritability of 0.28)
- **GWAS sig hits for ADHD should emerge with larger sample sizes.**
- Less than heritability estimates from twin studies (~0.75)
- **Suggests potential contribution from rarer DNA variants**

Endophenotypes for ADHD

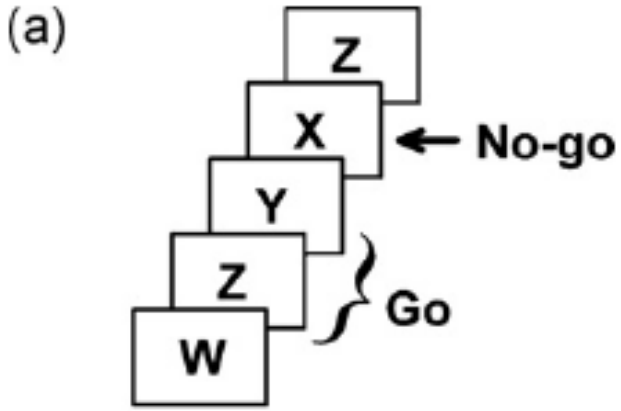


Executive function- response inhibition

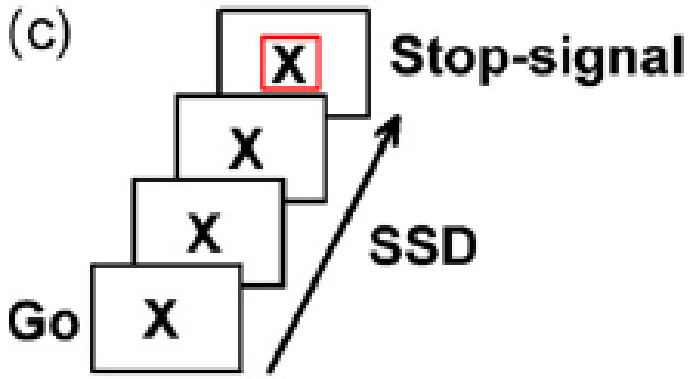
- Is an aspect of executive control that refers to the ability to inhibit action when it is no longer appropriate
- Usually measured using variants of the Go/No-go task or the stop-signal task

Measuring Inhibition

Go/No-Go



Stop-Signal

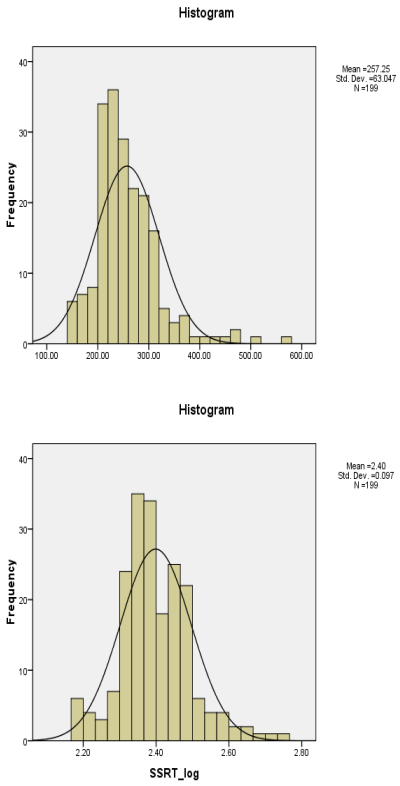


Commission Errors (% correct inhibition)- Inhibition
Omission Errors- Sustained Attention
Reaction Time Variability- Cognitive Control

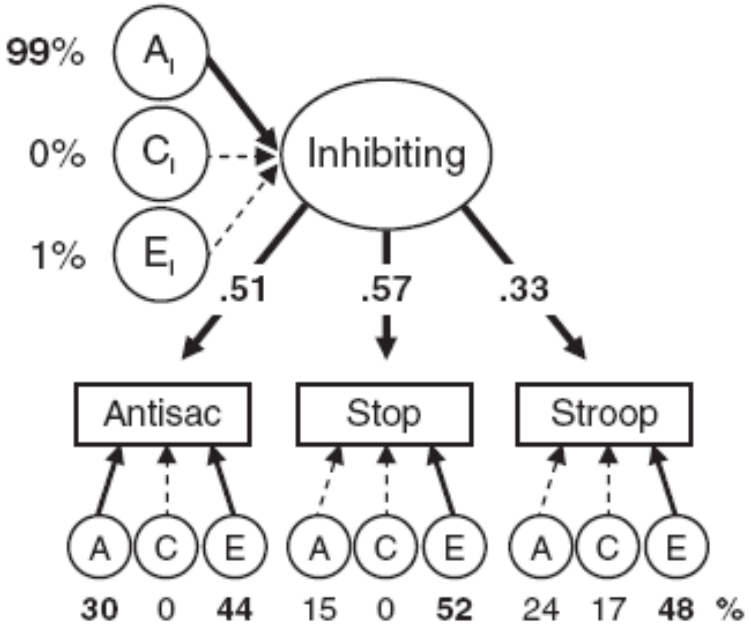
Stop-Signal Reaction Time (SSRT)- Speed of Inhibition

Behaviour Genetics of Inhibition

- Twin studies demonstrate high heritability for measures of response inhibition



Stop-signal Reaction Time (SSRT) can be transformed to a normal distribution



Freidman et al, 2008

Inhibitory deficits as a familial marker of ADHD

TABLE 2. Family History, Psychosocial Risk, and Neurobiological Risk in Children With Attention Deficit Hyperactivity Disorder (ADHD), Classified by Level of Inhibition,[a] and Healthy Comparison Children

Risk Variable	ADHD Group (N=54): Children With Poor Inhibition (N=27)		ADHD Group (N=54): Children With Good Inhibition (N=27)		Healthy Comparison Children (N=26)		Analysis
	N	%[b]	*N*	%[b]	*N*	%[b]	χ^2 (*df*=2)
Family history of ADHD	13	48.1	5	18.5	2	7.7	12.60*
Mother	5	18.5	1	3.8	1	3.8	4.57
Father	7	25.9	4	15.4	2	7.7	3.35
Sibling	5	18.5	1	3.8	1	3.8	4.57

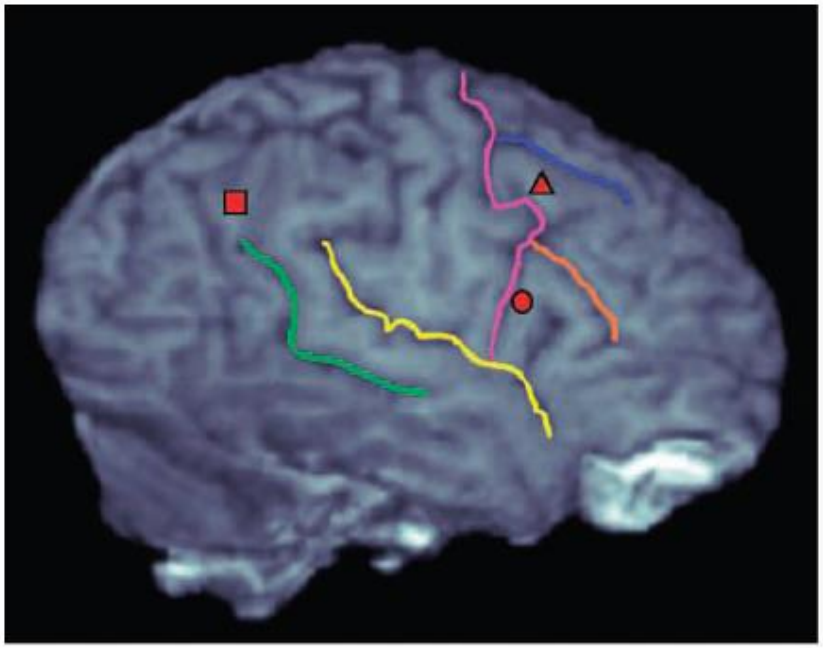
TABLE 2. Demographic Characteristics and Mean Stop-Signal Reaction Time Score for Children With ADHD and Their Biological Family Members Compared With Unrelated Healthy Comparison Groups of Children and Adults

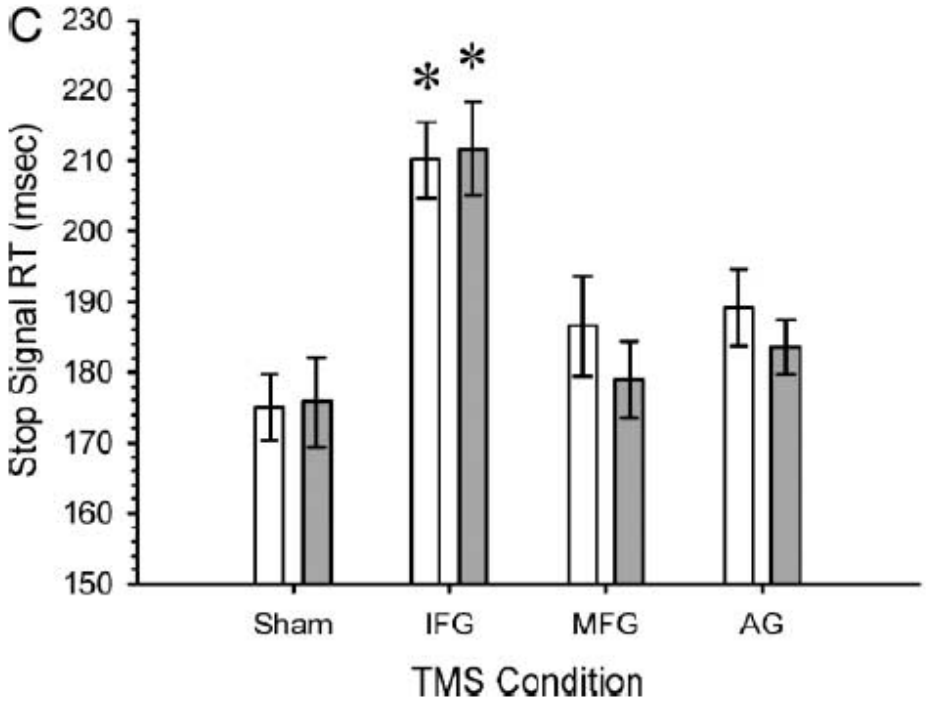
Characteristic	ADHD Children (N=79)		Unaffected Siblings (N=34)		Comparison Children (N=63)		Parents (N=104)		Comparison Adults (N=88)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age[a] (years)	9.1	2.1	9.8	2.8	9.9	2.8	42.83	4.9	41.38	8.3
IQ[b]	99.6	11.3	106.1	11.3	119.6	10.6				
Stop-signal reaction time[c] (msec)	354.6	154.7	298.2	130.3	263.2	105.2	251.1	69.6	205.9	53.6
	N	%	N	%	N	%	N	%	N	%
Male	62	79	16	47	28	44	32	31	41	47

Schachar et al, 2005

Cognitive Neuroanatomy of Response Inhibition

Transranial Magnetic Stimulation (TMS) of frontal cortex disrupts inhibition

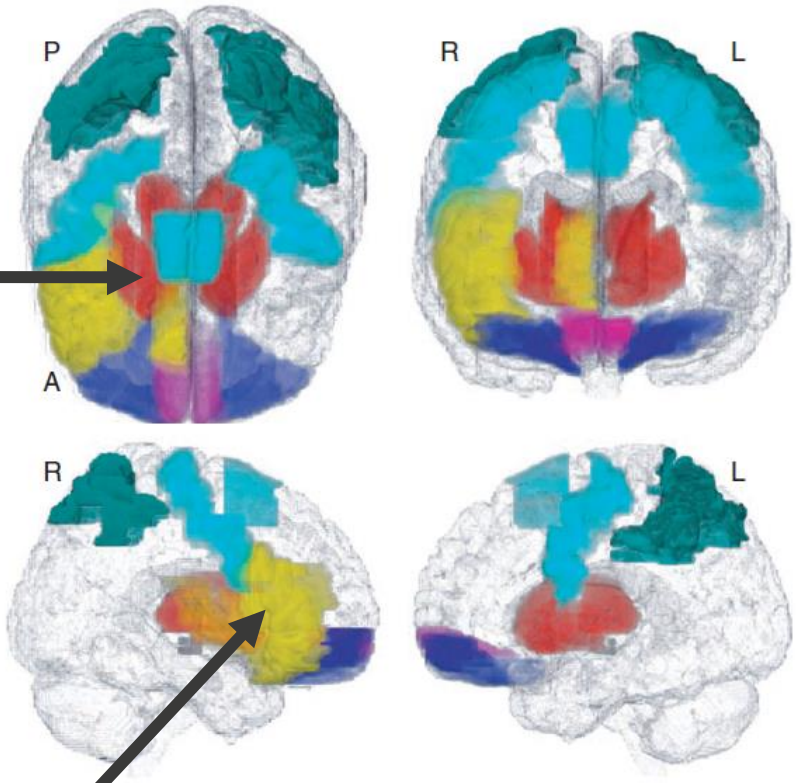




Chambers et al, 2006, JOCN

Brain Imaging

BG Stop Network



R Frontal Stop Network

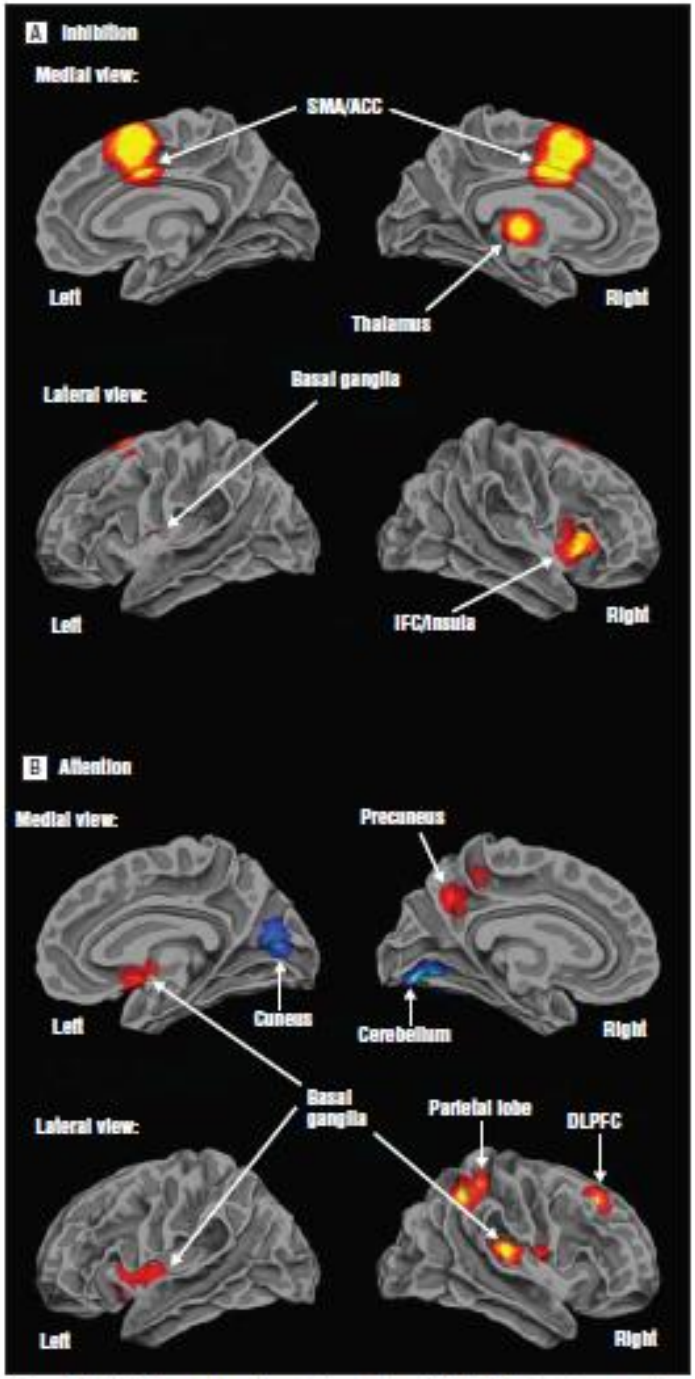
1,896 14 Year Old Adolescents

Whelan et al, 2012, Nat Neuro

Meta-analysis shows that response inhibition deficits are reliable in ADHD

Table 1. Illustrative Widely Used Neuropsychologic Measures Comparing ADHD (Combined Type) to Controls: Group Differences and Percent Impaired in 3 Samples

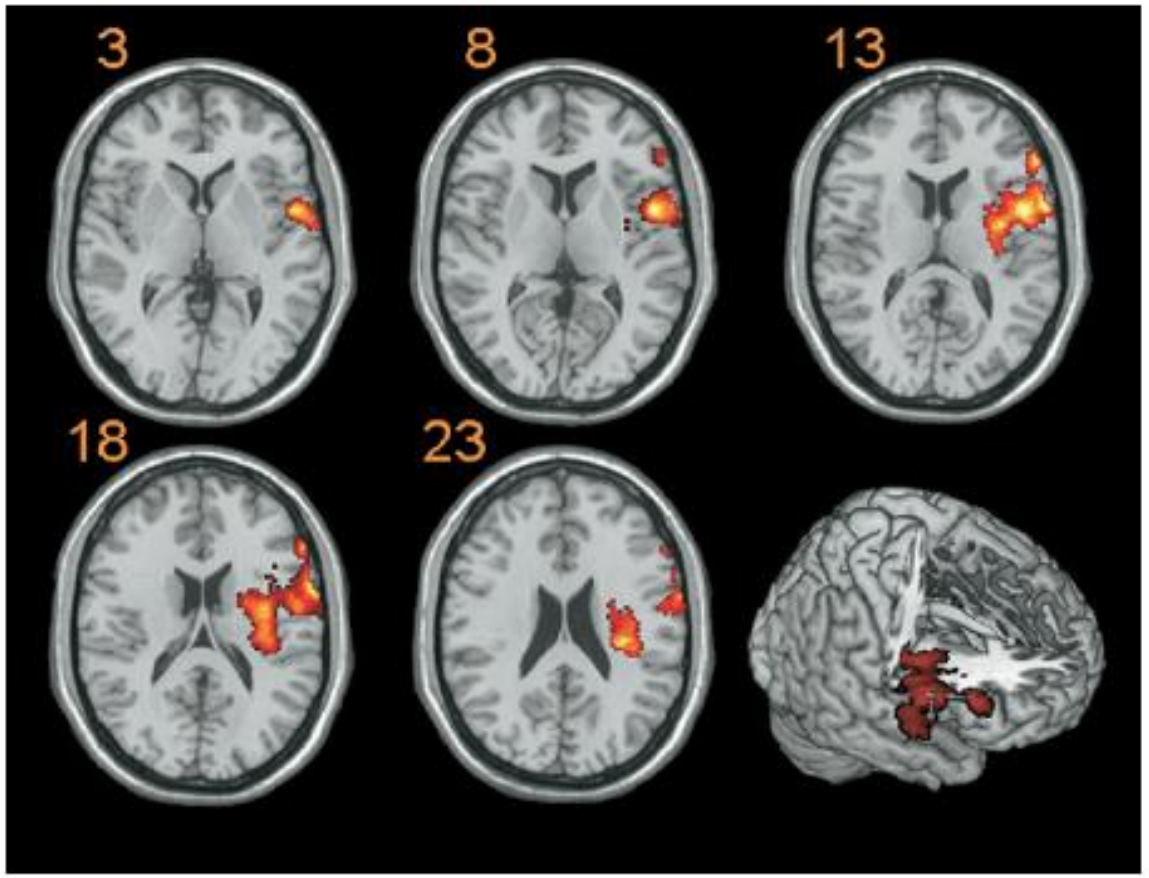
Measure	Sample	Effect Size (d)			% ADHD Beyond Control 90th Percentile
		d	η^2	*p*	
SSRT	MI (all)	.88	.133	<.001	51
	CO	.79	.101	<.001	45
RT Variability	MI	.75	.123	<.001	48
	CO	.77	.125	<.001	44
Stroop CW	MI	.50	.045	<.05	25
	CO	.84	.132	<.001	44
	MGH	.62	.09	<.001	25
CPT	MI	.91	.11	<.001	37
	CO	.54	.053	<.001	35
	MGH	.17	.01	.11	16
Trailmaking	MI	.35	.033	<.05	27
	CO	.35	.031	<.01	24



Meta analysis of functional brain imaging ADHD studies (Rubia et al)

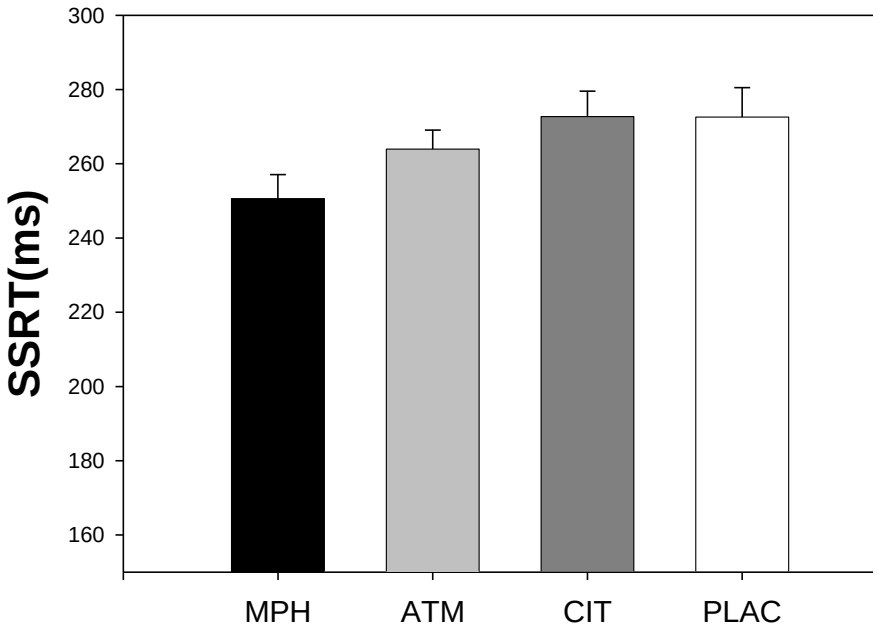
- Decreased activity in inhibition networks
- Decreased activity in attention networks

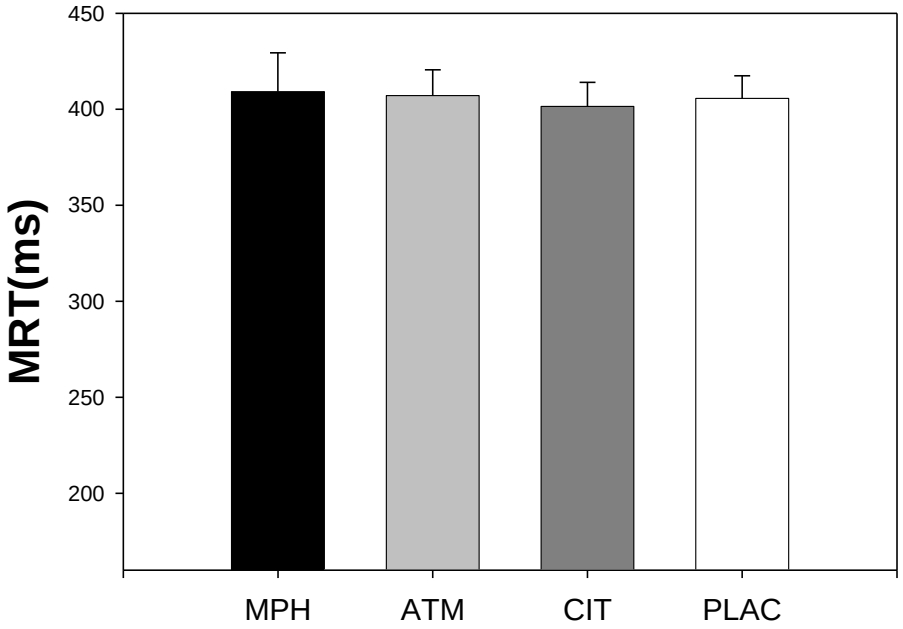
Atomoxetine improves inhibitory control and modulates IFG activity



Chamberlain et al 2008

MPH enhances inhibition
Nandam et al 2011, Biol Psychiatry





[F(3,69)=5.52, p=0.002]

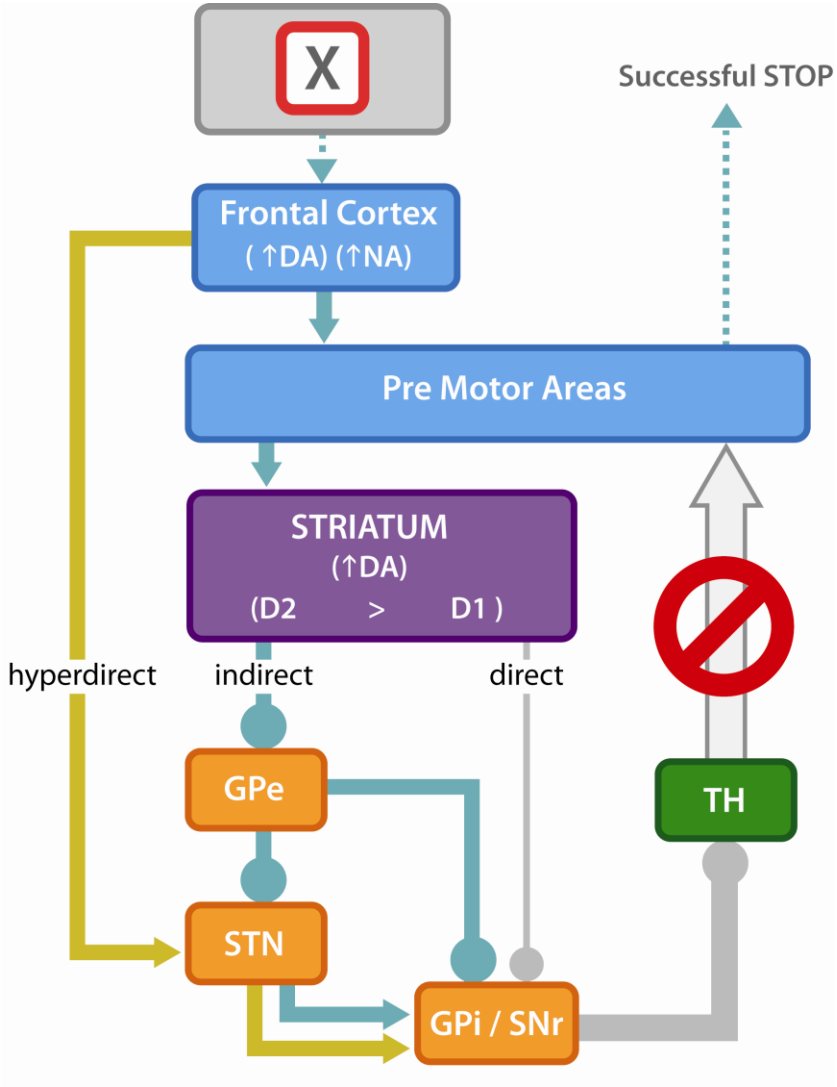
MPH<ATM, p<0.05

MPH< CIT, PLAC, p<0.01

[F(3,69)=0.14,p=0.935].

Both dopamine and noradrenaline appear important for inhibitory control

Neurochemistry of Inhibition



NET1, D4, D2,
α-2,

DAT1, D2

Molecular Targets Become Candidate Genes for Genetic Association

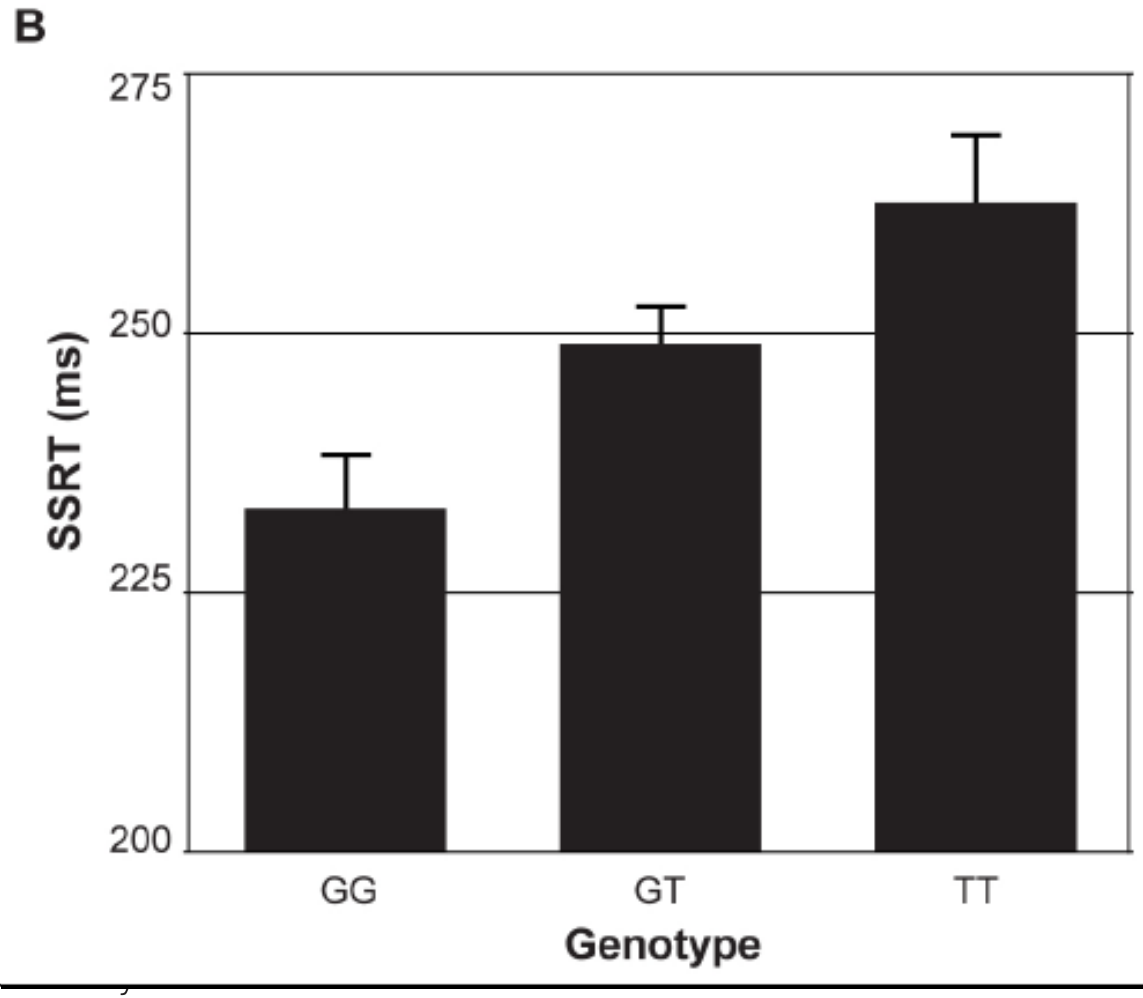
With Inhibition

Genetic Association Study of Inhibition

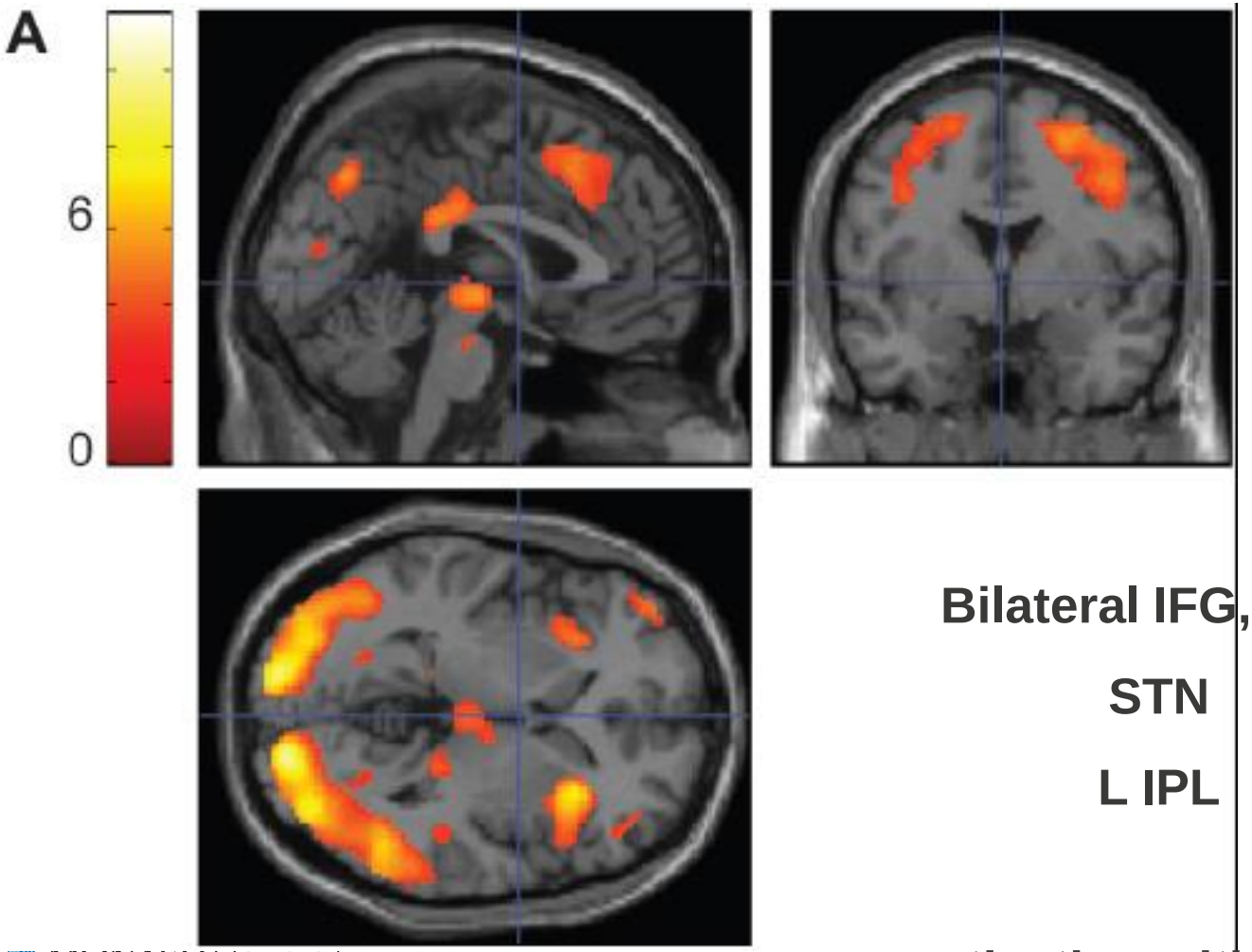
Cummins et al Mol Psych 2012

SNP ID	MAF	p value SSRT	p - GoRT	p - SDGoRT
rs40358	.14	.21	0.043*	0.56
rs37020	.45	.0002**	0.31	0.11
rs10053602	.23	.49	0.57	0.51
rs393795	.22	.0012*	0.065	.037*
rs11737901	.36	.007*	0.57	0.72
rs460000	.23	.0004**	0.086	0.02

Additive influence of T allele of DAT1 rs37020 on SSRT



Imaging Genetics of Inhibition



Bilateral IFG, MFG

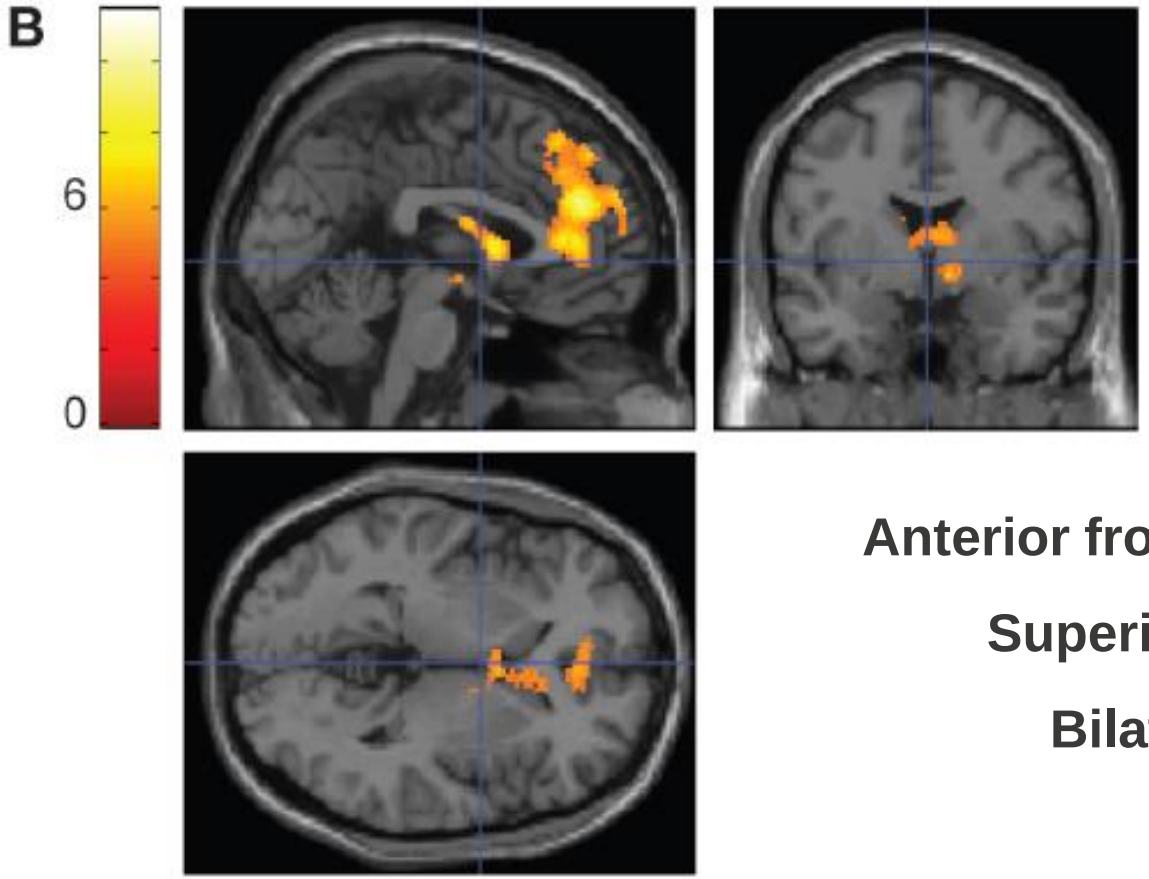
STN

L IPL

↑activation with ↓SSRT

Imaging Genetics of Inhibition

Influence of rs37020 genotype



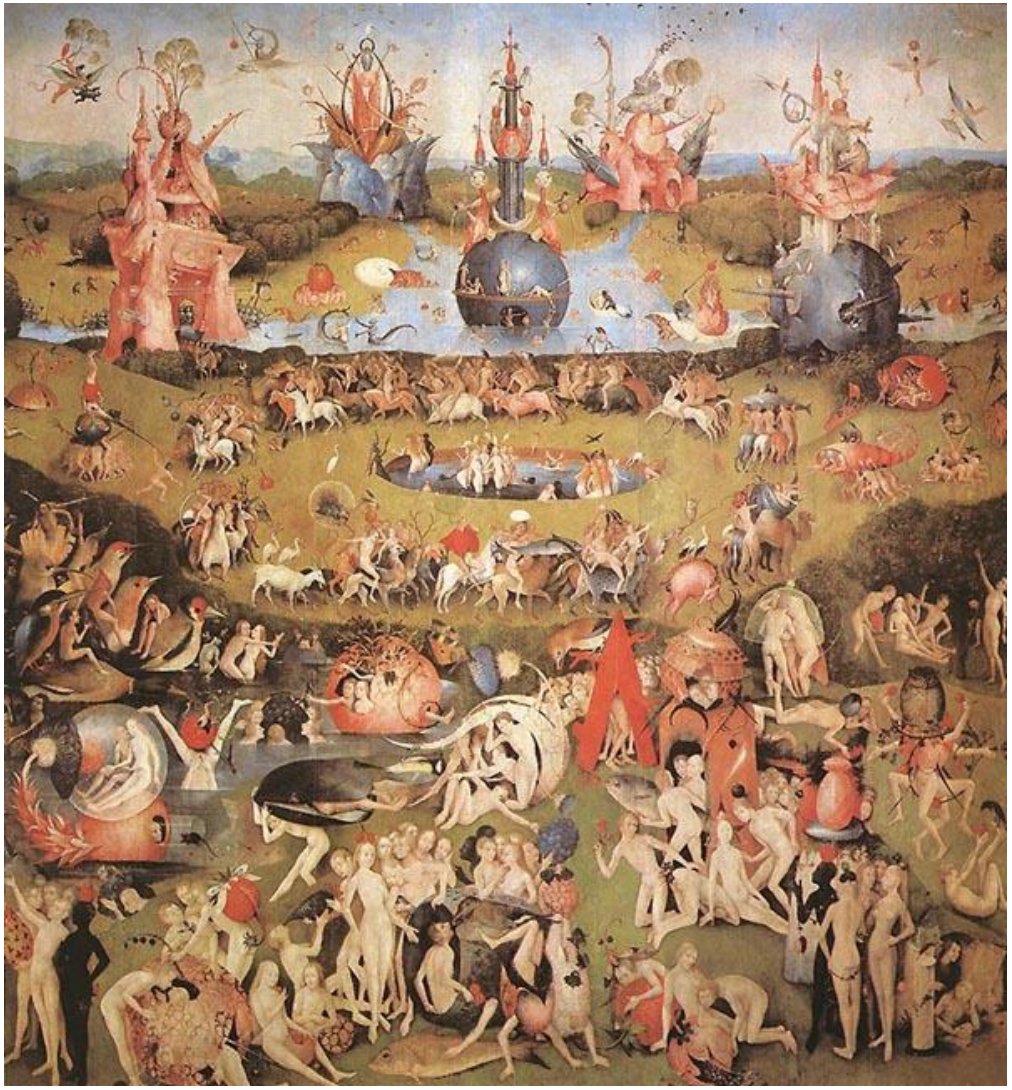
Anterior frontal, superior frontal

Superior medial gyrus

Bilateral Caudate

Inhibition-related activity increased additively from TT to GT to GG genotype

Spatially Selective Attention

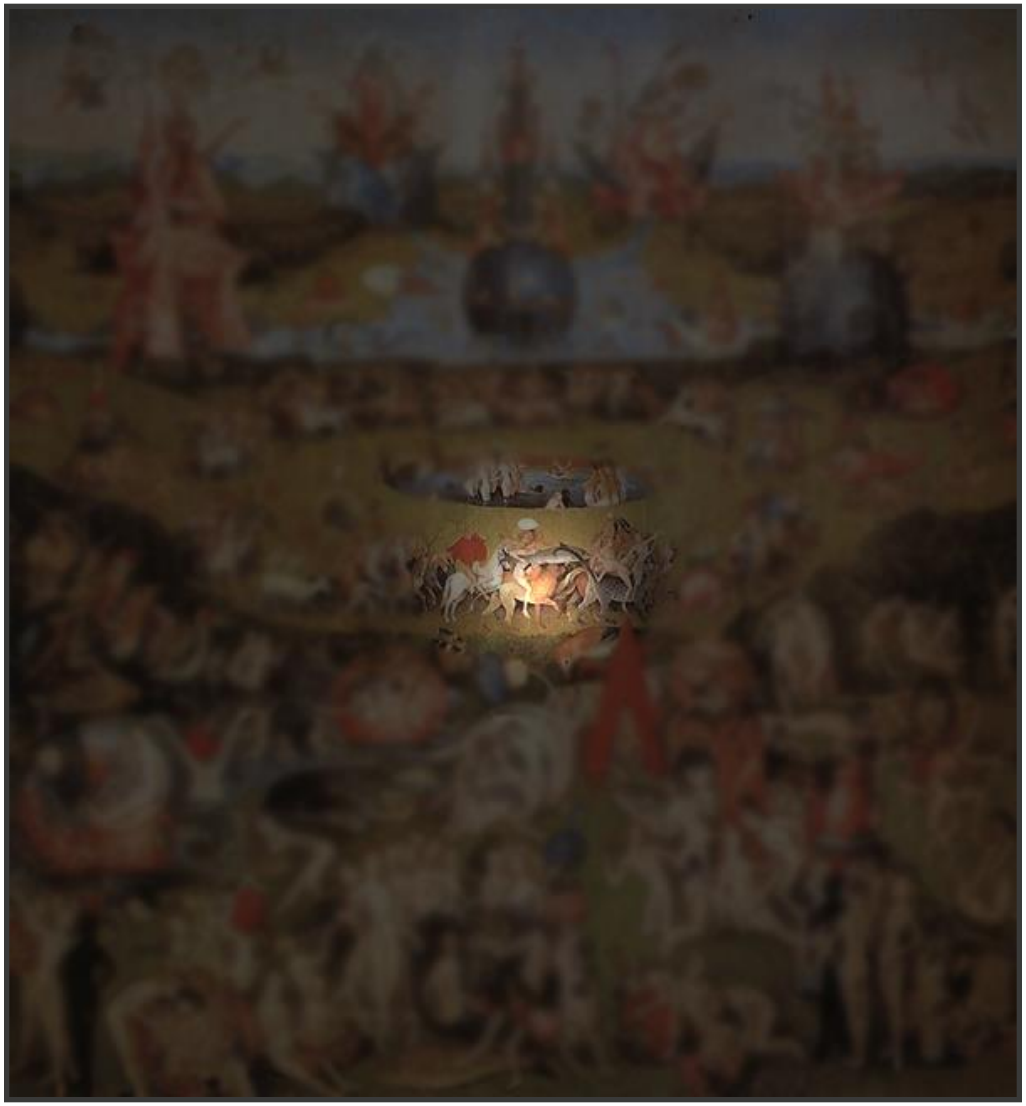


Attention is
spatially selective

Spatial selection can
occur **covertly**

– without eye movements

Spatially Selective Attention



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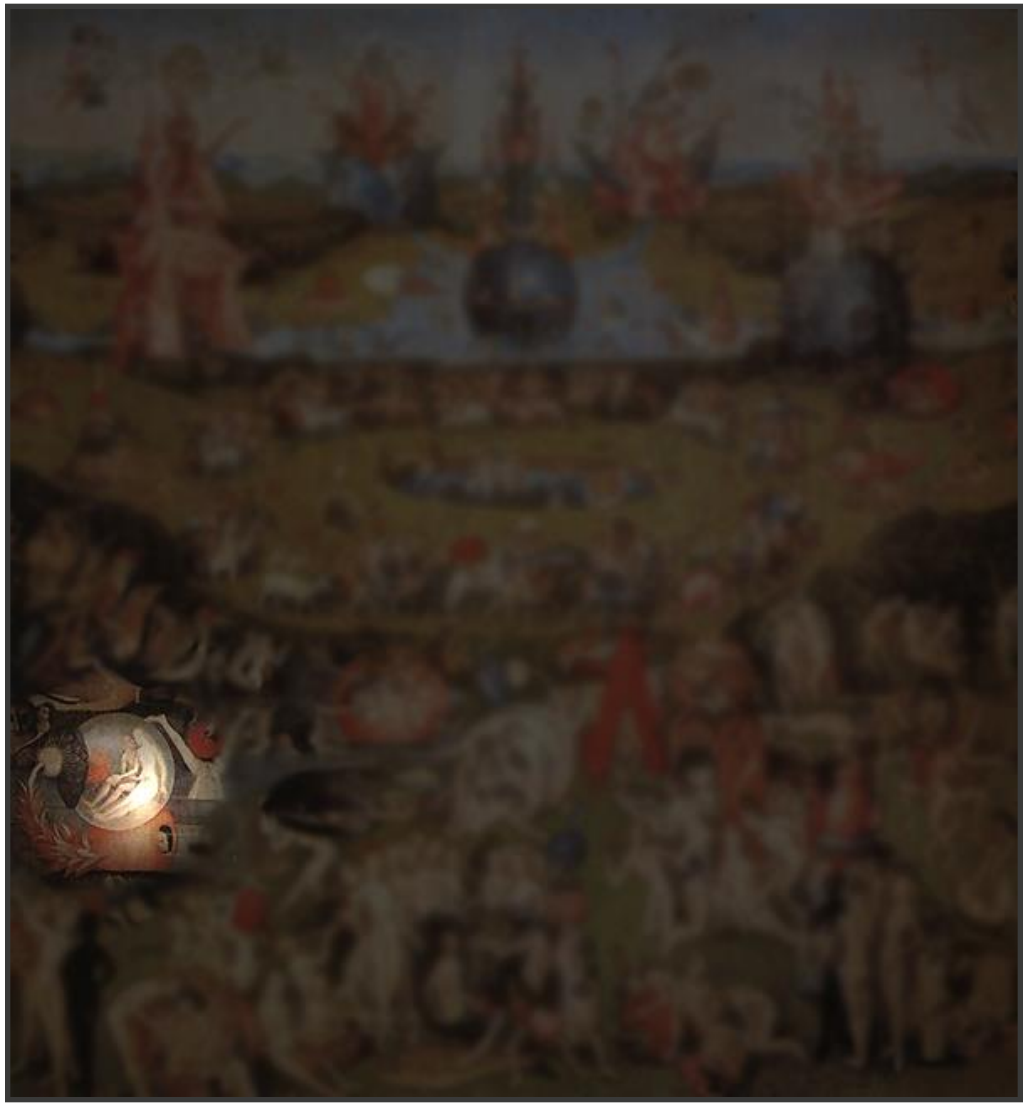
Spatially Selective Attention



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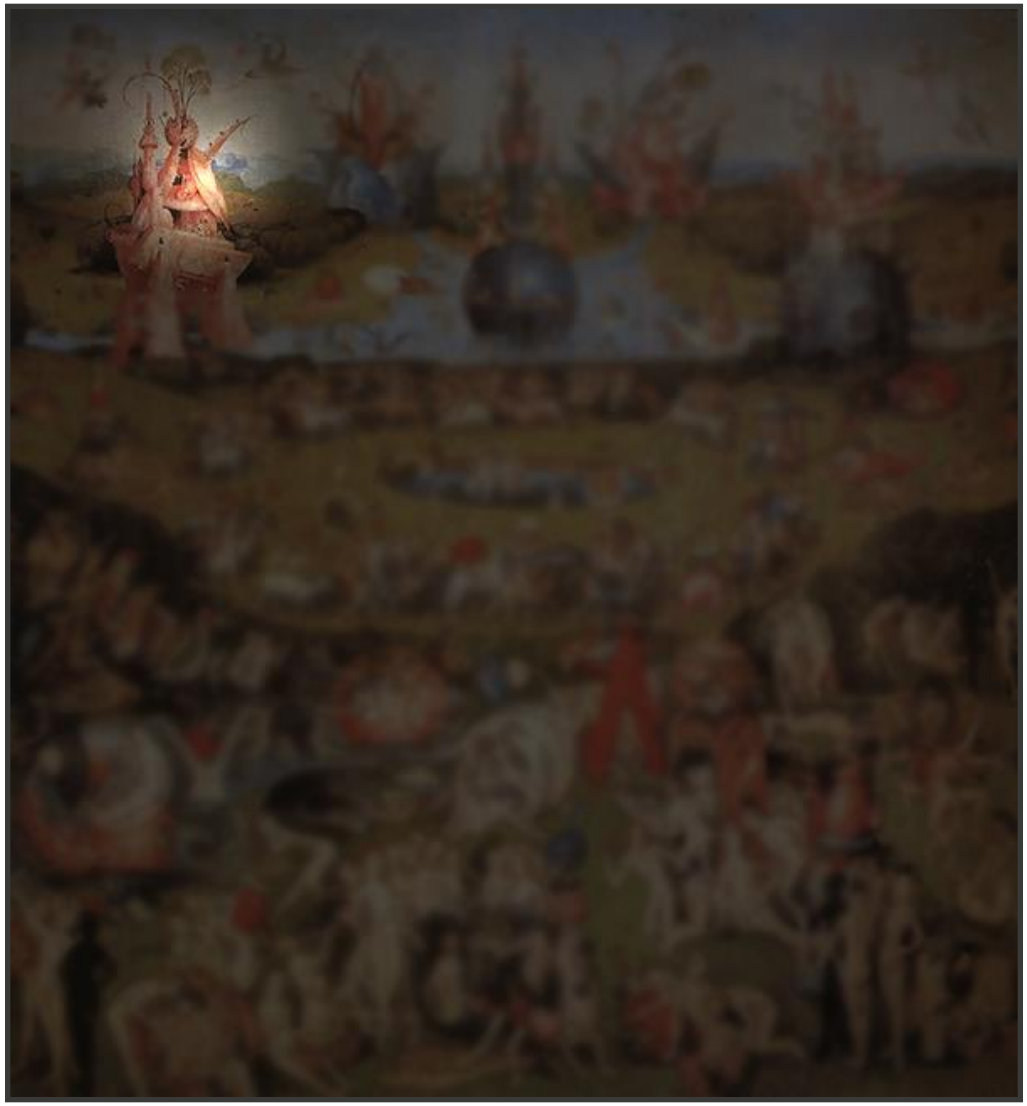
Spatially Selective Attention



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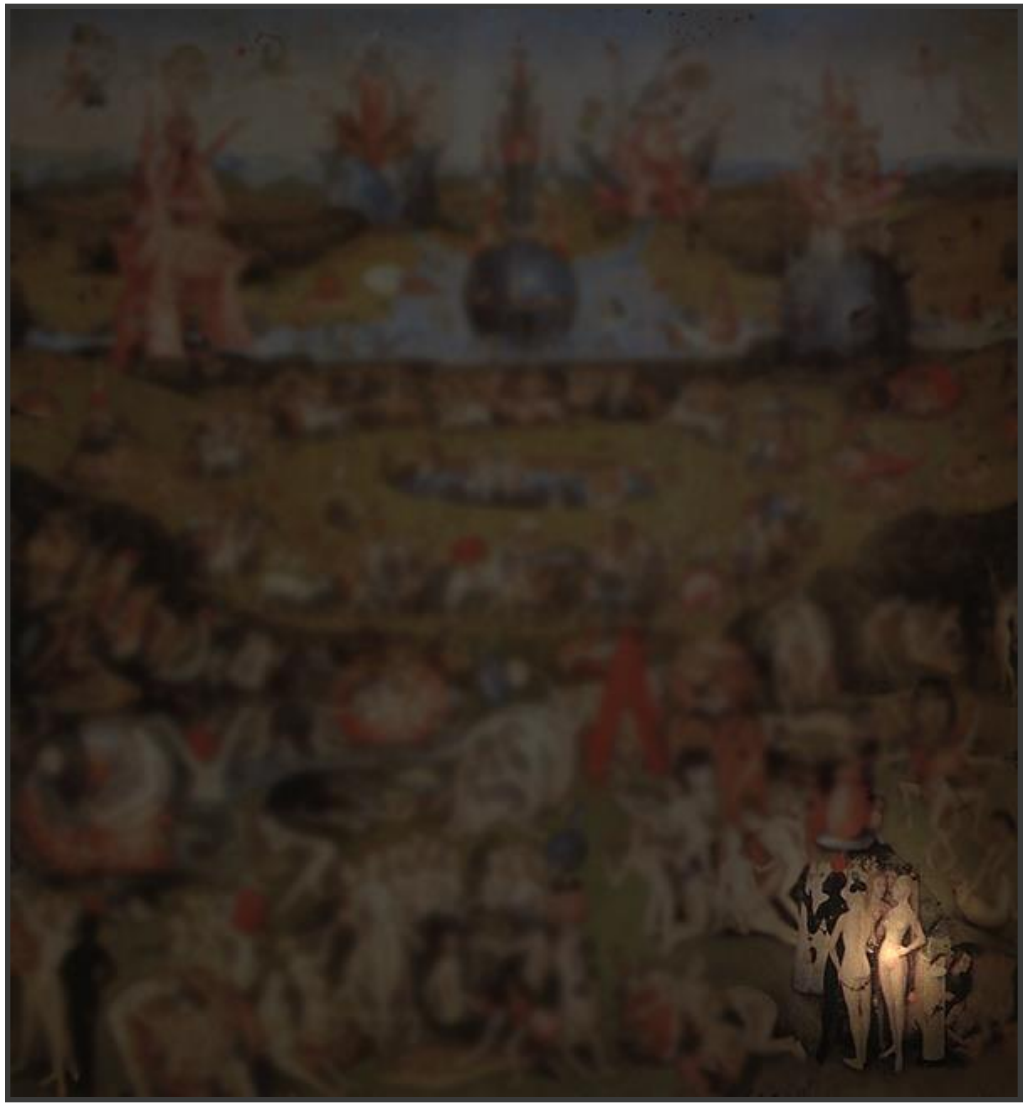
Spatially Selective Attention



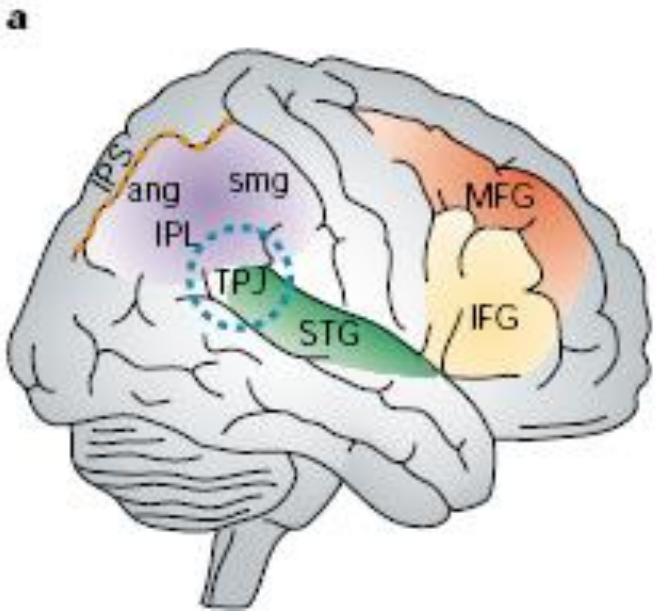
Attention is spatially selective

Spatial selection can occur **covertly**

– without eye movements

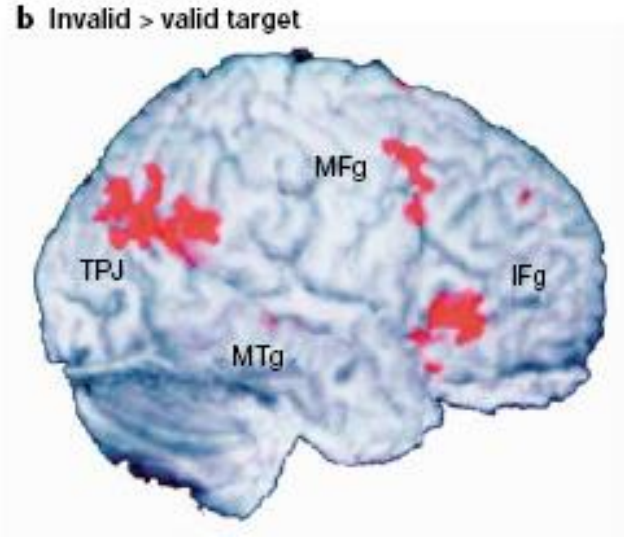
Neural Correlates of Spatially Selective Attention

Anatomy of Neglect



•Unilateral neglect arises typically from damage to RH regions, including TPG, STG and IFG, but also striatal areas.
•Ipsilateral bias of attention and reorienting deficits to contralateral space.

Neuroimaging of spatial attention



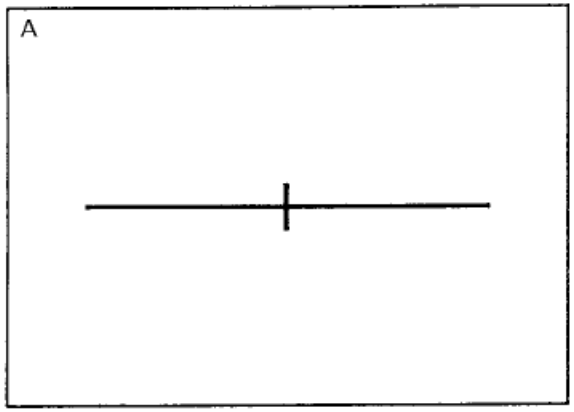
•Spatial reorienting to unattended targets activates a broad, largely bilateral network
•Activity within the TPJ appears more strongly lateralised to RH

Spatial selective attention and ADHD

- **Voeller and Heilman (1988) first proposed that ADHD could be a "neglect syndrome"**
  - **ADHD children made more left-sided errors resembling patients with right-hemisphere lesions**

- **Sheppard et al (1999) asked children with ADHD and healthy controls to perform a line bisection task**
  - **ADHD children showed a right bias or *asymmetry***
  - **The right bias resolved with methylphenidate (MPH)**

Line bisection- Subject is asked to Bisect the line



Off med's the ADHD children bisected to the right; the reverse of controls. This resolved with MPH

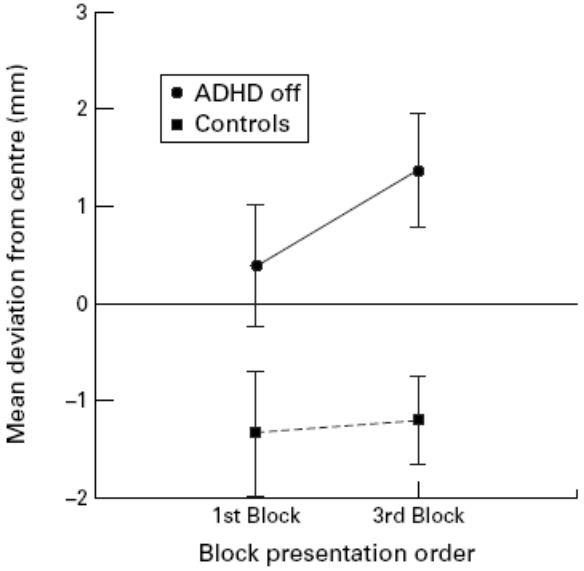


Figure 2 Mean deviation from centre (mm) for each background screen condition for children with ADHD off medication and controls.

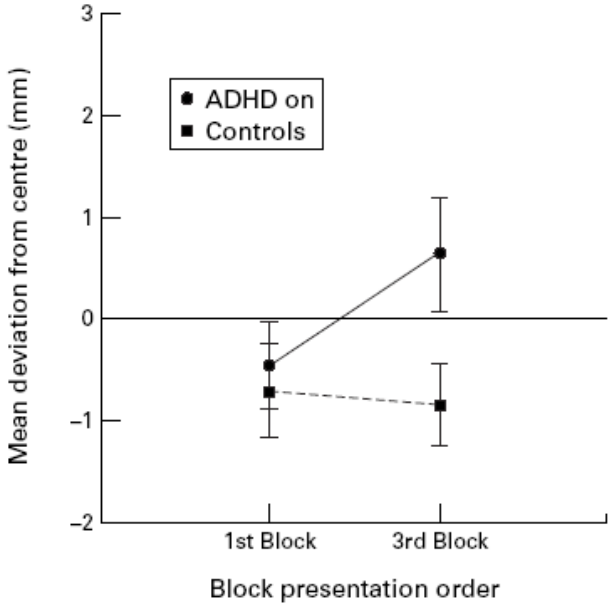
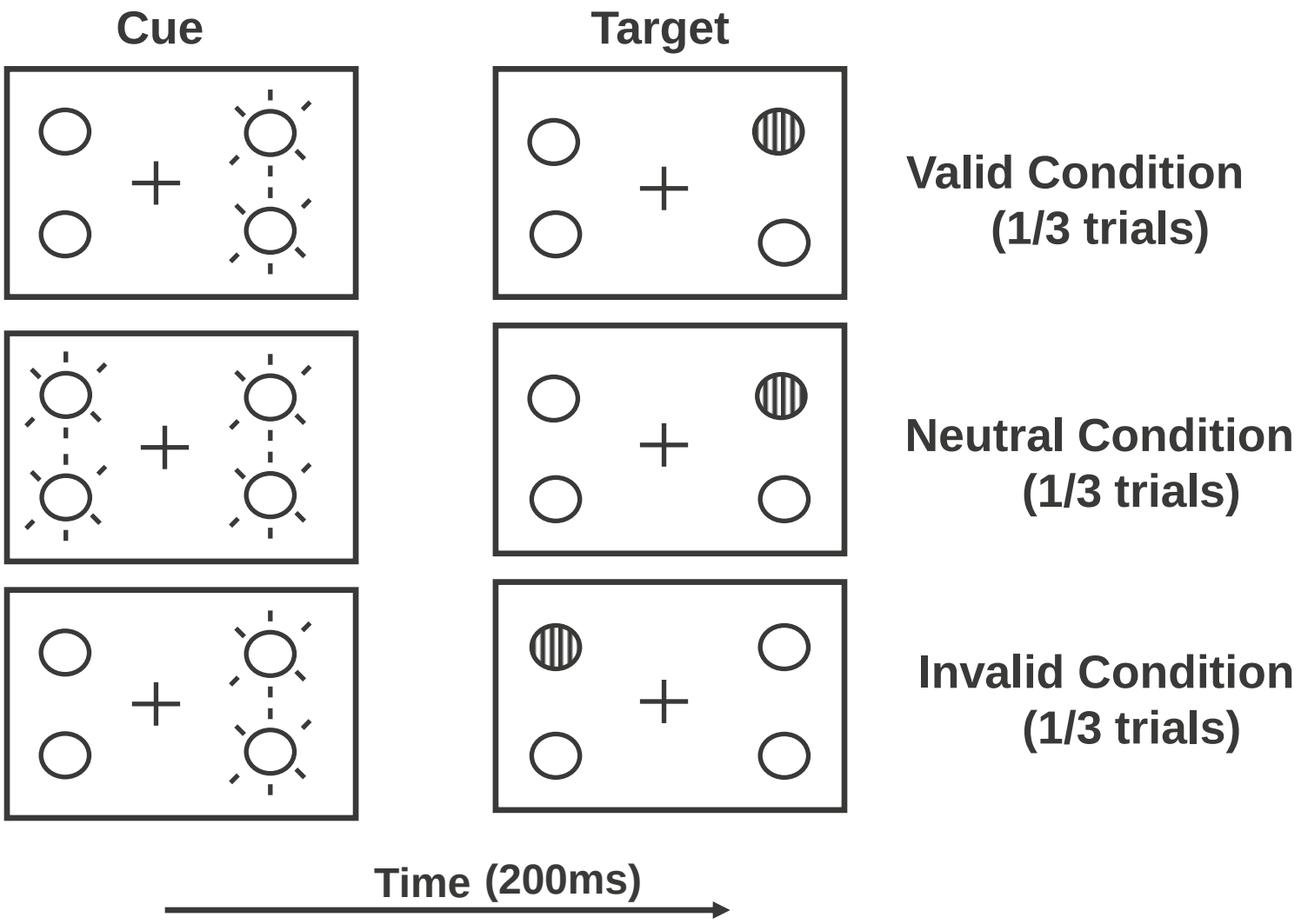


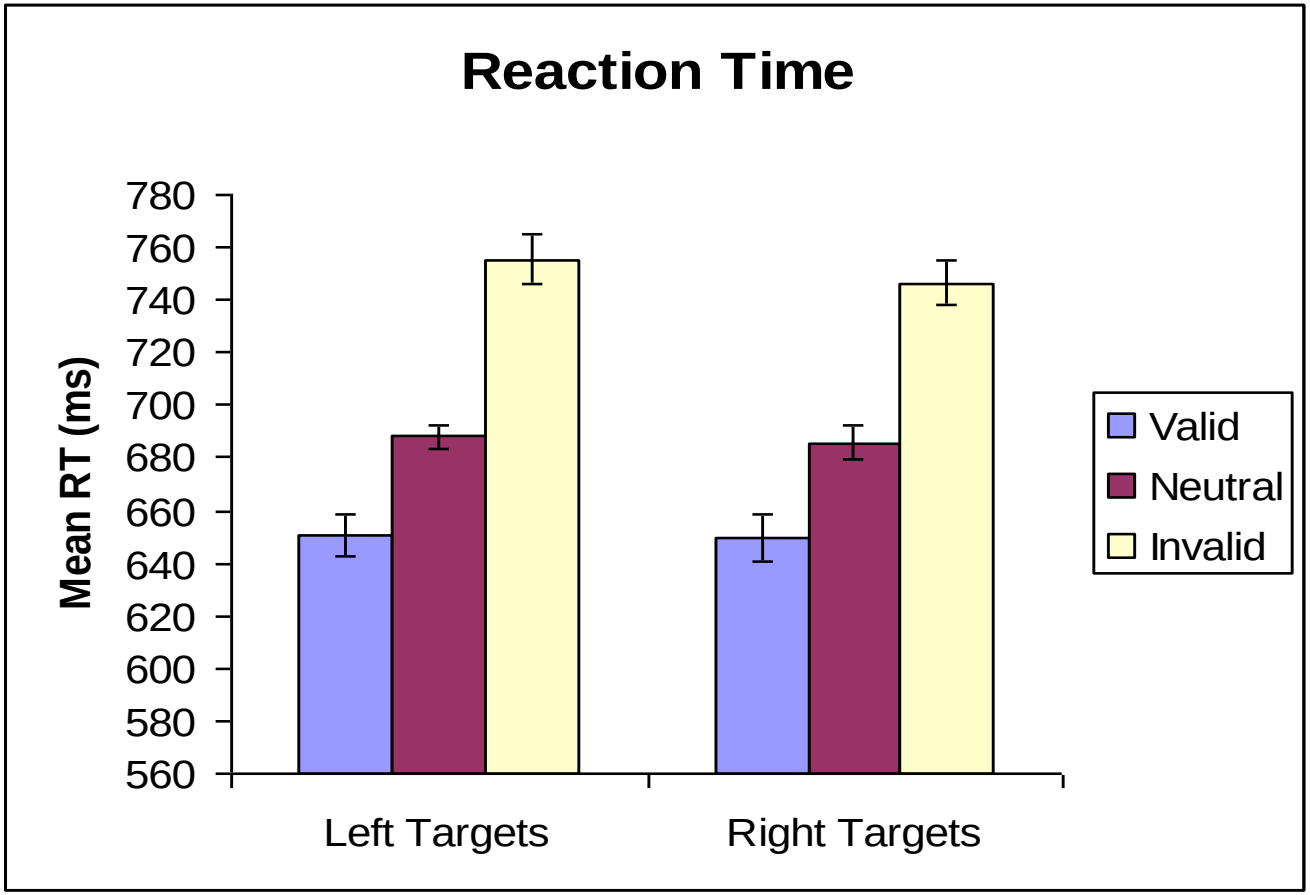
Figure 3 Mean deviation from centre (mm) for each background screen condition for children with ADHD on medication and controls.

Measuring Spatially Selective Attention

Reflexive or Exogenous Cuing



Measuring Spatially Selective Attention



Invalid RT > Neutral RT > Valid RT

Cuing Cost: Invalid RT – Neutral RT: Cost to RT of reorienting attention

Cuing Benefit: Neutral RT- Valid RT: Benefit to RT of spatial orienting

Spatial Selective Attention and ADHD (Bellgrove et al, 2009, Arch Gen Psych)

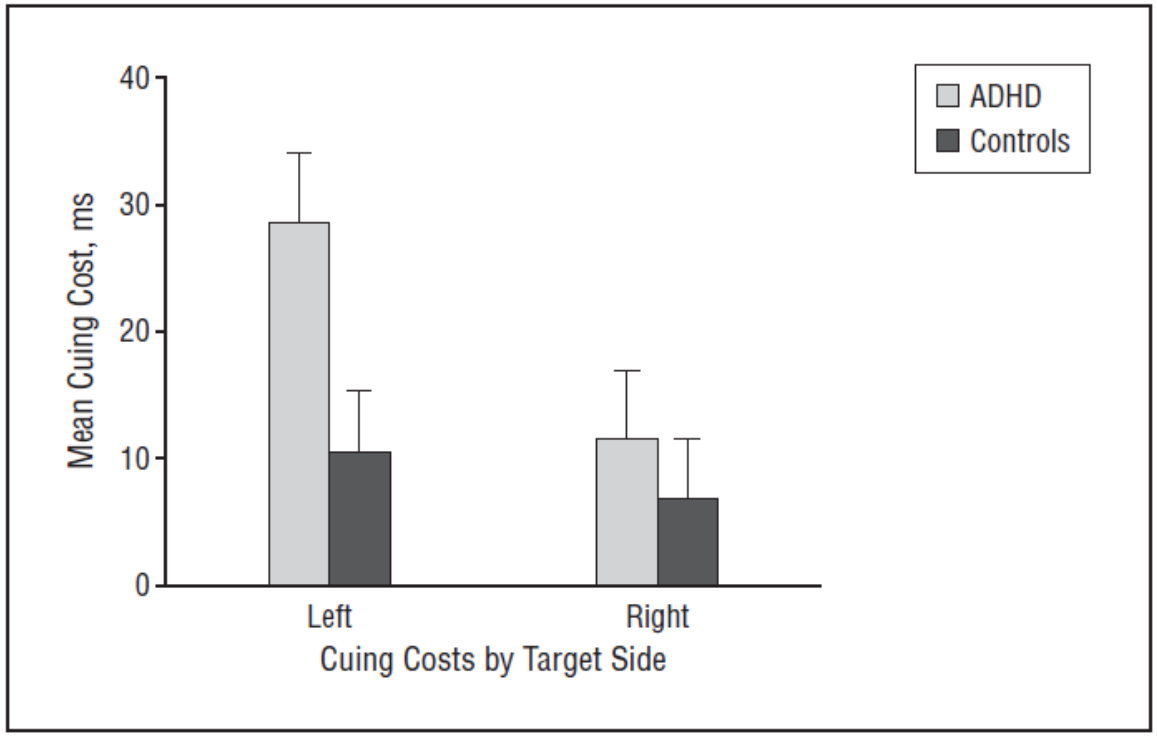


Figure 2. Mean cuing cost (invalid reaction time – neutral reaction time) as a function of target side and diagnosis at the 200-millisecond stimulus onset asynchrony for the exogenous cuing task. ADHD indicates attention-deficit/hyperactivity disorder.

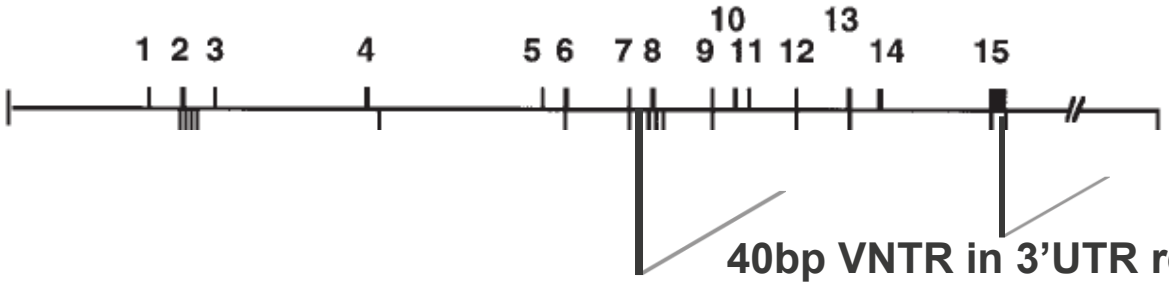
Children with ADHD were slower to reorient their attention to the left when invalidly cued to the right, compared to controls

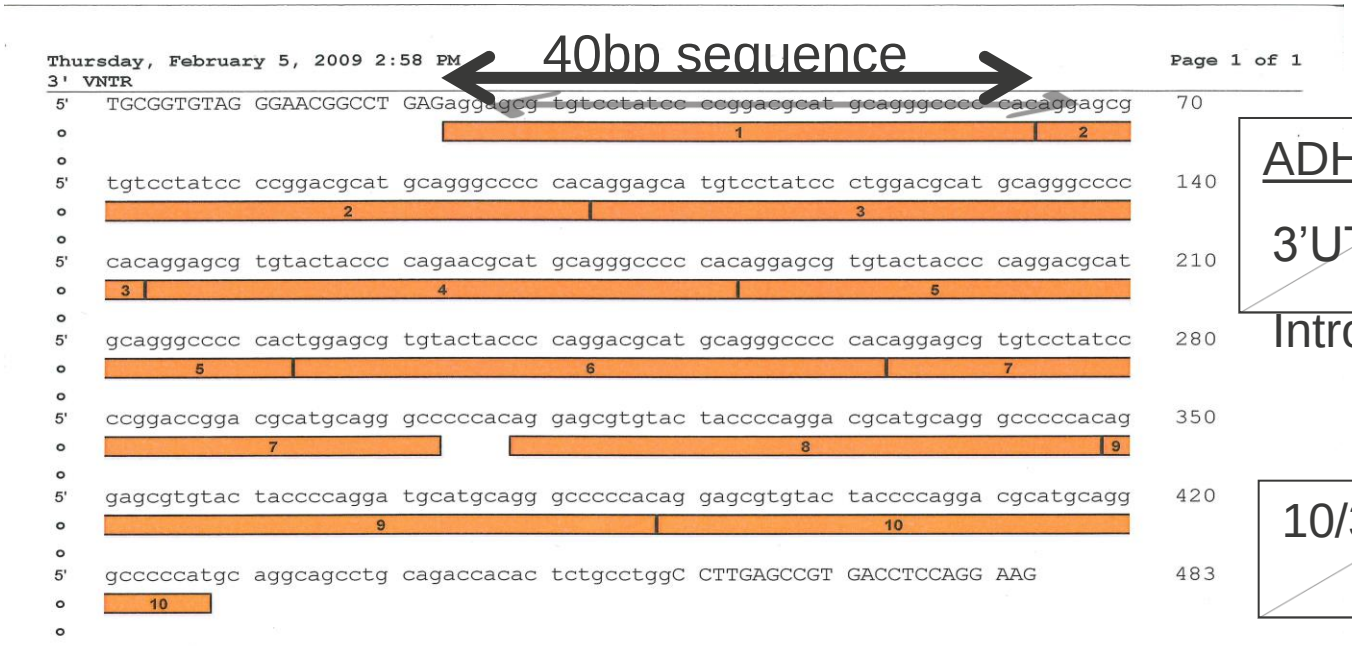
Hypothesis:

Is asymmetry of attention in ADHD linked to dopamine functioning?

Dopamine Transporter Gene (DAT1)

- DAT1- 5p15.3, 15 exons, ~64kb long.





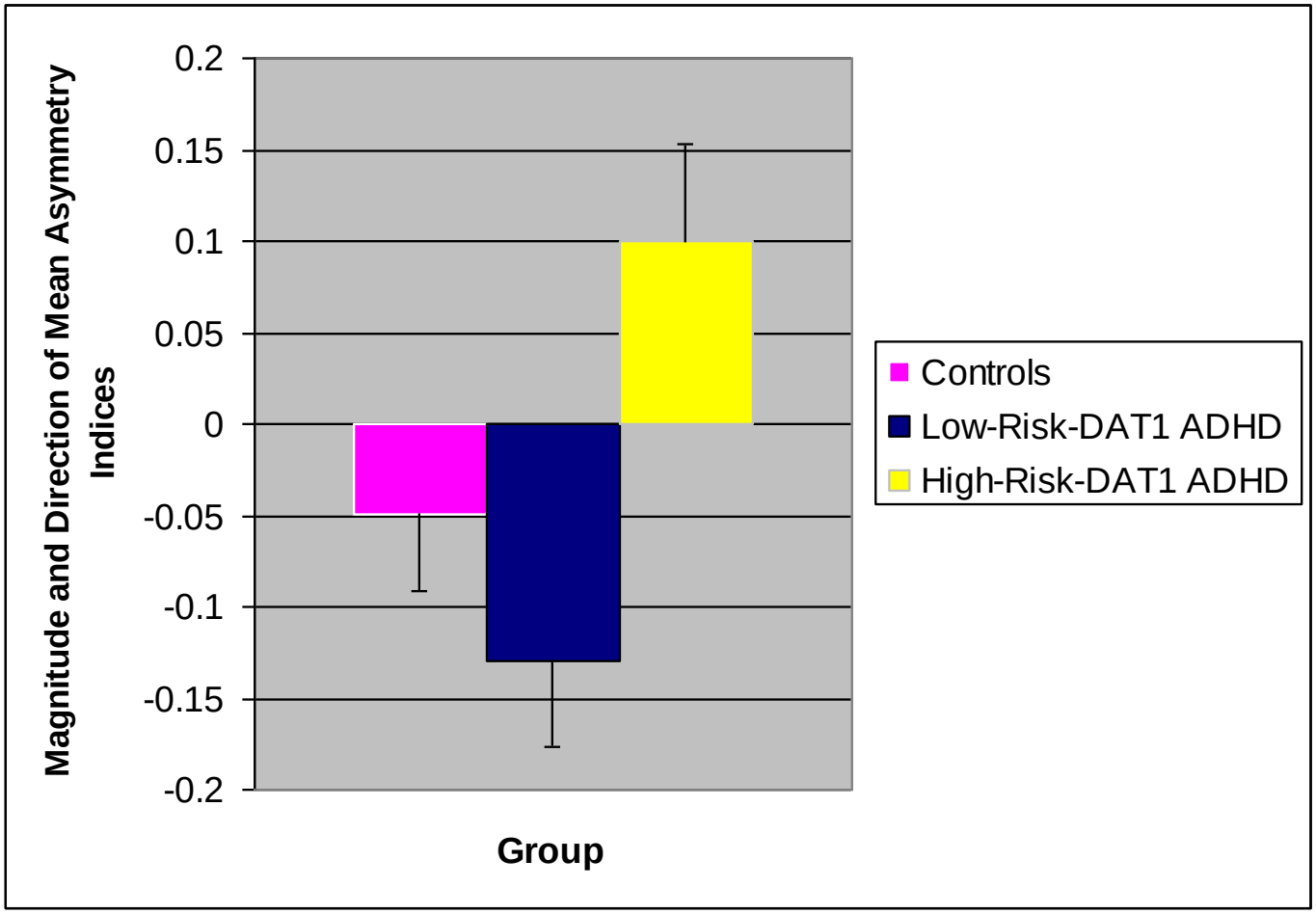
ADHD Associated Alleles

3'UTR VNTR- 10-repeat

Intron 8 VNTR- 3-repeat

10/3 DAT1 Haplotype

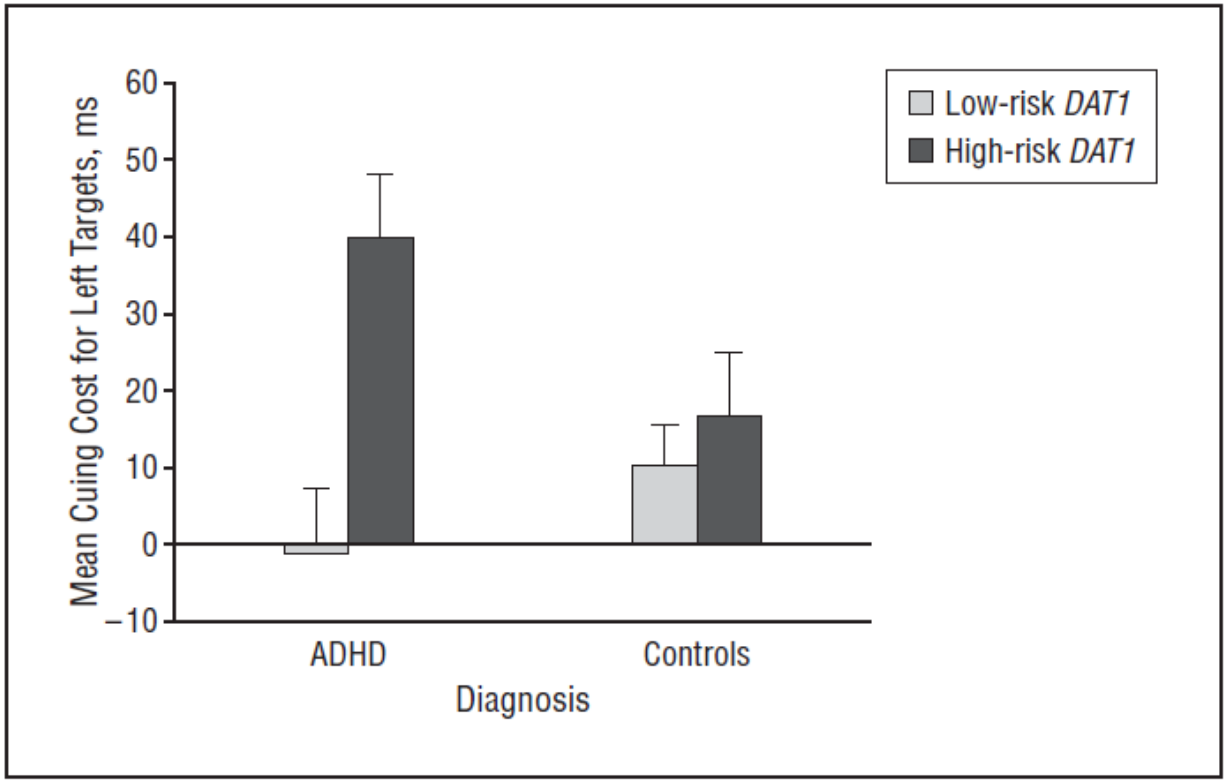
OR~2.5



Bellgrove et al (2005), *Neuropsychopharmacology*

Bellgrove et al (2007), *Neuropsychopharmacology*

Spatially selective attention deficits are modified by Dopamine Transporter Genotype (DAT1)



Bellgrove et al 2009, *Archives of General Psychiatry*

Influence of DAT1 genotype on spatial attention in healthy adults
Newman et al, 2012, *Neuropsychologia*

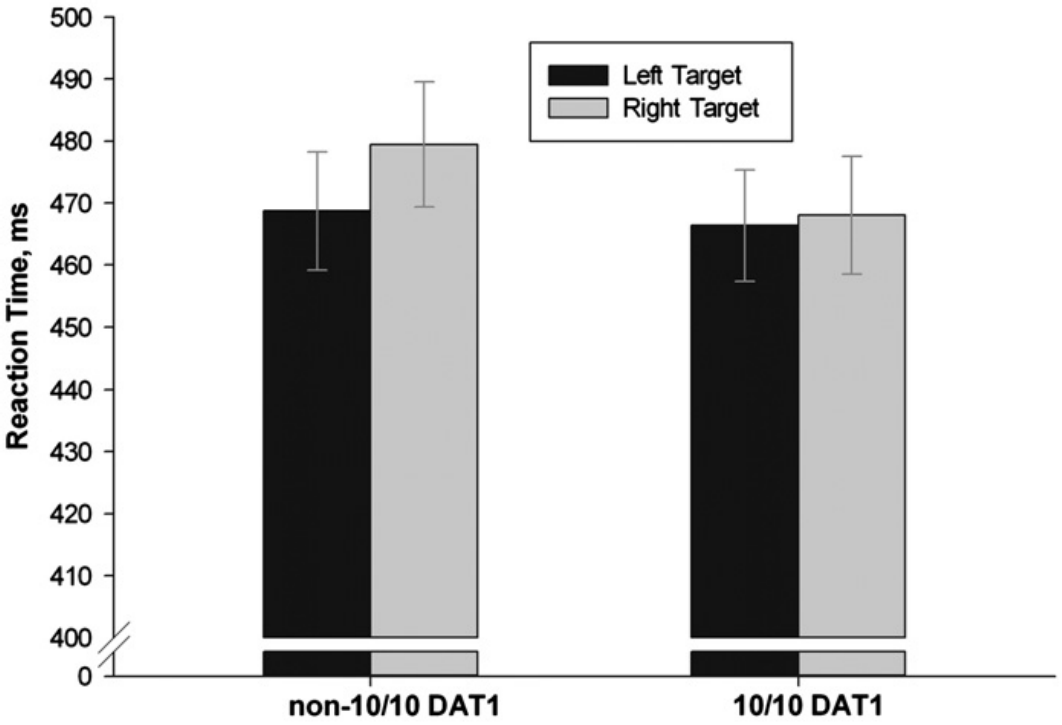
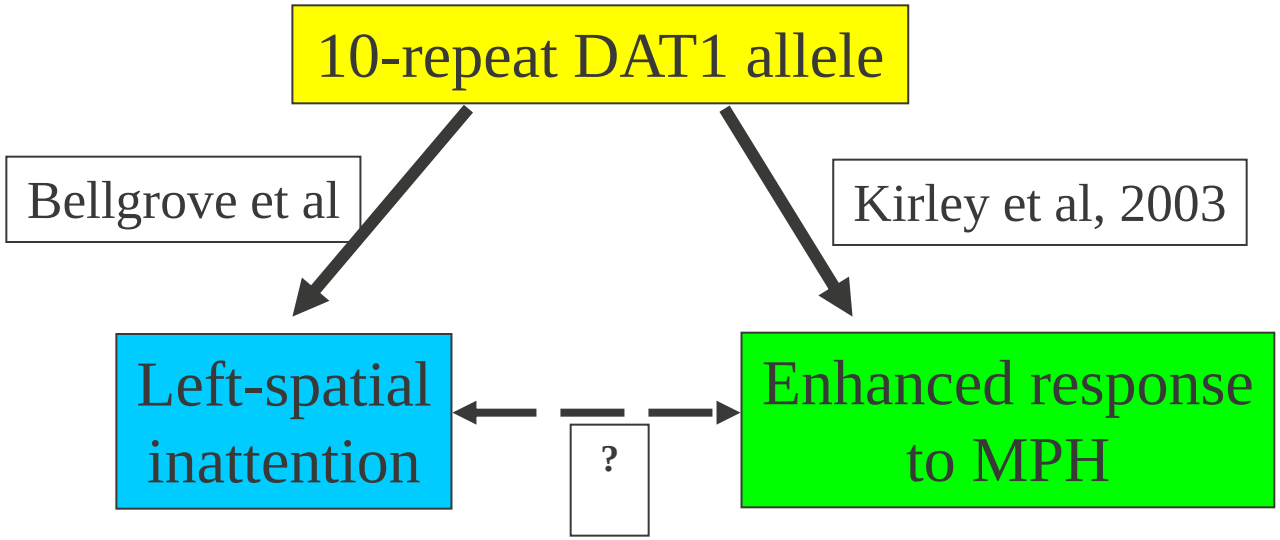
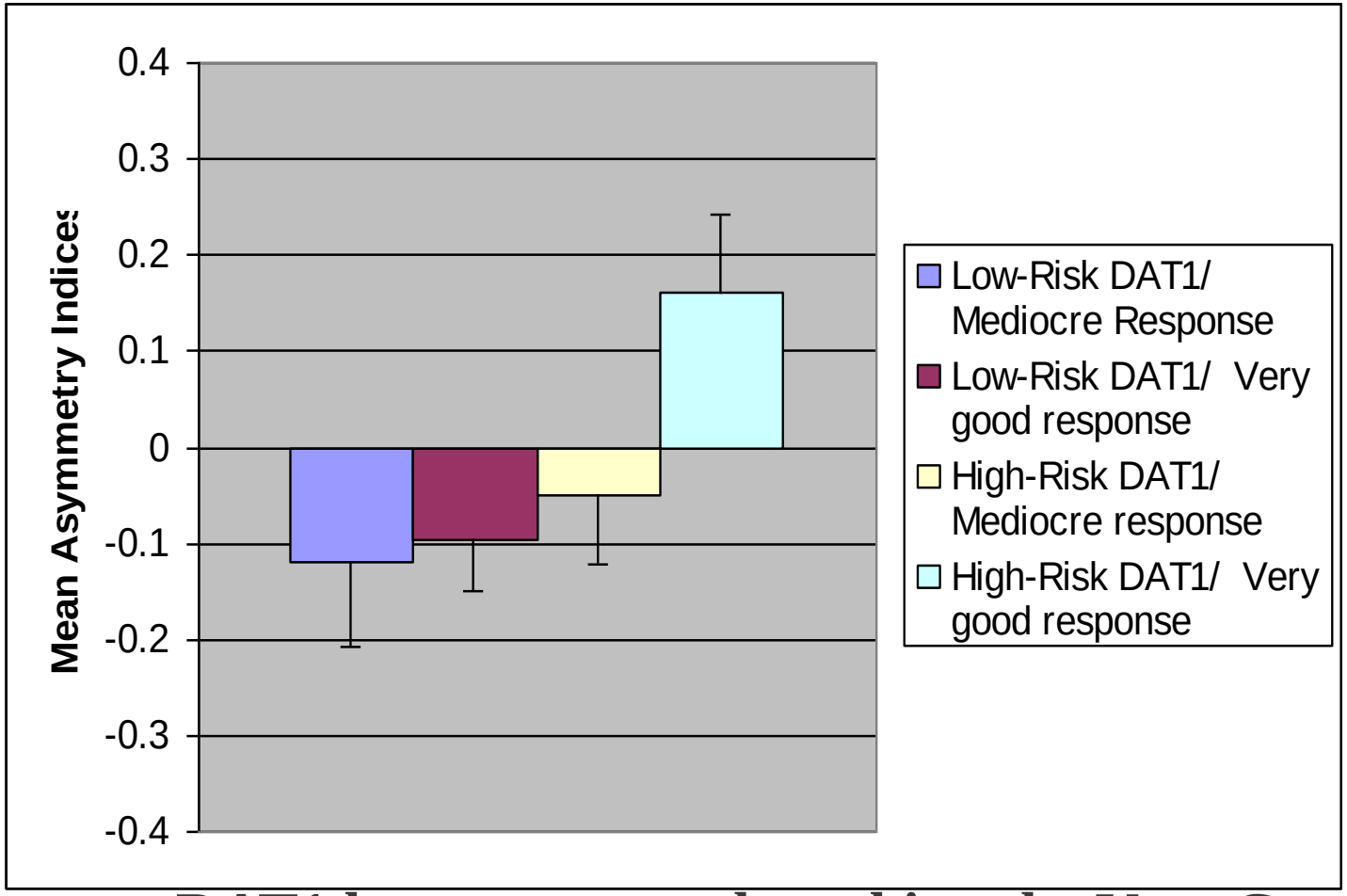


Fig. 2. Mean peripheral target RT as a function of target-side and DAT1 genotype group. The non-10/10 DAT1 group displayed significantly faster responses to left than right peripheral targets, whereas those with the 10/10 genotype showed no significant asymmetry between response times for left and right targets. Error bars reflect the standard error of the mean.

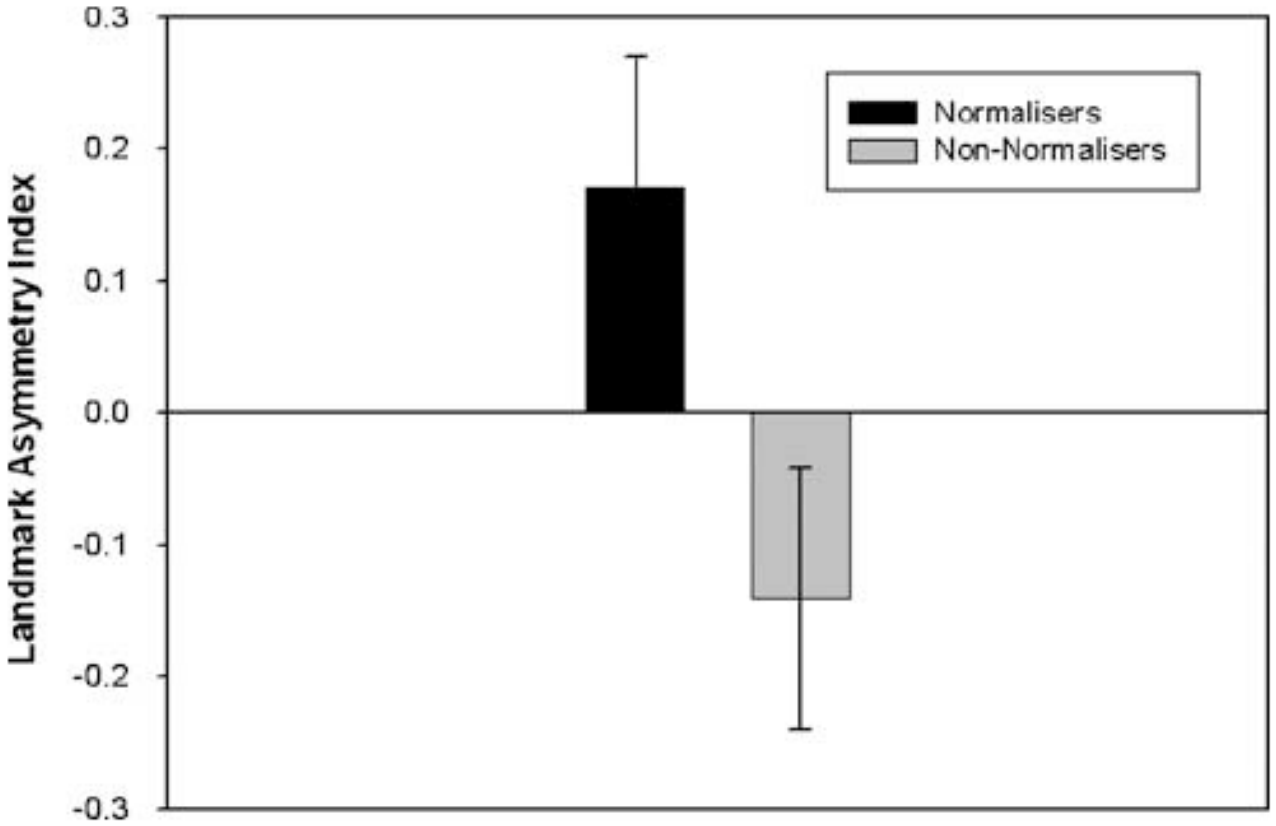
Hypothesis: **attentional asymmetry will predict an enhanced therapeutic response to MPH**





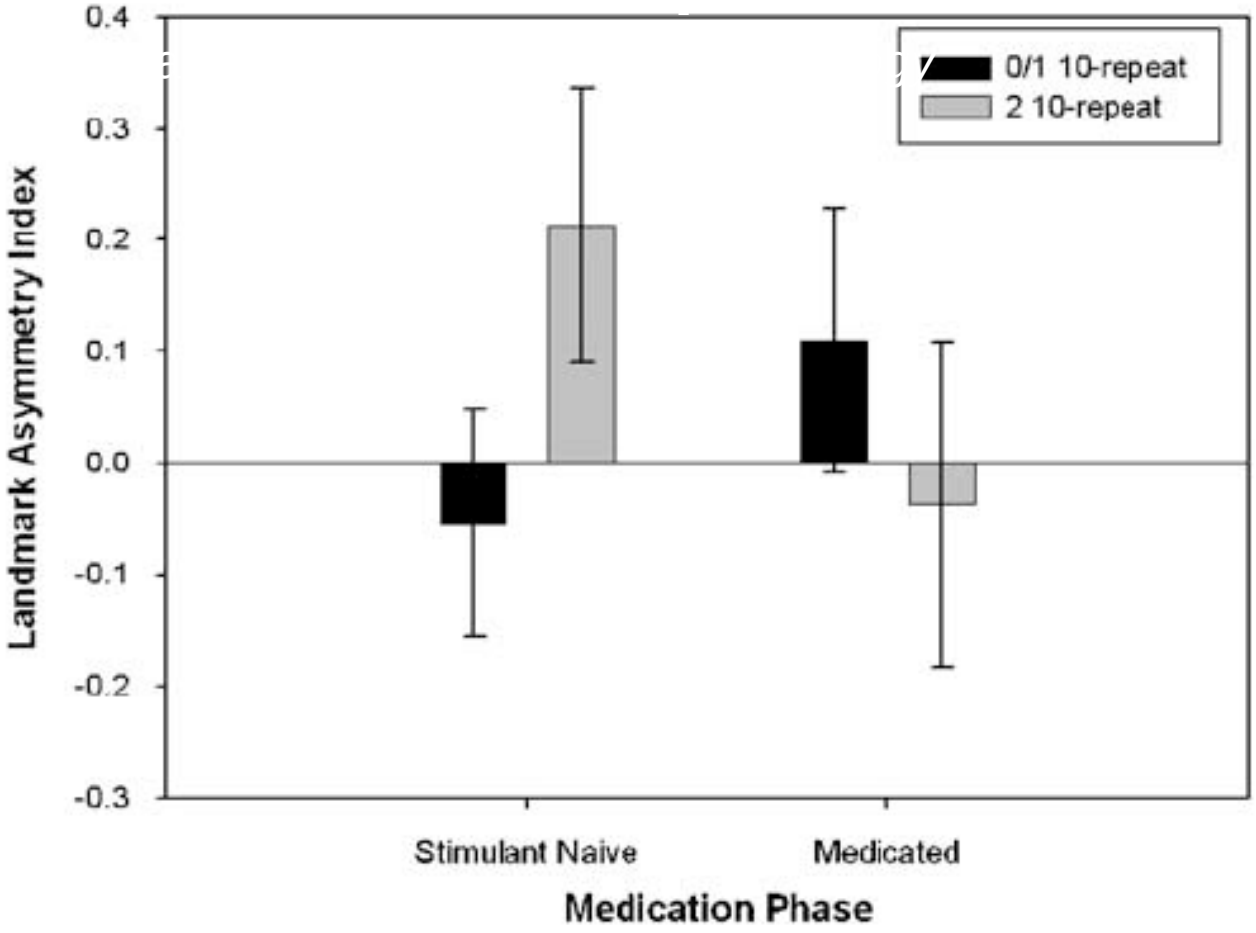
10-repeat DAT1 homozygotes who achieved a Very Good Response to MPH, displayed left-spatial inattention

Bellgrove et al (2005), *Neuropsychopharmacology*

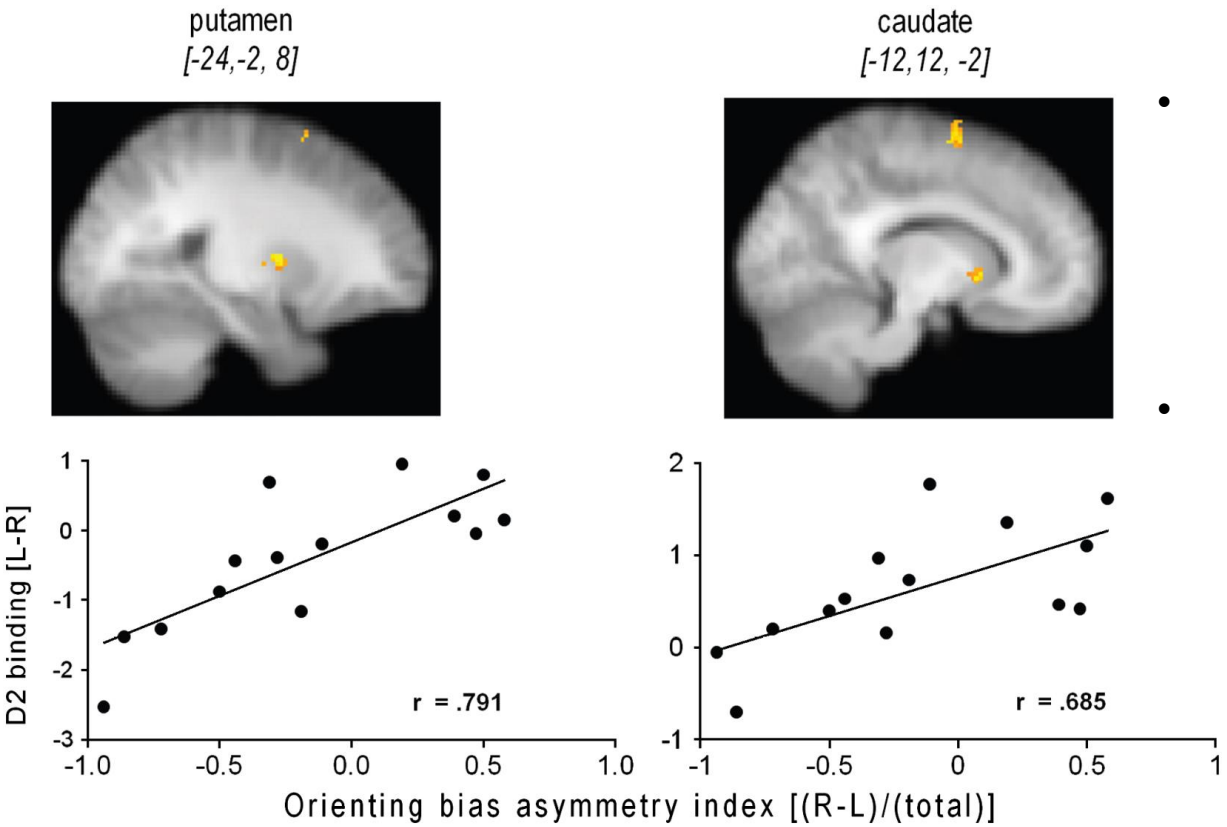


η^2=.18

Attentional asymmetry at baseline predicted normalisation of symptoms with MPH after 6 weeks

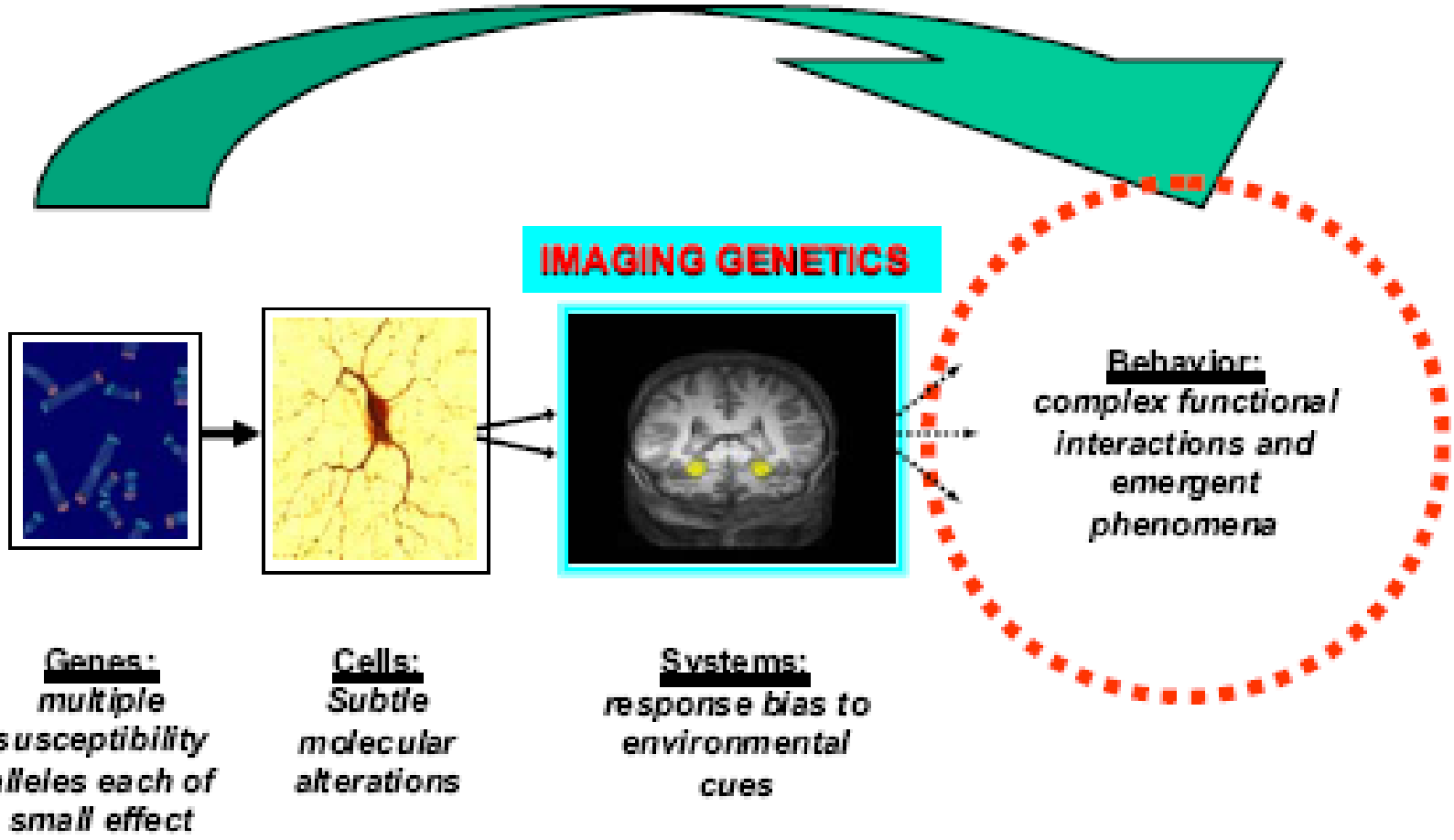

0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
Landmark Asymmetry Index
0/1 10-repeat
2 10-repeat
Stimulant Naive
Medicated
Medication Phase

Spatial asymmetry linked to striatal dopamine Tomer et al, 2012, *Cerebral Cortex.*



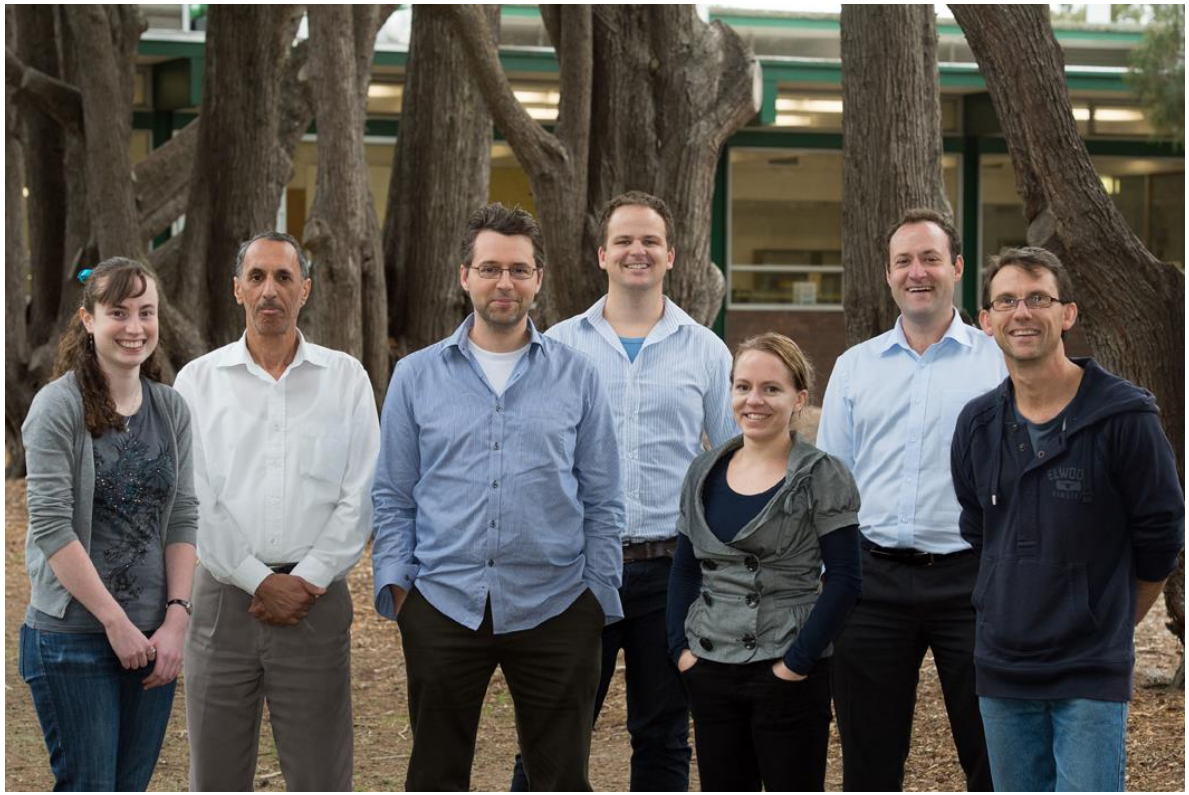
- **Attentional asymmetry reflects individual differences in the lateralisation of dopamine systems**
- **Orienting directed contra-laterally to hemisphere with >D2 binding**

The path from here to there…



DAT1 → ? → Frontal-Striatal Circuits → Inhibition → ADHD

Acknowledgements



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