Neurobiology of Attention Deficit Hyperactivity Disorder (ADHD)

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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

- Methylphenidate or Ritalin
- Atomoxetine
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 Methylphenidate or Ritalin
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.
Individual 1 differs from 2 at a single Base-pair location C / T SNP.

Within a Population, you have:
- C/C genotypes
- C/T genotypes
- TT genotypes

Single nucleotide polymorphisms (SNPs)

C- Allele

T-Allele

Non-ADHD

Inattention

ADHD

Frequency

Within a Population, you have:
- C/C genotypes
- C/T genotypes
- TT genotypes

Single nucleotide polymorphisms (SNPs)
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

Tong et al, AJMG: Neuropsychiatric Genetics, 2015

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

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

Abbreviations: ADHD, attention deficit hyperactivity disorder; GPCR, G-protein-coupled receptors; GWAS, genome wide association studies; UTR, untranslated region; VNTR, variable number tandem repeat. aFirst reported by. bMeta-analysis article. cGWAS finding. dAssociation in large sample or validation using animal model.
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. 1 vs. 2 copies of an allele) against the phenotype, across the whole genome.

**Problem?**
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 $\alpha=0.05$, 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

- 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

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.
Endophenotypes for ADHD

Symptom Domains
  Cognitive Systems
    Neural Systems
      Genes

Hyperactivity/Impulsivity
  Inhibitory Deficits
    Fronto-Striatal Circuits
      Genes

Increasing Strength of Genetic Effects
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

<table>
<thead>
<tr>
<th>Risk Variable</th>
<th>ADHD Group (N=54)</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children With Poor Inhibition (N=27)</td>
<td>Children With Good Inhibition (N=27)</td>
</tr>
<tr>
<td>N</td>
<td>( %^b )</td>
<td>N</td>
</tr>
<tr>
<td>Family history of ADHD</td>
<td>13</td>
<td>48.1</td>
</tr>
<tr>
<td>Mother</td>
<td>5</td>
<td>18.5</td>
</tr>
<tr>
<td>Father</td>
<td>7</td>
<td>25.9</td>
</tr>
<tr>
<td>Sibling</td>
<td>5</td>
<td>18.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ADHD Children (N=79)</th>
<th>Unaffected Siblings (N=34)</th>
<th>Comparison Children (N=63)</th>
<th>Parents (N=104)</th>
<th>Comparison Adults (N=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.1</td>
<td>2.1</td>
<td>9.8</td>
<td>2.8</td>
<td>9.9</td>
</tr>
<tr>
<td>IQ</td>
<td>99.6</td>
<td>11.3</td>
<td>106.1</td>
<td>11.3</td>
<td>119.6</td>
</tr>
<tr>
<td>Stop-signal reaction time (ms)</td>
<td>354.6</td>
<td>154.7</td>
<td>298.2</td>
<td>130.3</td>
<td>263.2</td>
</tr>
<tr>
<td>Male</td>
<td>62</td>
<td>79</td>
<td>16</td>
<td>47</td>
<td>28</td>
</tr>
</tbody>
</table>

*Schaenar et al, 2005*
Cognitive Neuroanatomy of Response Inhibition

Transcranial Magnetic Stimulation (TMS) of frontal cortex disrupts inhibition

Chambers et al, 2006, JOCN
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

<table>
<thead>
<tr>
<th>Measure</th>
<th>Sample</th>
<th>Effect Size (d)</th>
<th>η²</th>
<th>p</th>
<th>% ADHD Beyond Control 90th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSRT</td>
<td>MI (all)</td>
<td>.88</td>
<td>.133</td>
<td>&lt;.001</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td>.79</td>
<td>.101</td>
<td>&lt;.001</td>
<td>45</td>
</tr>
<tr>
<td>RT Variability</td>
<td>MI</td>
<td>.75</td>
<td>.123</td>
<td>&lt;.001</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td>.77</td>
<td>.125</td>
<td>&lt;.001</td>
<td>44</td>
</tr>
<tr>
<td>Stroop CW</td>
<td>MI</td>
<td>.50</td>
<td>.045</td>
<td>&lt;.05</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td>.84</td>
<td>.132</td>
<td>&lt;.001</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>MGH</td>
<td>.62</td>
<td>.09</td>
<td>&lt;.001</td>
<td>25</td>
</tr>
<tr>
<td>CPT</td>
<td>MI</td>
<td>.91</td>
<td>.11</td>
<td>&lt;.001</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td>.54</td>
<td>.053</td>
<td>&lt;.001</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>MGH</td>
<td>.17</td>
<td>.01</td>
<td>.11</td>
<td>16</td>
</tr>
<tr>
<td>Trailmaking</td>
<td>MI</td>
<td>.35</td>
<td>.033</td>
<td>&lt;.05</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>CO</td>
<td>.35</td>
<td>.031</td>
<td>&lt;.01</td>
<td>24</td>
</tr>
</tbody>
</table>
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

Both dopamine and noradrenaline appear important for inhibitory control
Neurochemistry of Inhibition

Molecular Targets Become Candidate Genes for Genetic Association With Inhibition

NET1, D4, D2, α-2,

DAT1, D2
# Genetic Association Study of Inhibition

Cummins et al Mol Psych 2012

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>MAF</th>
<th>p value SSRT</th>
<th>p - GoRT</th>
<th>p - SDGoRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs40358</td>
<td>.14</td>
<td>.21</td>
<td>0.043*</td>
<td>0.56</td>
</tr>
<tr>
<td>rs37020</td>
<td>.45</td>
<td>.0002**</td>
<td>0.31</td>
<td>0.11</td>
</tr>
<tr>
<td>rs10053602</td>
<td>.23</td>
<td>.49</td>
<td>0.57</td>
<td>0.51</td>
</tr>
<tr>
<td>rs393795</td>
<td>.22</td>
<td>.0012*</td>
<td>0.065</td>
<td>.037*</td>
</tr>
<tr>
<td>rs11737901</td>
<td>.36</td>
<td>.007*</td>
<td>0.57</td>
<td>0.72</td>
</tr>
<tr>
<td>rs4600000</td>
<td>.23</td>
<td>.0004**</td>
<td>0.086</td>
<td>0.02</td>
</tr>
</tbody>
</table>
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
Attention is spatially selective

Spatial selection can occur **covertly**

– without eye movements
Attention is spatially selective.

Spatial selection can occur **covertly** – without eye movements.
Attention is spatially selective

Spatial selection can occur **covertly**
– without eye movements
Attention is spatially selective

Spatial selection can occur **covertly**

— without eye movements
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

Valid Condition (1/3 trials)

Neutral Condition (1/3 trials)

Invalid Condition (1/3 trials)
Measuring Spatially Selective Attention

**Reaction Time**

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

Cuing Benefit: Neutral RT- Valid RT: Benefit to RT of spatial orienting
Children with ADHD were slower to reorient their attention to the left when invalidly cued to the right, compared to controls.

**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.
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

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 allele
  - Bellgrove et al
  - Kirley et al, 2003
- Left-spatial inattention
- Enhanced response to MPH
10-repeat DAT1 homozygotes who achieved a Very Good Response to MPH, displayed left-spatial inattention

Bellgrove et al (2005), *Neuropsychopharmacology*
Attentional asymmetry at baseline predicted normalisation of symptoms with MPH after 6 weeks

$\eta^2 = .18$
Interaction of DAT1 genotype and Medication Phase for spatial bias

Bellgrove et al, 2007, Neuropsychopharmacology
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...

IMAGING GENETICS

Behavior: complex functional interactions and emergent phenomena

Genes: multiple susceptibility alleles each of small effect
Cells: Subtle molecular alterations
Systems: response bias to environmental cues

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

MONASH University
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