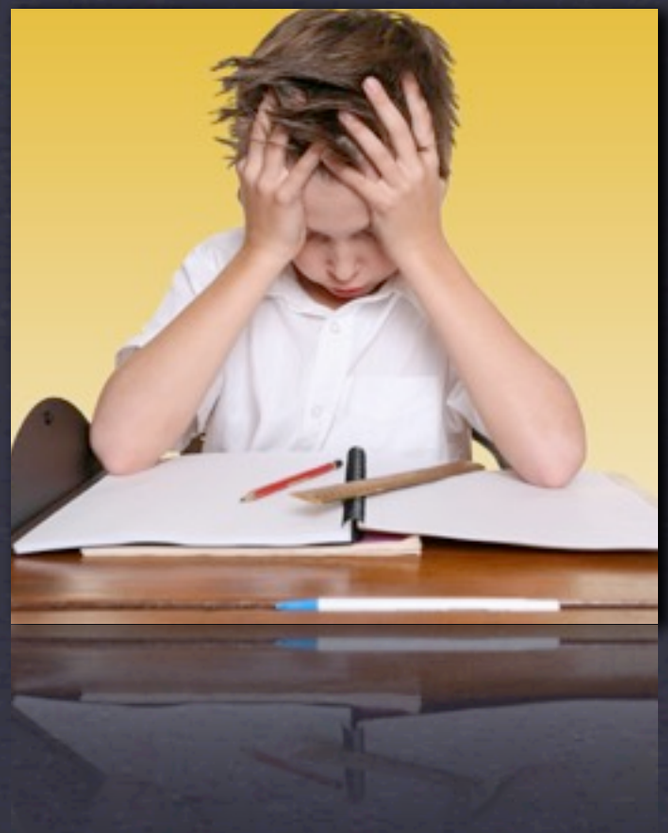
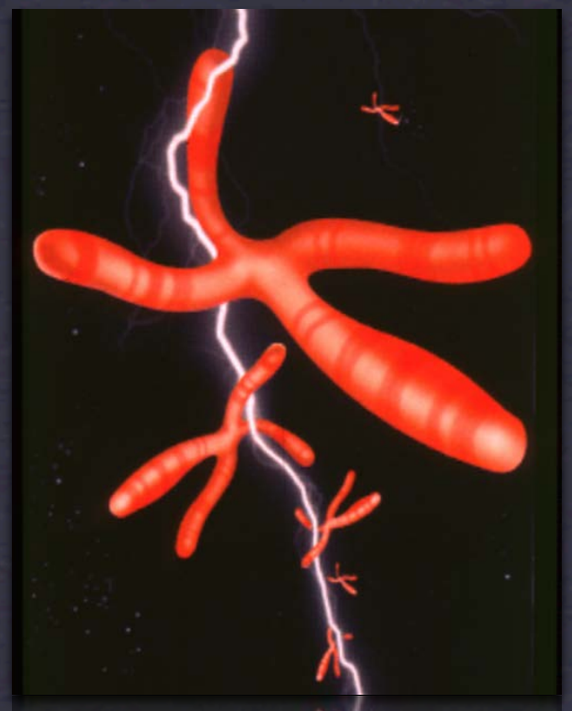
RARE MISSPELLINGS OF THE GENOME, DOPAMINE MISHANDLING, AND ADHD





RANDY D. BLAKELY, PH.D.

FLORIDA ATLANTIC UNIVERSITY BRAIN INSTITUTE

Dr. Blakely declares no conflicts of interest derived from financial support of the research to be discussed

Dr. Blakely has consulted on research projects in the past for Amgen, Forest Research Institute, Lundbeck, Jubilant Innovation, Pfizer, Prexa, and Wyeth

CONFLICTS OF INTEREST

ACKNOWLEDGEMENTS

Blakely Lab

Lorena Areal
Gwynne Davis
Raaj Gowrishankar
Paul Gresch
Maureen Hahn
Rania Katamish
Michelle Mazei-Robison
Felix Mayer
Michael Gill
Peter Hamilton
Marc Mergy
Rodenia Pert
Max Rabil
Nathan Richtand
Justin Riele
DJ Sakrikar
Keeley Spiess
Adele Stewart
Jeremy Veenstra-VanderWeele
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Michael Freissmuth
Aurelio Galli
Stephanie Gantz
Jonathan Javitch
Doug McMahon
Richard Shelton
Harald Sitte
Gregg Stanwood
Mark Stein
Roxanne Vaughan
John Williams

BRAIN & BEHAVIOR RESEARCH FOUNDATION
Awarding NARSAD Grants

National Institute of Mental Health

NIH National Institute on Drug Abuse

FAU BRAIN INSTITUTE
Florida Atlantic University

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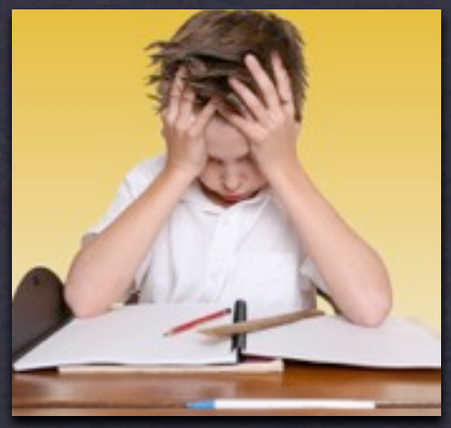
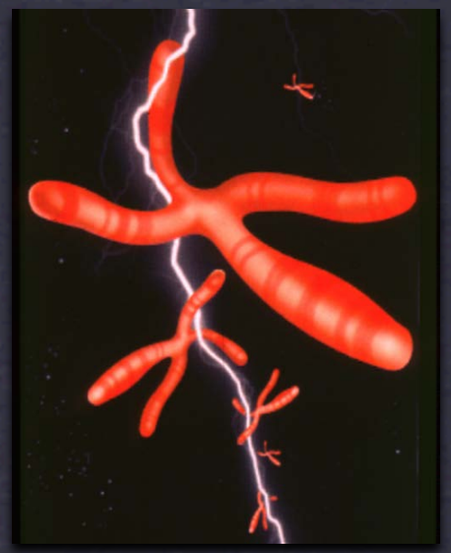
OUTLINE



ADHD: Diagnosis, Genetics and Dopamine Involvement



Identification of Mutations in the Human Dopamine Transporter (DAT) in Subjects with ADHD

The In Vivo Consequences of DAT Val559: From Biochemistry to Behavior and Novel Biomarkers

ATTENTION-DEFICIT/HYPERACTIVITY DISORDER

- The most commonly diagnosed neurobehavioral disorder in children (8.4% ages 2-17, 5.4 million) in the U.S. have a formal ADHD diagnosis (National Survey of Children's Health 2016)
- Diagnosed solely through behavioral observation, with presentation of symptoms of inattention, impulsivity and hyperactivity (by age 12, DSM-5). Male bias in diagnosis (~4:1)
- Approximately 60% are treated by medication, 25% receive no treatment of any form
- Higher incidence of substance abuse, conduct disorders, unemployment, incarceration if left untreated

BASIC FACTS AND STATISTICS

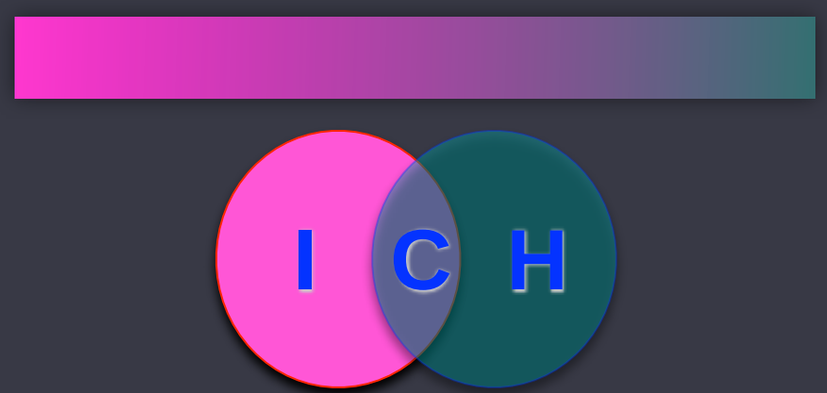
ADHD: DSM-5 Diagnostic Criteria

Inattentive Symptoms

- Careless Mistakes
- Difficulty Sustaining Attention
- Not Seeming To Listen
- Failing To Finish Tasks
- Difficulty Organizing
- Avoiding Tasks That Require Sustained Attention
- Losing Things
- Easily Distracted
- Forgetful

Hyperactive/Impulsive Symptoms

- Fidgeting
- Inability To Stay Seated
- Moving Excessively
- Difficulty Playing Quietly
- On the Go
- Talks Excessively
- Blurting Out Answers
- Difficulty Waiting Turns
- Interrupting



- 6 or more symptons per category for 6 months or more
- symptoms present in two or more settings and prior to age 12
- Symptoms disrupt normal function/quality of life

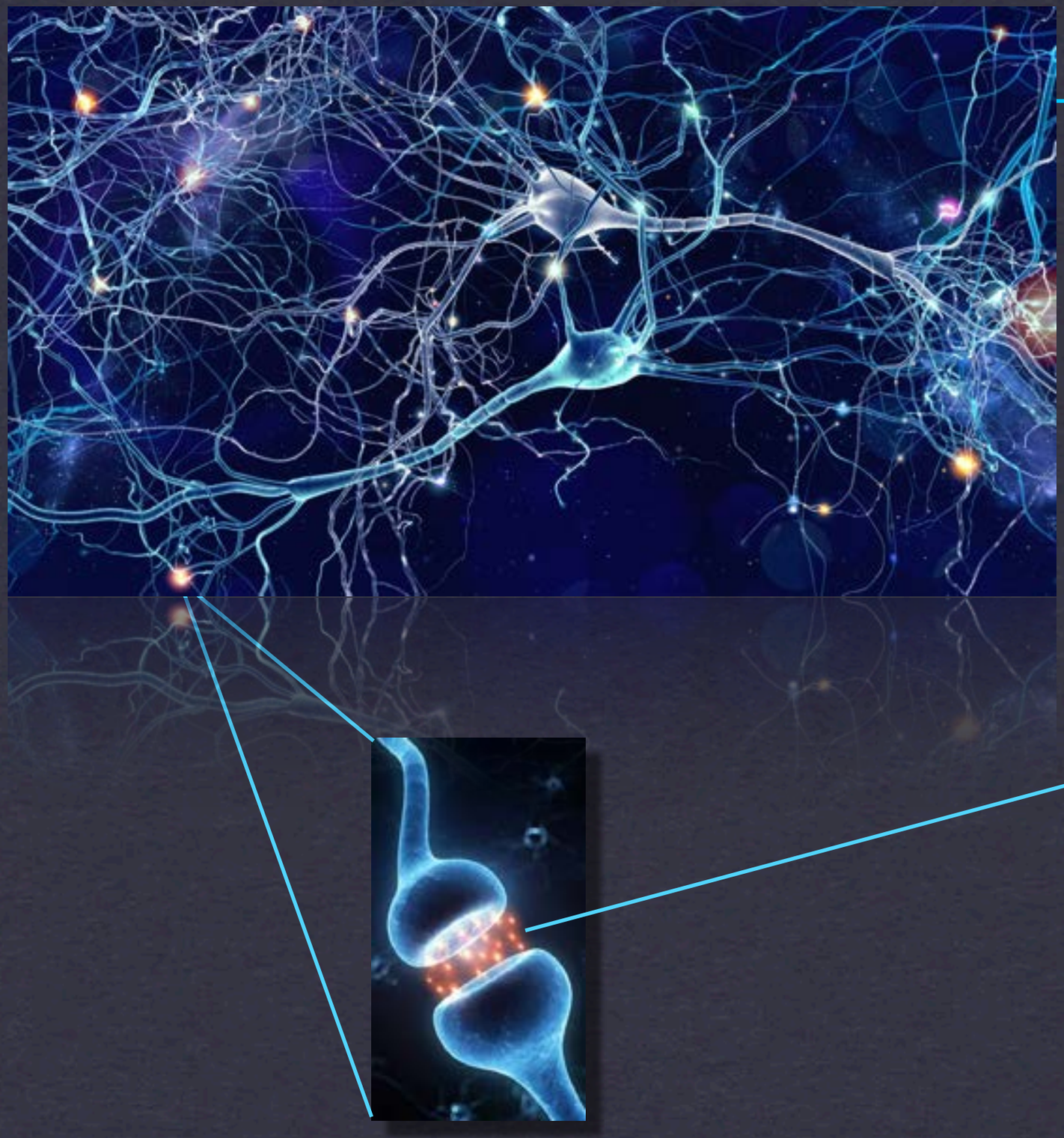
ATTENTION-DEFICIT/HYPERACTIVITY DISORDER

- **Heritability** estimated at 60-90%, common and rare gene variation linked to neuronal development and synaptic function
- Co-morbidity and co-occurrence: **Bipolar Disorder and Autism**
- **Shared genetic heritability** with Bipolar Disorder, Major Depression, and Schizophrenia (Brainstorm Consortium, 2018)

GENETIC ARCHITECTURE

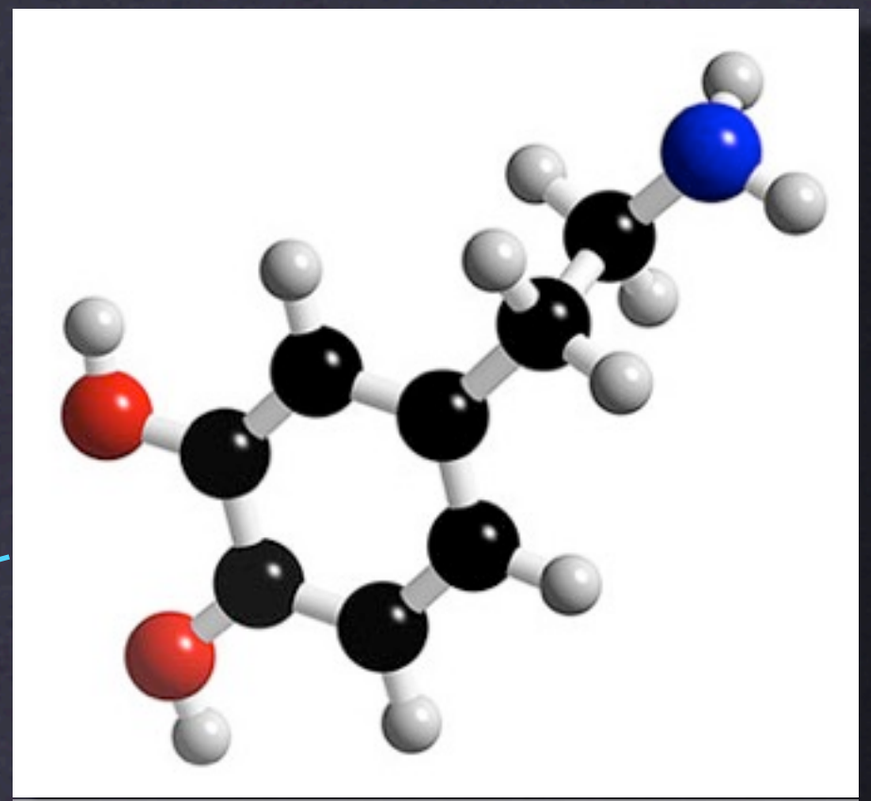
- Common genetic variation of small effect (GWAS)
- Rare genetic variation of large effect (e.g. Fragile X mutation)
- Rare genetic variation of small to moderate effect (risk factor)

ADHD GENETICS



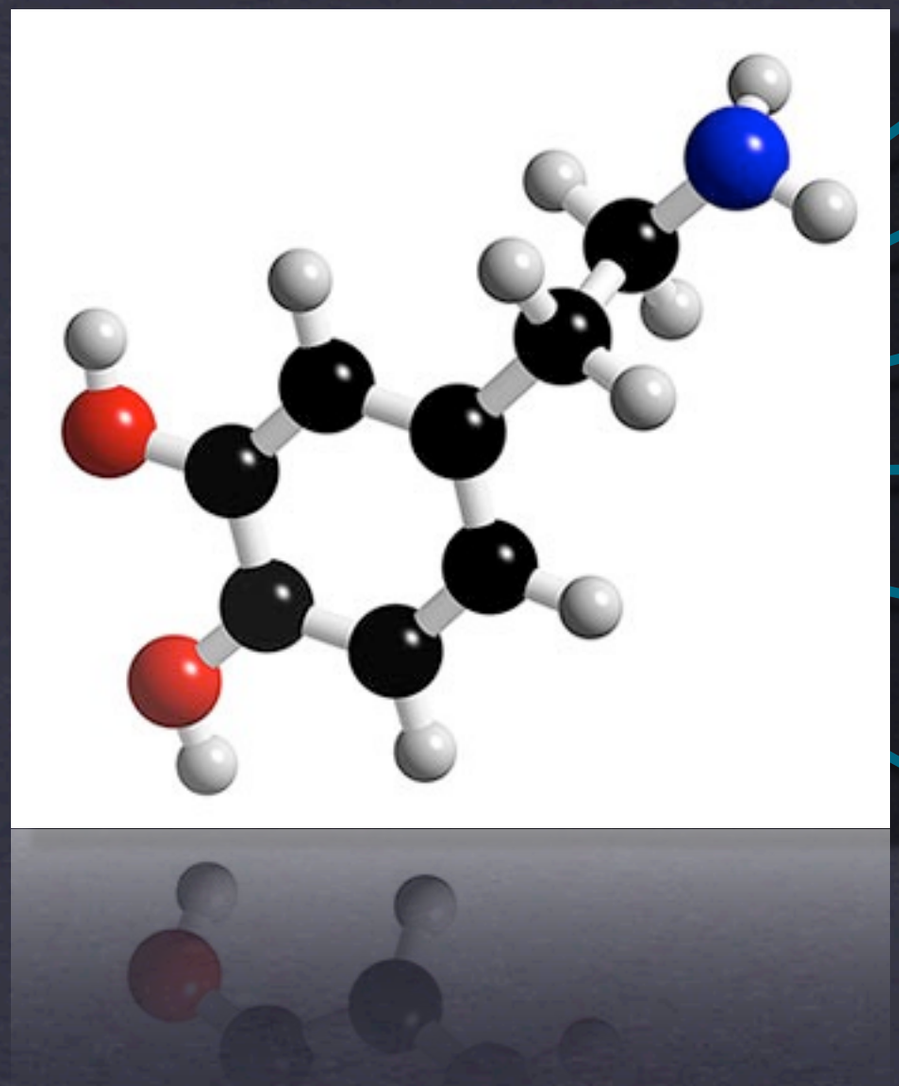
HUMAN BRAIN
100 BILLION NEURONS

DOPAMINE



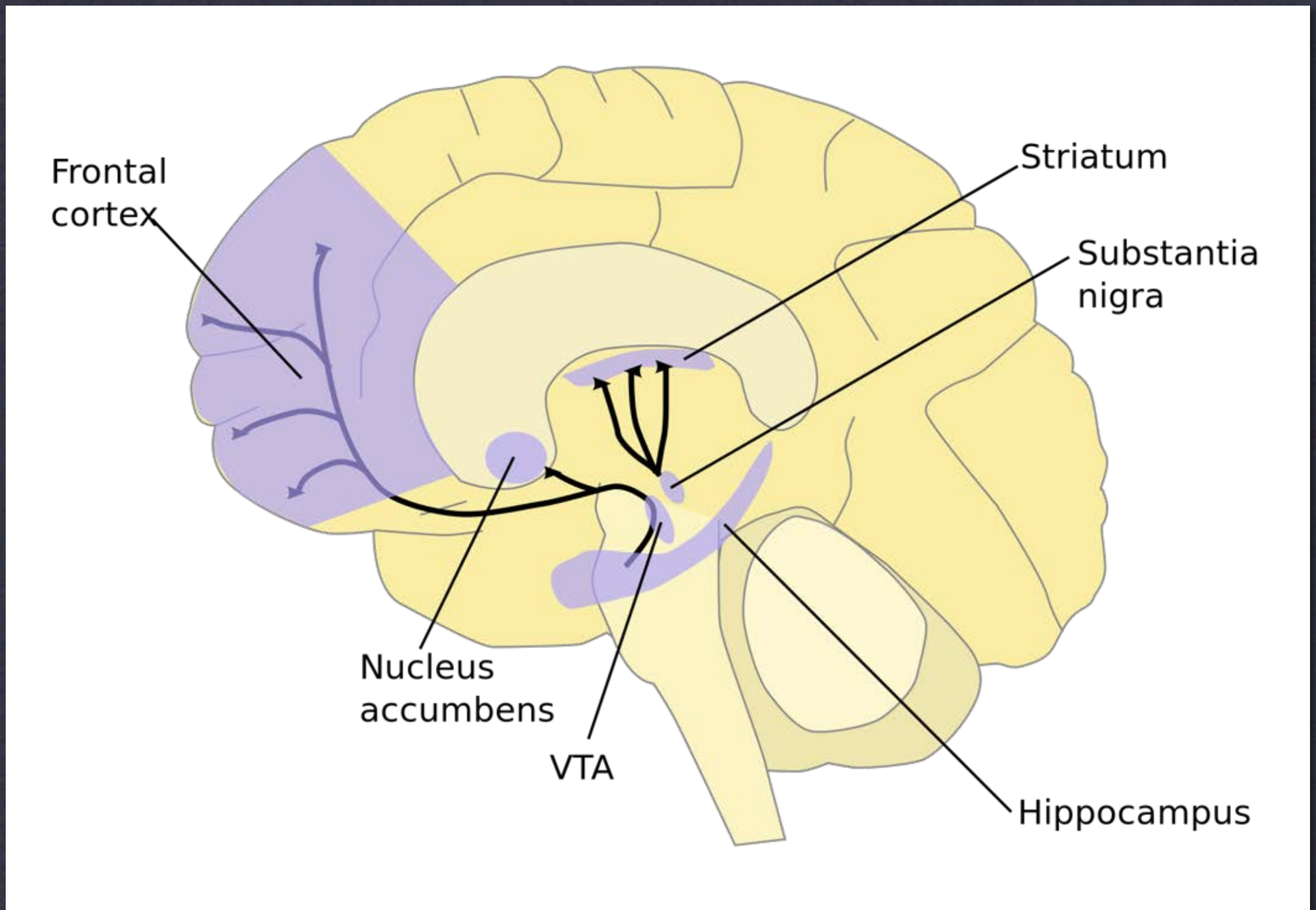
DOPAMINE: KEY BRAIN NEUROTRANSMITTER

DOPAMINE

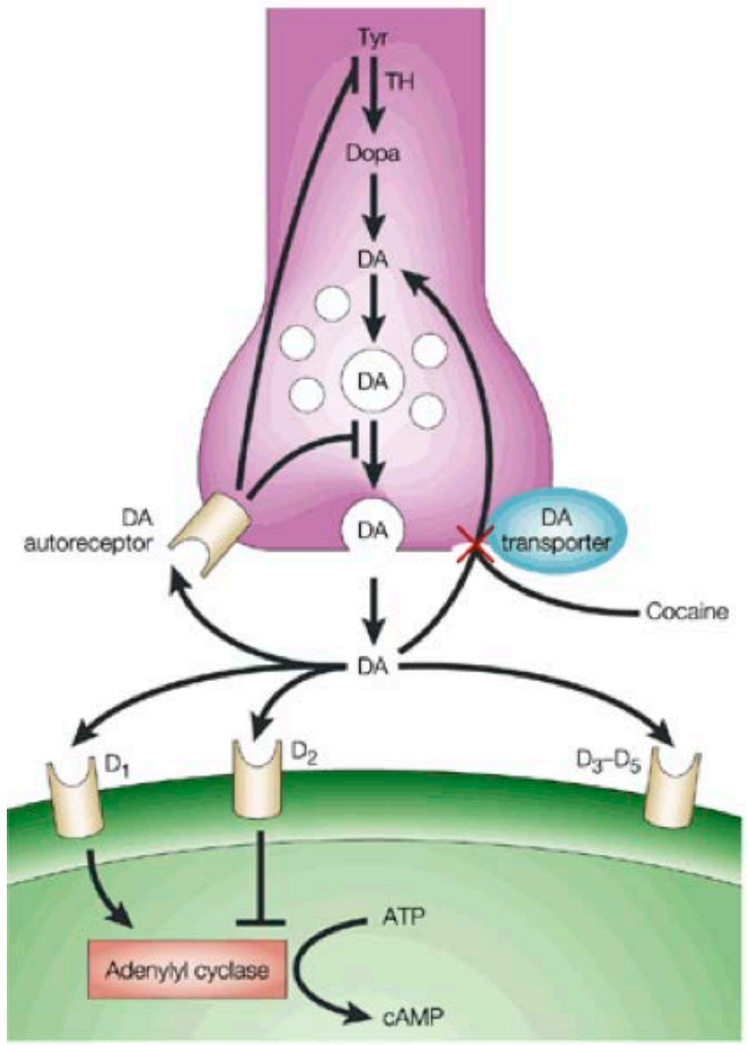


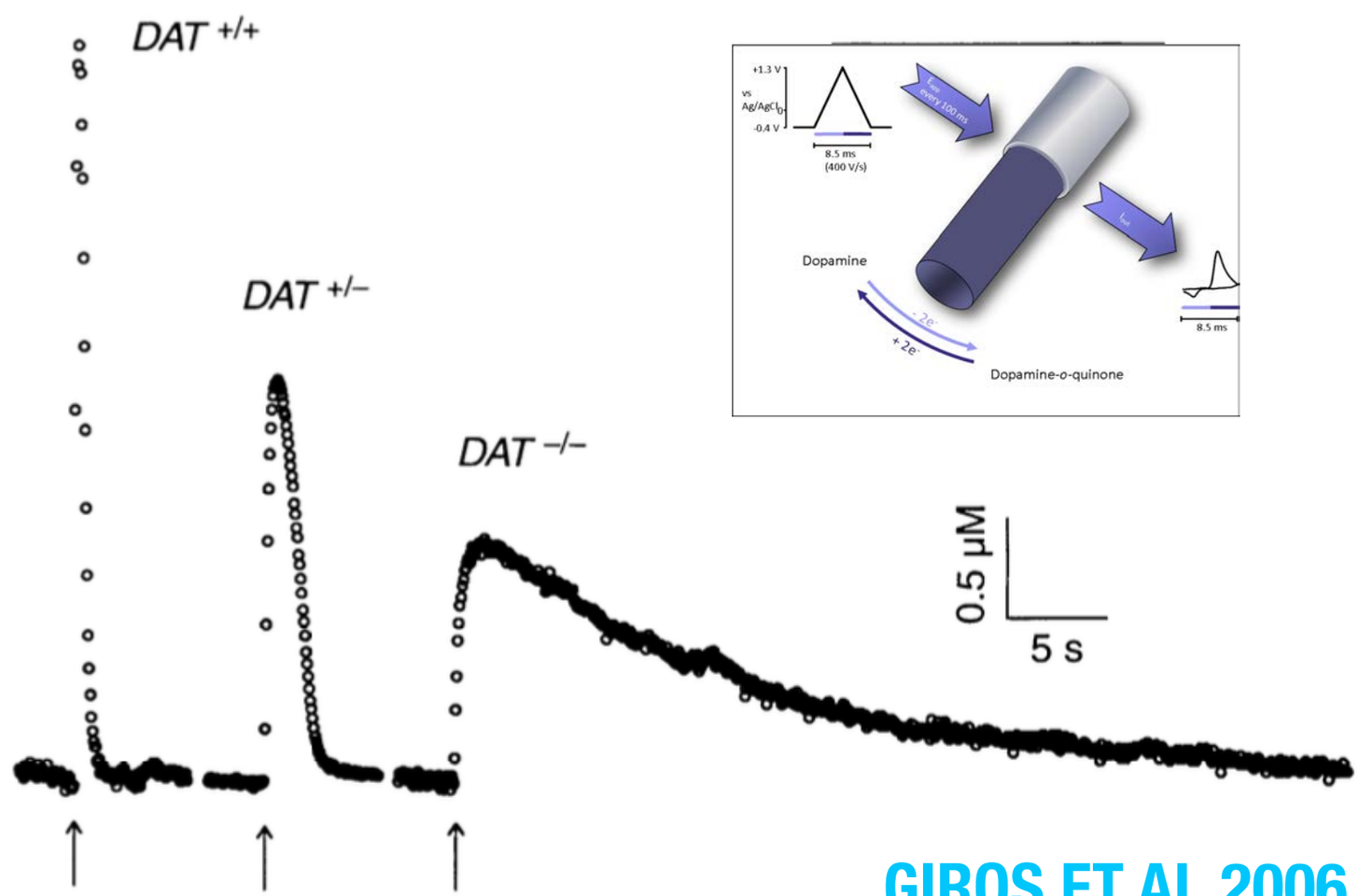
- Cue Saliency
- Attention
- Executive Function
- Learning
- Reward and Motivation
- Locomotor Activity

DA CORE FUNCTIONS PERTURBED IN ADHD



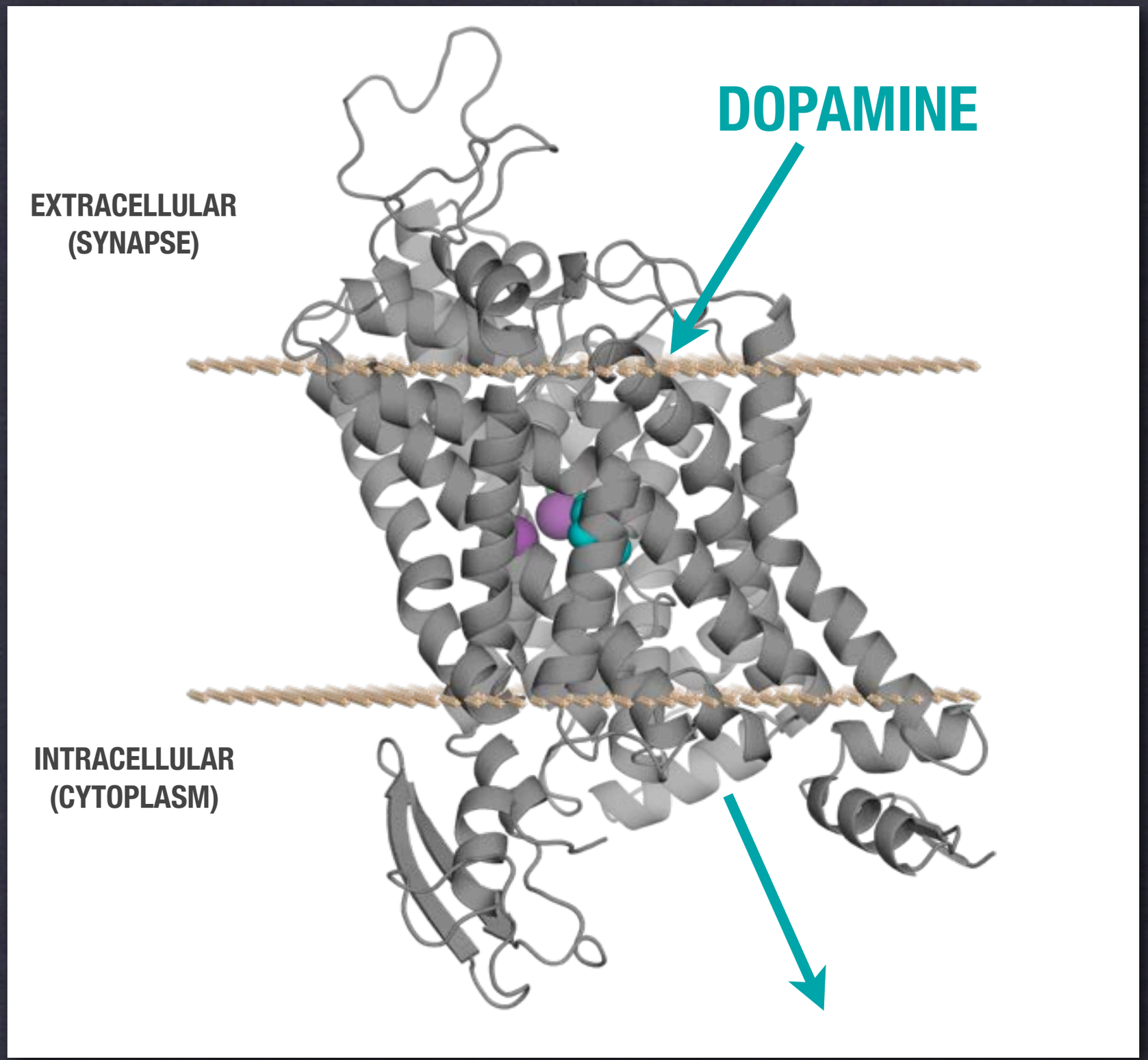
BRAIN DOPAMINE PATHWAYS





GIROS ET AL 2006

CONTROL OF DOPAMINE AVAILABILITY BY THE DOPAMINE TRANSPORTER (DAT)



DAT Model Courtesy of Cristina Fenollar-Ferrer

DOPAMINE TRANSPORTER STRUCTURE

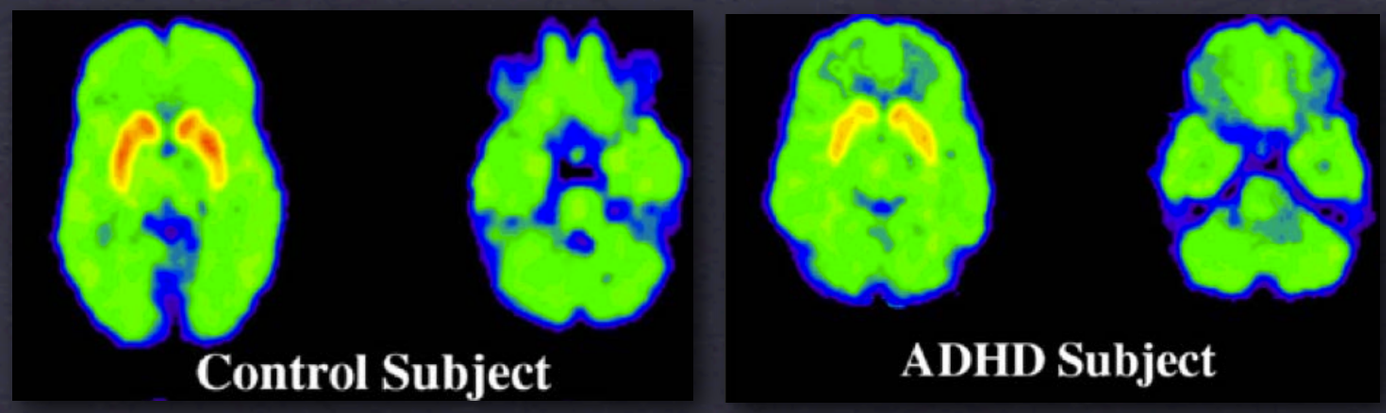
Evaluating Dopamine Reward Pathway in ADHD:

Clinical Implications

Dr. Nora D. Volkow, MD, Dr. Gene-Jack Wang, MD, Dr. Scott H. Kollins, PhD, Dr. Tim L. Wigal, PhD, Dr. Jeffrey H. Newcorn, MD, Dr. Frank Telang, MD, Dr. Joanna S. Fowler, PhD, Dr. Wei Zhu, PhD, Dr. Jean Logan, PhD, Dr. Yeming Ma, PhD, Dr. Kith Pradhan, MS, Dr. Christopher Wong, MS, and **Dr. James M. Swanson, PhD**
National Institute on Drug Abuse (Dr Volkow) and Laboratory of Neuroimaging, National Institute on Alcohol Abuse and Alcoholism (Drs Volkow, Telang, and Ma), Bethesda, Maryland; Medical and Chemistry Departments, Brookhaven National Laboratory, Upton, New York (Drs Wang, Fowler, and Logan, Messrs Pradhan and Wong); Department of Psychiatry, Mount Sinai Medical Center, New York, New York (Drs Wang, Newcorn, and Fowler); Department of Psychiatry, Duke University Medical Center, Durham, North Carolina (Dr Kollins); Child Development Center, University of California, Irvine (Drs Wigal and Swanson); Department of Applied Mathematics and Statistics, State University of New York at Stony Brook, Stony Brook (Dr Zhu)

VOLKOW ET AL 2009

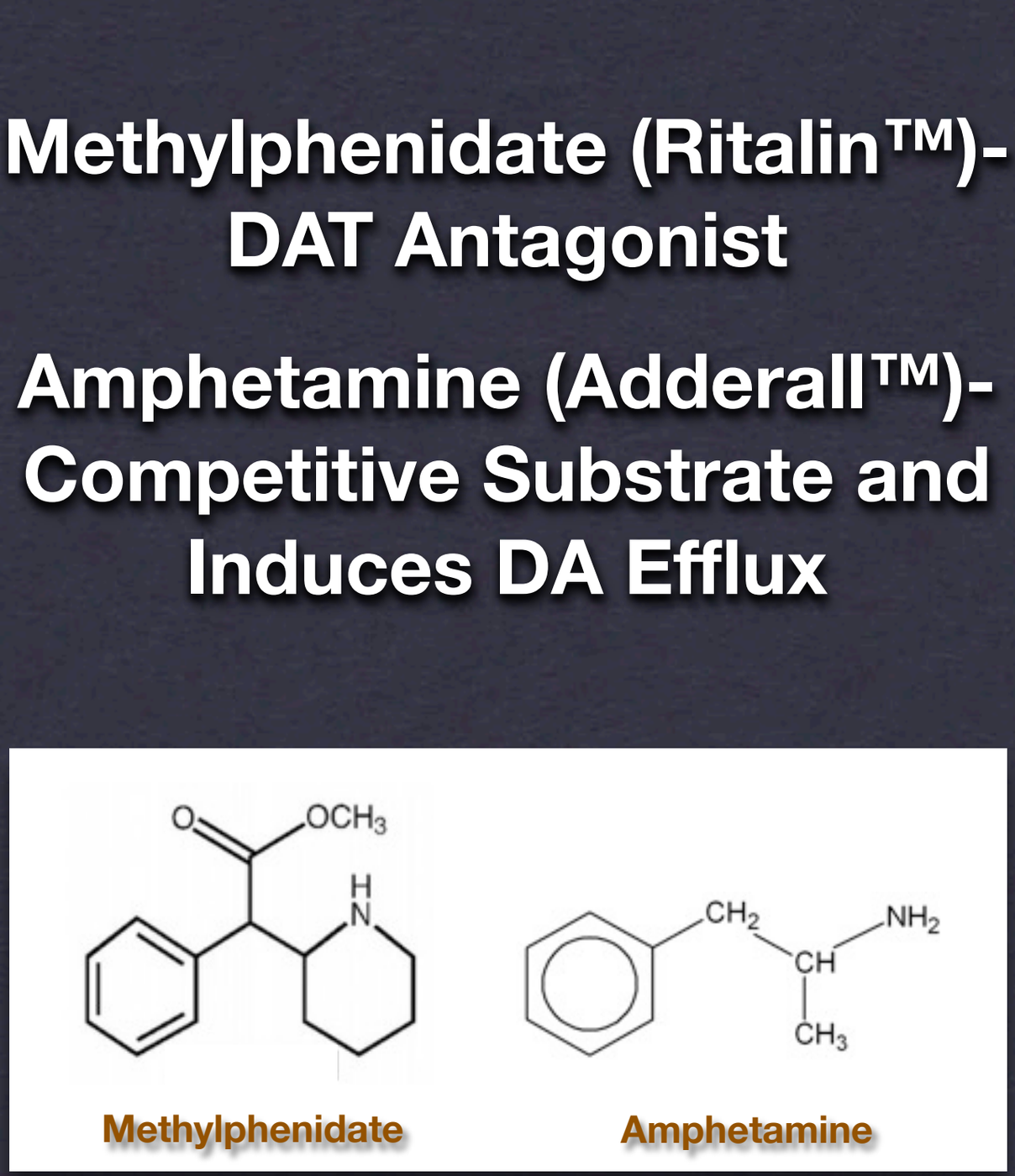
DA TRANSPORTER DENSITY

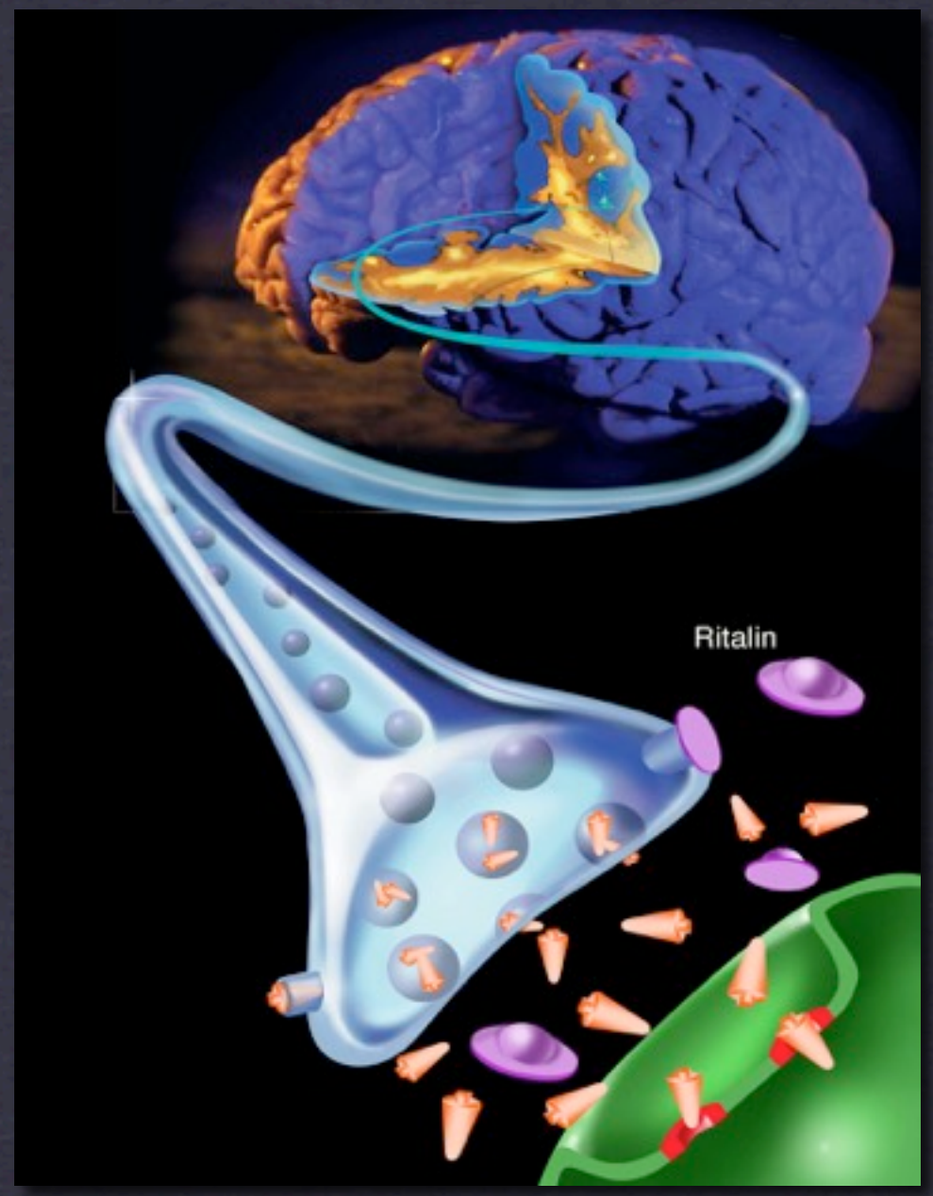


VOLKOW ET AL 2007

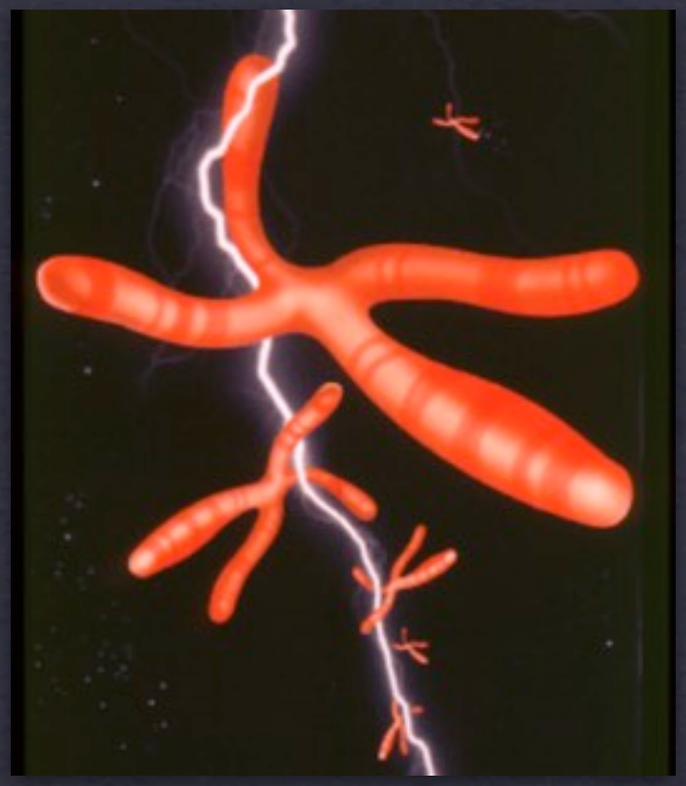
REDUCED DAT LEVELS IN ADHD - TONIC ELEVATION IN DA?

- **Methylphenidate (Ritalin™)- DAT Antagonist**
- **Amphetamine (Adderall™)- Competitive Substrate and Induces DA Efflux**



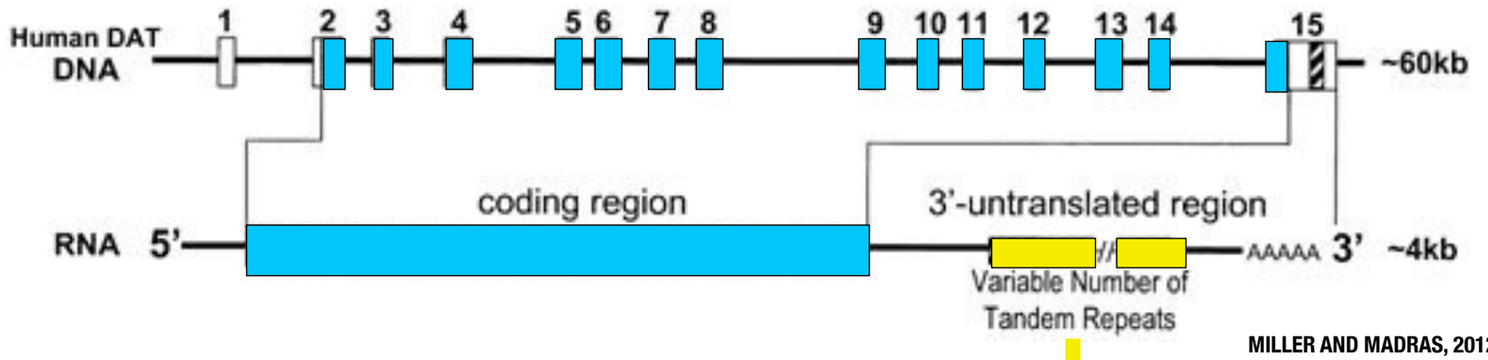


MAJOR MEDICATIONS TO TREAT ADHD TARGET DAT



HUMAN DAT GENE - *SLC6A3*

CHROMOSOME 5



MILLER AND MADRAS, 2012

Am. J. Hum. Genet. 56:993–998, 1995

Association of Attention-Deficit Disorder and the Dopamine Transporter Gene

Edwin H. Cook, Jr.,[1,2,3] Mark A. Stein,[1,3] Matthew D. Krasowski,[1,2] Nancy J. Cox,[4] Deborah M. Olkon,[1] John E. Kieffer,[1] and Bennett L. Leventhal[1,2,3]

[1] Child and Adolescent Psychiatry, Department of Psychiatry, [2]Laboratory of Developmental Neuroscience, Harris Center for Developmental Studies, [3]Department of Pediatrics, and [4]Department of Medicine, University of Chicago, Chicago

JOURNAL OF CHILD PSYCHOLOGY AND PSYCHIATRY

Journal of Child Psychology and Psychiatry 55:8 (2014), pp 914–923 doi:10.1111/jcpp.12212

Genetics of preparation and response control in ADHD: the role of DRD4 and DAT1

Björn Albrecht,[1,2,*] Daniel Brandeis,[2,3,4,5,*] Henrik Uebel- von Sanderslehen,[1] Lilian Valko,[2] Hartmut Heinrich,[6,7] Xiaohui Xu,[8] Renate Drechsler,[9] Alexander Heise,[1] Jonna Kuntsi,[8] Ueli C. Müller,[5,2] Philip Asherson,[8] Hana-Christoph Steinhausen,[5,10,11] Aribert Rothenberger,[1] and Tobias Banaschewski[2]

[1]Child and Adolescent Psychiatry, University Medical Center Göttingen, Göttingen, Germany; [2]Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim/ Heidelberg University, Mannheim, Germany; [3]Department of Child and Adolescent Psychiatry, University of Zürich, Zürich, Switzerland; [4]Center for Integrative Human Physiology, University of Zürich, Zürich, Switzerland; [5]Neuroscience Center Zurich, University of Zurich and ETH Zurich, Zürich, Switzerland; [6]Department of Child and Adolescent Mental Health, University of Erlangen, Erlangen, Germany; [7]Hochacher- Klinik, München, Germany; [8]King's College London, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, London, UK; [9]Interkantonale Hochschule für Heilpädagogik, Zürich, Switzerland; [10]Research Unit of Child and Adolescent Psychiatry, Aalborg University Hospital, Aalborg, Denmark; [11]Clinical Psychology and Epidemiology, University of Basel, Basel, Switzerland

Šerý *et al. Behavioral and Brain Functions* (2015) 11:21
DOI 10.1186/s12993-015-0066-8

BBF BEHAVIORAL AND BRAIN FUNCTIONS

RESEARCH Open Access

A 40-bp VNTR polymorphism in the 3′-untranslated region of *DAT1/SLC6A3* is associated with ADHD but not with alcoholism

Omar Šerý[1,2*], Ivo Pacl[3], Ivana Drtílková[4], Pavel Theiner[4], Marta Kopečková[3], Petr Zvolský[3] and Vladimír J. Balcar[3]

COMMON GENETIC VARIATION IN DAT GENE AND ADHD

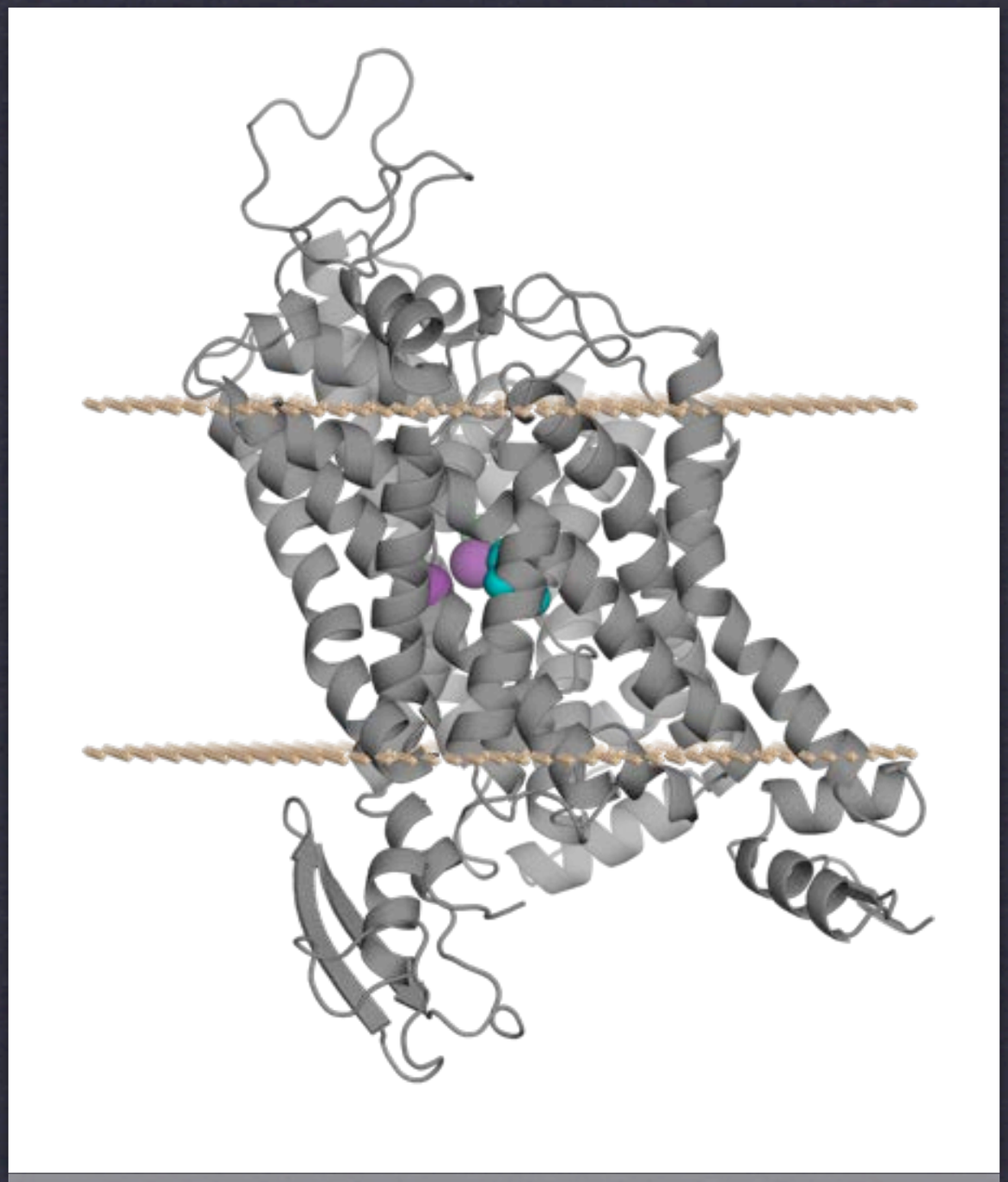
HYPOTHESIS

- ADHD is a brain disorder that may possess, and even be enriched for, highly penetrant but overall rare, genetic variants that target DA signaling including DAT.

- Characterization of such variants *in vitro* and *in vivo* is needed to support the hypothesis that a perturbation of DA signaling can contribute to the traits of ADHD

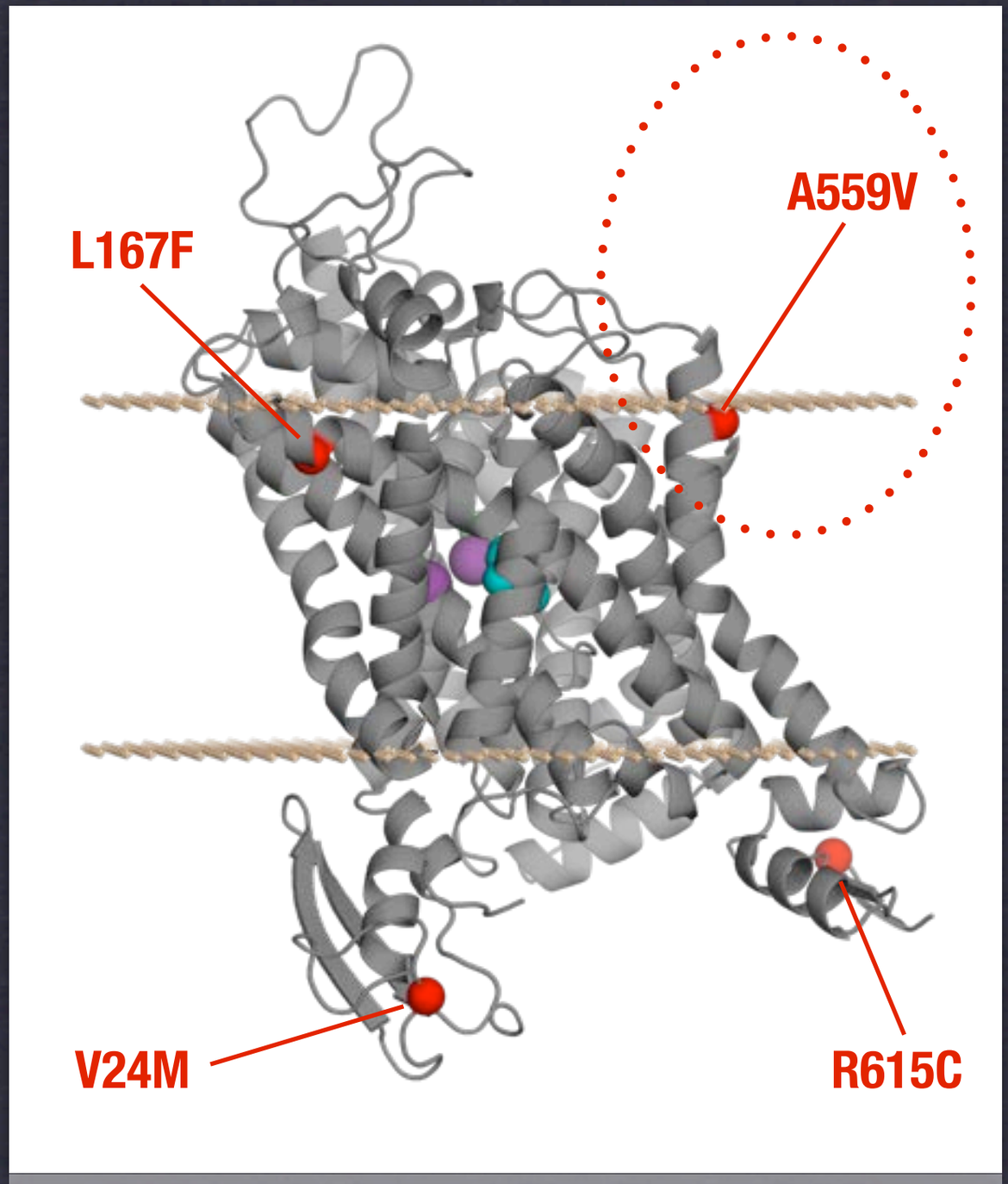
- Identification of rare, conserved genetic variation in DAT may help build useful animal models that can elucidate molecular, cellular and circuit level mechanisms linked to ADHD.

DAT CODING VARIATION: RARE OPPORTUNITIES FOR PROGRESS?



MAZEI-ROBISON ET AL., 2005
SAKRIKAR ET AL, 2012
MERGY ET AL, 2014

MULTIPLE DAT CODING VARIANTS IDENTIFIED IN ADHD



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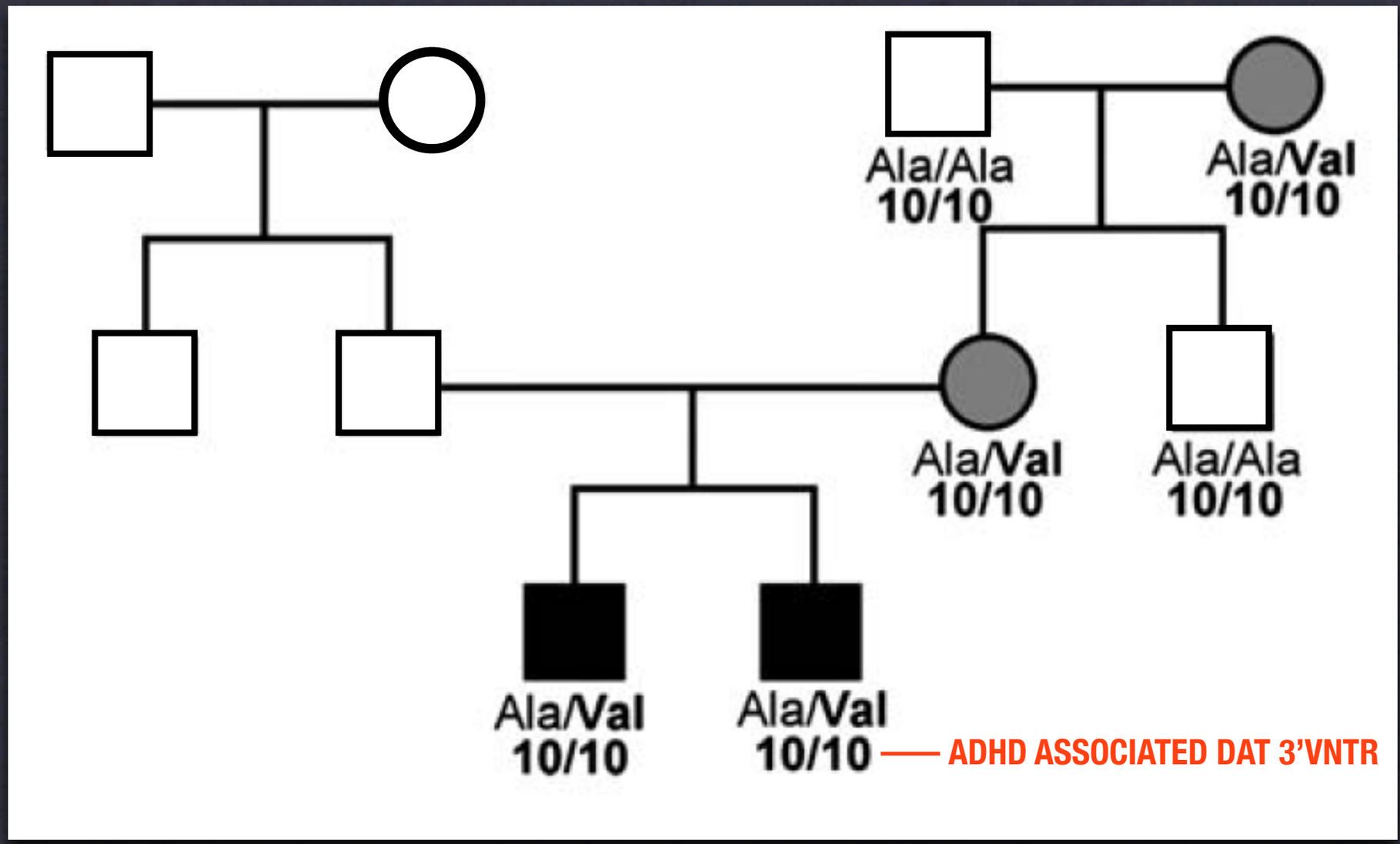


- BIPOLAR DISORDER: GRUNHAGE ET AL 2000
- AUTISM: BOWTON ET AL, 2014

MAZEI-ROBISON ET AL., 2005, 2008

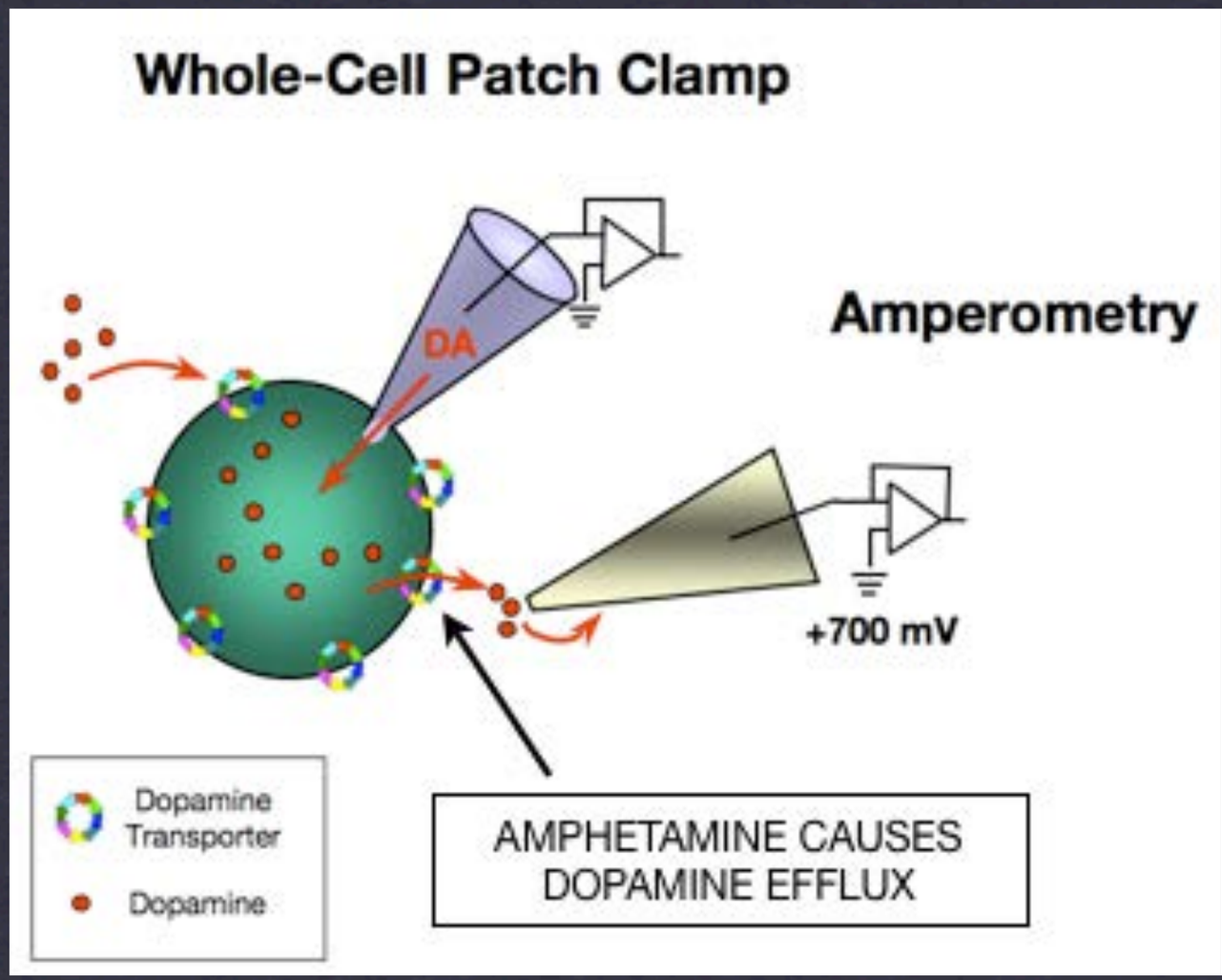
IDENTIFICATION OF DAT VAL559 IN TWO MALE ADHD SIBLINGS

AND NOW FOR SOMETHING COMPLETELY NORMAL...

- Normal DAT total protein levels
- Normal DAT surface expression
- Normal dopamine transport activity
- Normal affinity for dopamine
- Normal affinity for amphetamine and methylphenidate
- Normal despondent reaction of graduate student

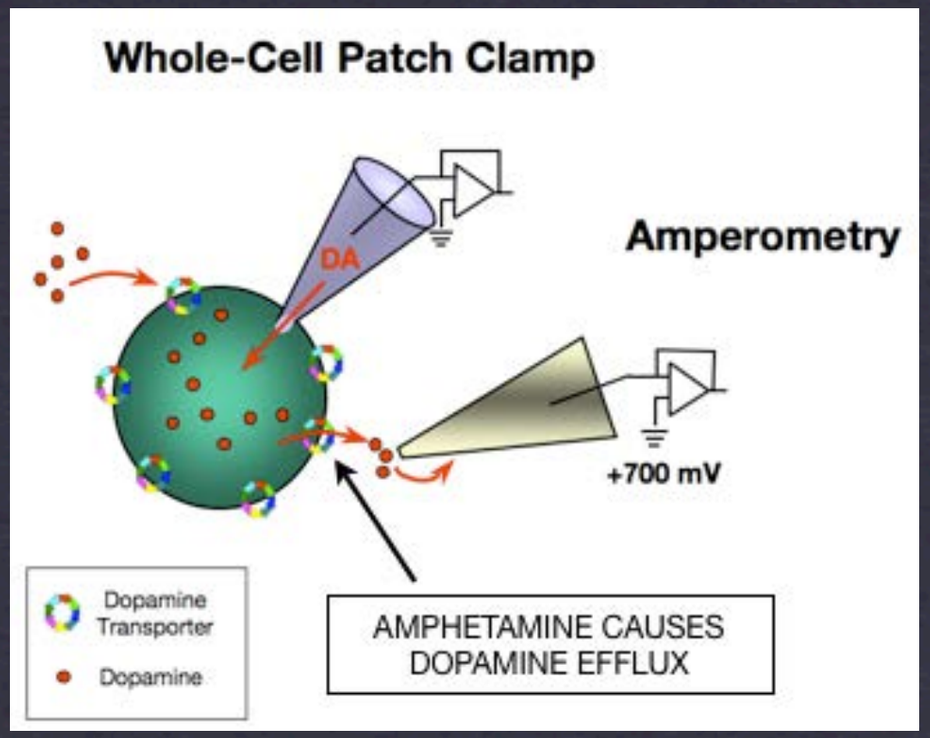
MAZEI-ROBISON ET AL 2008

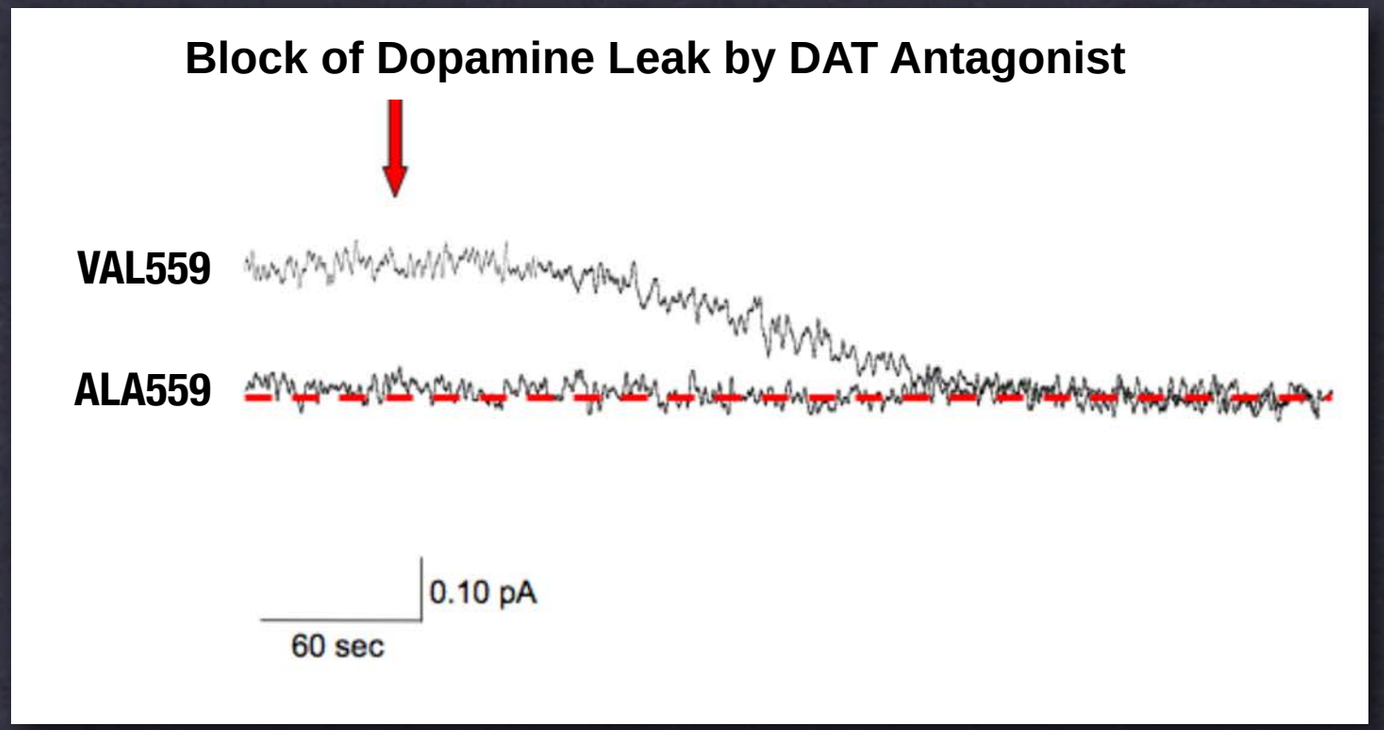
DAT VAL559: INITIAL IN VITRO STUDIES COME UP DRY

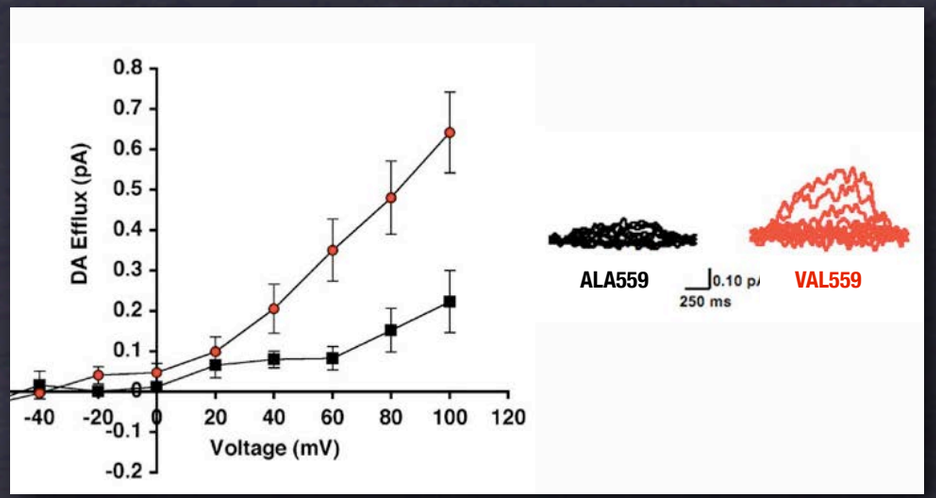


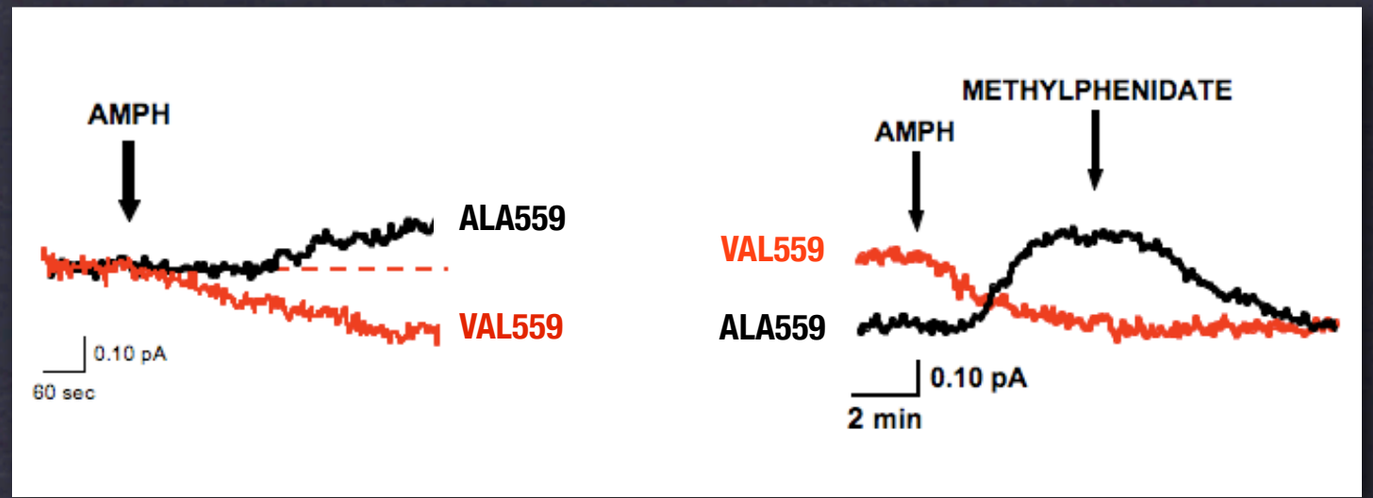
MAZEI-ROBISON ET AL 2008

DAT VAL559 DISPLAYS ANOMALOUS DA EFFLUX (ADE)





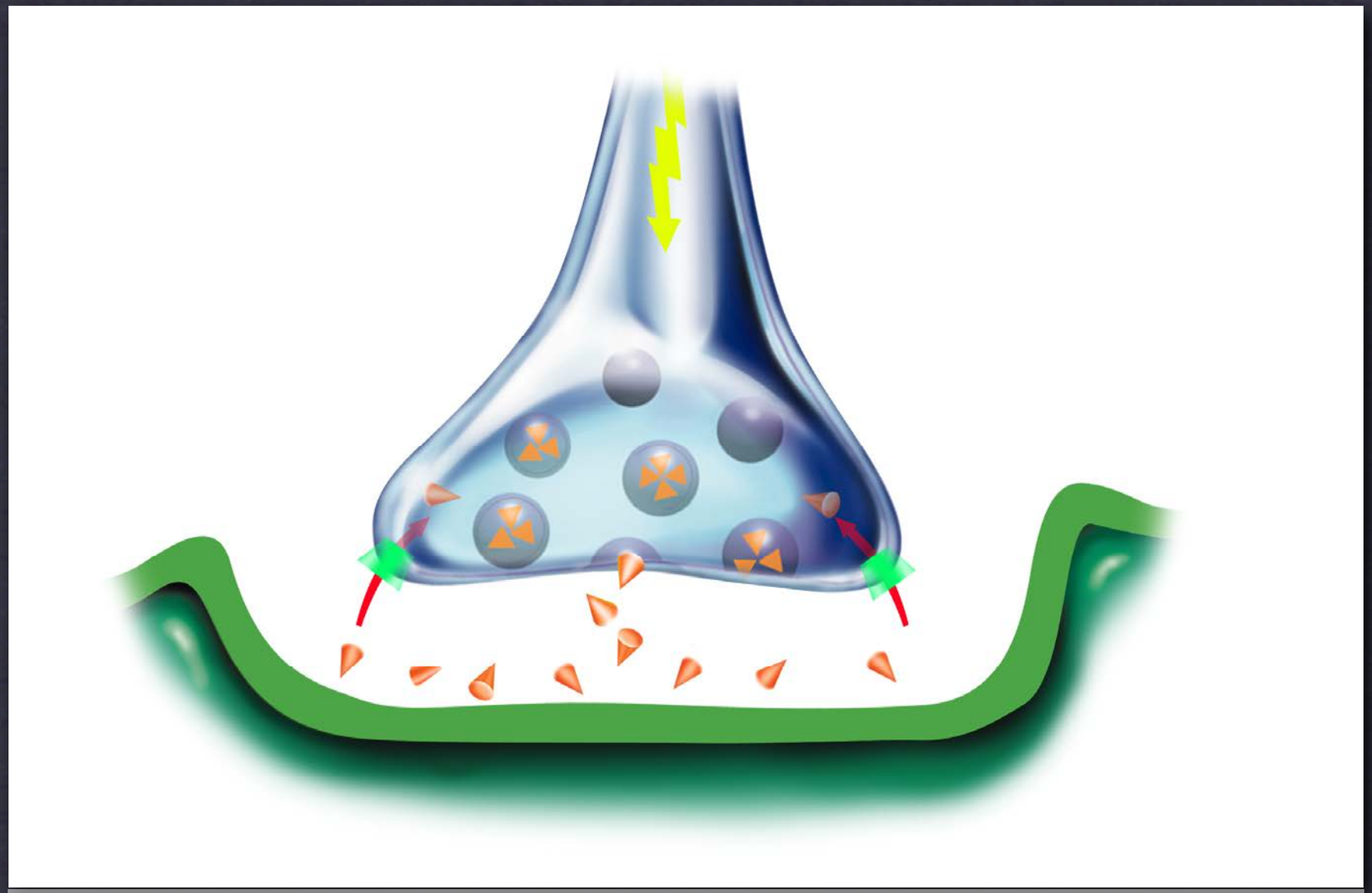




MAZEI-ROBISON ET AL 2008

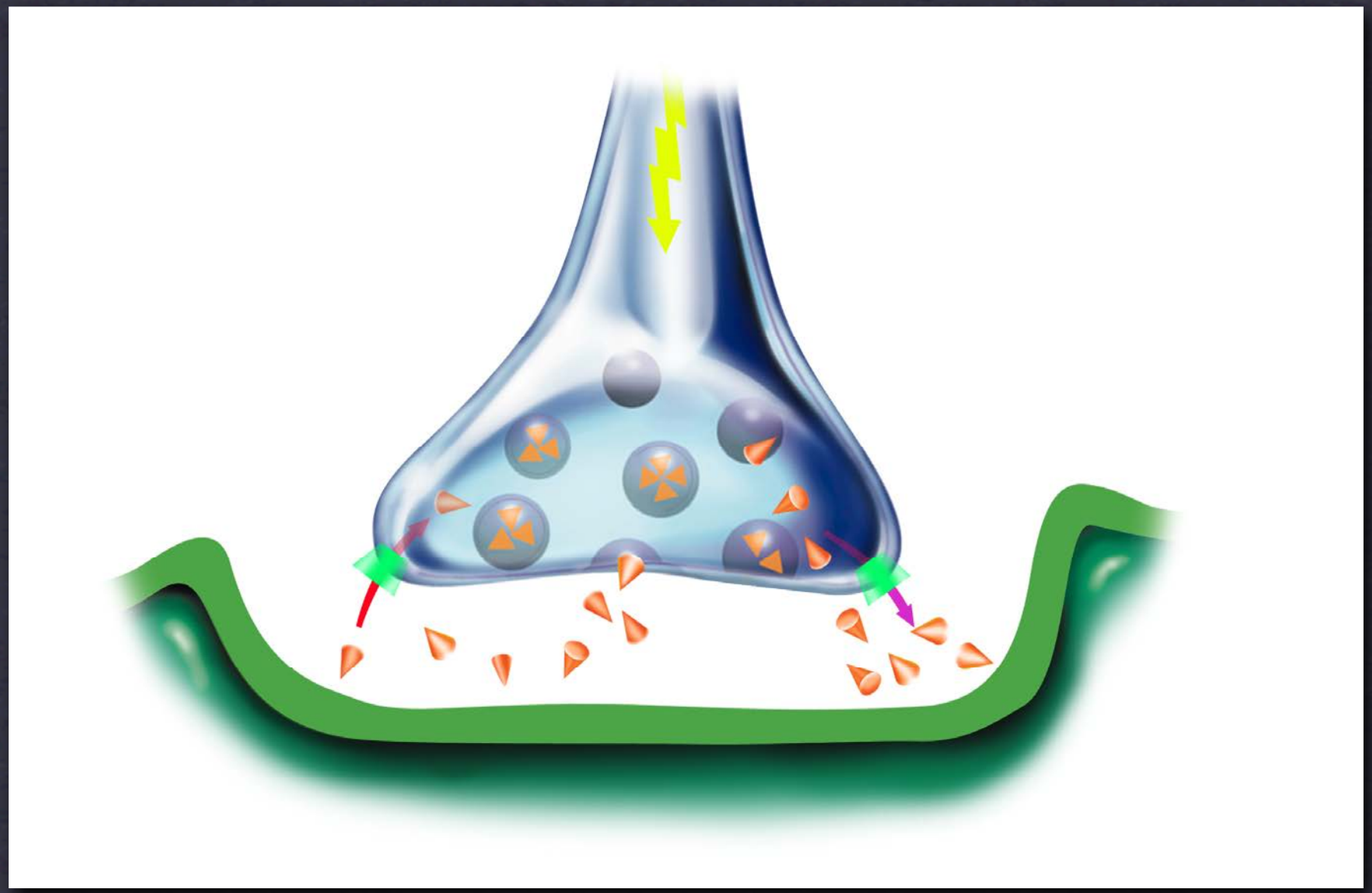
DAT VAL559 DISPLAYS ANOMALOUS DA EFFLUX (ADE)

NORMAL DAT

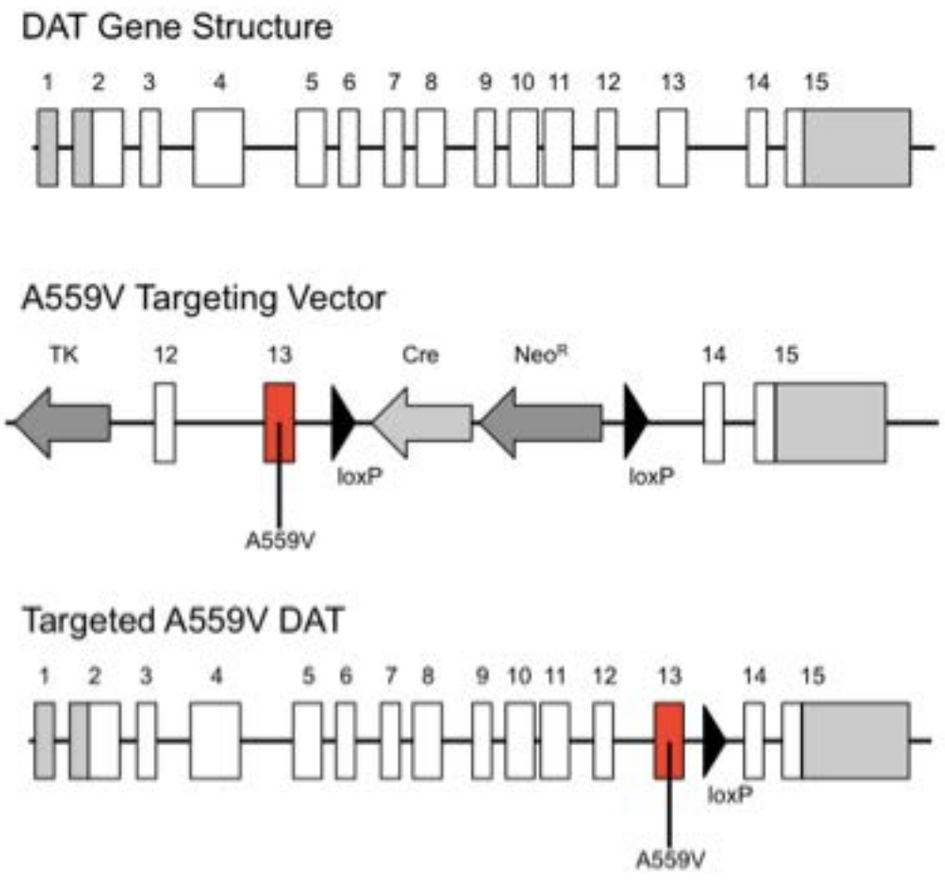


PROPOSED SYNAPTIC IMPACT OF DAT VAL559

LEAKY MUTANT DAT = VAL559



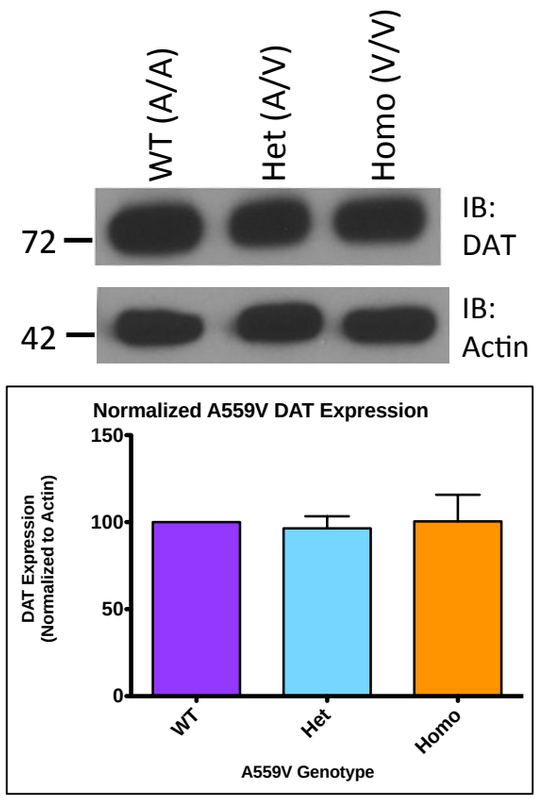
PROPOSED SYNAPTIC IMPACT OF DAT VAL559



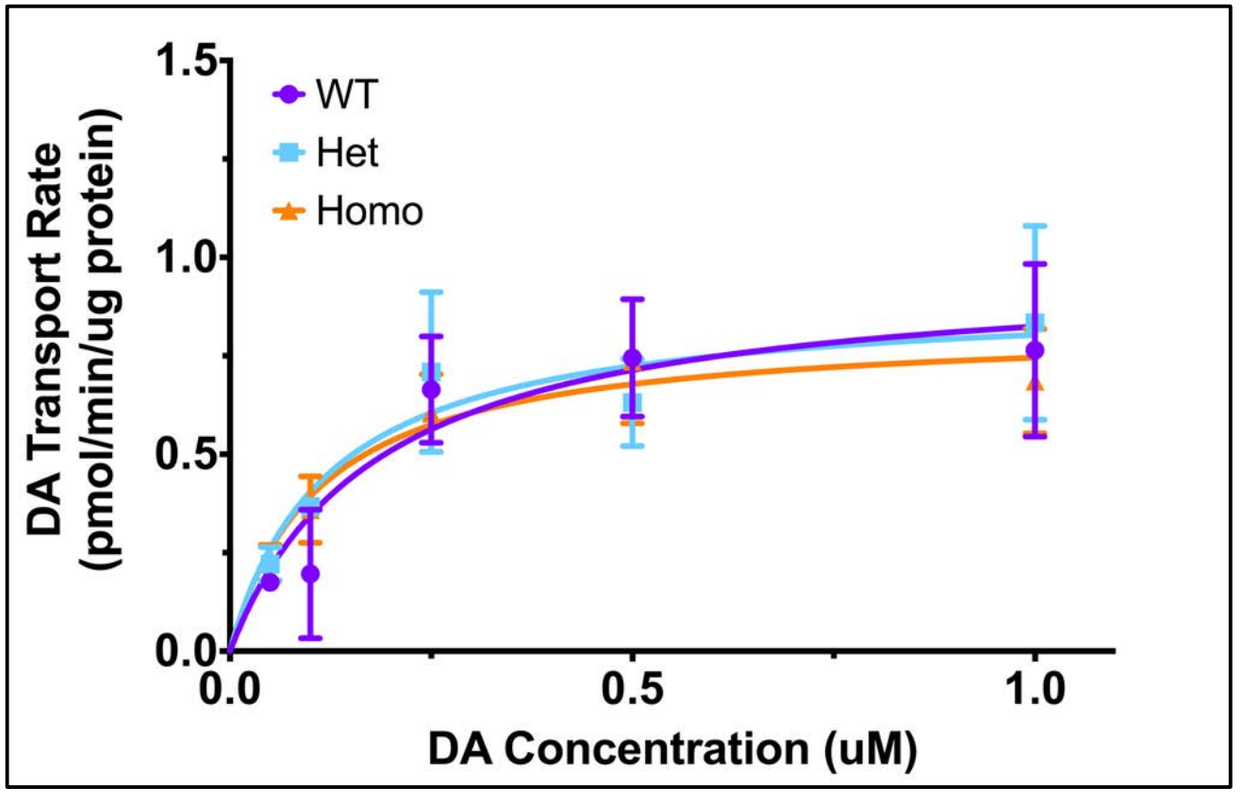
MERGY ET AL 2014

DAT VAL559 MICE: MICE WITH ADHD TRAITS?

DAT PROTEIN



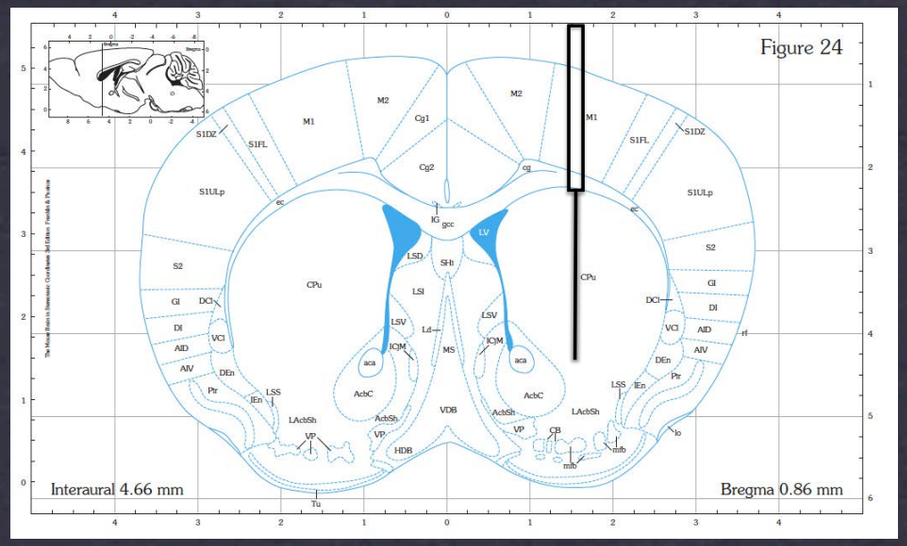
DA UPTAKE

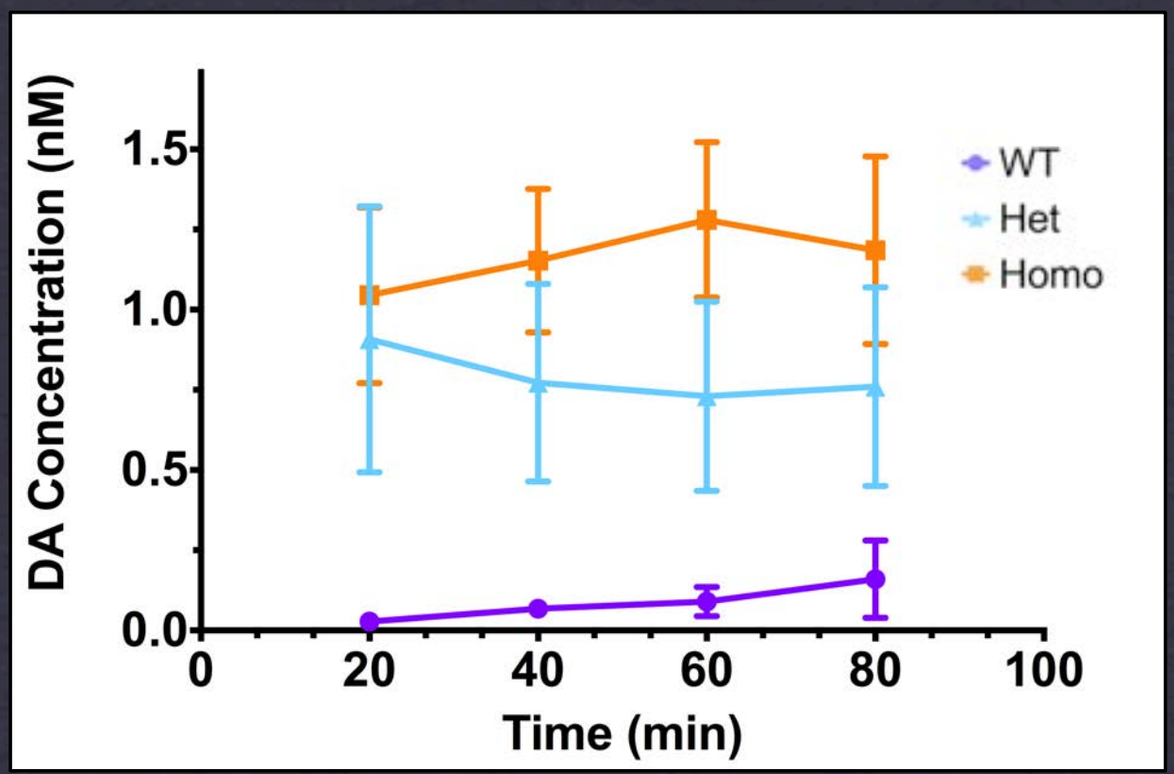


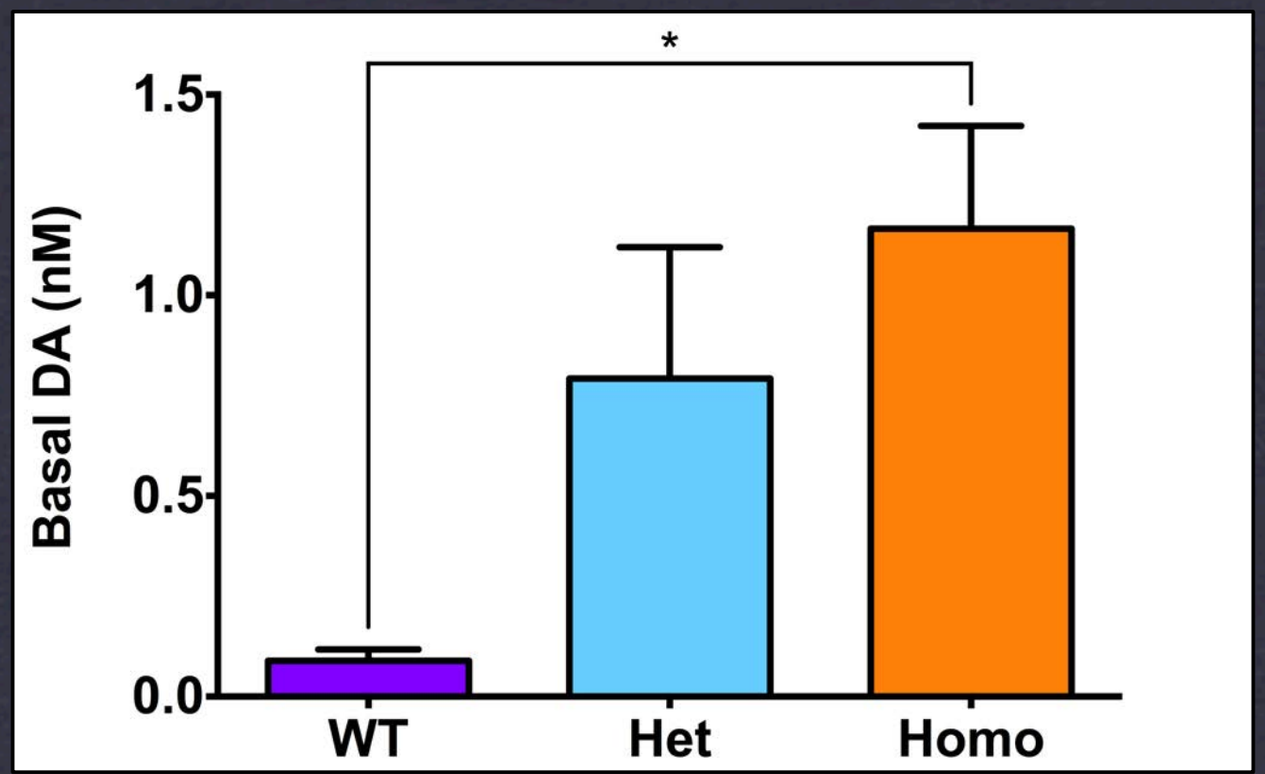
MERGY ET AL 2014

DAT VAL559 MICE: NORMAL TRANSPORTER PROTEIN LEVELS AND DOPAMINE UPTAKE INTO NERVE TERMINALS

MICRODIALYSIS SAMPLING OF EXTRACELLULAR DA LEVELS

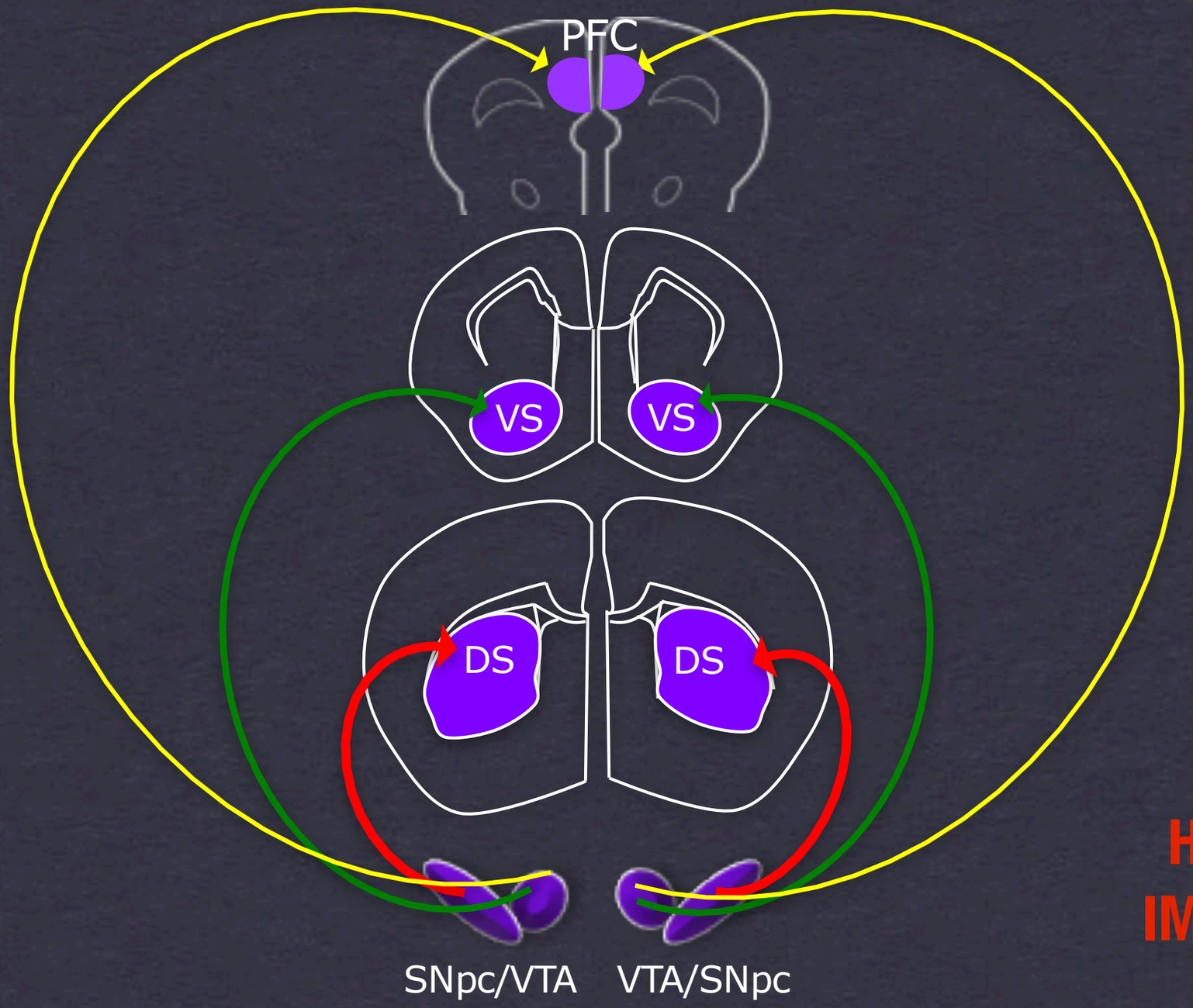






MERGY ET AL 2014

ELEVATED EXTRACELLULAR DOPAMINE IN DAT VAL559 MICE



EXECUTIVE FUNCTION ATTENTION

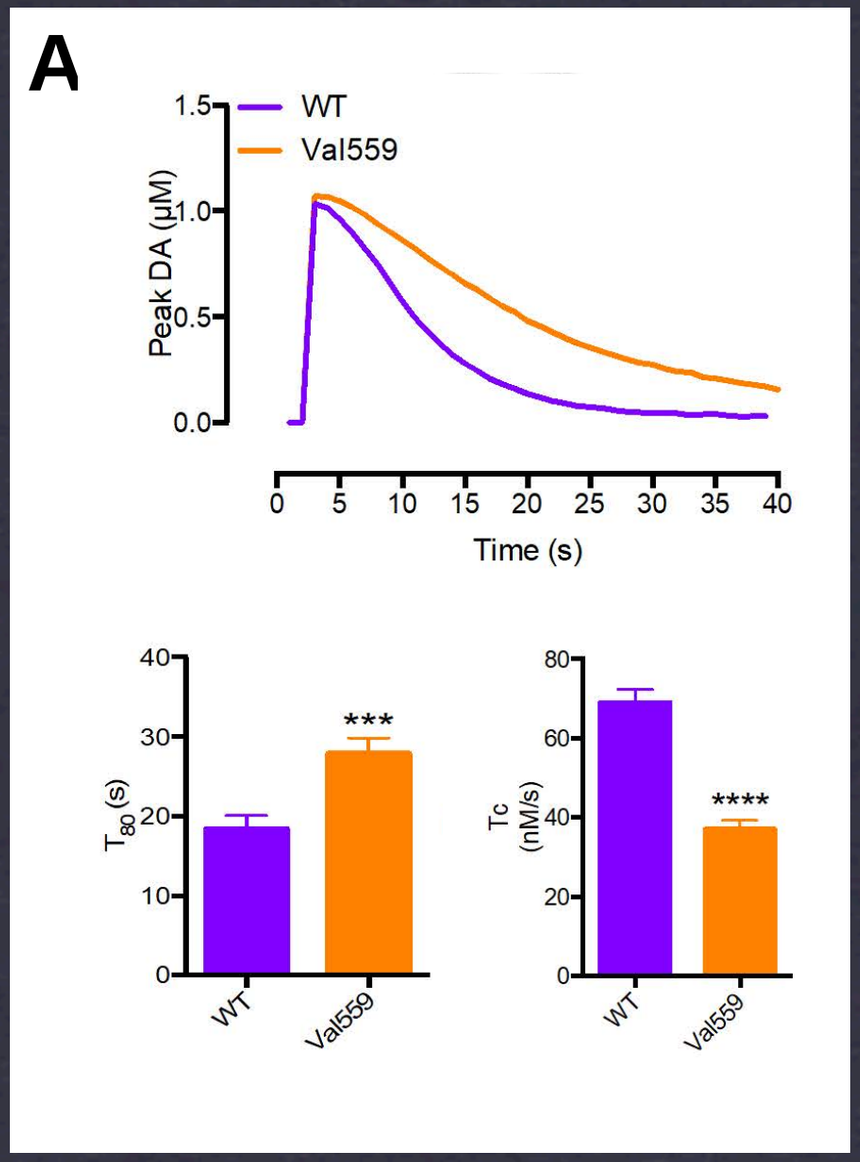
REWARD SALIENCE

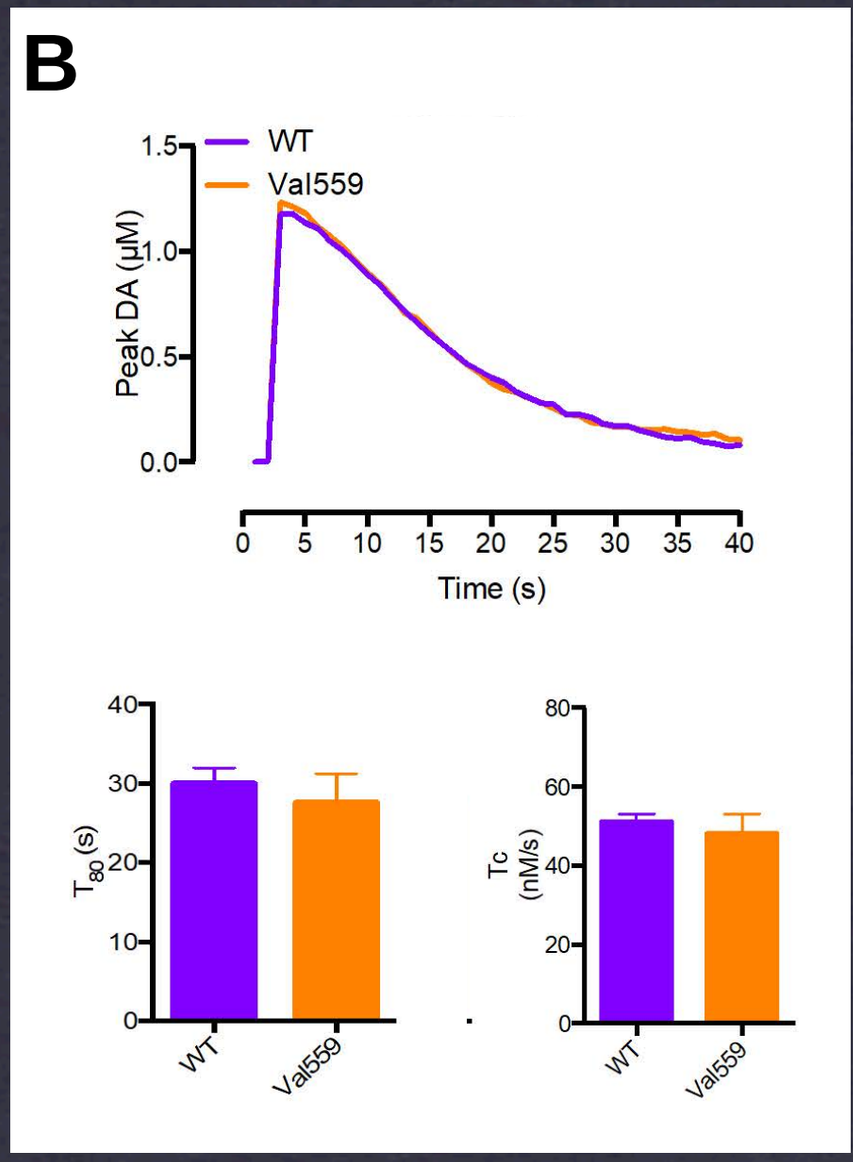
LOCOMOTION HABIT LEARNING IMPULSE CONTROL

DISTINCT DA PROJECTIONS SUBSERVE DIFFERENT BEHAVIORAL DOMAINS

DORSAL STRIATUM

VENTRAL STRIATUM



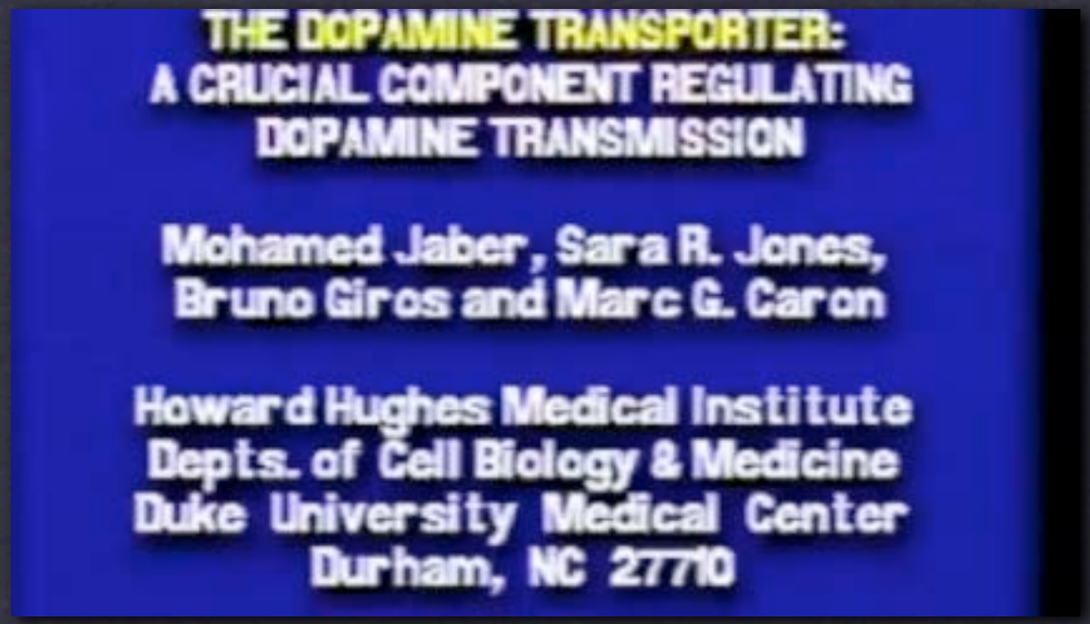


GOWRISHANKAR ET AL, 2018

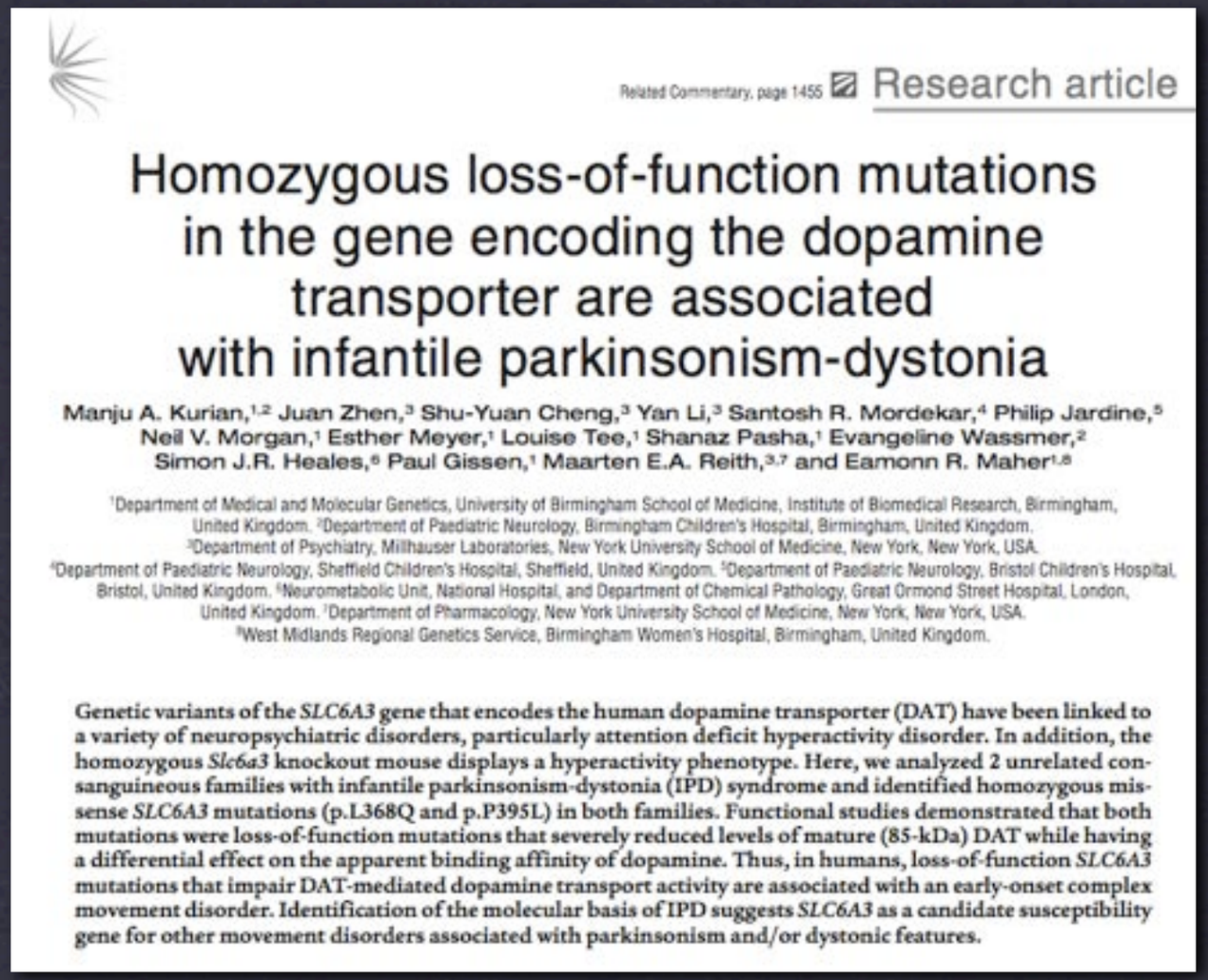
MUTANT DAT IN ALL DA BRAIN REGIONS BUT DAT VAL559 EFFECTS ONLY IN SOME

DAT VAL559 MOUSE: BEHAVIOR

Spontaneous and Drug-Modulated Locomotor Activation



GIROS ET AL, 1996



Related Commentary, page 1455 Research article

Homozygous loss-of-function mutations in the gene encoding the dopamine transporter are associated with infantile parkinsonism-dystonia

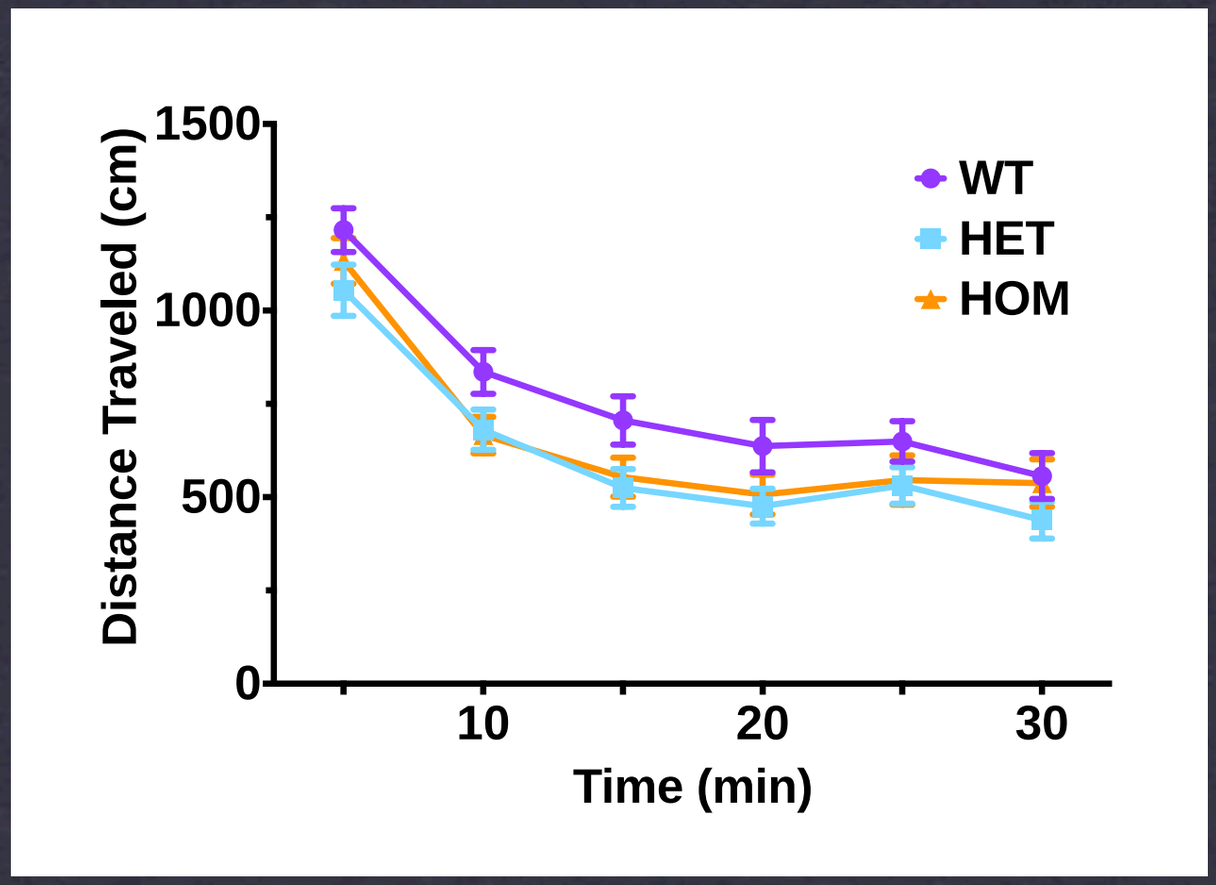
Manju A. Kurian,[1,2] Juan Zhen,[3] Shu-Yuan Cheng,[3] Yan Li,[3] Santosh R. Mordekar,[4] Philip Jardine,[5] Neil V. Morgan,[1] Esther Meyer,[1] Louise Tee,[1] Shanaz Pasha,[1] Evangeline Wassmer,[2] Simon J.R. Heales,[6] Paul Gissen,[1] Maarten E.A. Reith,[3,7] and Eamonn R. Maher[1,8]

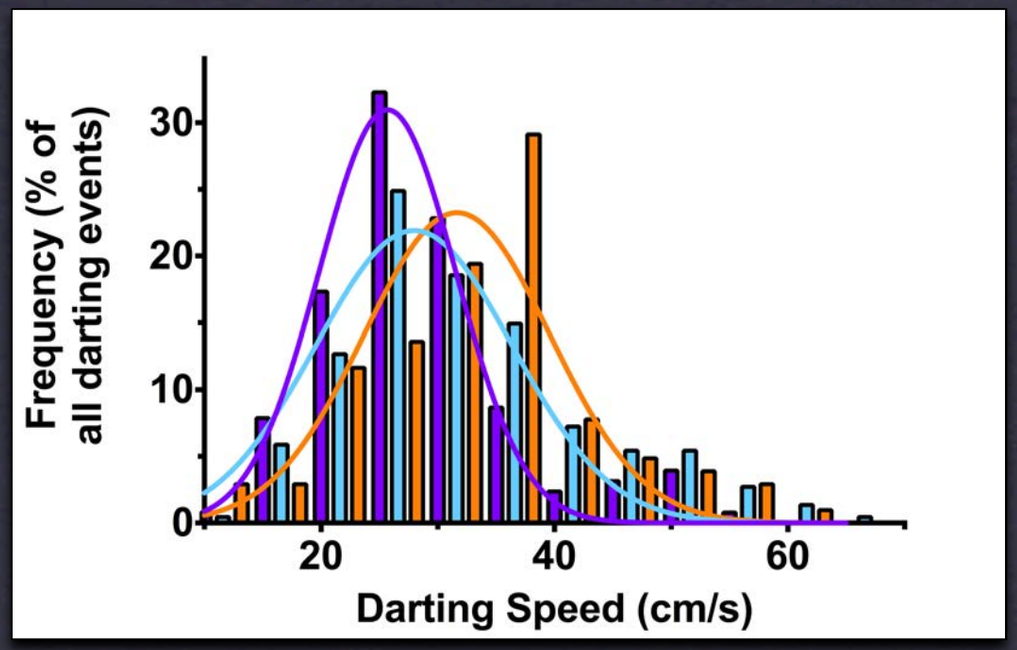
[1]Department of Medical and Molecular Genetics, University of Birmingham School of Medicine, Institute of Biomedical Research, Birmingham, United Kingdom. [2]Department of Paediatric Neurology, Birmingham Children's Hospital, Birmingham, United Kingdom. [3]Department of Psychiatry, Millhauser Laboratories, New York University School of Medicine, New York, New York, USA. [4]Department of Paediatric Neurology, Sheffield Children's Hospital, Sheffield, United Kingdom. [5]Department of Paediatric Neurology, Bristol Children's Hospital, Bristol, United Kingdom. [6]Neurometabolic Unit, National Hospital, and Department of Chemical Pathology, Great Ormond Street Hospital, London, United Kingdom. [7]Department of Pharmacology, New York University School of Medicine, New York, New York, USA. [8]West Midlands Regional Genetics Service, Birmingham Women's Hospital, Birmingham, United Kingdom.

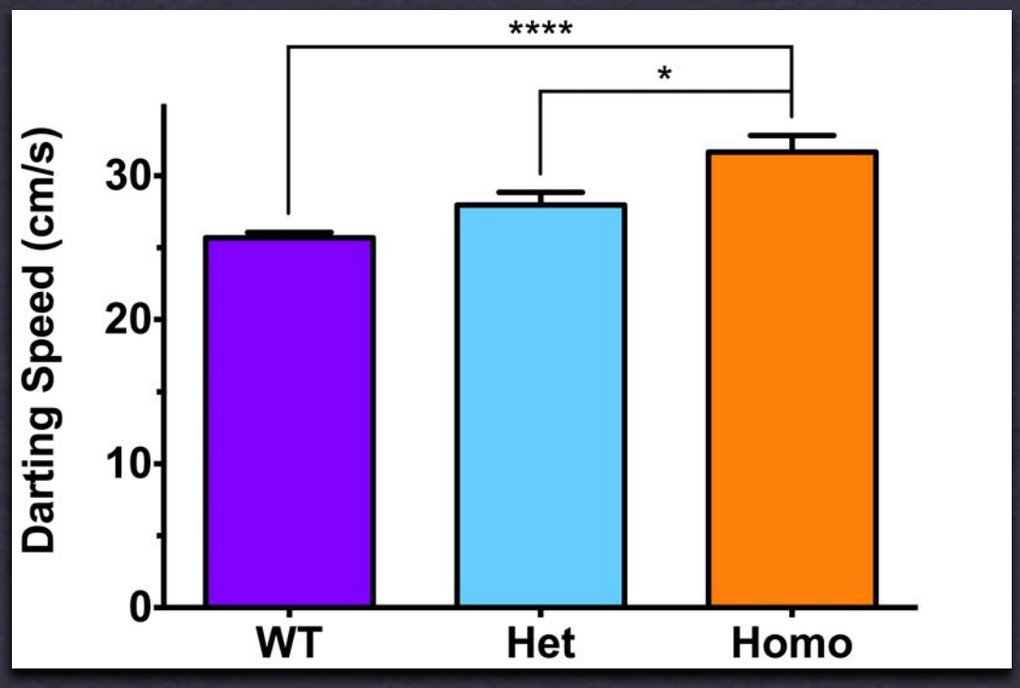
Genetic variants of the *SLC6A3* gene that encodes the human dopamine transporter (DAT) have been linked to a variety of neuropsychiatric disorders, particularly attention deficit hyperactivity disorder. In addition, the homozygous *Slc6a3* knockout mouse displays a hyperactivity phenotype. Here, we analyzed 2 unrelated consanguineous families with infantile parkinsonism-dystonia (IPD) syndrome and identified homozygous missense *SLC6A3* mutations (p.L368Q and p.P395L) in both families. Functional studies demonstrated that both mutations were loss-of-function mutations that severely reduced levels of mature (85-kDa) DAT while having a differential effect on the apparent binding affinity of dopamine. Thus, in humans, loss-of-function *SLC6A3* mutations that impair DAT-mediated dopamine transport activity are associated with an early-onset complex movement disorder. Identification of the molecular basis of IPD suggests *SLC6A3* as a candidate susceptibility gene for other movement disorders associated with parkinsonism and/or dystonic features.

KURIAN ET AL, 2009

FULL LOSS OF DAT IN MICE: PROFOUND HYPERACTIVITY



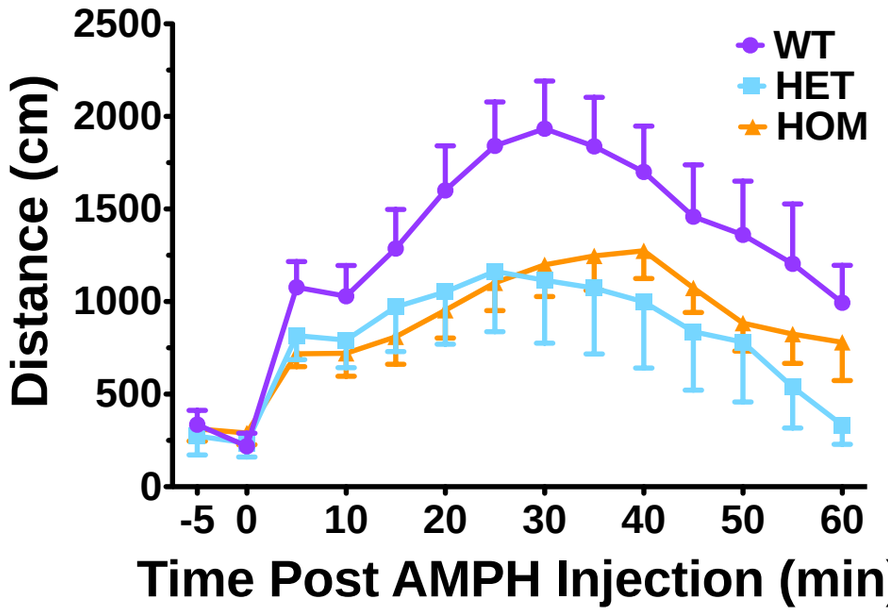




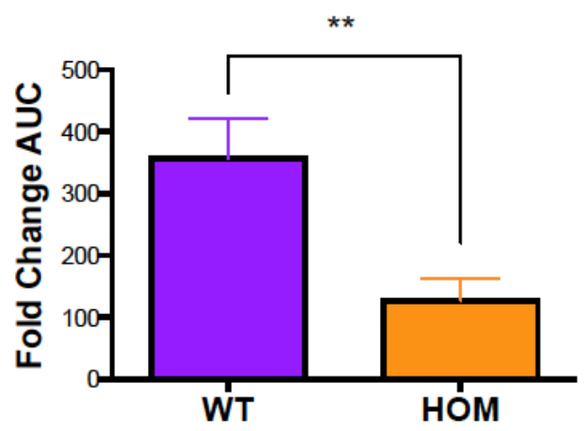
MERGY ET AL 2014

DAT VAL559 MICE:
A SURPRISE - LACK OF SPONTANEOUS HYPERACTIVITY

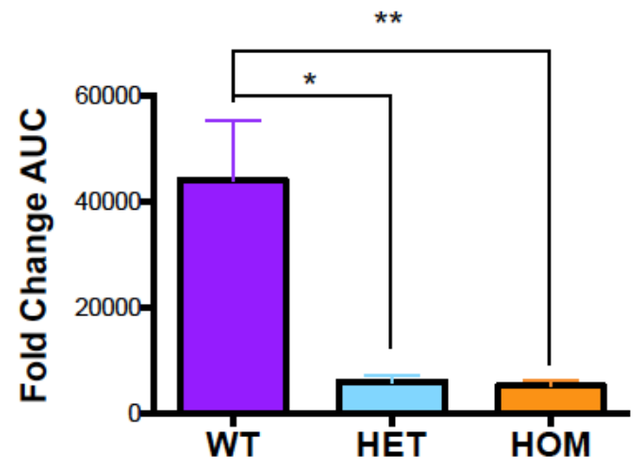
AMPH- 3 MG/KG I.P.



AMPH- 3 MG/KG I.P.



AMPH- 0.1µM IN VIVO



MICRODIALYSIS DA-LEVELS

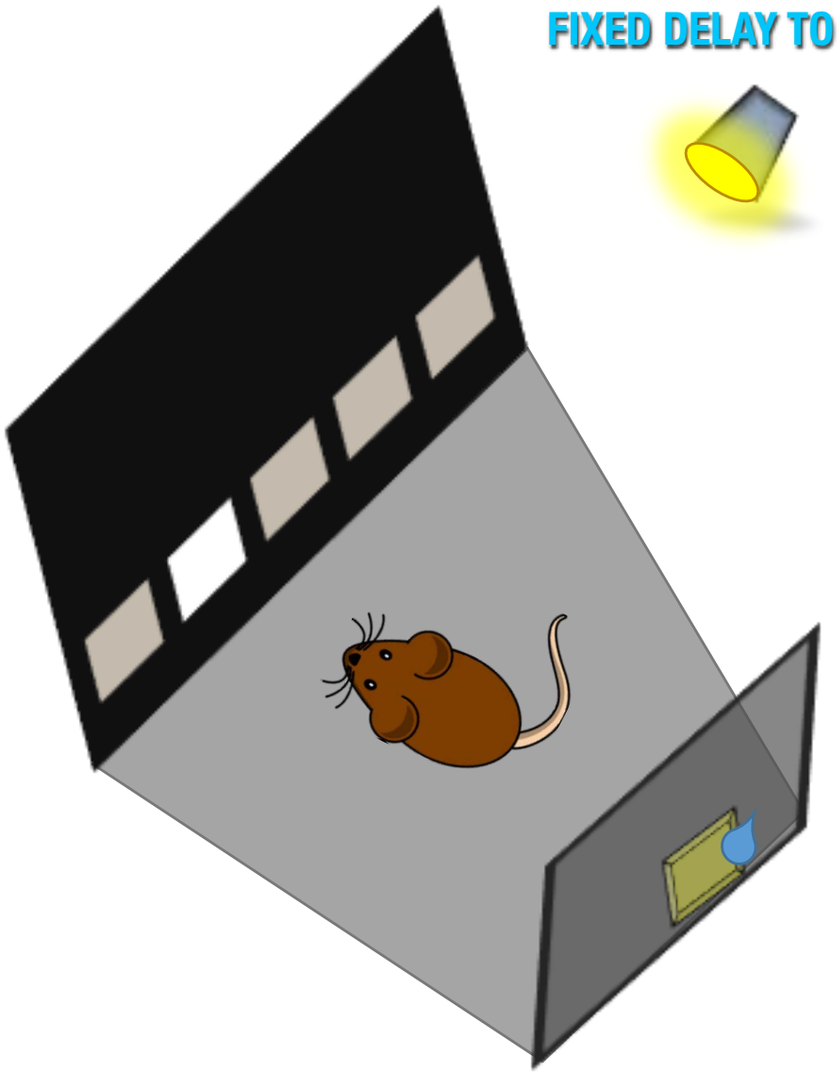
MERGY ET AL 2014

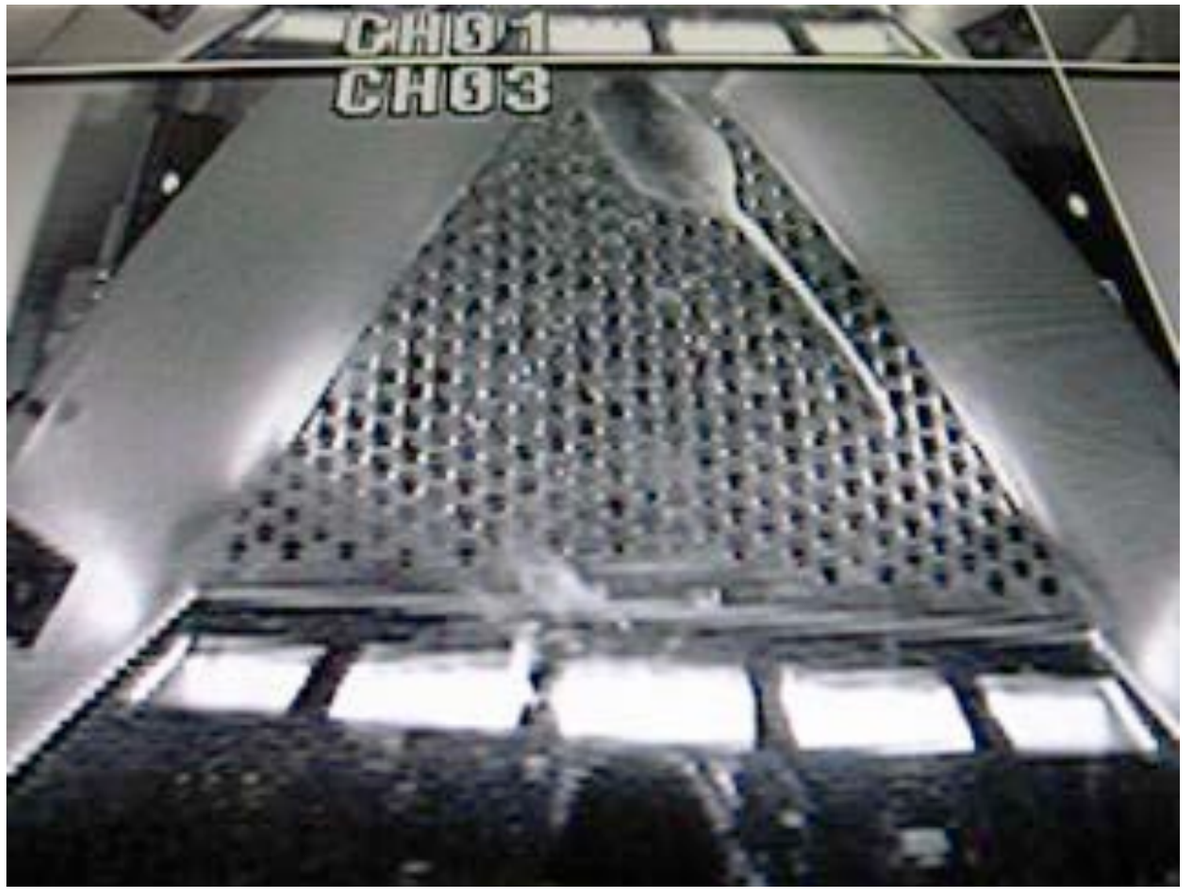
DAT VAL559 KI MICE: BLUNTED LOCOMOTOR AND DOPAMINE RESPONSE TO AMPHETAMINE

DAT VAL559 MOUSE: BEHAVIOR

Attention, Learning and Impulsivity

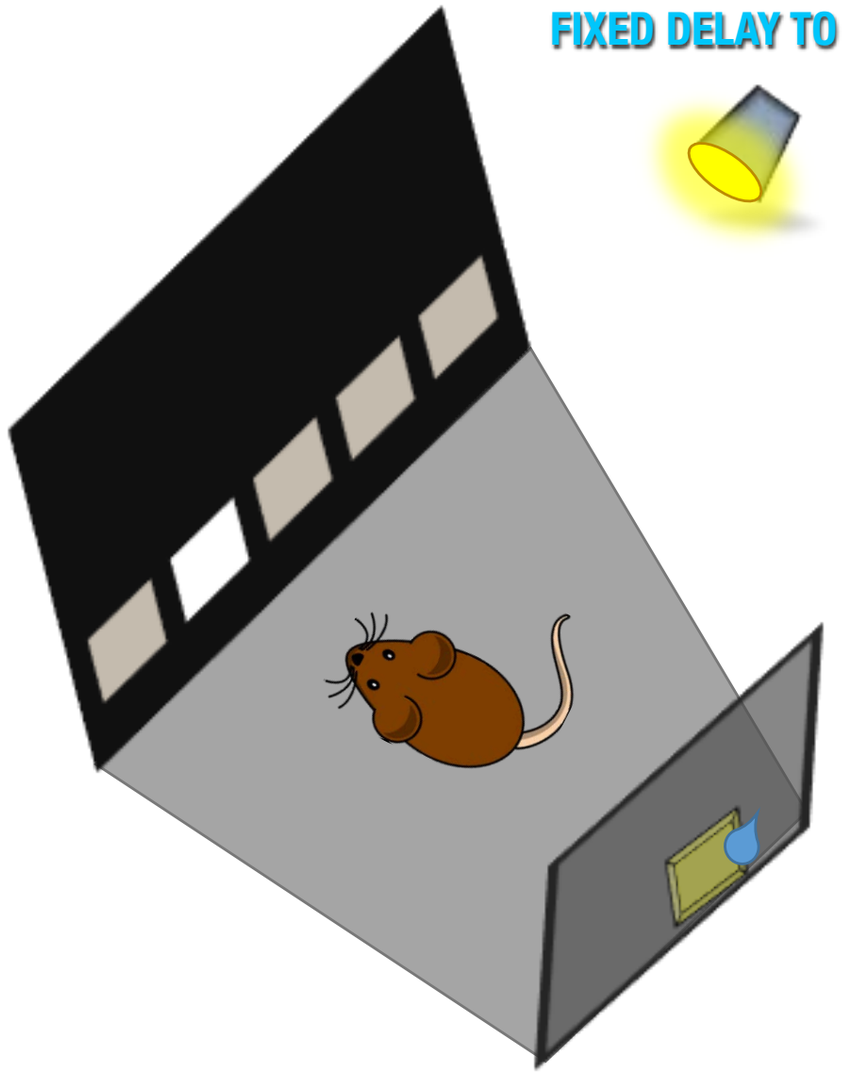
START SIGNAL
FIXED DELAY TO CHOOSE

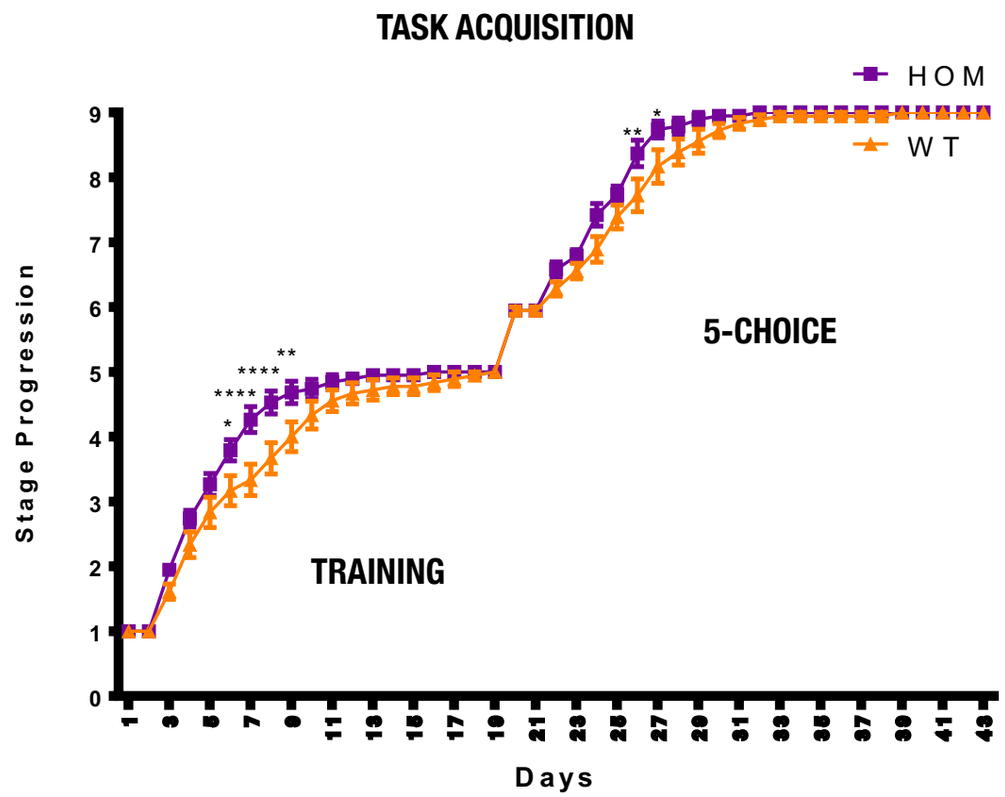




DAVIS ET AL 2017

A TASK TO EVALUATE THE LEARNING CAPACITY AND IMPULSIVITY OF DAT VAL559

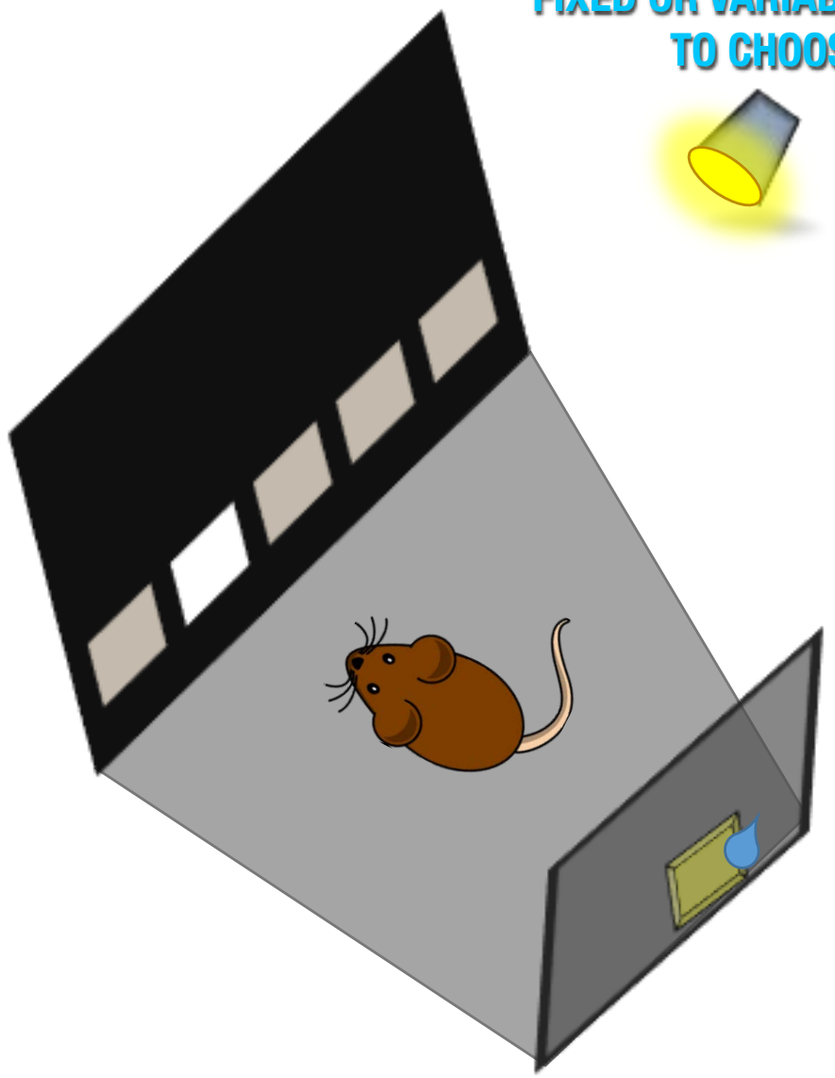




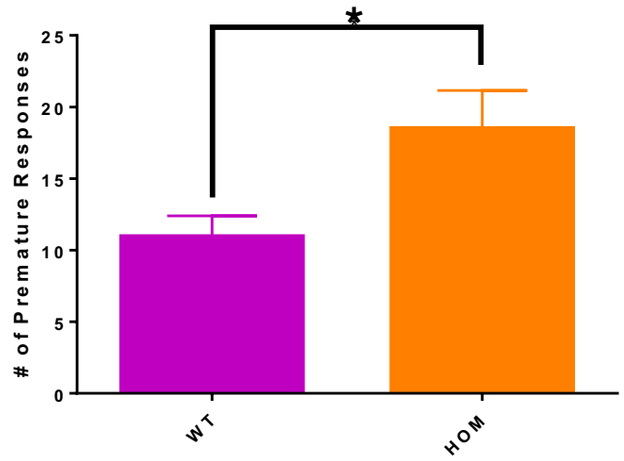
DAVIS ET AL 2017

A TASK TO EVALUATE THE LEARNING CAPACITY AND IMPULSIVITY OF DAT VAL559

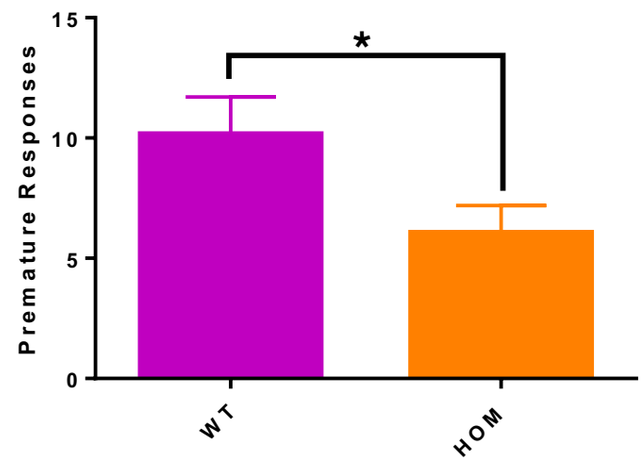
START SIGNAL
FIXED OR VARIABLE DELAY
TO CHOOSE



MORE PREMATURE RESPONSES AFTER TRAINING
ON FIXED DELAY



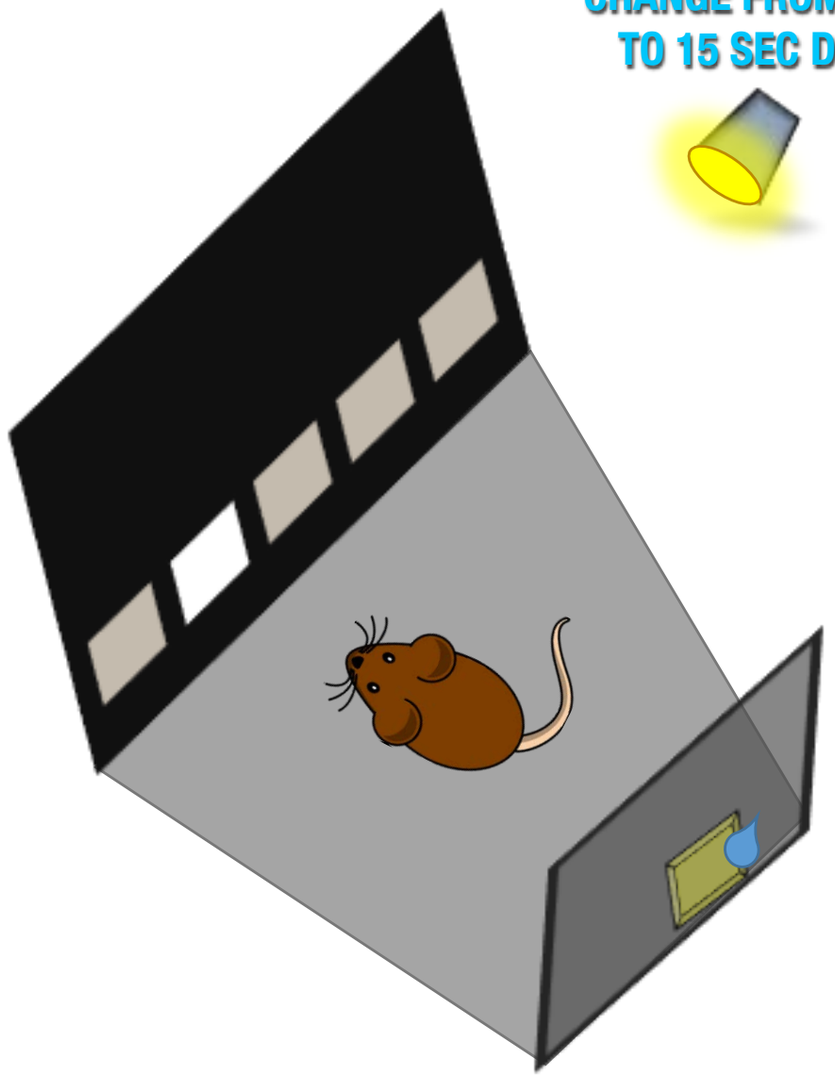
FEWER PREMATURE RESPONSES AFTER TRAINING
ON VARIABLE DELAY



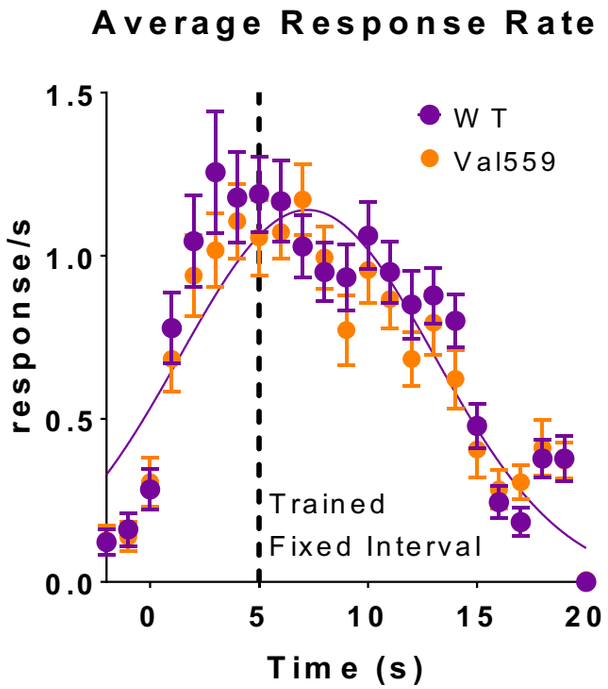
DAVIS ET AL 2017

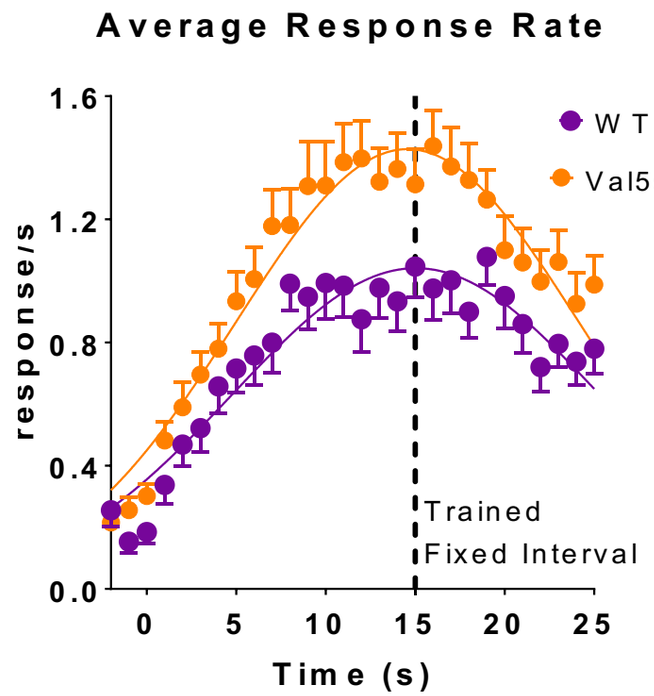
A TASK TO EVALUATE THE LEARNING CAPACITY AND IMPULSIVITY OF DAT VAL559

START SIGNAL
CHANGE FROM 5 SEC
TO 15 SEC DELAY



PEAK INTERVAL TESTING





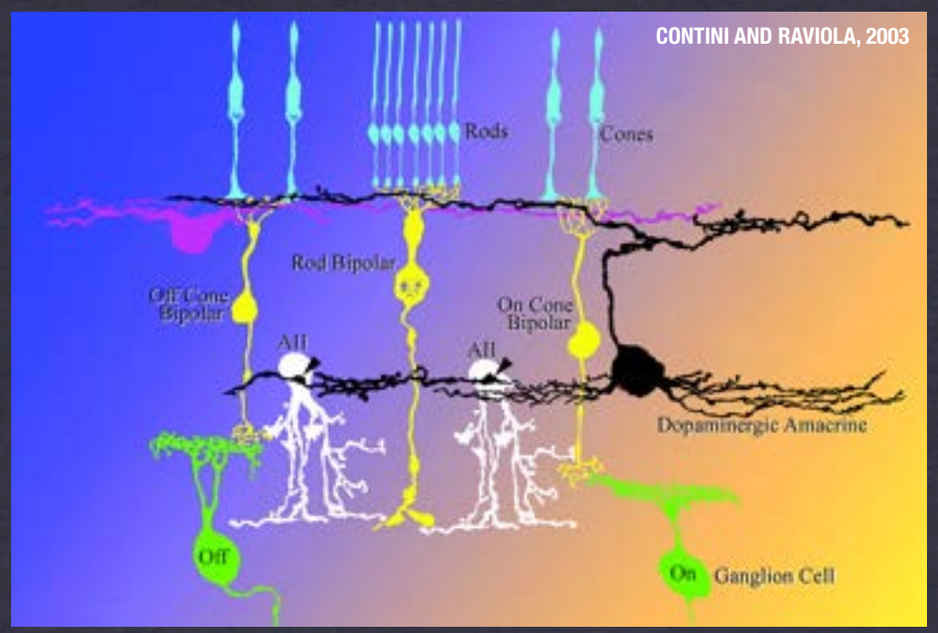
DAVIS ET AL 2018

DAT VAL559 MICE CAN ACCURATELY CALCULATE ELAPSED TIME BUT EXHIBIT ELEVATED RESPONSE RATES AS DELAYS INCREASE

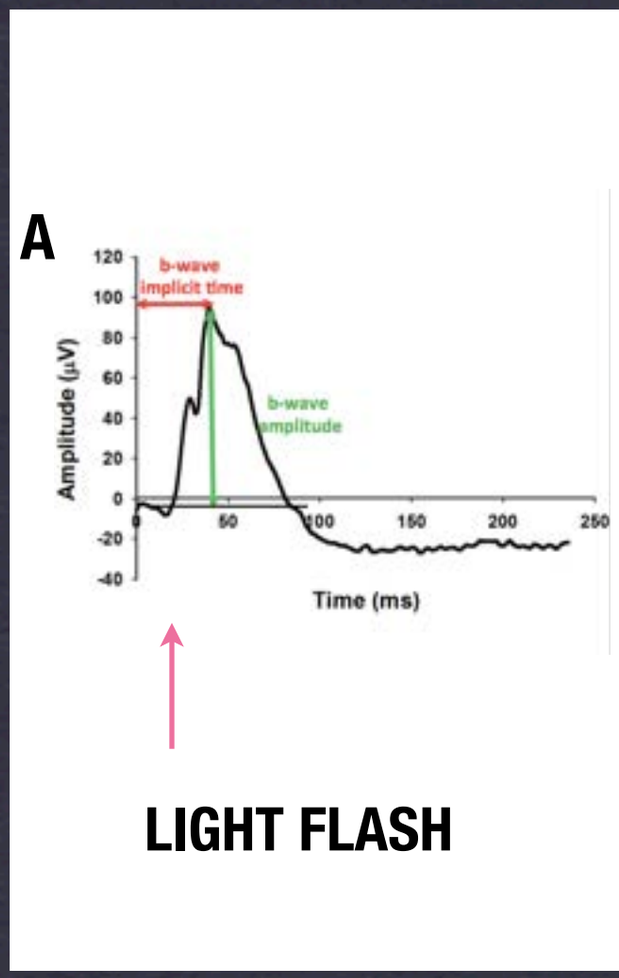
DAT VAL559 MOUSE: BIOMARKERS

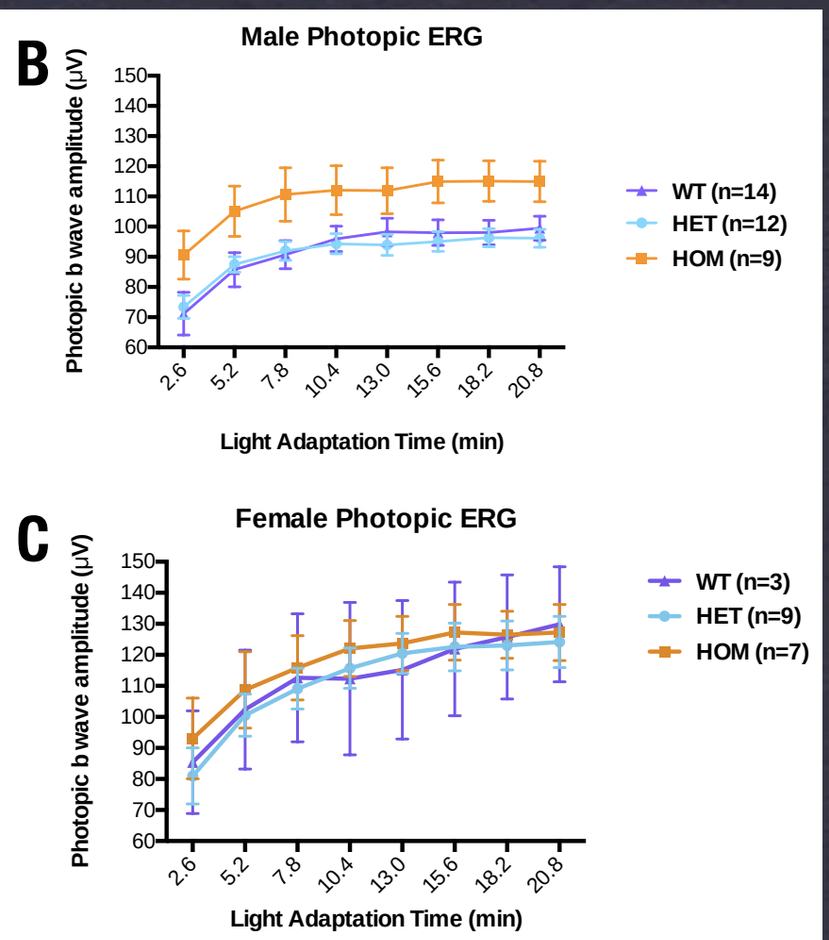
The Eyes Have It: Retinal Changes in DAT and Dopamine

RETINAL AMACRINE CELLS MAKE AND RELEASE DOPAMINE



DAT VAL559 MICE SHOW ALTERATIONS IN LIGHT-EVOKED ELECTRICAL RESPONSES



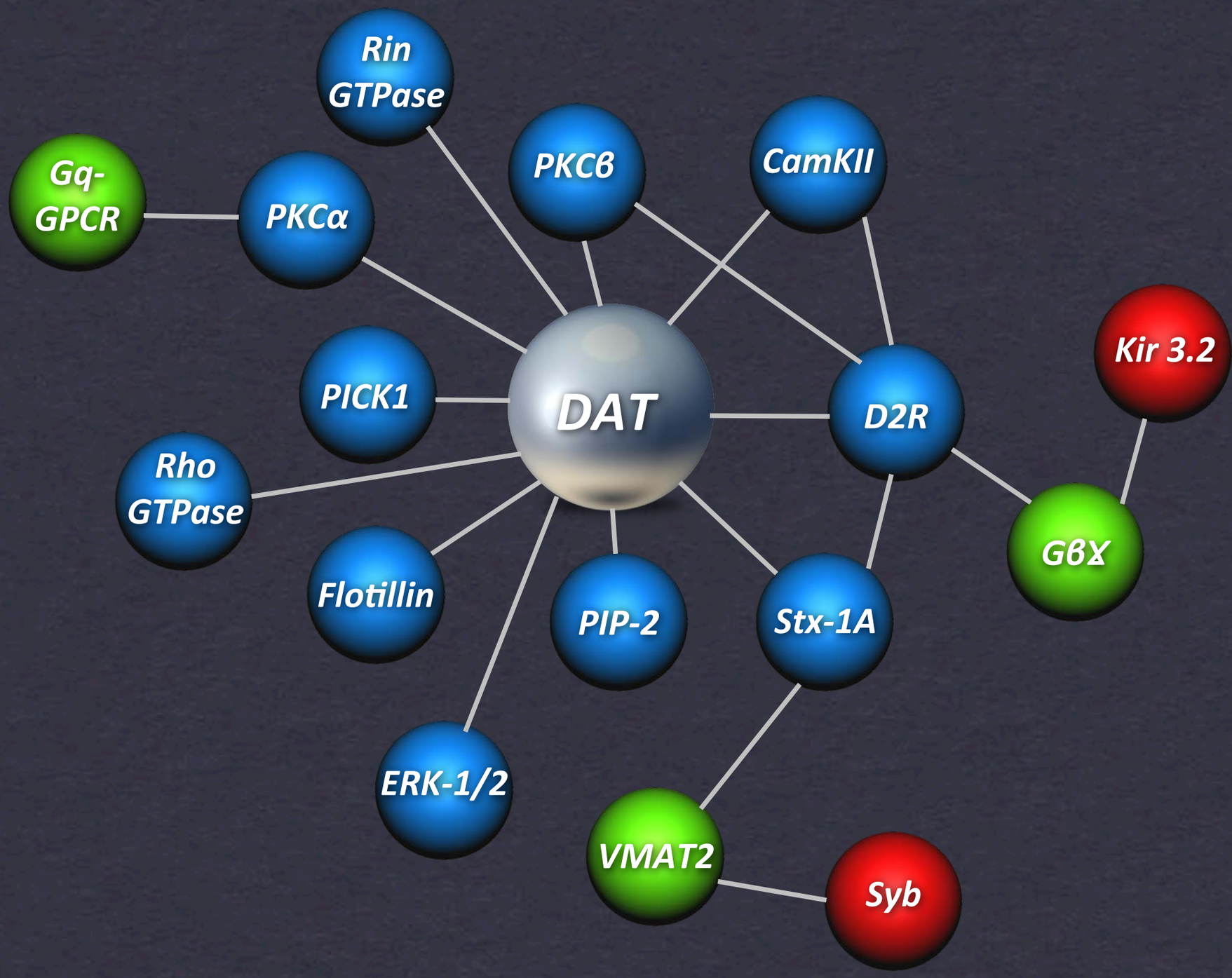


DAI ET AL IN 2017

MONITORING DOPAMINE IN THE RETINA: BIOMARKER FOR ADHD?

DAT VAL559 MOUSE: THE FUTURE

Is DAT All There Is?



NO DAT IS AN ISLAND - AN ADHD GENE NETWORK?

- Rare variation in the human dopamine transporter (DAT) gene has been identified in individuals with ADHD and ADHD comorbid disorders (BPD, ASD).

- The DAT Val559 mutation exhibits an abnormal outward leak of dopamine that can be suppressed by the two most commonly used ADHD medications.

- Adolescent male DAT Val559 mice are not spontaneously hyperactive, but display stress-induced darting behavior and increased struggling when handled, suggesting deficits in aversive learning or impulse control

- DAT Val559 mice display increased motivation for reward, elevated reward-based learning, and waiting impulsivity when trained on predictable outcomes

- Although the DAT Val559 mutation is expressed throughout the brain, dopamine handling by DAT in the dorsal striatum, a region involved in reward learning, habit formation and goal directed behavior, is particularly impacted

- DAT Val559 mice may be useful in identifing biomarkers that could provide for improved diagnosis as well as treatments tailored to to those with dopamine disturbances

DAT VAL559: SUMMARY OF FINDINGS

FAU™

BRAIN INSTITUTE

Florida Atlantic University

WHERE DISCOVERY COMES TO MIND