Oxidative stress and impaired methylation in autism: Focus on vitamin B12

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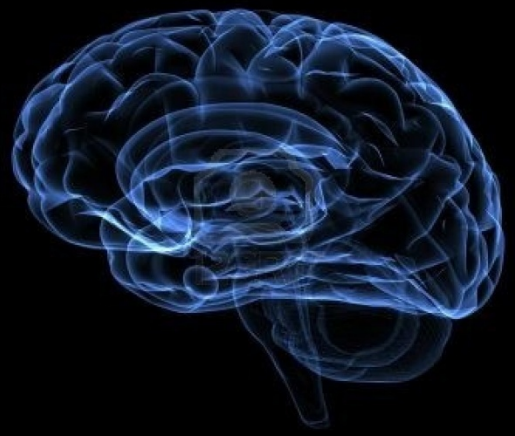


FINANCIAL DISCLOSURE:

1. Member of the scientific advisory board of Immunotec Inc.

2. Received research grant support from A2 Milk Corporation

OUTLINE:

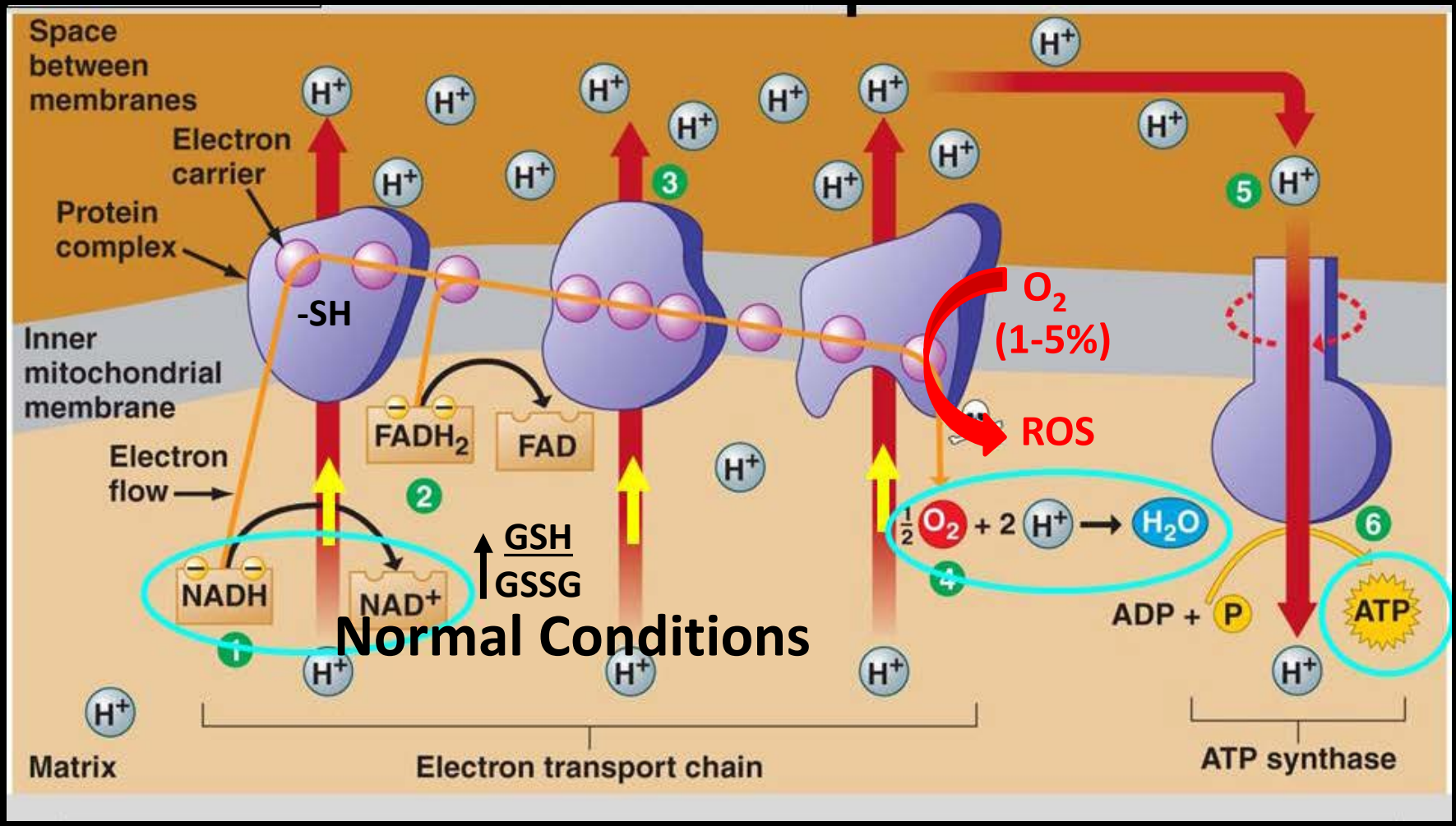
1. Brief Review of Redox/Methylation Metabolism

2. DNA Methylation, Epigenetic Regulation

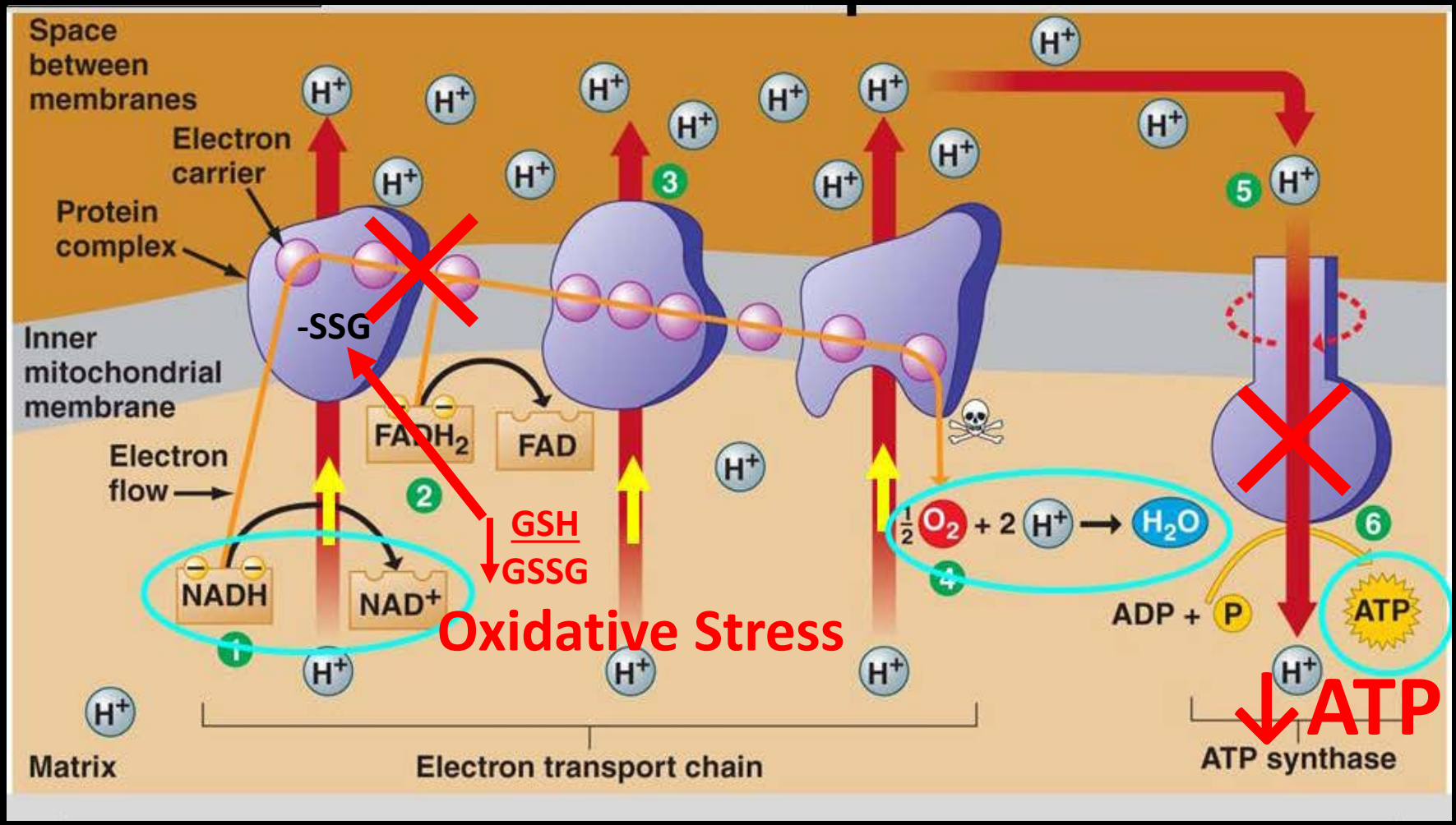
3. Gluten/Casein-derived Opiate Peptides

3. Vitamin B12 in Human Brain

Mitochondrial Electron Transport Chain Activity Provides ATP and Maintains Energy But Also Generates Reactive Oxygen Species (ROS)



Oxidative Stress Turns Off The Electron Transport Chain: Mitochondrial Dysfunction



Epigenetic changes can help restore redox equilibrium

Antioxidant Demand
(ROS formation)

Antioxidant Supply
(Glutathione; GSH)

Homeostatic Equilibrium

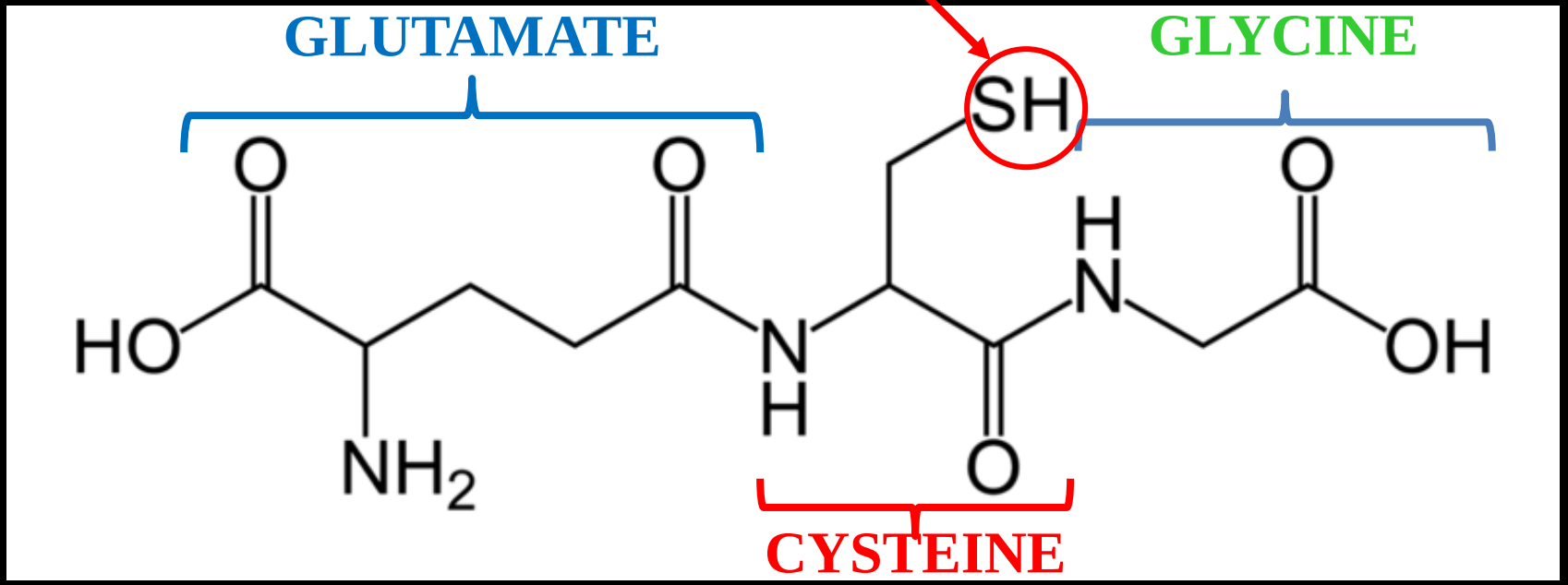
Restoration of Homeostasis

↑ Antioxidant demand

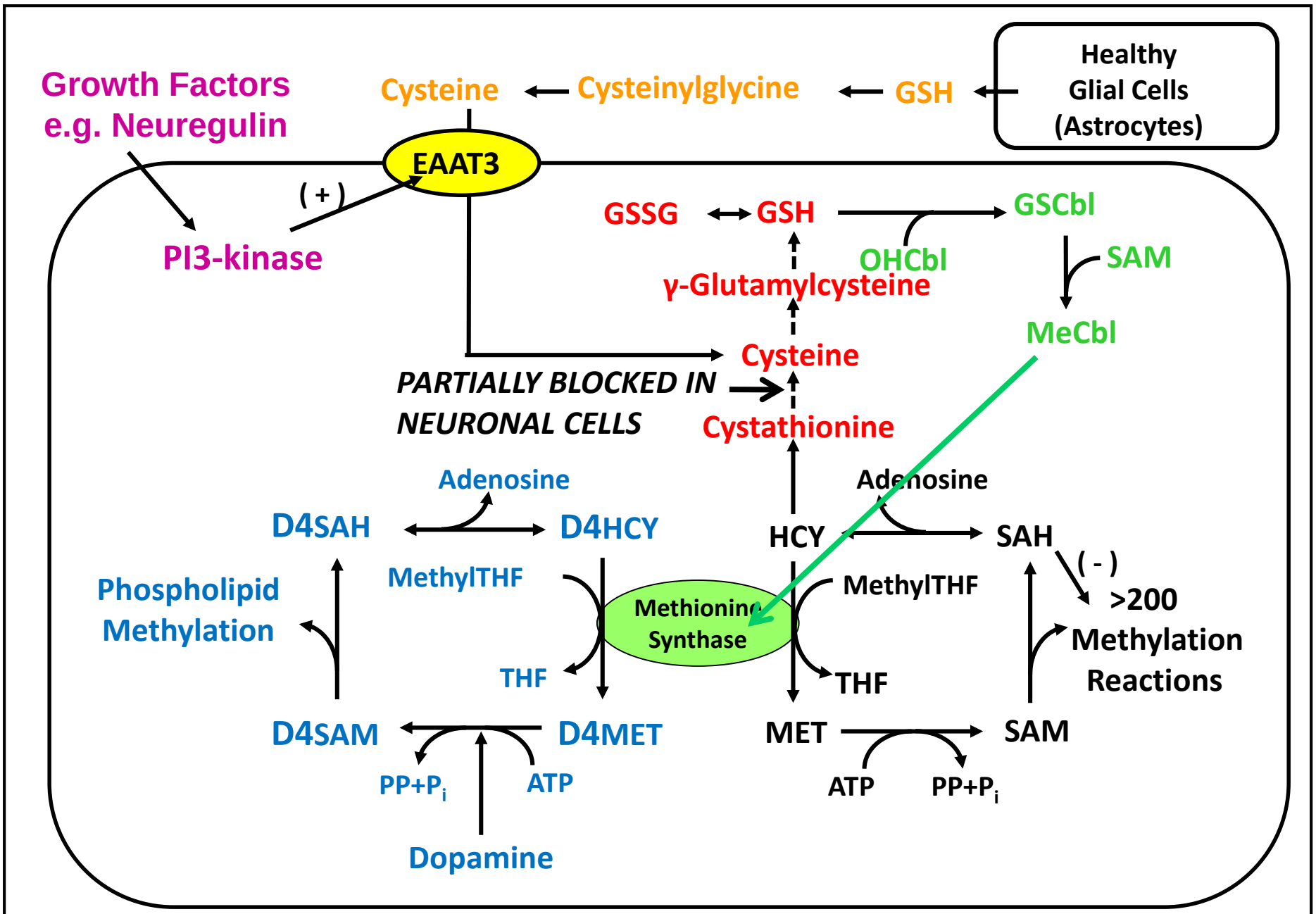
↓ Antioxidant Supply

Oxidative Stress → Adaptive Epigenetic Changes

Glutathione:
The primary antioxidant in cells



Redox and Methylation Metabolic Pathways



REDOX:

- **The balance between reduced *vs.* oxidized states**

- **The balance between antioxidant supply and demand**
  e.g. the antioxidant glutathione (GSH)

  GSH (reduced form)] **REDOX**
  GSSG (oxidized form)] **STATUS**

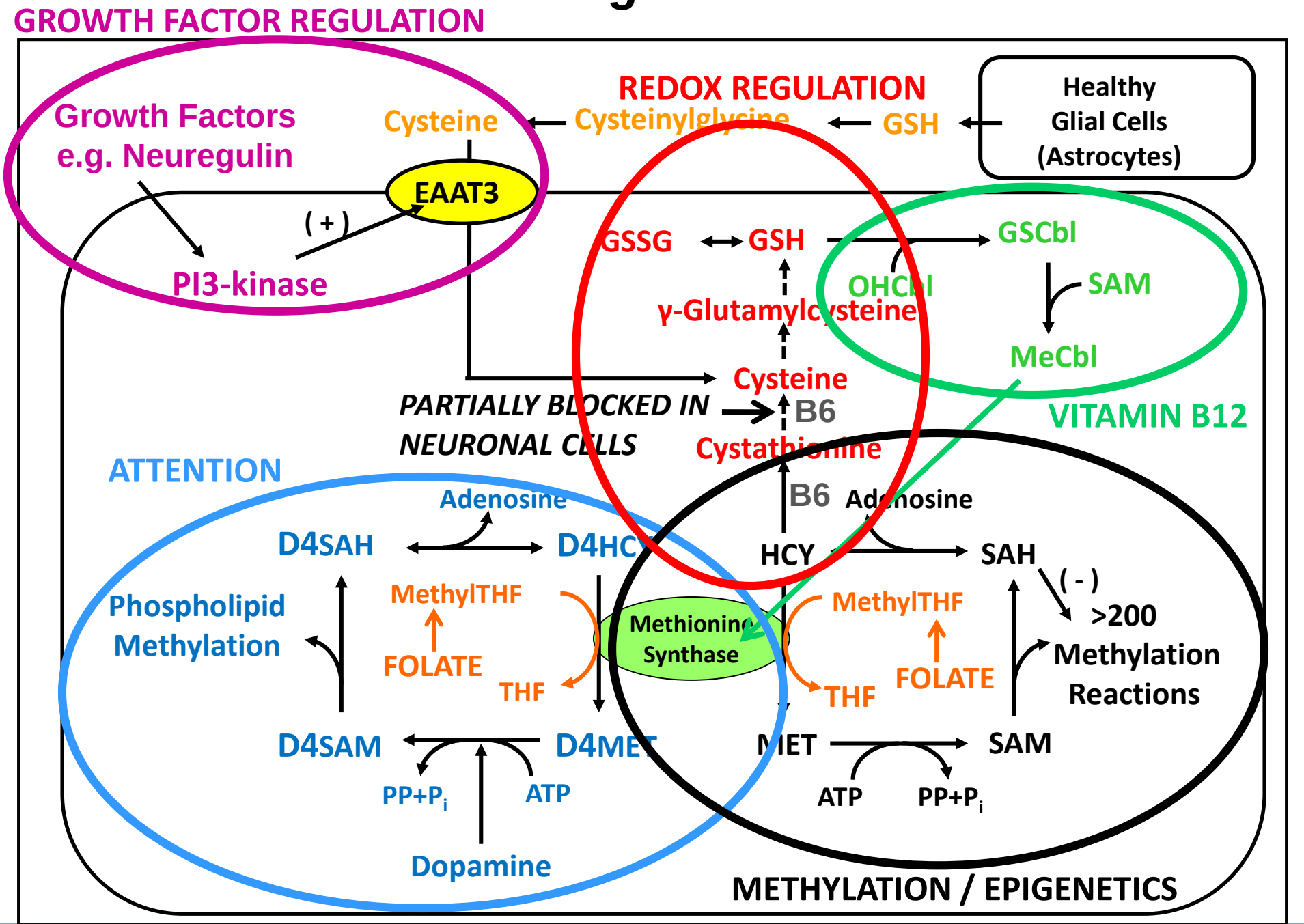
METHYLATION:

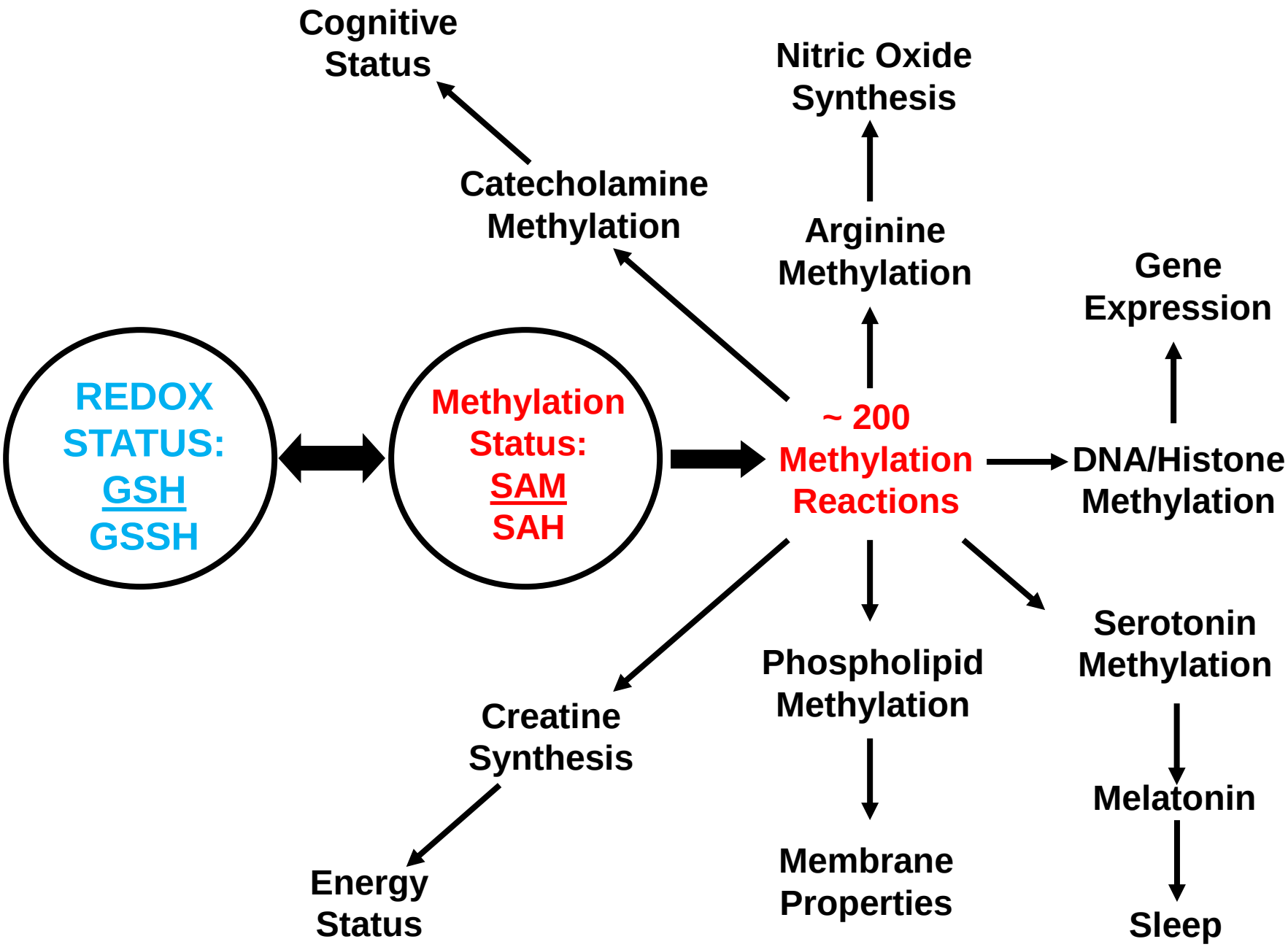
- **Addition of a carbon atom (CH_3- methyl group)**
  e.g. DNA methylation

- **S-Adenosylmethionine (SAM) is the universal donor**

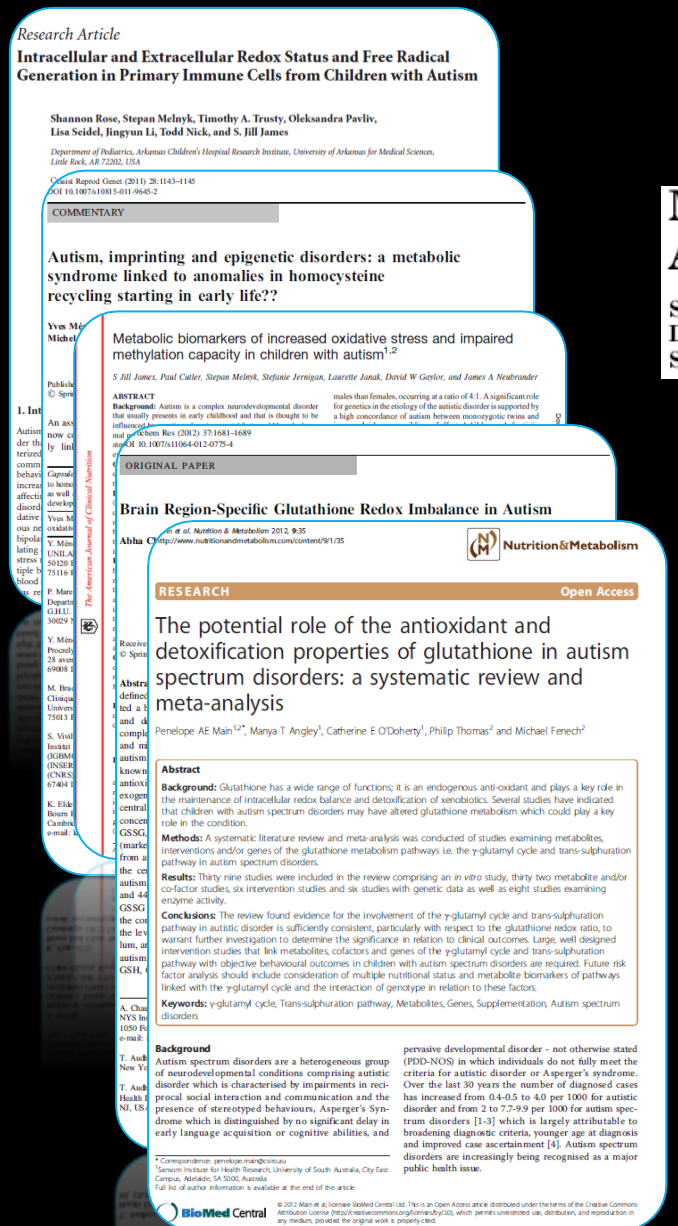
  SAM (methyl donor] **METHYLATION**
  SAH (methylation inhibitor)] **STATUS**

Integrated Metabolic Activities




Cognitive Status
Nitric Oxide Synthesis
Catecholamine Methylation
Arginine Methylation
Gene Expression
REDOX STATUS: GSH GSSH
Methylation Status: SAM SAH
~ 200 Methylation Reactions
DNA/Histone Methylation
Serotonin Methylation
Creatine Synthesis
Phospholipid Methylation
Melatonin
Energy Status
Membrane Properties
Sleep

>40 studies link ASD to oxidative stress, low levels of the antioxidant glutathione (GSH) and impaired methylation



Research Article

Intracellular and Extracellular Redox Status and Free Radical Generation in Primary Immune Cells from Children with Autism

Shannon Rose, Stepan Melnyk, Timothy A. Trusty, Oleksandra Pavliv, Lisa Seidel, Jingyun Li, Todd Nick, and S. Jill James

Department of Pediatrics, Arkansas Children's Hospital Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR 72202, USA

Autism Reprod Genet (2011) 28:1143–1145
DOI 10.1007/s10815-011-9645-2

COMMENTARY

Autism, imprinting and epigenetic disorders: a metabolic syndrome linked to anomalies in homocysteine recycling starting in early life??

Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism[1,2]

S Jill James, Paul Cutler, Stepan Melnyk, Stefanie Jernigan, Laurette Janak, David W Gaylor, and James A Neubrander

ABSTRACT

Background: Autism is a complex neurodevelopmental disorder that usually presents in early childhood and that is thought to be influenced

males than females, occurring at a ratio of 4:1. A significant role for genetics in the etiology of the autistic disorder is supported by a high concordance of autism between monozygotic twins and

Neurochem Res (2012) 37:1681–1689
DOI 10.1007/s11064-012-0775-4

ORIGINAL PAPER

Brain Region-Specific Glutathione Redox Imbalance in Autism

Abha Ch

et al. Nutrition & Metabolism 2012, 9:35
http://www.nutritionandmetabolism.com/content/9/1/35

Nutrition&Metabolism

RESEARCH Open Access

The potential role of the antioxidant and detoxification properties of glutathione in autism spectrum disorders: a systematic review and meta-analysis

Penelope AE Main[1,2*], Manya T Angley[1], Catherine E O'Doherty[1], Philip Thomas[2] and Michael Fenech[2]

Abstract

Background: Glutathione has a wide range of functions; it is an endogenous anti-oxidant and plays a key role in the maintenance of intracellular redox balance and detoxification of xenobiotics. Several studies have indicated that children with autism spectrum disorders may have altered glutathione metabolism which could play a key role in the condition.

Methods: A systematic literature review and meta-analysis was conducted of studies examining metabolites, interventions and/or genes of the glutathione metabolism pathways i.e. the γ-glutamyl cycle and trans-sulphuration pathway in autism spectrum disorders.

Results: Thirty nine studies were included in the review comprising an *in vitro* study, thirty two metabolite and/or co-factor studies, six intervention studies and six studies with genetic data as well as eight studies examining enzyme activity.

Conclusions: The review found evidence for the involvement of the γ-glutamyl cycle and trans-sulphuration pathway in autistic disorder is sufficiently consistent, particularly with respect to the glutathione redox ratio, to warrant further investigation to determine the significance in relation to clinical outcomes. Large, well designed intervention studies that link metabolites, cofactors and genes of the γ-glutamyl cycle and trans-sulphuration pathway with objective behavioural outcomes in children with autism spectrum disorders are required. Future risk factor analysis should include consideration of multiple nutritional status and metabolite biomarkers of pathways linked with the γ-glutamyl cycle and the interaction of genotype in relation to these factors.

Keywords: γ-glutamyl cycle, Trans-sulphuration pathway, Metabolites, Genes, Supplementation, Autism spectrum disorders

Background

Autism spectrum disorders are a heterogeneous group of neurodevelopmental conditions comprising autistic disorder which is characterised by impairments in reciprocal social interaction and communication and the presence of stereotyped behaviours, Asperger's Syndrome which is distinguished by no significant delay in early language acquisition or cognitive abilities, and pervasive developmental disorder - not otherwise stated (PDD-NOS) in which individuals do not fully meet the criteria for autistic disorder or Asperger's syndrome. Over the last 30 years the number of diagnosed cases has increased from 0.4-0.5 to 4.0 per 1000 for autistic disorder and from 2 to 7.7-9.9 per 1000 for autism spectrum disorders [1-3] which is largely attributable to broadening diagnostic criteria, younger age at diagnosis and improved case ascertainment [4]. Autism spectrum disorders are increasingly being recognised as a major public health issue.

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Metabolic Endophenotype and Related Genotypes are Associated With Oxidative Stress in Children With Autism

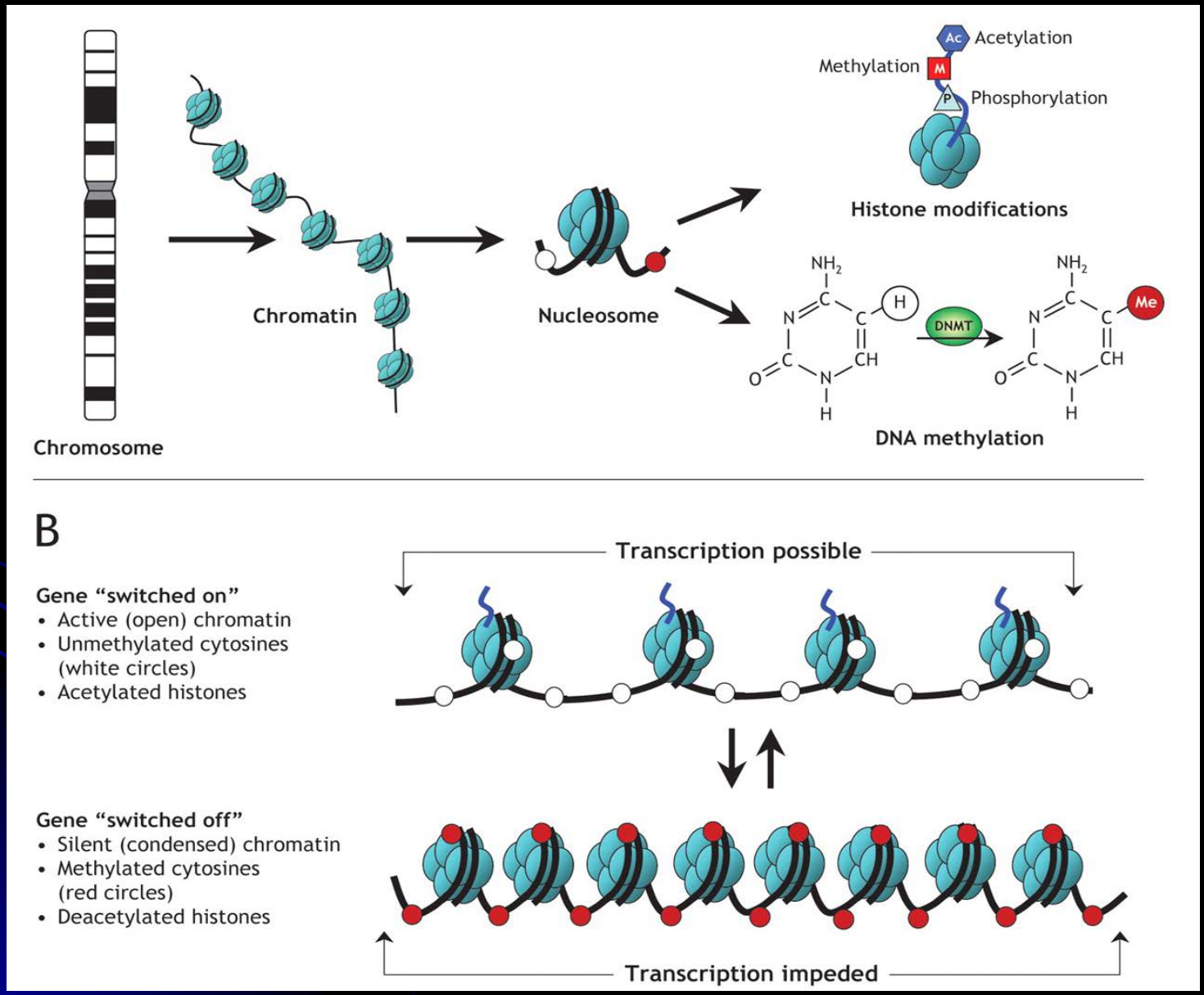
S. Jill James,[1]* Stepan Melnyk,[1] Stefanie Jernigan,[1] Mario A. Cleves,[1] Charles H. Halsted,[2] Donna H. Wong,[2] Paul Cutler,[3] Kenneth Bock,[4] Marvin Boris,[5] J. Jeffrey Bradstreet,[6] Sidney M. Baker,[7] and David W. Gaylor[8]

TABLE II. Transmethylation and Transsulfuration Metabolites in Autistic Cases and Controls

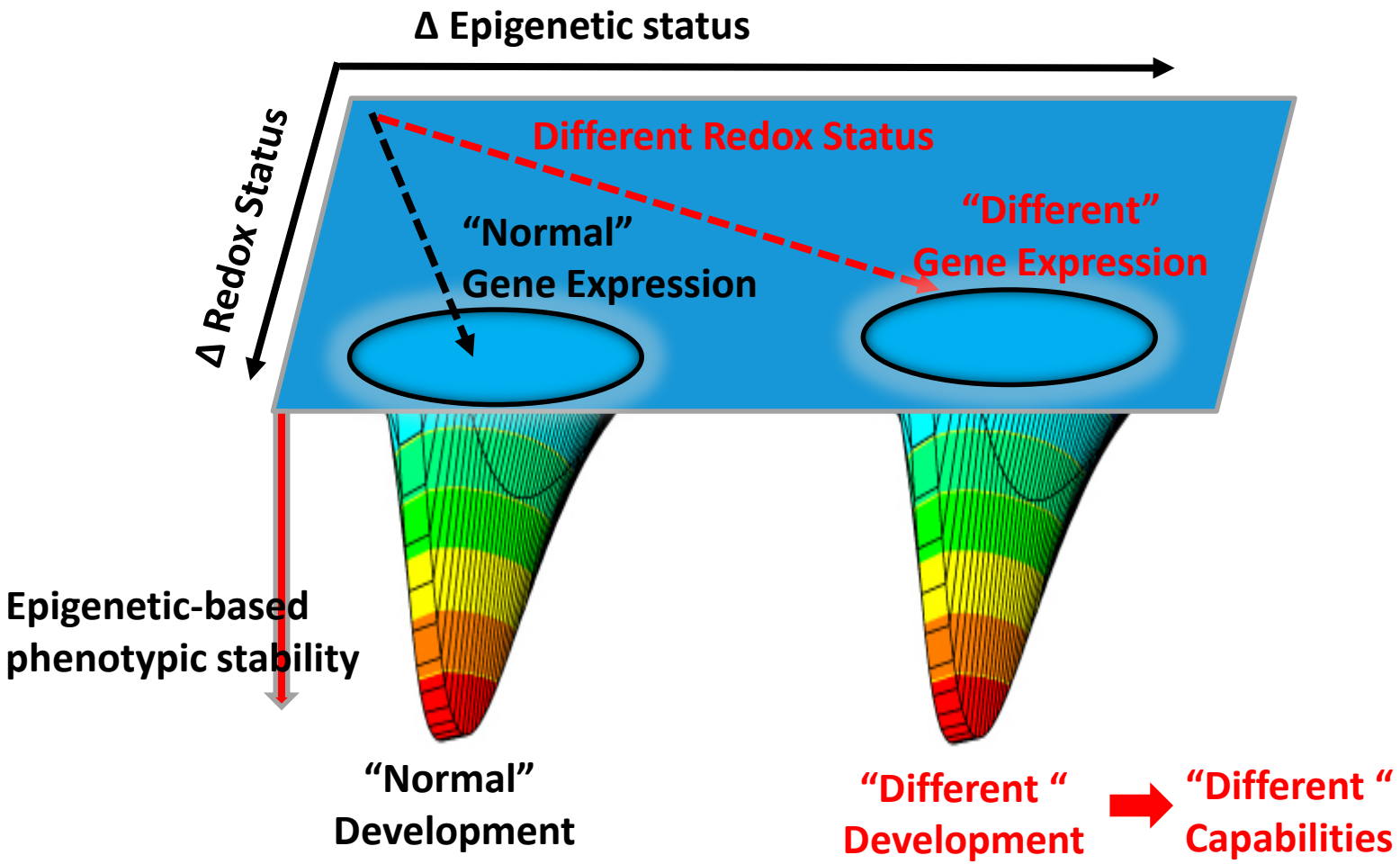
	Control[a] (n = 73)	Autistic[a] (n = 80)	*P*-value
Methionine (μmol/L)	28.0 ± 6.5	20.6 ± 5.2	<0.0001
SAM (nmol/L)	93.8 ± 18	84.3 ± 11	<0.0001
SAH (nmol/L)	18.8 ± 4.5	23.3 ± 7.9	<0.0001
SAM/SAH ratio	5.5 ± 2.8	4.0 ± 1.7 ← 28% ↓	<0.0001
Adenosine (μmol/L)	0.19 ± 0.13	0.28 ± .13	0.001
Homocysteine (μmol/L)	6.0 ± 1.3	5.7 ± 1.2	0.03v
Cystathionine (μmol/L)	0.19 ± 0.1	0.24 ± 0.1	<0.0001
Cysteine (μmol/L)	207 ± 22	165 ± 14	<0.0001
Cysteinylglycine (μmol/L)	39.4 ± 7.3	38.9 ± 11	0.78
Total GSH (μmol/L)	7.53 ± 1.7	5.1 ± 1.2	<0.0001
Free GSH (μmol/L)	2.2 ± 0.9	1.4 ± 0.5 ← 36% ↓	<0.0001
GSSG (μmol/L)	0.24 ± 0.1	0.40 ± 0.2	<0.0001
Total GSH/GSSG ratio	28.2 ± 7.0	14.7 ± 6.2	<0.0001
Free GSH/GSSG ratio	7.9 ± 3.5	4.9 ± 2.2 ← 38% ↓	<0.0001

SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; GSH, glutathione; GSSG, glutathione disulfide.
[a]Means ± SD.

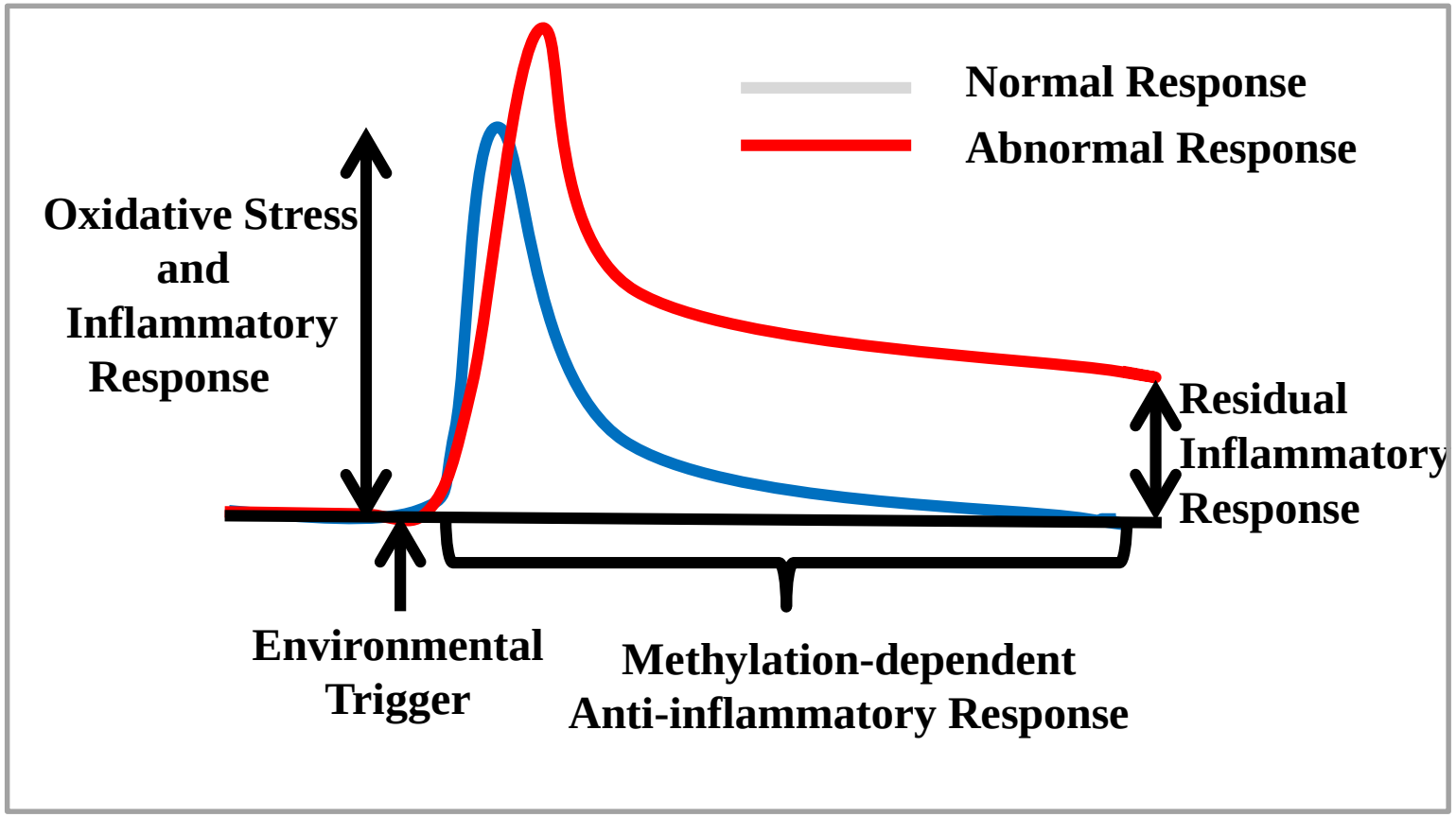
Methylation of DNA and histones is fundamental to epigenetic regulation of gene expression during development



Differences in Redox Status Can Influence Epigenetic Status Leading To Different Developmental Outcomes

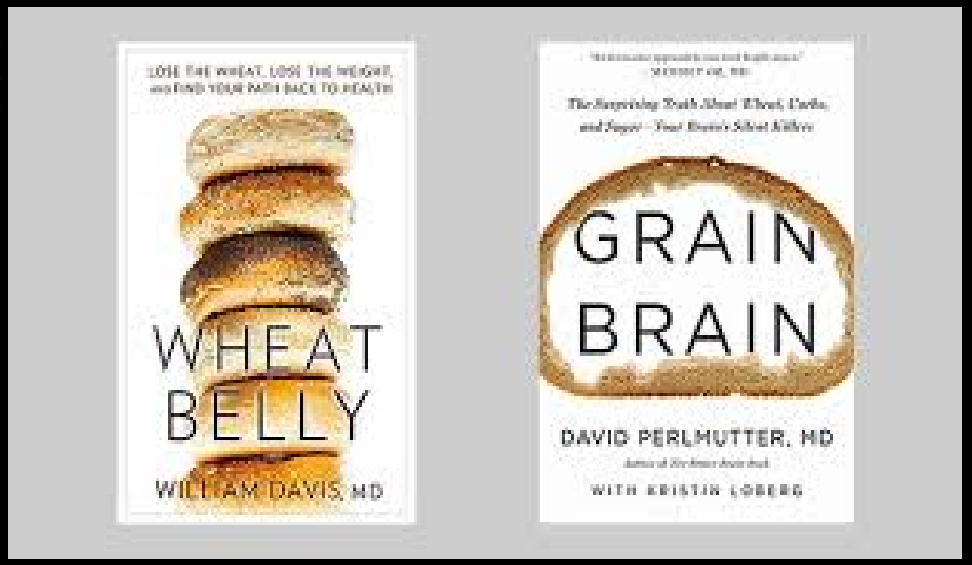


Inadequate resolution of inflammation and/or oxidative stress in vulnerable individuals can contribute to chronic inflammation



Redox and Epigenetic Effects of Gluten/Casein-derived Opiate Peptides

Dr. Malav Trivedi



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Journal of Nutritional Biochemistry xx (2014) xxx–xxx
Journal of Nutritional Biochemistry
ELSEVIER

Food-derived opioid peptides inhibit cysteine uptake with redox and epigenetic consequences☆

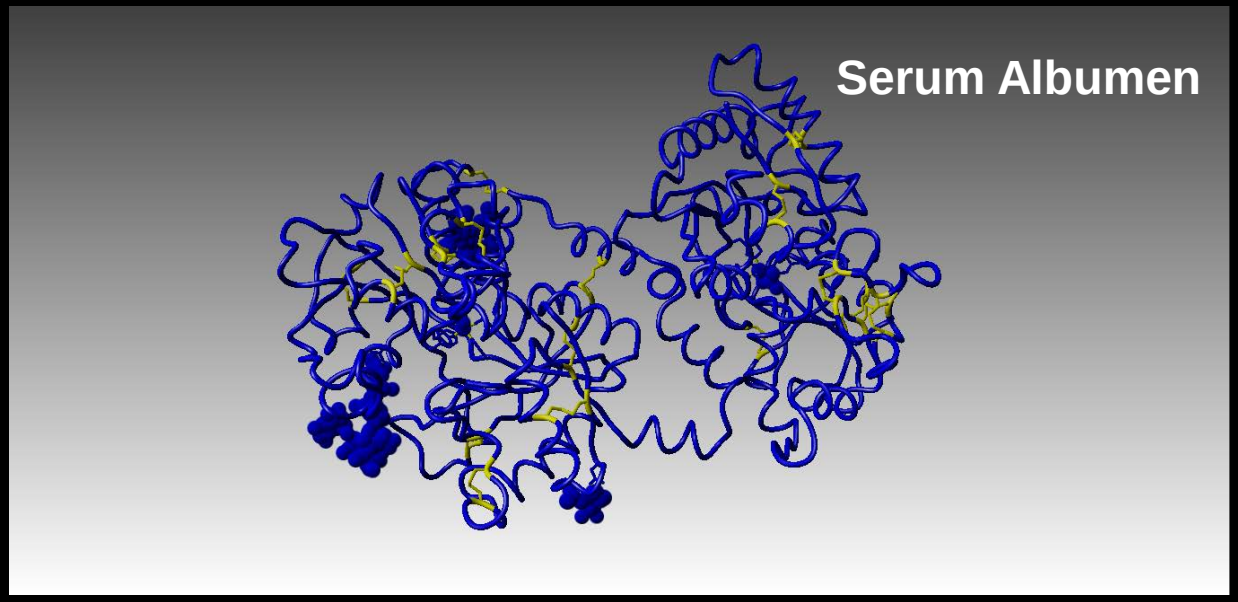
Malav S. Trivedi[a], Jayni S. Shah[a], Sara Al-Mughairy[a], Nathaniel W. Hodgson[a], Benjamin Simms[a], Geert A. Trooskens[b], Wim Van Criekinge[b], Richard C. Deth[a,*]

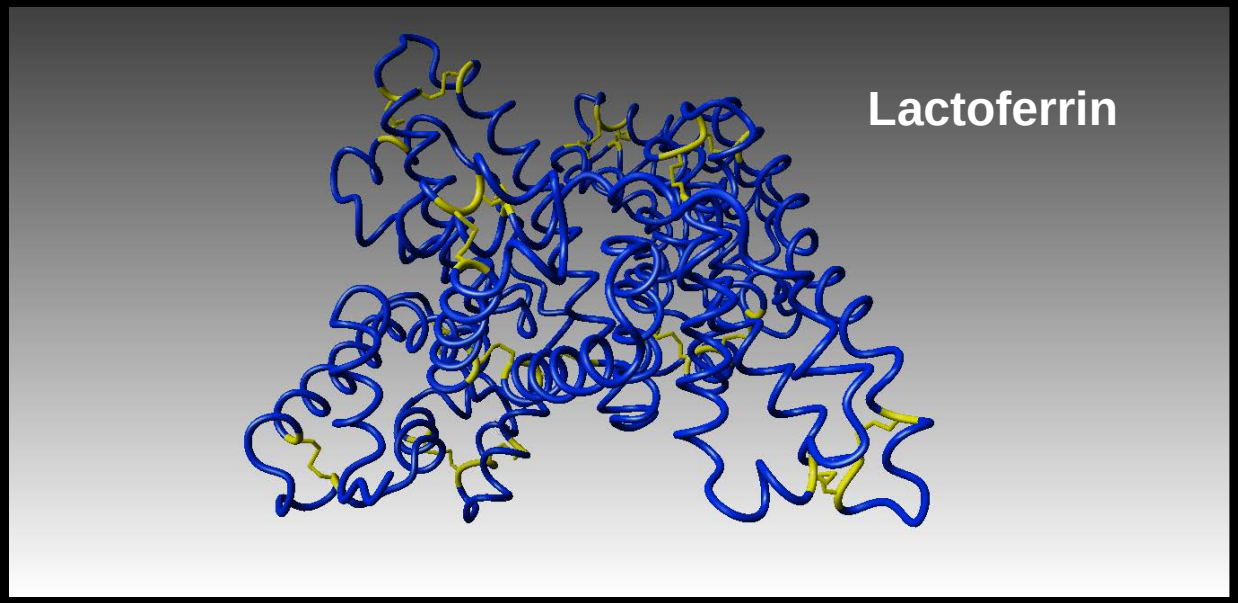
MILK: It's all about the curds and whey

CURDS = Caseins

WHEY = Cystine-rich soluble proteins

Whey proteins are rich in cysteine (cystine)





NOT ALL COWS ARE THE SAME: SOME ARE A1, SOME ARE A2

Sources of milk and wheat-derived opiate peptides.

Human breast milk

β-Casein (40 % of milk protein)

Human β-Casomorphin -7

Human BCM7

Tyr-Pro-Phe-Val-Glu-Pro-Ile

Casein

β-Casein (12g/L of milk)

59 66 -67

-L- V- Y- P- F- P- G- P- I -X

pepsin

LAP

elastase

A1

(His at 67)

A2

(Pro at 67)

Bovine β-Casomorphin -7

(0.4 g/L milk)

Bovine BCM7

Tyr-Pro-Phe-Pro-Gly-Pro-Ile

Wheat/Barley

Albumin Globulin Prolamin Glutelin

Gliadins

α- gliadin-7

Gluten GM7

Tyr-Pro-Gln-Pro-Gln-Pro-Phe


SILK ORIGINAL SOYMILK
$3.99
NON GMO
SILK VANILLA SOYMILK
$3.99
SILK UNSWEETENED ORGANIC SOYMILK
$3.99
NON GMO
VEGAN
0%
new
The milk that might change everything
a2 Milk
FAT-FREE MILK
0%
new
The milk that might change everything
a2 Milk
LOWFAT MILK
1%
new
The milk that might change everything
a2 Milk
REDUCED FAT MILK
2%
new
The milk that might change everything
a2 Milk
MILK
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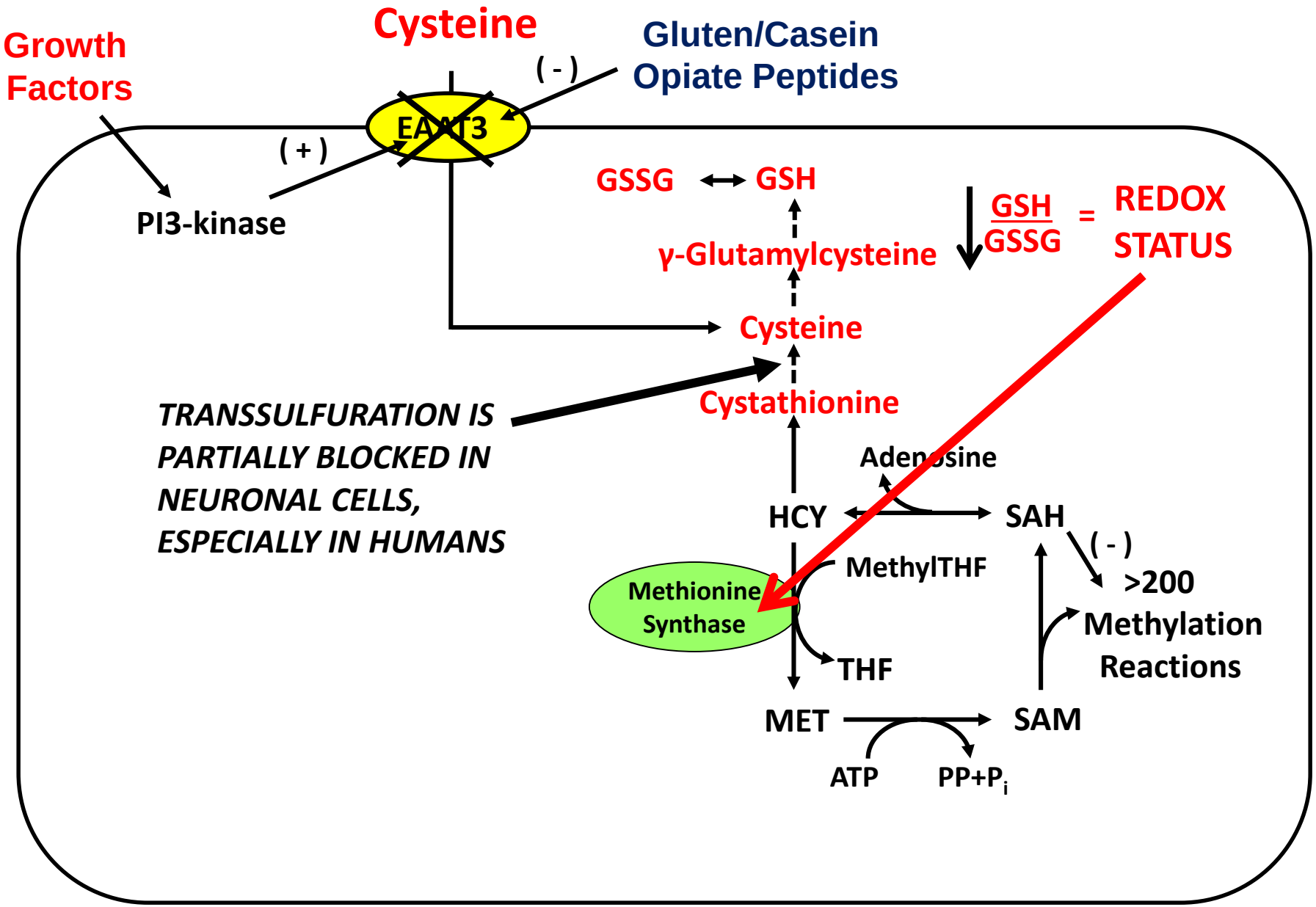
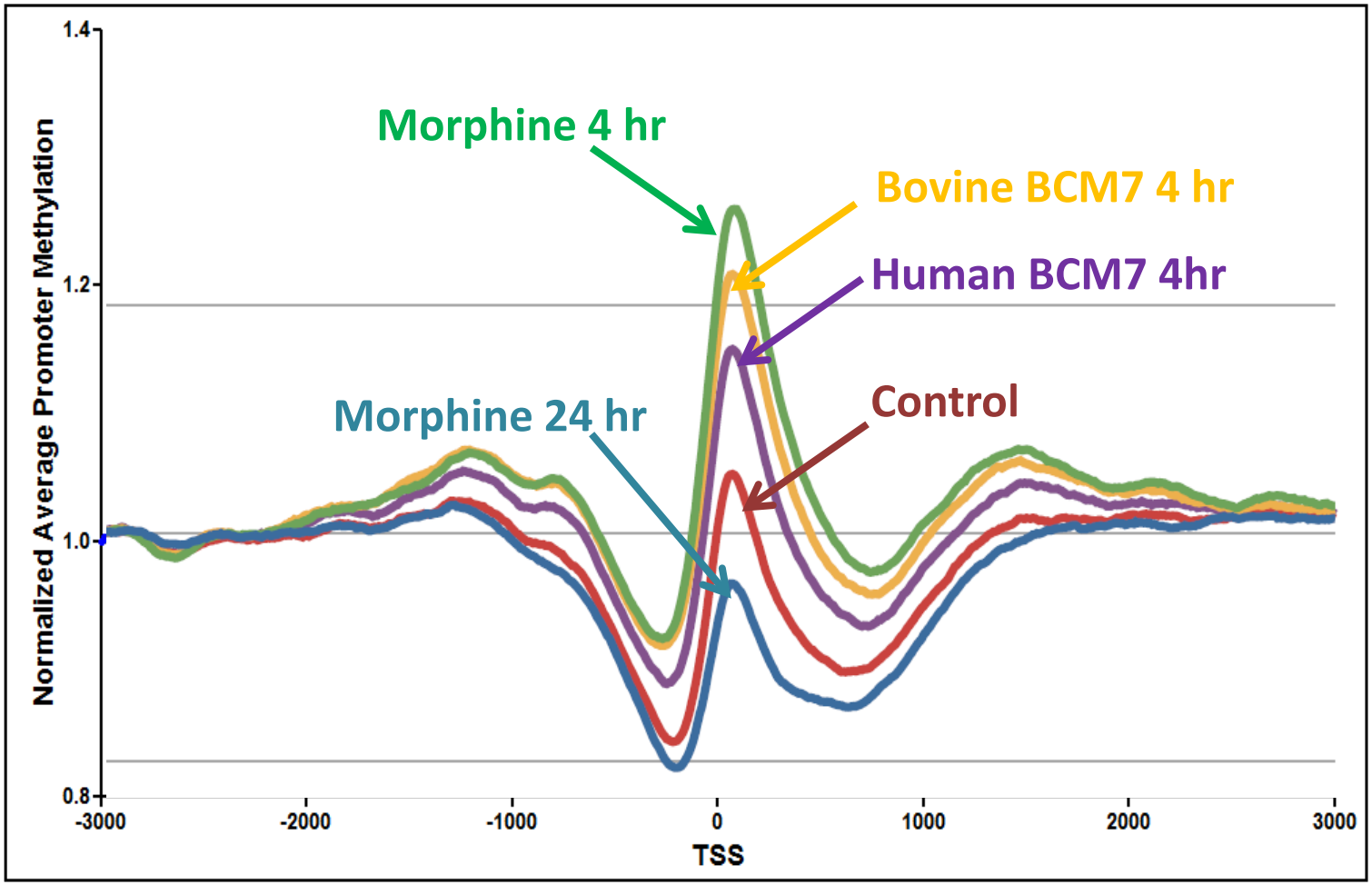
Opiates inhibit-mediated cysteine uptake



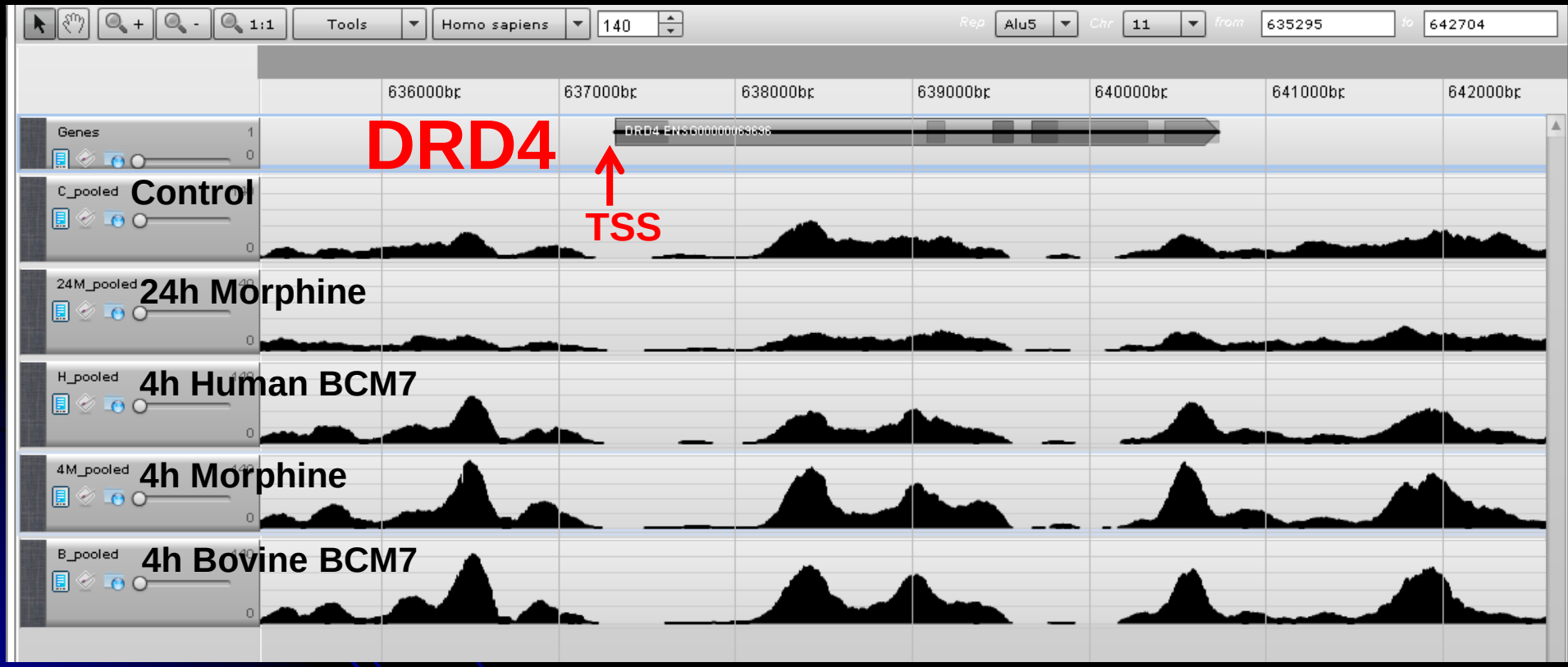
Global Promoter Methylation- SH-SY5Y cells

Morphine > bBCM7>hBCM7

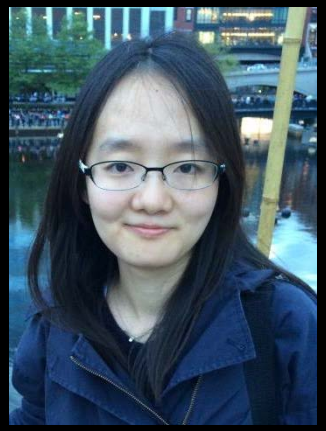


SH-SY5Y human neuroblastoma cells were treated with 1µM morphine, bBCM7 or hBCM7 for 4 hr (n = 5) and DNA methylation was analyzed by MBD-seq. 53,561 genes were aligned at their transcription start site (TSS) and average methylation between -3000bp and +3000bp was computed and normalized to values at -3000bp.

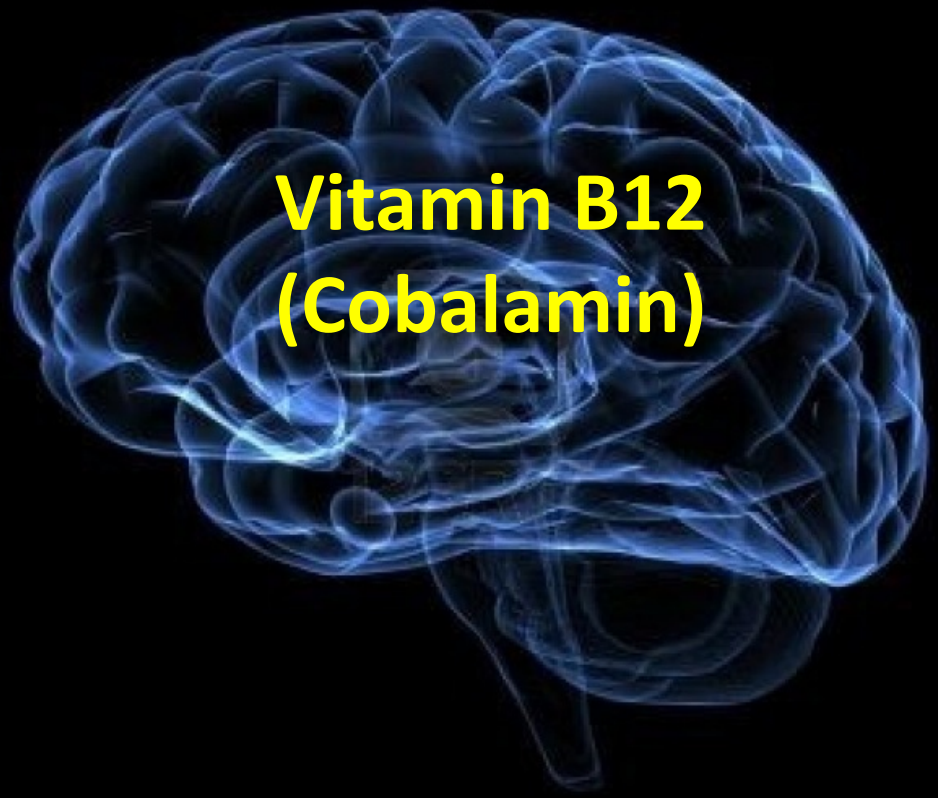
Changes in DNA methylation in the D4 Dopamine Receptor region following treatment with morphine or human/bovine BCM7



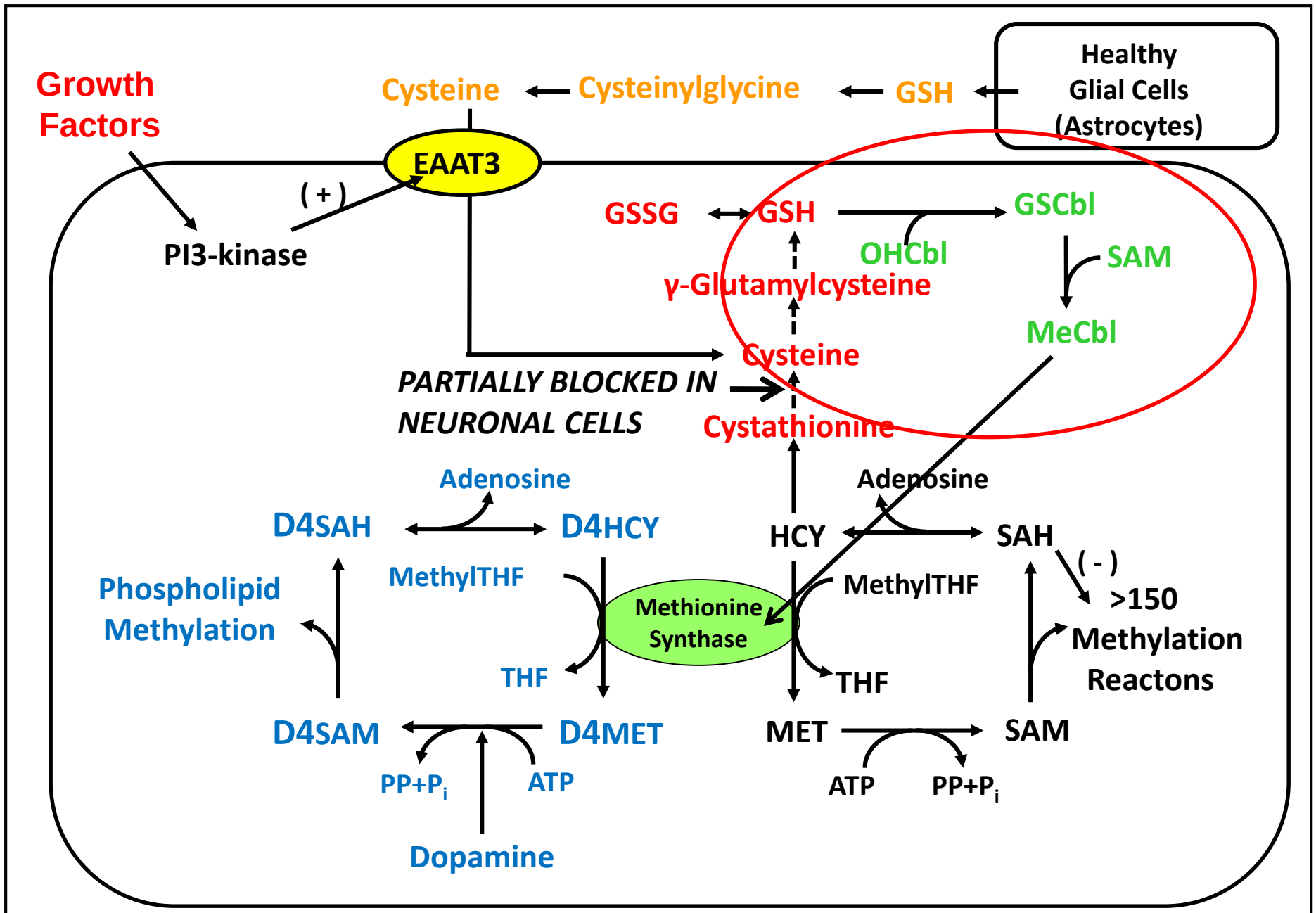
Brain-specific Aspects of Redox /Methylation Metabolism



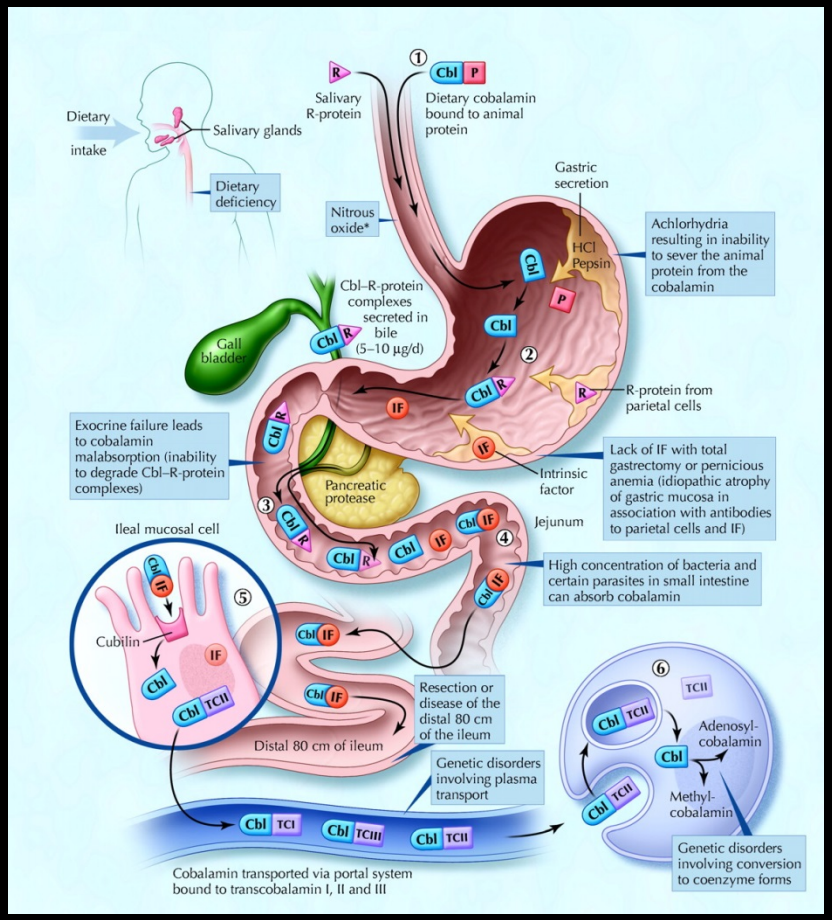
Yiting Zhang

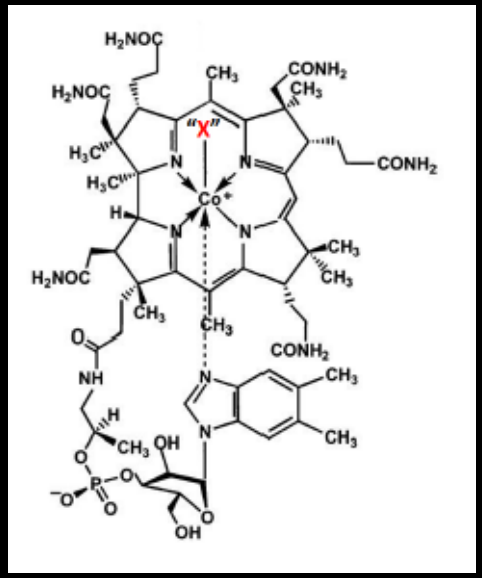


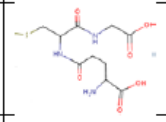
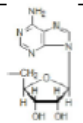
Vitamin B12 (Cobalamin) in the Human Frontal Cortex



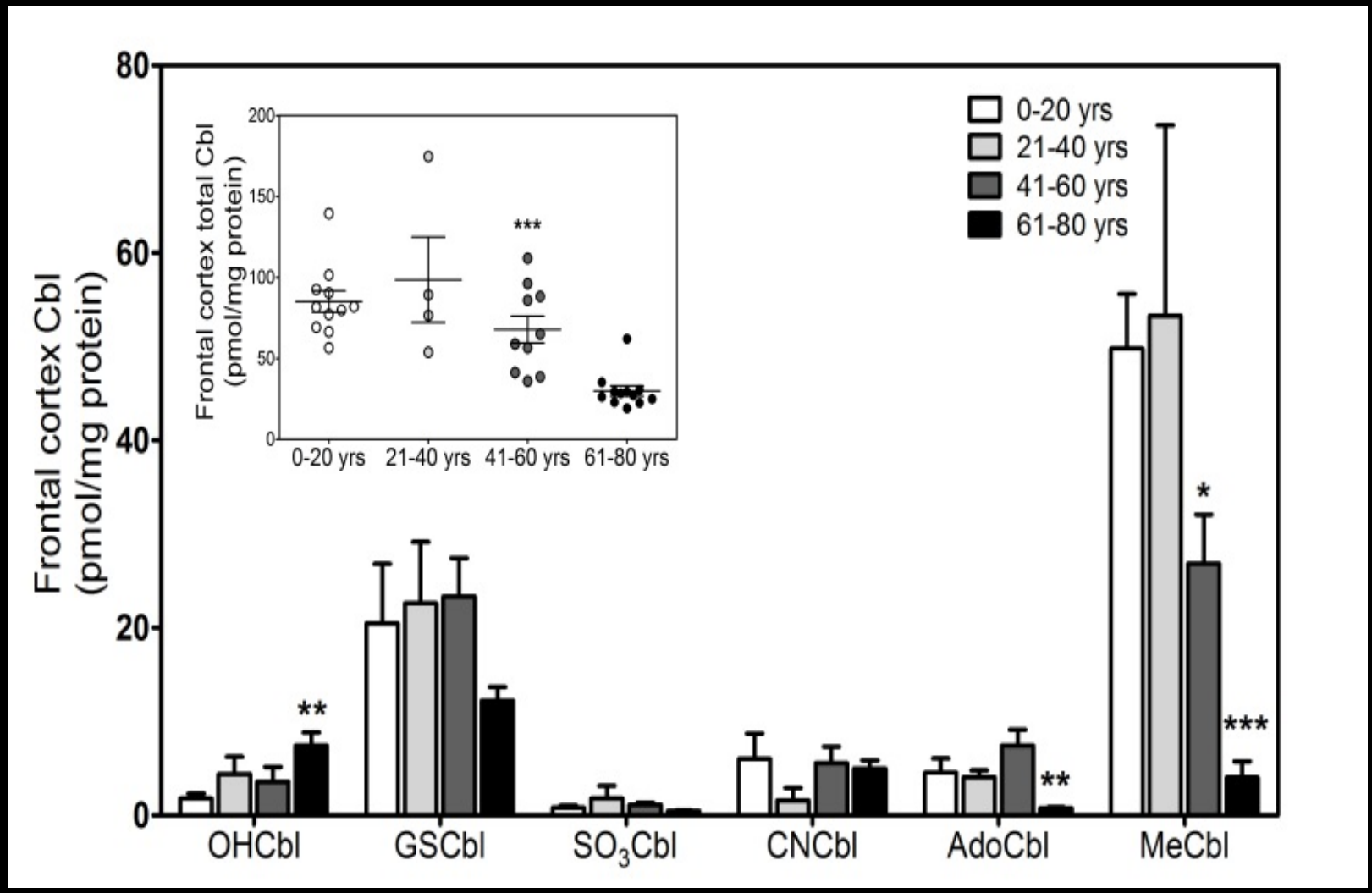
Vitamin B12 status in postmortem human brain during aging, autism and schizophrenia



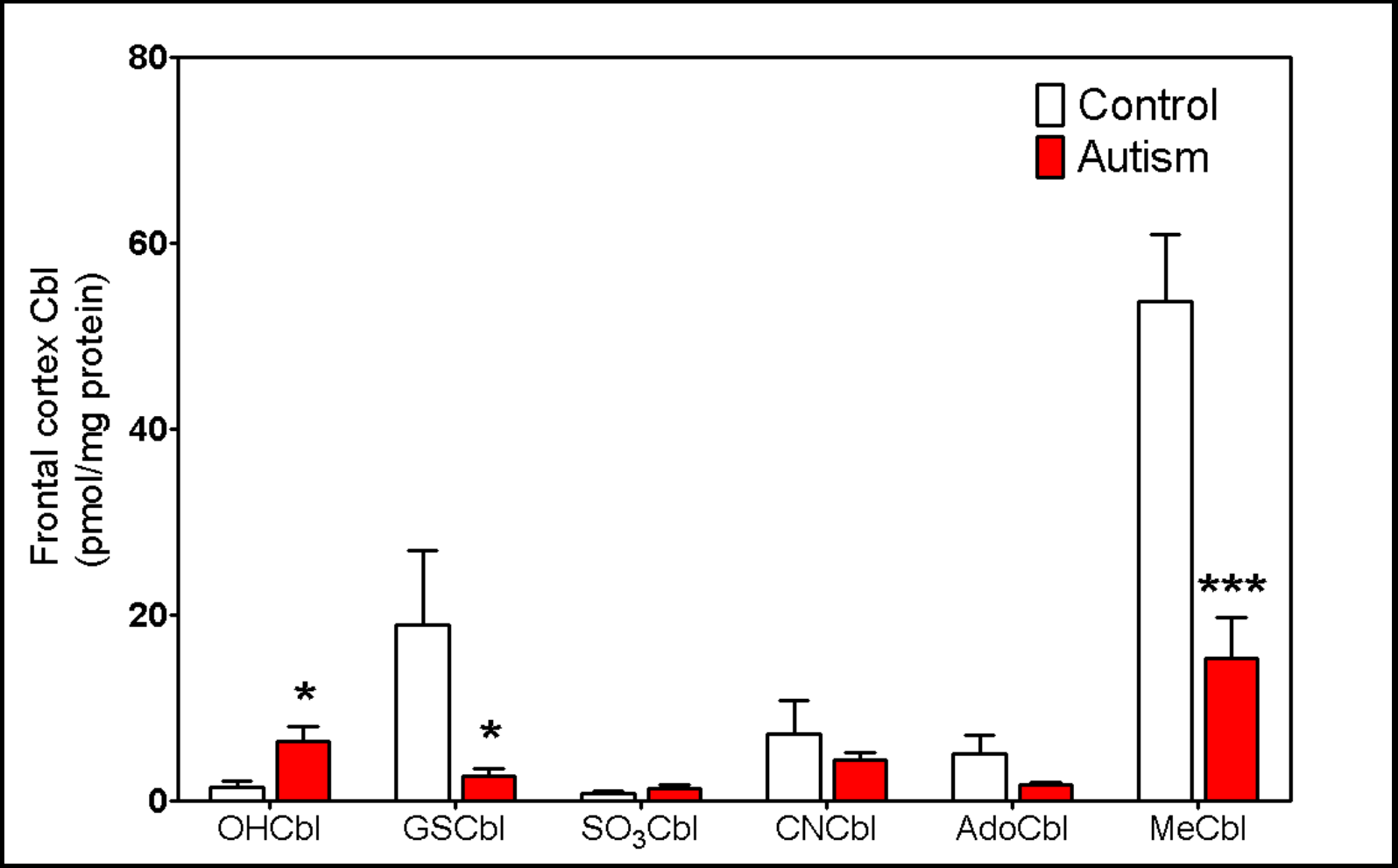


Cbl species		
X=	-hydroxo	-OH
X=	-glutathionyl	
X=	-sulfito	$-SO_3$
X=	-cyano	-CN
X=	-deoxyadenosyl	
X=	-methyl	$-CH_3$

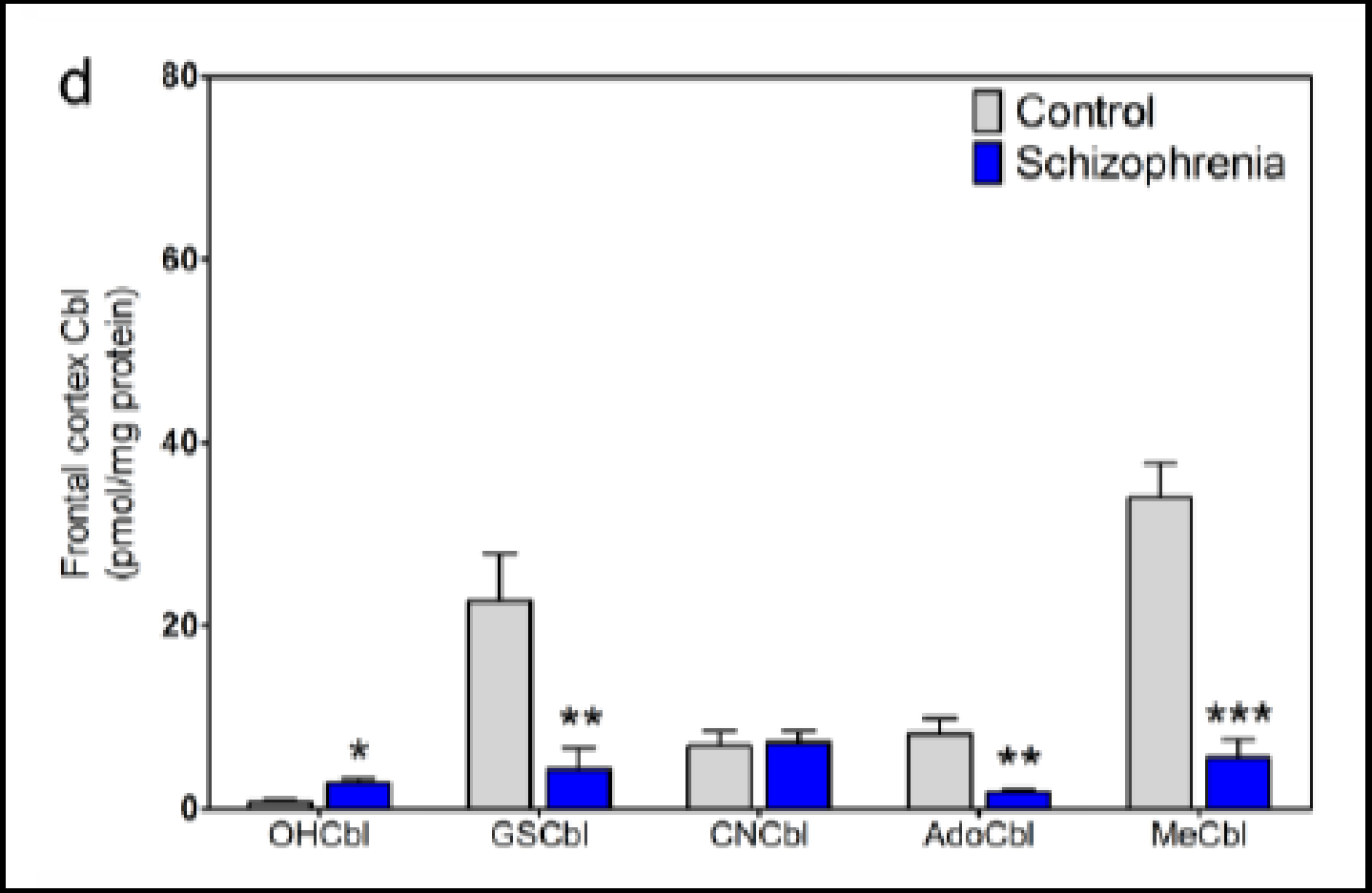
Levels of MethylB12 (MeCbl) and total Cbl decrease with age in frontal cortex



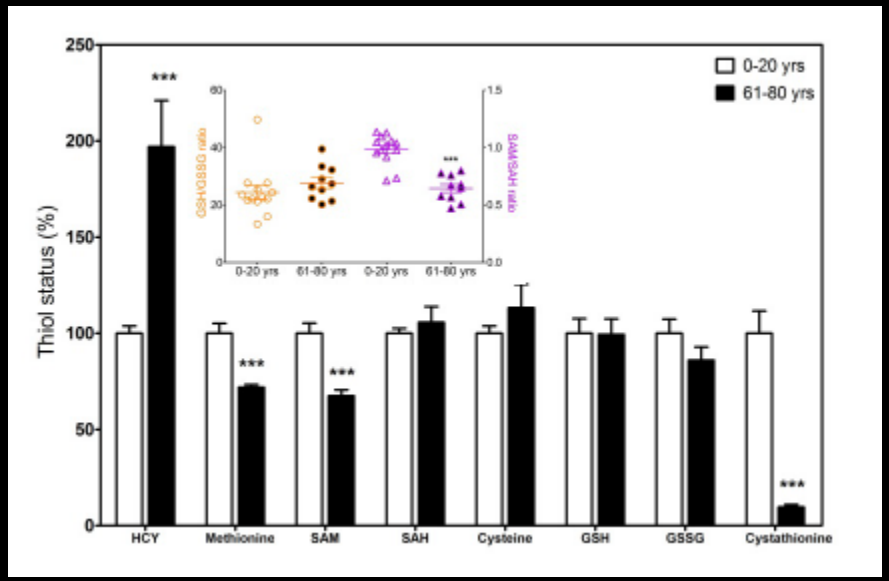
MethylB12 (MeCbl) and total B12 are decreased in autism compared to age-matched control subjects

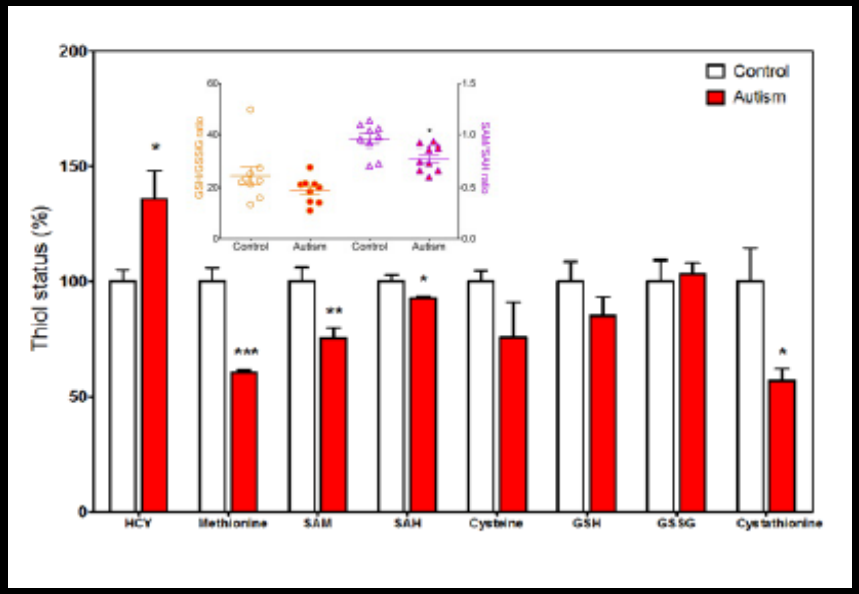


MethylB12 (MeCbl) and total B12 are decreased in schizophrenia, similar to the decrease in autism



Brain levels of redox and methylation metabolites change with age and are abnormal in autistic subjects





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Oxidative Medicine and Cellular Longevity
Article ID 704068

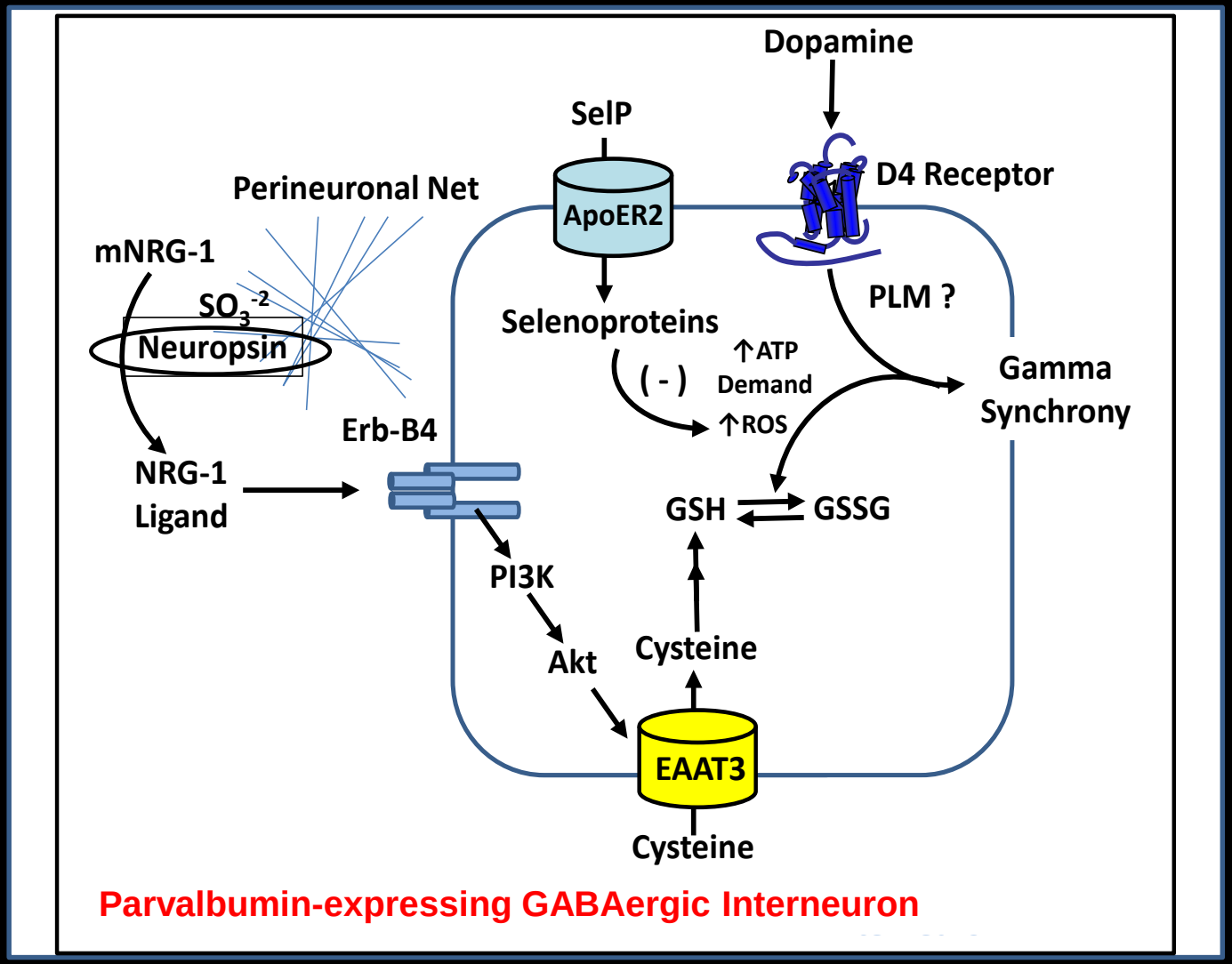
Research Article

Neuregulin 1 Promotes Glutathione-Dependent Neuronal Cobalamin Metabolism by Stimulating Cysteine Uptake

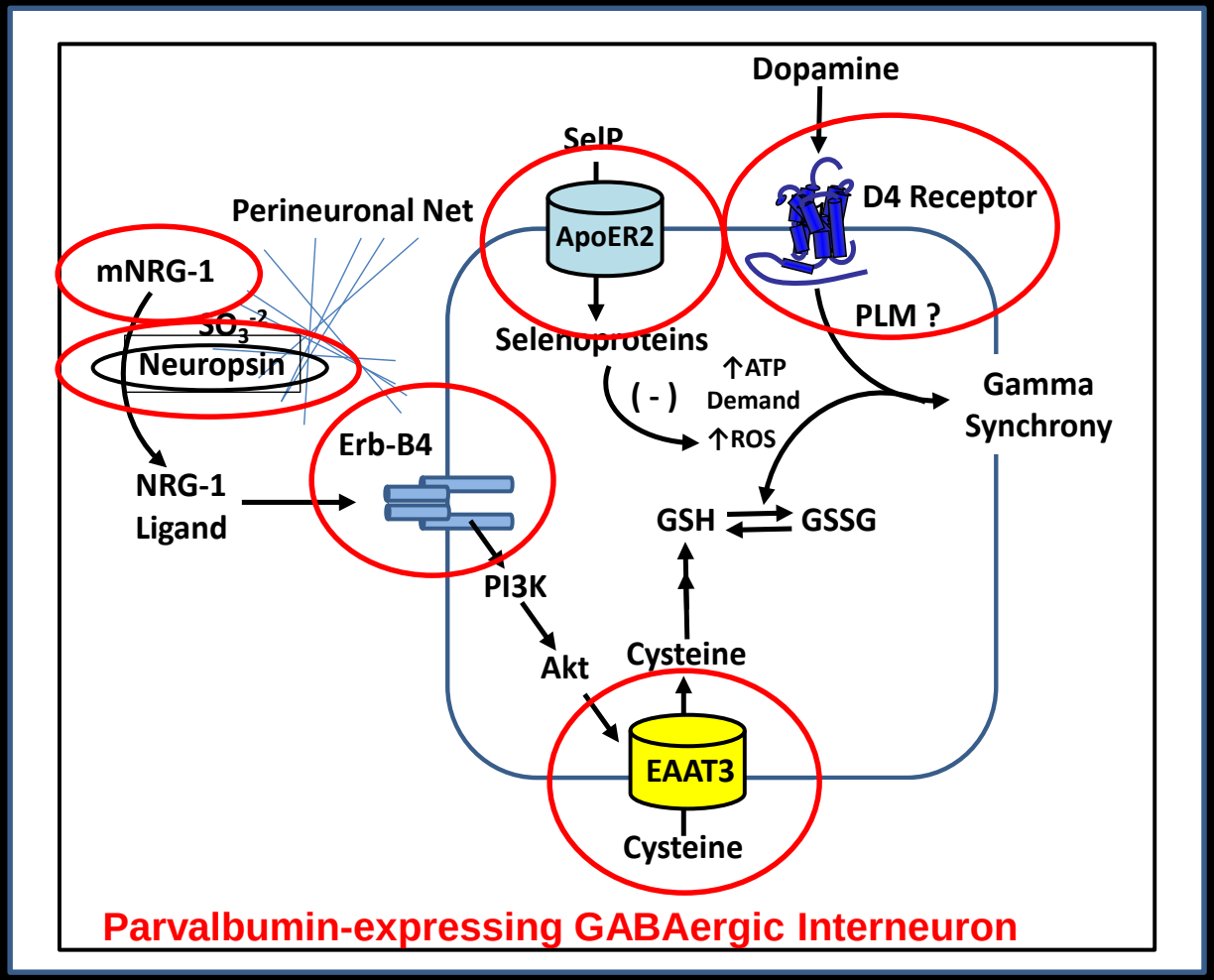
Yiting Zhang,[1] Nathaniel Hodgson,[2] Malav Trivedi,[3] and Richard Deth[1,3]

[1]*Department of Pharmaceutical Sciences, Northeastern University, Boston, MA, USA*
[2]*Department of Neurology, F.M. Kirby Neurobiology Center, Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA*
[3]*Department of Pharmaceutical Sciences, Nova Southeastern University, Fort Lauderdale, FL 33328, USA*

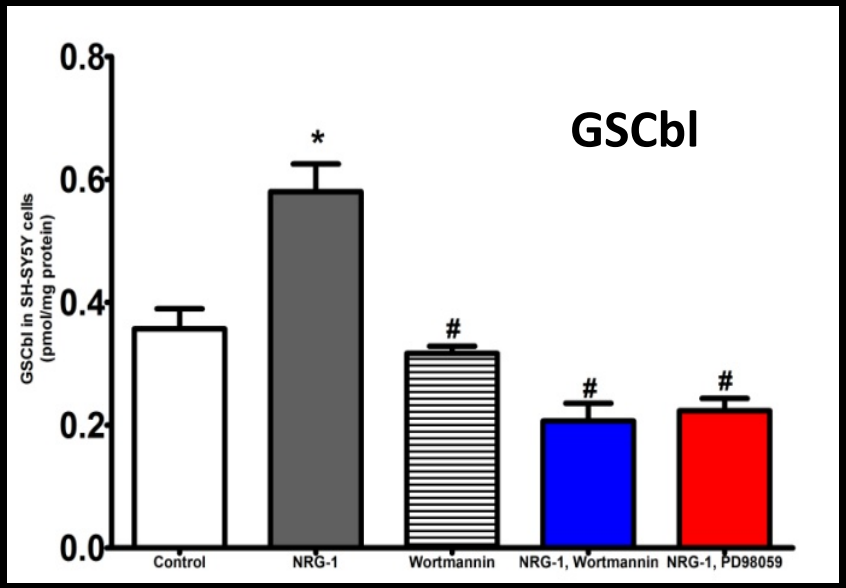
Neuregulin-1 (NRG-1) stimulates cysteine uptake and GSH synthesis in parvalbumin-expressing interneurons

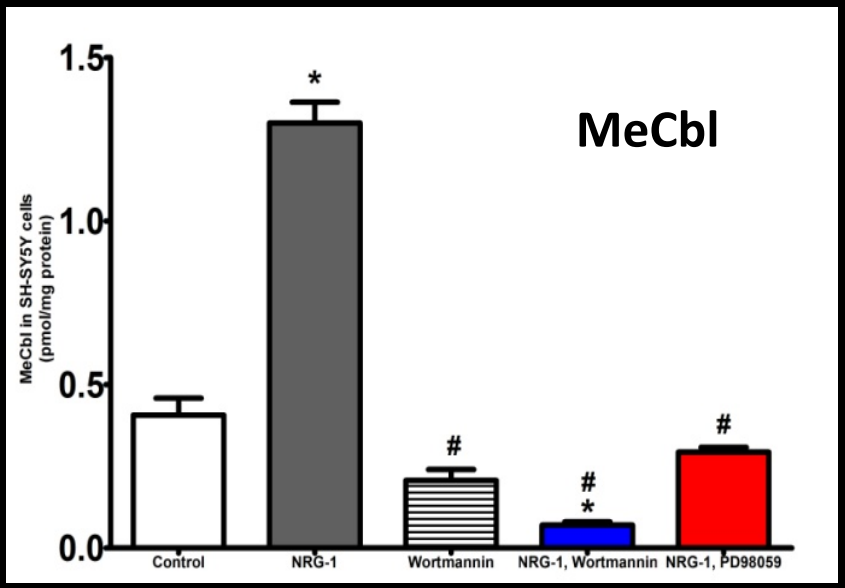


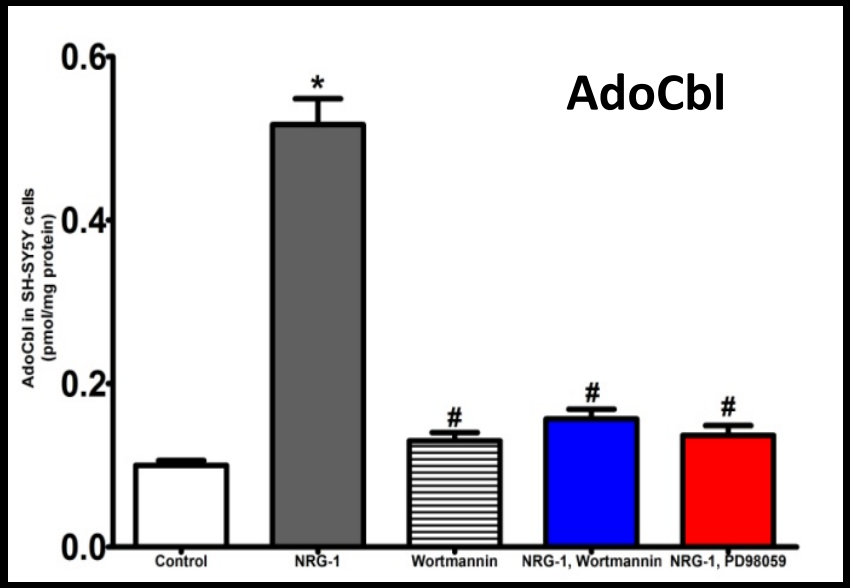
Each of these genes have been linked to schizophrenia

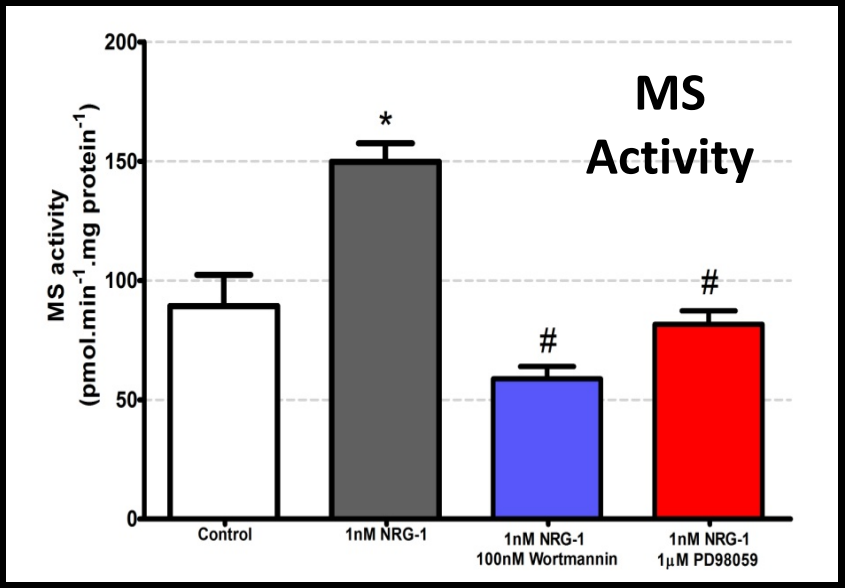


NRG-1 increases GSCbl, MeCbl and AdoCbl in association with increased MS activity

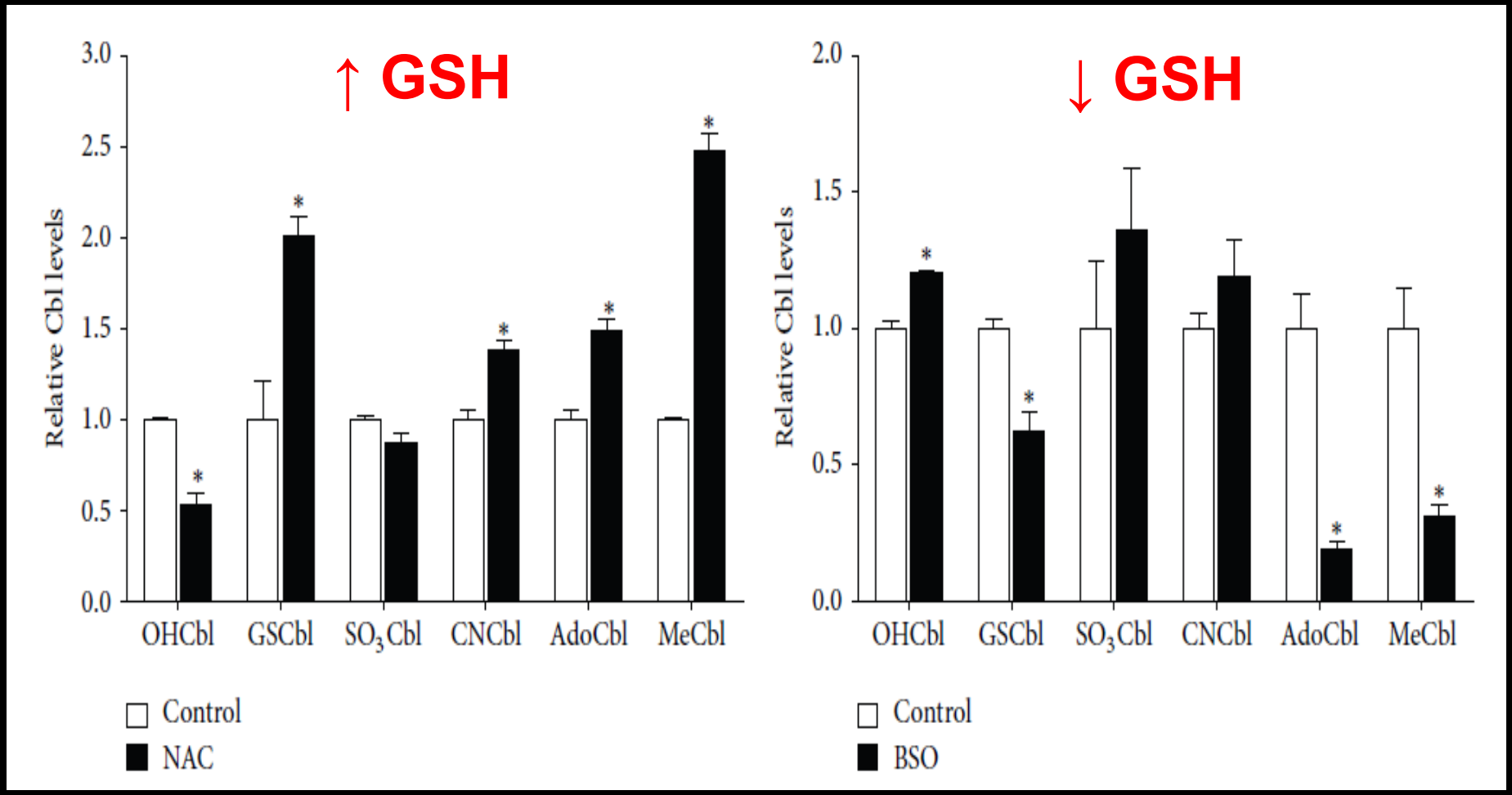




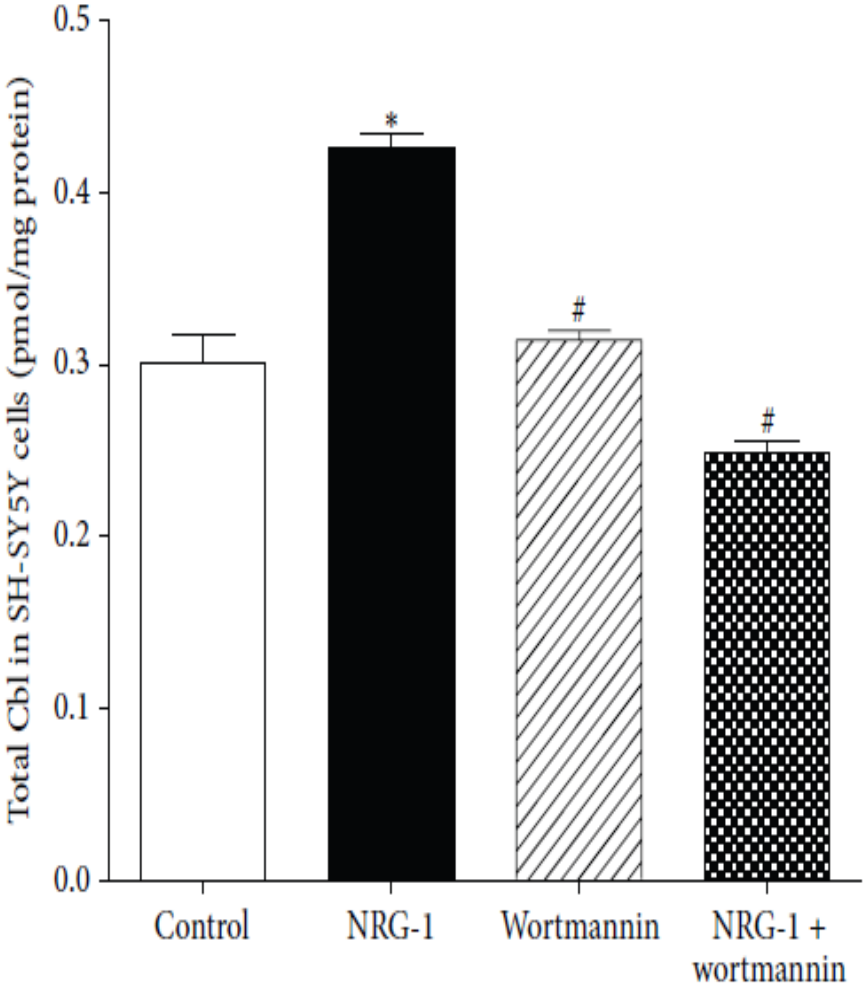


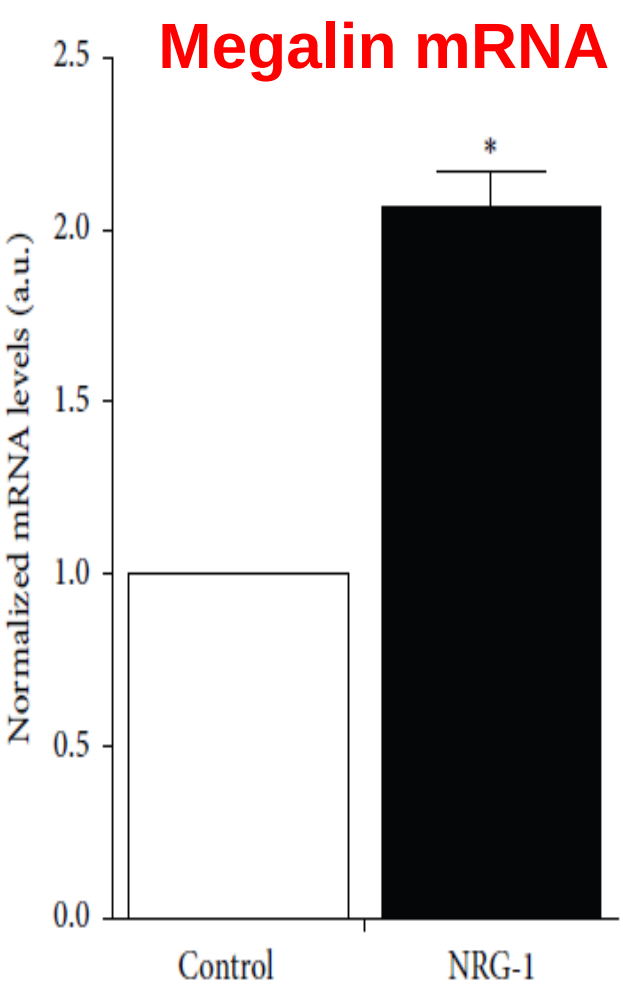


Increasing or decreasing GSH increases or decreases active forms of B12

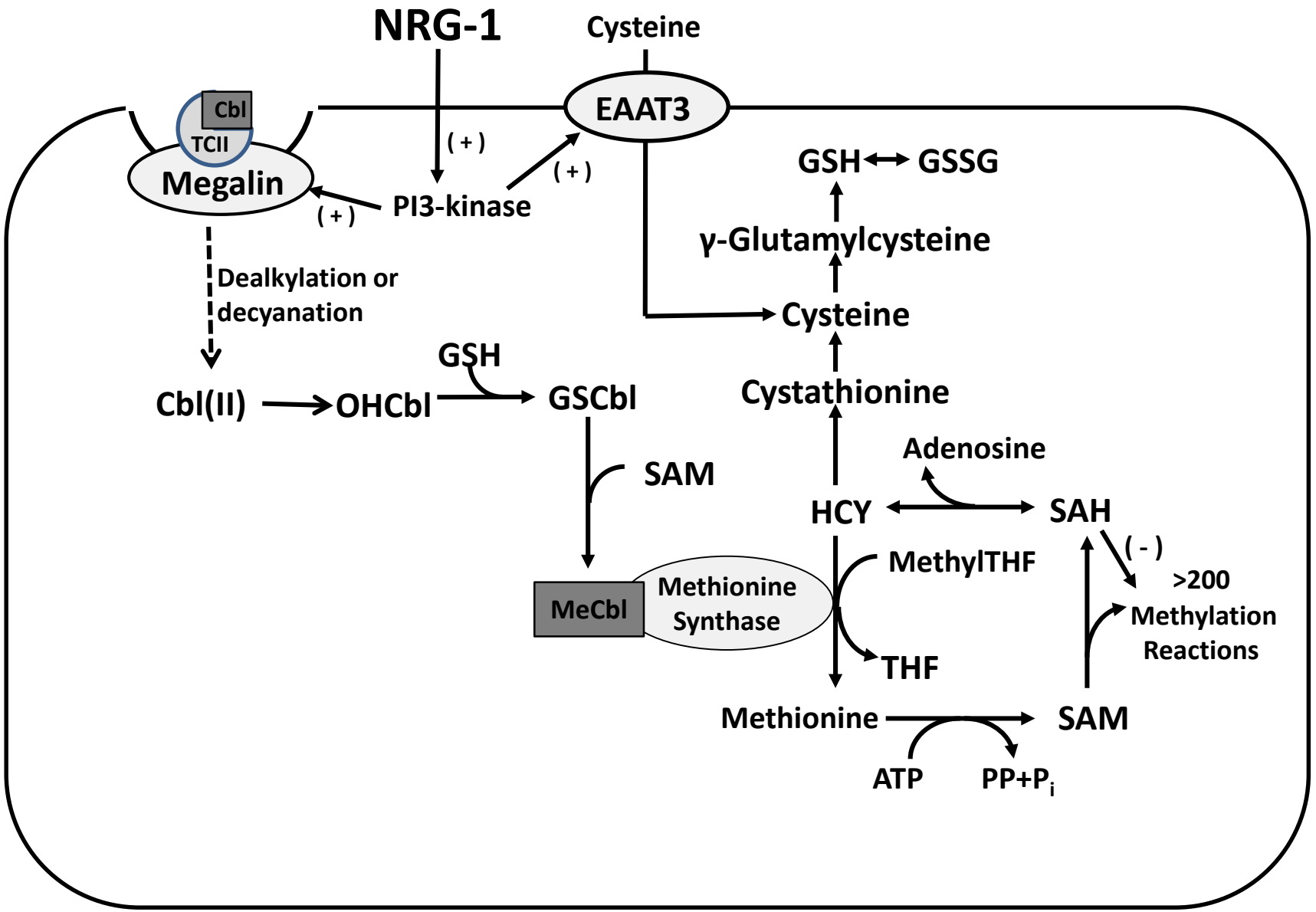


The NRG-1-induced increase of Cbl is accompanied by an increase in megalin mRNA



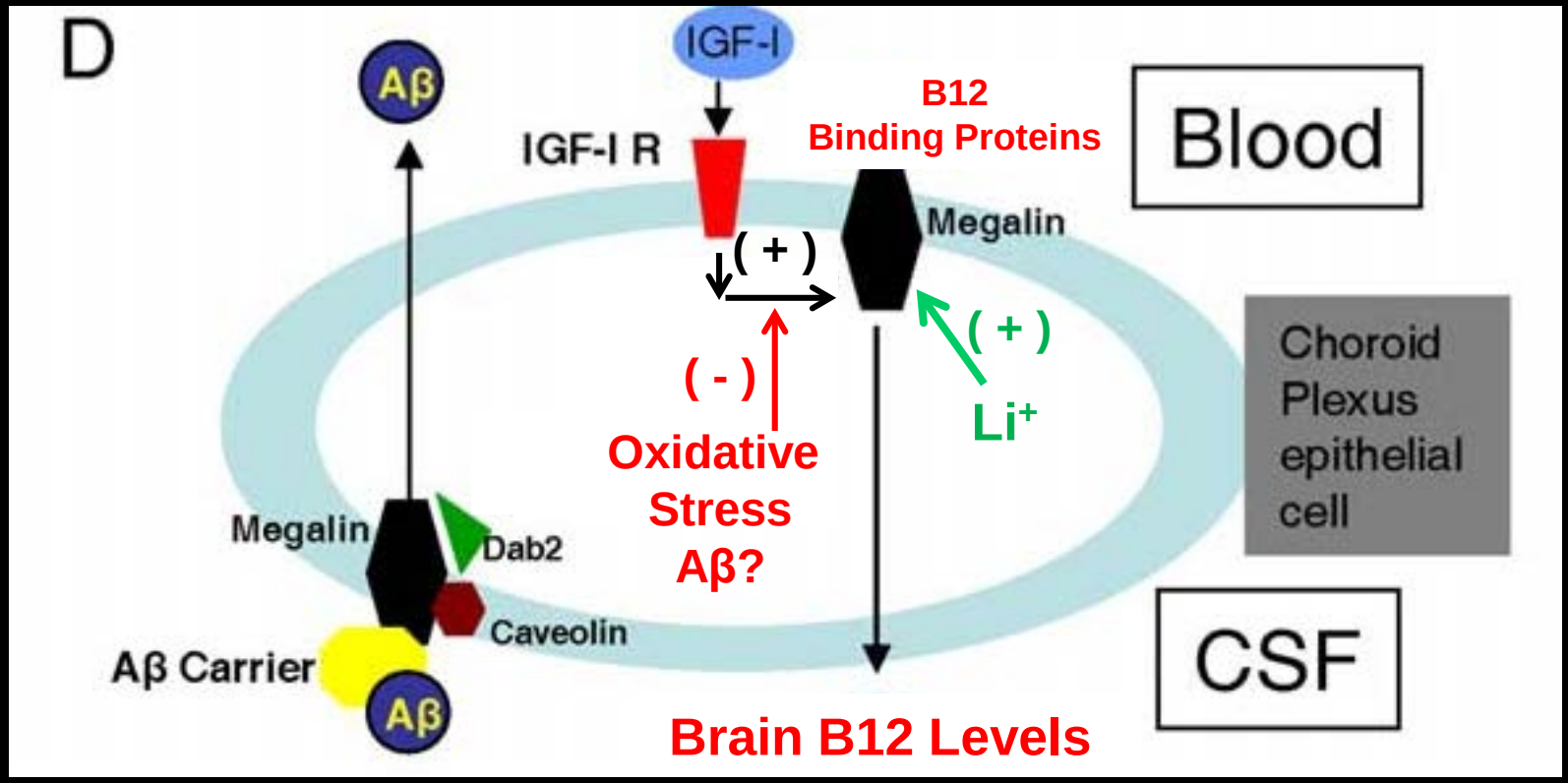


Neuregulin promotes uptake of cysteine and vitamin B12

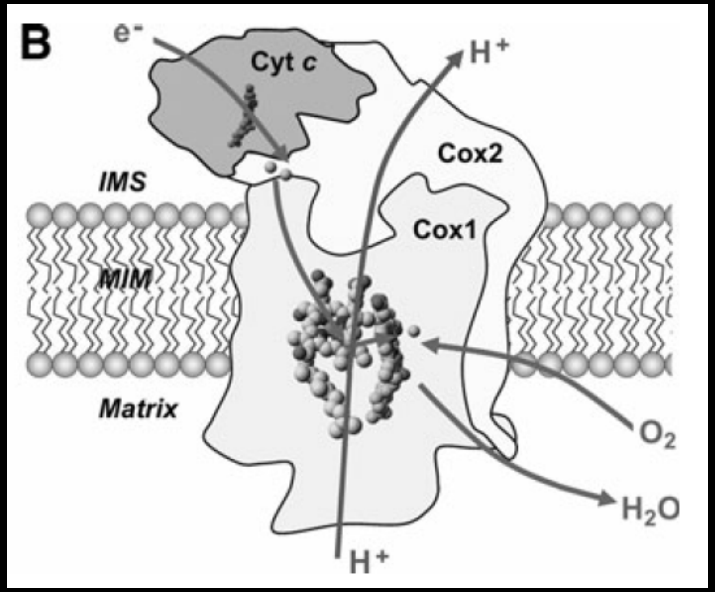


A Hypothesis:

Megalin transports B12 into the brain in the choroid plexus



Cytochrome C Oxidase :

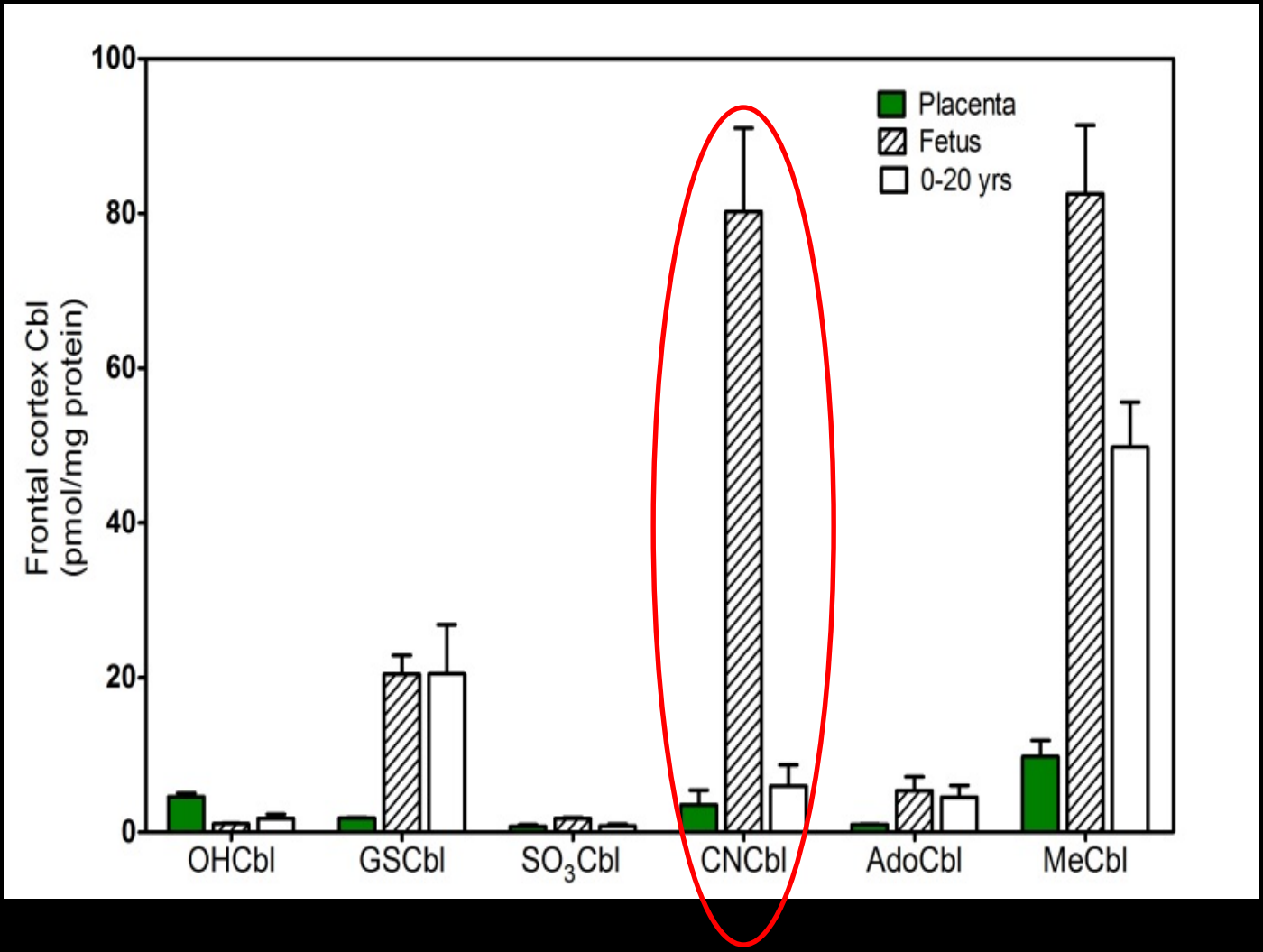


In bacteria and mitochondria

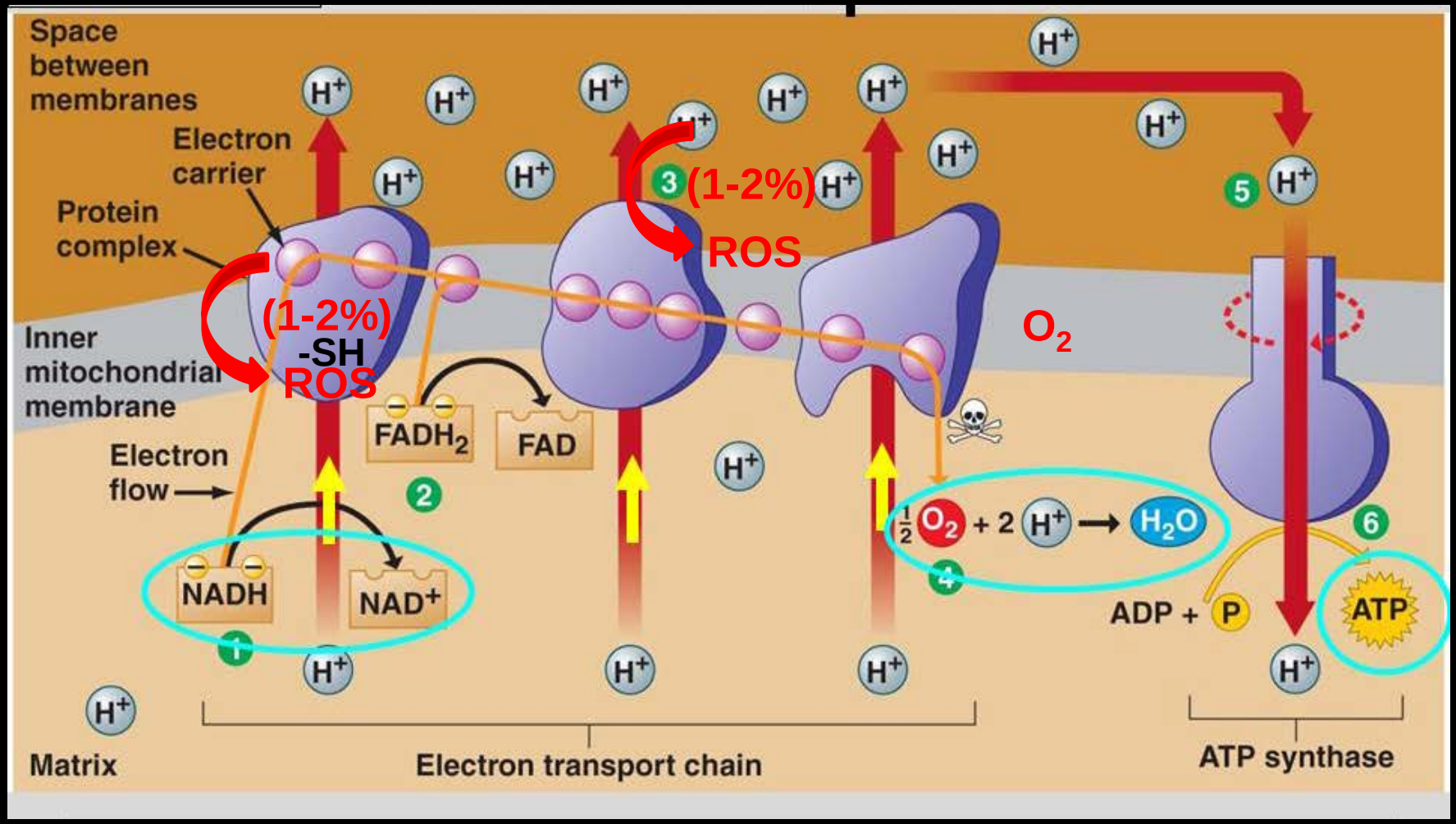
Reduces O_2 to water:

$4\ Fe^{2+}$-cytochrome *c* + $8\ H^+_{in} + O_2 \rightarrow 4\ Fe^{3+}$-cytochrome *c* + $2\ H_2O + 4\ H^+_{out}$

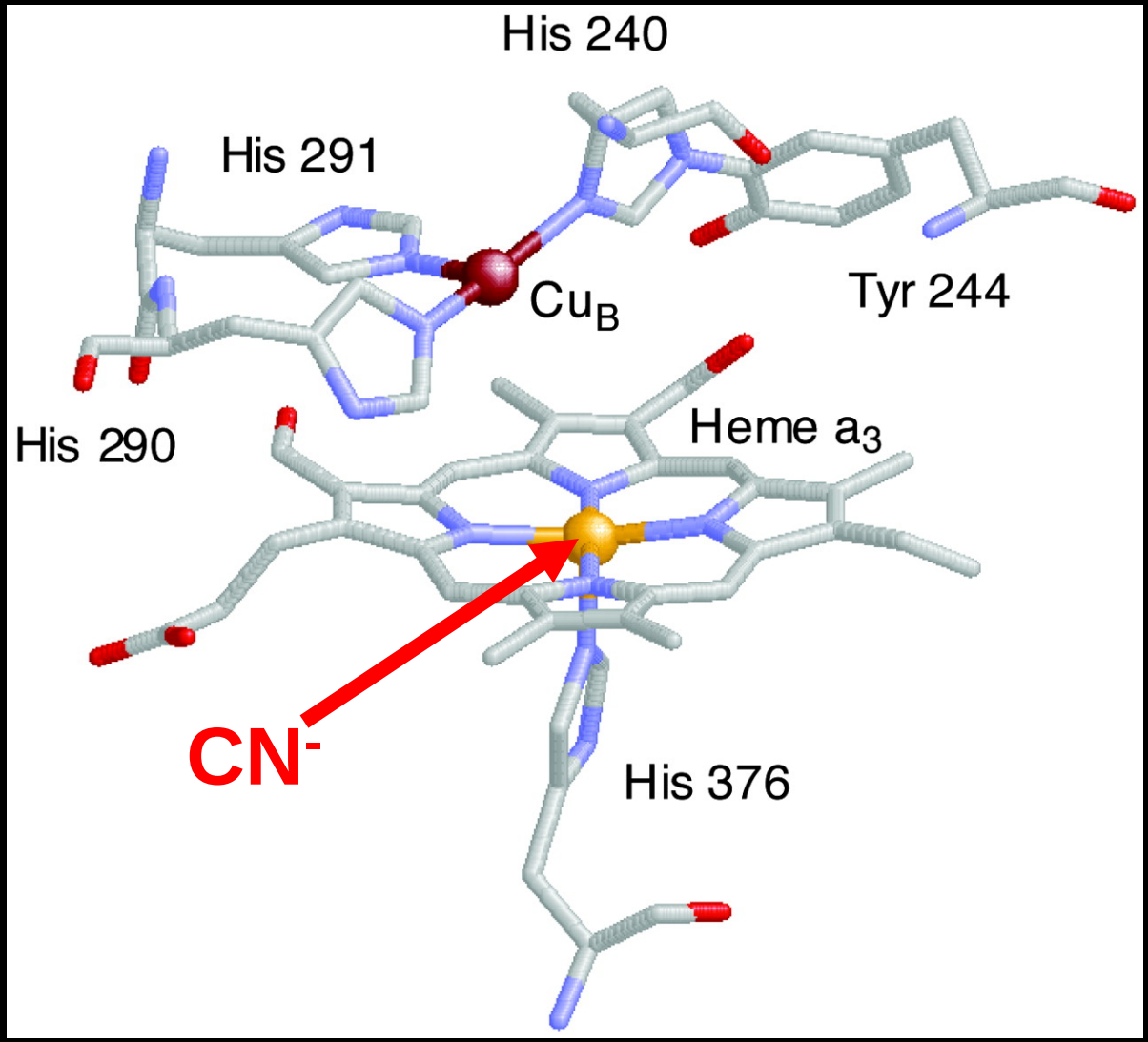
Levels of cyanocobalamin (CNCbl) are remarkably higher in fetal brain



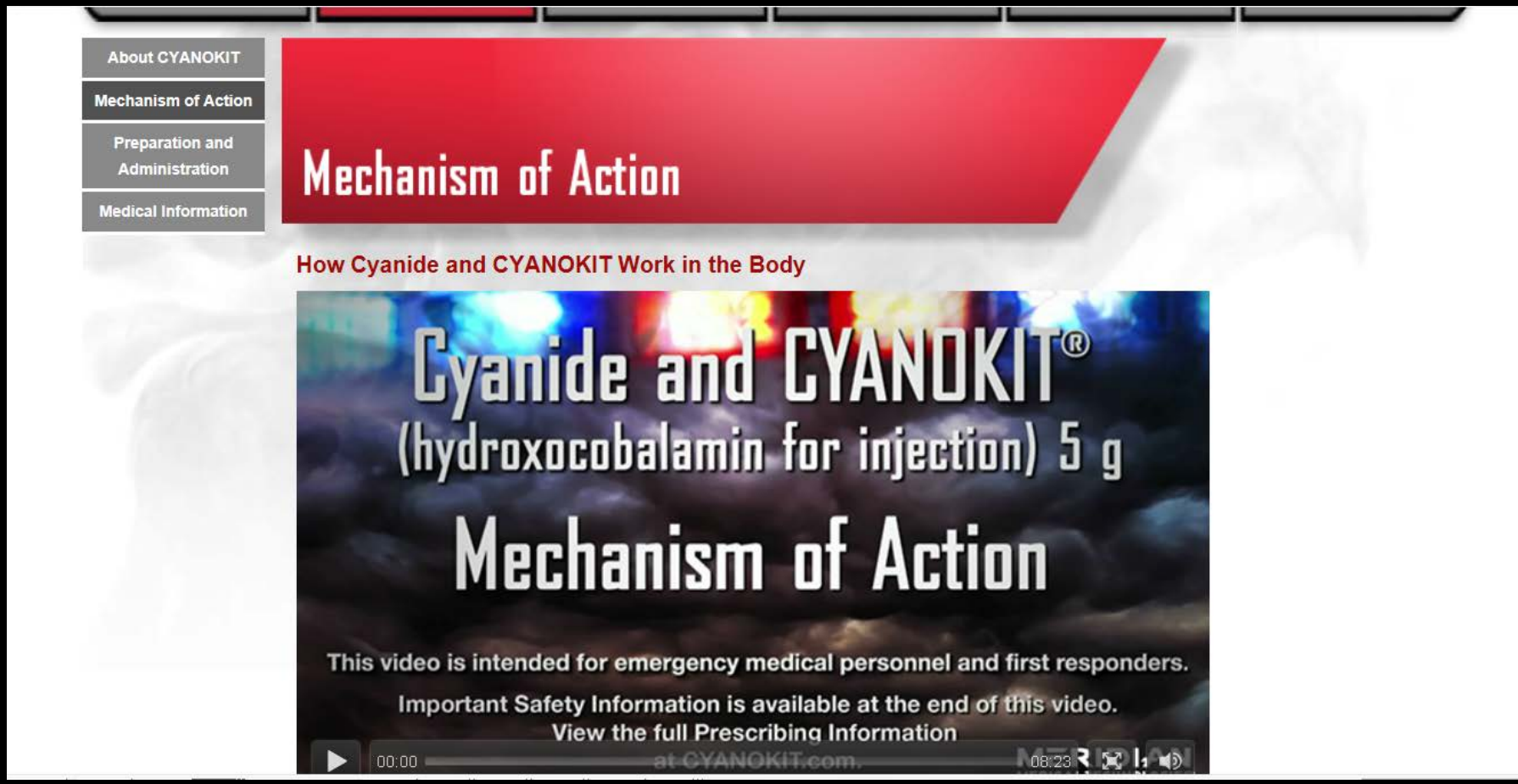
Complex IV (Cytochrome C Oxidase) reduces O_2 and creates H^+ gradient for ATP formation



Heme groups in cytochrome C oxidase are the target of cyanide poisoning



Hydroxocobalamin is an antidote for cyanide poisoning



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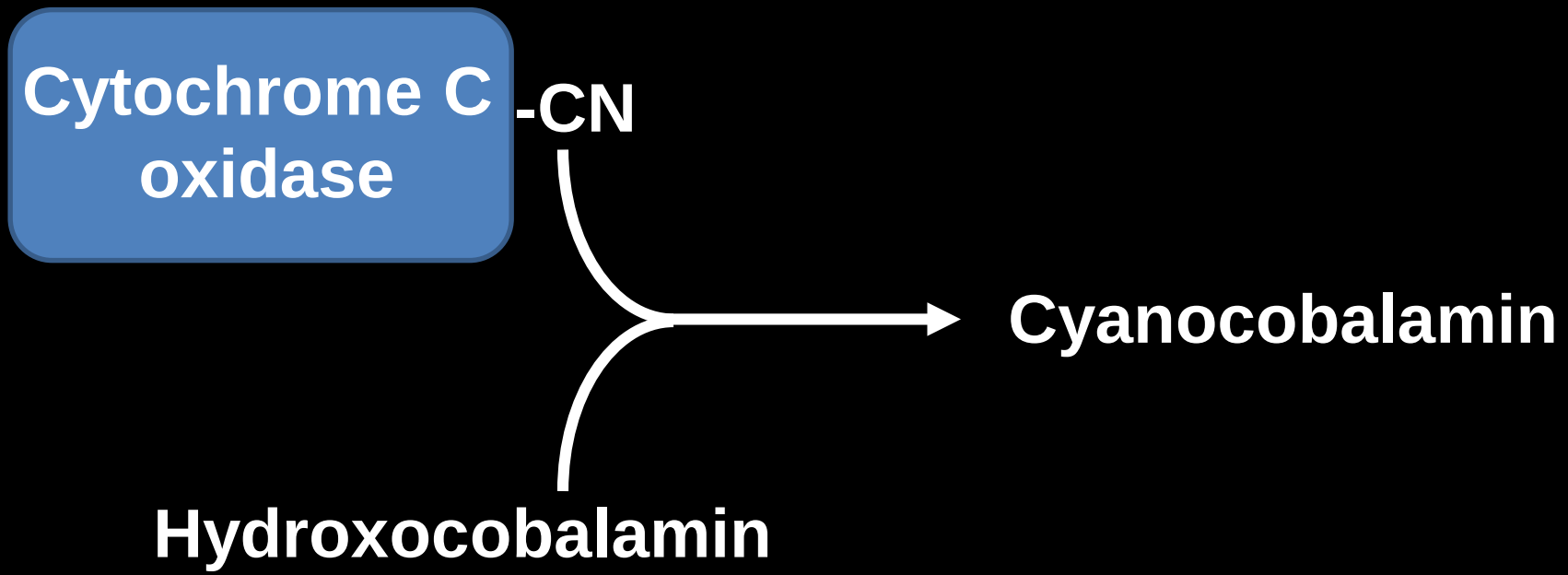
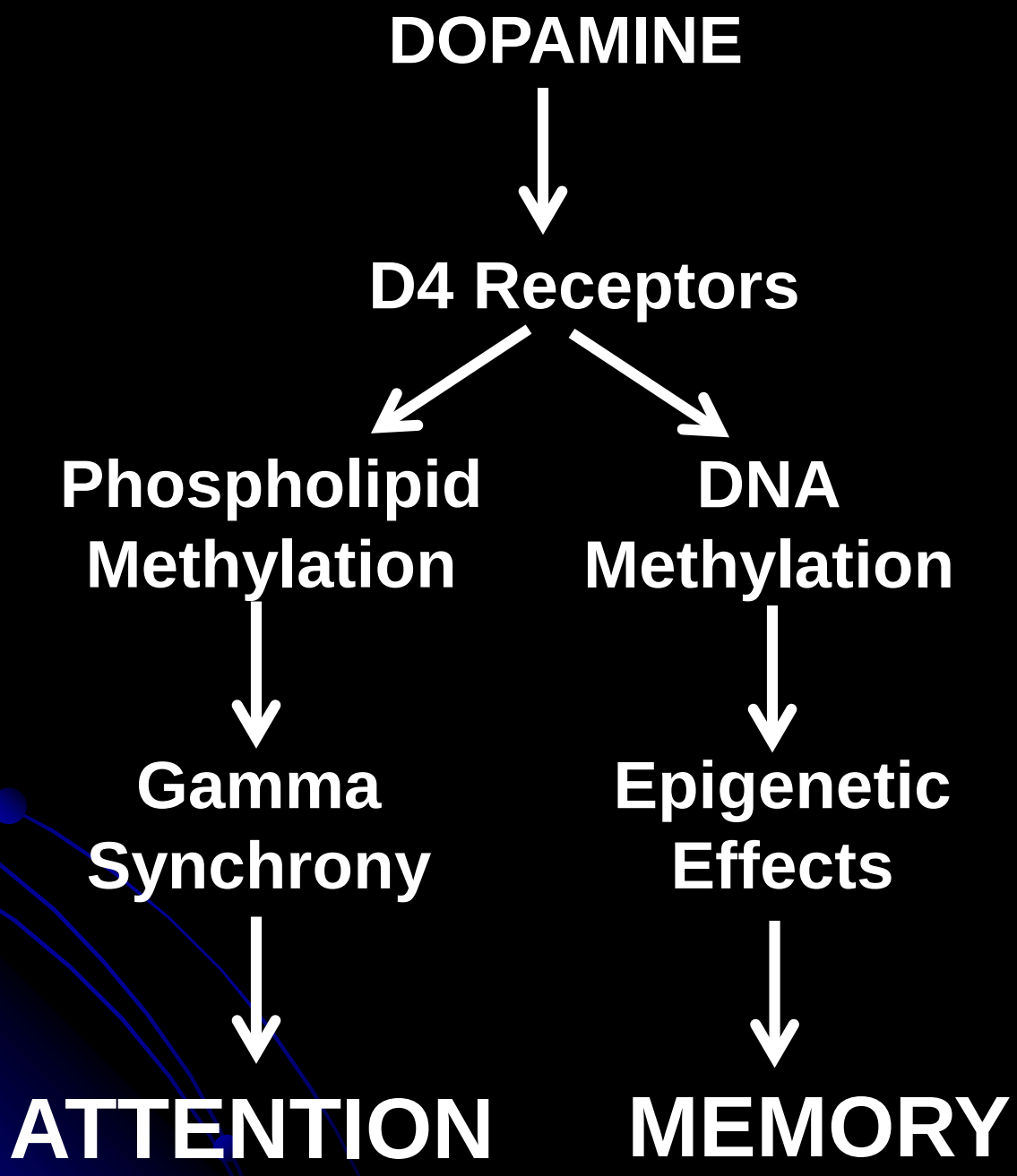
Effect of hydroxycobalamin on the inhibition of cytochrome c oxidase by cyanide. II - In isolated cytochrome c oxidase.*

L.C.Vieira Lopes and A.P.Campello




DOPAMINE
D4 Receptors
Phospholipid Methylation
DNA Methylation
Gamma Synchrony
Epigenetic Effects
ATTENTION
MEMORY

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