OF MERCURY, MICROBES, MICE AND MEN:
CLUES FOR AUTISM FROM ANIMAL MODELS OF
NEURODEVELOPMENTAL DAMAGE

Autism One
Chicago, IL
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www.greeneidlab.columbia.edu
“Many authors assure us that mental alienation is epidemic. \textit{It is certain that there are years when, independently of moral causes, insanity seems suddenly to extend to a great number of individuals}.”

- Esquirol, 1845
THREE STRIKES:
GENES, ENVIRONMENT & TIMING DEFINE PATHOGENESIS

Stage 1
Week 1

Stage 10
Week 4

FERTILIZED OOCYTE

NEURAL FOLD FUSES

FETUS
(a la da Vinci)

Developmental disorders (autism, AD/HD)

Psychiatric & demyelinating disorders

Degenerative disorders

GENETIC FACTORS

ENVIRONMENTAL FACTORS

GENETIC FACTORS

ENVIRONMENTAL FACTORS

infections
toxins
malnutrition stressors

child
early adult
late adult
Epidemiology  Animal Models
CLUES TO THE NEUROBIOLOGY OF AUTISM

Infantile autism: Kanner (1943); Asperger (1944)
Prevalence ↑ 10x since 1985
Male:female ratio ≥ 4:1
Regressive subsets?
Macrocephaly/abnormal brain growth
Autopsy data suggest developmental lesion
Neurochemical disturbances - dopamine, serotonin, glutamate
Seasonal, viral and immune links
Genetic and epigenetic contributions - up to 15 genes
(BROAD) disease concordance: MZ = 90%; DZ = 35%; sibs = 4%
MeCP2 (Rett syndrome) gene expression is subject to transcriptional and post-transcriptional modulation (role for exogenous agents?)
→ autism spectrum disorders, Prader-Willi, Angelman (Samaco et al., 2004)
CLINICAL FEATURES OF AUTISM

Is autism one or several disorders?

SOCIAL SKILLS
- nonverbal interactions
- friendship
- joint attention
- reciprocity

COMMUNICATION
- language
- conversation
- perseveration
- play

UNUSUAL ACTIVITIES
- obsessive interests
- rigid rituals
- stereotypies
- preoccupation with parts of objects

DSM-IV: ≥ 2

DSM-IV: ≥ 1

DSM-IV: > 1
ENDOPHENOTYPIC FEATURES OF AUTISM

Which features of autism help us understand its causes and complexity?

- Social Reciprocity
- Obsessive-Compulsive
- Imagination
- Attention
- Mood
- Disinhibition

- Lax Ligaments

- Elimination
  - Inflammatory Bowel Disease

- Autoimmunity
  - Infection

- Head & Brain Size
  - Height
  - Weight

- Connective Tissue

- Gi

- Immune

- Growth

- Seizures

- Psychiatric
  - Mood
  - Disinhibition

- Cognitive
  - Learning & Memory
  - Abstract Thought
  - Mental Retardation
  - Savant Skills

- Visual
  - Eye Gaze
  - Peripheral Vision
  - Oculomotor

- Sensory
  - Hearing
  - Taste
  - Crossmodal

- Motor
  - Activity Level
  - Muscle Tone
  - Posture
  - Coordination
  - Motor Programs
  - Autonomic Reflexes

- Gastrointestinal

- Immune

- Endocrine
EPIDEMIOLOGIC INVESTIGATIONS IN AUTISM

Reported prevalence - at least 10x ↑ since 1985

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<th></th>
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<th>broad</th>
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<td>1-2 / 10,000</td>
<td>4-5 / 10,000</td>
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<td>&gt; 1985</td>
<td>40 / 10,000</td>
<td>67 / 10,000</td>
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Hypotheses

diagnostic substitution; wider case definition and awareness

environmental exposures

infectious and immune challenges

toxins: cumulative mercury burden, PCBs, PBDEs

vaccines/vaccine components: MMR, thimerosal/ethylmercury

xenobiotic-induced epigenetic effects: DNA methylation

others: changes in pediatric antipyretic/pain reliever usage > 1985
INCREASE IN AUTISM PREVALENCE IS NOT EXPLAINED FULLY BY DIAGNOSTIC SUBSTITUTION

Fig. 1. Probability of becoming a California DDS client by age 4 for autism and mental retardation of unknown cause.

Croen and Grether, J Autism Dev Disord 2003;33:227-229
Fig 1. Prevalence (cases per 10,000 population) of select special education classifications among US children according to age and birth cohort.

Newschaffer et al., 2005
DO ENVIRONMENTAL AND/OR IMMUNE FACTORS PLAY A ROLE IN SOME CASES OF AUTISM?

**Genetic studies**
Monozygotic twin concordance < 100% and with diverse phenotypes
Failure to find consistent linkage with any single gene, role for epigenetic influences
Linkage to immune response genes (HLA, C4b null) only in simplex families, not multiplex
↑ family history autoimmune disease, psoriasis, allergies, asthma

**Geographic clusters**
Leominster, MA; Brick Township, NJ; N. California
Texas (61% ↑ in autism/1,000 lb Hg released into environment; Palmer 2005)

**Infection/immune challenge/inflammation**
Excess of March and August births
Viral associations (rubella, herpesviruses, others)
Toxins, vaccines/vaccine components (PCBs, PBDEs, thimerosal/ethylmercury; MMR)
Immune disturbance: Th1→Th2 cytokines (*except* ↑ IFNγ, IL-12 as in Hg-related autoimmunity);
↑ IgG1, IgG4, IgE; anti-CNS antibodies; ↓ C4b

**Neuroinflammation**
↑ neuroglial activation, neuroinflammation (↑ MCP-1, TGFβ1) - Vargas et al. (2005))

**Hypothesis:** Neural-immune pathways may define an ASD endophenotype
Moderation of the Effect of Adolescent-Onset Cannabis Use on Adult Psychosis by a Functional Polymorphism in the Catechol-O-Methyltransferase Gene: Longitudinal Evidence of a Gene X Environment Interaction

Avshalom Caspi, Terrie E. Moffitt, Mary Cannon, Joseph McClay, Robin Murray, Honalee Harrington, Alan Taylor, Louise Arseneault, Ben Williams, Antony Braithwaite, Richie Poulton, and Ian W. Craig

**Background:** Recent evidence documents that cannabis use by young people is a modest statistical risk factor for psychotic symptoms in adulthood, such as hallucinations and delusions, as well as clinically significant schizophrenia. The vast majority of cannabis users do not develop psychosis, however, prompting us to hypothesize that some people are genetically vulnerable to the deleterious effects of cannabis.

**Methods:** In a longitudinal study of a representative birth cohort followed to adulthood, we tested why cannabis use is associated with the emergence of psychosis in a minority of users, but not in others.

**Results:** A functional polymorphism in the catechol-O-methyltransferase (COMT) gene moderated the influence of adolescent cannabis use on developing adult psychosis. Carriers of the COMT valine<sup>158</sup> allele were most likely to exhibit psychotic symptoms and to develop schizophreniform disorder if they used cannabis. Cannabis use had no such adverse influence on individuals with two copies of the methionine allele.

**Conclusions:** These findings provide evidence of a gene × environment interaction and suggest that a role of some susceptibility genes is to influence vulnerability to environmental pathogens.
**Key points**

Adult cannabis use did not have the same effect, suggesting window of vulnerability

Early cannabis use alone NOT a major cause of schizophreniform disorder

Risk of exposure only significant in context of susceptibility gene
IMPORTANCE OF TIMING IN PATHOGENESIS

*Lessons from Borna Disease Virus*

Adult vs newborn rats infected with the same virus have different disease due to variable maturity of the central nervous and immune systems.
BORNAVIRUS MODELS OF NEUROPSYCHIATRIC DISEASES

DEVELOPMENTAL CONTEXT: maturity of CNS and immune systems

ADULT RATS

Biphasic disease

Acute syndrome
- hyperactivity
- exaggerated startle responses

Chronic syndrome
- stereotyped motor behaviors
- dyskinesias, dystonias

Pathology
- encephalitis
- massive cell loss

Neurotransmitter lesion
- dopamine

NEONATAL RATS (primate 3rd trimester)

Disorder unfolds with CNS development

Pathology
- hyperactivity
- learning deficits
- disturbed social interactions
  - vocalizations
  - play behavior
- unusual taste preferences

Neurotransmitter lesion
- 5HT and glutamate

DEVELOPMENTAL CONTEXT: maturity of CNS and immune systems
AGE-DEPENDENT PATHOLOGY IN INFECTED RATS
COMPLEX COMPULSIVE BEHAVIORS IN ADULT BD RATS
DOPAMINE RECEPTOR IMBALANCE IN ADULT BD RAT STRIATUM

NORMAL, D2

BDV, D2

40% decrease in D2 receptor expression

NORMAL, D1

BDV, D1

20% increase in D1 receptor expression
SELF-MUTILATION IN ADULT BD RATS: Response to D1 Receptor Blockade (SCH23390)

Tail biting *(before drug)*

Ambulation/abrogation of tail biting *(response to drug)*

Tail biting resumes *(clearance of drug)*
## Bornavirus Models of Neuropsychiatric Diseases

**Developmental Context:** Maturity of CNS and immune systems

### Adult Rats

**Biphasic Disease**

- **Acute Syndrome**
  - Hyperactivity
  - Exaggerated startle responses

- **Chronic Syndrome**
  - Stereotyped motor behaviors
  - Dyskinesias, dystonias

**Pathology**

- Encephalitis
- Massive cell loss

**Neurotransmitter lesion**

- Dopamine

### Neonatal Rats

**Disorder unfolds with CNS development**

- Hyperactivity
- Learning deficits
- Disturbed social interactions
  - Vocalizations
  - Play behavior
- Unusual taste preferences

**Pathology**

- No encephalitis
- Dysgenesis of hippocampus & cerebellum

**Neurotransmitter lesion**

- 5HT and glutamate

---

**Developmental Context:** Maturity of CNS and immune systems
MOTOR ABNORMALITIES IN AUTISM AND NEONATALLY-INFECTED RATS

normal righting reflex

abnormal righting movements in infant later diagnosed with autism

(Teitelbaum 1999)

development of righting reflex in rodents
TRANSIENT EARLY MOTOR DISTURBANCES IN NEONATALLY-INFECTED RATS

FALLS DURING AMBULATION

% FALLING ONTO BACK

DAY AFTER INOCULATION

Infected (n=10)  
Control (n=22)

$\chi^2 = 19.2, \ p < 0.0001$

*  $p = 0.007$

**  $p = 0.0015$
DISTURBED SOCIAL COMMUNICATION IN NEONATALLY-INFECTED RATS

MATERNAL SEPARATION PARADIGM

CONTROL

INFECTED

30 seconds
HYPERACTIVITY IN NEONATALLY-INFECTED RATS

* *, p = 0.029
†, p = 0.001
‡, p = 0.0009
CYTOKINE DYSREGULATION IN PREFRONTAL CORTEX OF NEONATALLY-INFECTED RATS

**CORRECTED VOLUME (MEAN)**

- IL1α
- IL1β
- IL6
- TNFα

- Infected (4 wk p.i.)
- Control

* p = 0.042
** p = 0.0006
APOPTOSIS-RELATED GENE EXPRESSION IN HIPPOCAMPUS OF NEONATALLY-INFECTED RATS

APOPTOSIS-RELATED GENE EXPRESSION IN HIPPOCAMPUS OF NEONATALLY-INFECTED RATS

CORRECTED VOLUME (MEAN)

Infected (4 wk p.i.)
Control

* p = 0.032
** p = 0.044
*** p = 0.017

FAS (Caspase-1)
ICE (Caspase-3)
YAMA
bcl-x
APOPTOSIS OF GRANULE CELLS IN DENTATE GYRUS OF NBD RATS

CONTROL

NBD, 4 wk pi

5 wk pi

NBD

NBD, 4 wk pi
APOPTOTIC LOSSES IN CEREBELLUM OF NBD RATS

GRANULE CELL LAYER

CONTROL
4 wk pi

NBD
4 wk pi

PURKINJE CELL LAYER


ABNORMAL DENDRITIC BRANCHING AND VARICOSITIES IN DENTATE GYRUS OF NEONATALLY-INFECTED RATS

Control

Infected

Infected

Similar findings in CA1 pyramidal cells of hippocampus in autism

Raymond et al. 1996
GLUR1 expression is altered in dentate gyrus of neonatally-infected rats.

Control

Infected, 4 wk p.i.

Redistribution of GluR1 receptors from molecular layer to soma of granule cells

4 wk timepoint coincides with developmental switch from flip to flop isoforms

Hypothesis: developmental arrest in flip isoform → prolonged glutamate sensitivity
AMPA RECEPTOR ANTAGONISM REDUCES HYPERACTIVITY IN NEONATALLY-INFECTED RATS

ANOVA

† Infected vs Control:  p = 0.0001
** NBQX(L) vs NBQX(S):  p = 0.0005
* NBQX(L) vs PBS(S):  p = 0.0007
NBQX(S) vs PBS(S):  p = ns
POSSIBLE PROMOTERS OF APOPTOSIS/NECROSIS

IDO ↑ BY IFN-γ
TDO ↑ BY GC’s

IFN-γ = induced by a wide variety of infections

ALSO ↑ BY IL-12 + IL-18?

ENZYMES OF KYN PATHWAY = PREFERENTIALLY LOCALIZED TO ASTROCYTES + MICROGLIA, BUT ALSO IN NEURONAL SUBSETS

POSSIBLE PROMOTERS OF APOPTOSIS/NECROSIS

reduction in 5HT synthesis also → independently to apoptosis

KYNURENINE PATHWAY

NMDAR + α7 NICOTINIC AchR ANTAGONIST
acts at glycine modulatory site assoc with NMDAR

INHIB OF KYN-3-OHase → ↓ NEURONAL DEATH POST-ISCHEMIC OR EXCITOTOXIC INSULTS

NMDAR AGONIST (PROMOTES EXCITOTOXICITY)
Selectivity at NMDAR2A +2B

→ ↓ SYNAPTIC ACTIVITY IN Hc, Cx

NMDAR AGONIST (PROMOTES EXCITOTOXICITY)
Selectivity at NMDAR2A +2B
LESSONS FROM BORNAVIRUS INFECTION MODELS

Phenotype depends on interaction of:

*genes x environment x timing*

Other infectious agents
rubella, influenza virus, lymphocytic choriomeningitis virus, herpesviruses

Xenobiotics
thalidomide, valproic acid, mercury, other heavy metals, PCBs, PBDEs (flame retardants), phthalates (plasticizers)

Focus on immune and neural system convergence (e.g., kynurenine pathway) and epigenetically-influenced developmental gene expression may shed light on determinants of vulnerability
BUILDING A GENERIC MODEL OF INFECTION-ASSOCIATED GESTATIONAL DAMAGE

*Lessons from polyinosine:polycytidylic acid* [*poly(I:C)*]

Behavioral and neuropathologic disturbances following late gestational immune challenge vary with genetic contributions of mother and offspring.

Timetables for CNS and immune system maturation vary across mouse strains.
Two mouse strains with different susceptibilities to immune damage: SJL (high), C57 (low)
SOLUBLE FACTORS IN SERUM OF DAMS
4h AFTER POLY(I:C)

% of no-injection control

*p<0.05   **p<0.005   ***p<0.0005

Haptoglobin
IL-1α
IL-3
IL-5
IL-6
IL-10
IL-17
IgA
IFNγ
Eotaxin
GCP-2/LIX
M-CSF
MDC
MIP-1γ
OSM
SCF
TIMP-1
TPO
VCAM-1
VEGF
vWF

C57 - PBS   C57 - Poly(I:C)   SJL - PBS   SJL - Poly(I:C)
POLY(I:C) INDUCES STRAIN-SPECIFIC DISTURBANCES OF EARLY LOCOMOTOR DEVELOPMENT (P8)

- **Movement Episodes**: C57: n=10, SJL: n=10, p=0.030
- **Velocity**: C57: n=12, SJL: n=10, p=0.036
- **Distance**: C57: n=12, SJL: n=10, p=0.021
- **Ambulatory Move Time**: C57: n=12, SJL: n=10, p=0.009
POLY(I:C) INDUCES POSTPUBERTAL HYPERACTIVITY IN C57 AND SJL MICE (WEEK 12)

**move episodes**

**move time**

**distance**

**velocity**

C57

Poly(I:C) High

Poly(I:C) Low

PBS

SJL

Poly(I:C) High

Poly(I:C) Low

PBS

* p < 0.05

** p < 0.005
CENTER AVOIDANCE (THIGMOTAXIS) IN PROGENY OF DAMS EXPOSED TO INFLUENZA H1N1 AT GD9.5

Shi, J Neurosci 2003
HYPERACTIVITY IN GD16 POLY(I:C) PUPS

Track Plot of XY plane activity over 90 minutes

Poly(I:C)

PBS

0-30 30-60 60-90
REELIN mRNA EXPRESSION IS INCREASED IN HIPPOCAMPUS OF C57 POLY(I:C) MICE

WEEK 8

WEEK 16

Poly(I:C) High
Poly(I:C) Low
PBS

* p < 0.02
LESSONS FROM POLY(I:C) MODEL

Phenotype depends on interaction of:

\[ \text{genes} \times \text{environment} \times \text{timing} \]

Multiple soluble mediators induced in utero may alter the course of neural development - mechanisms underlying common phenotypes may differ.

Delineation of developmental trajectory may elucidate pathways involved in pathogenesis of neuropsychiatric disorders.

What mechanisms contribute to the specificity of brain regions to damage or dysfunction?

- developmentally-sensitive susceptibility of receptors to soluble mediators?
- crossreactive antibodies that target specific brain components?
ARE AUTOIMMUNE REACTIONS DIRECTED AGAINST CNS COMPONENTS IMPLICATED IN THE PATHOGENESIS OF NEURODEVELOPMENTAL DISORDERS?

Lessons from Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infection (PANDAS)

Misdirected immune response to exogenous antigens (bacteria, viruses, xenobiotics, food products) may result in neuropsychiatric syndromes in susceptible hosts
PANDAS: A BACTERIAL AUTOIMMUNE NEUROPSYCHIATRIC DISORDER

Pediatric Autoimmune Neuropsychiatric Disorders Assoc w/ Strep infx (Swedo et al., 1998)

Sydenham’s chorea (St. Vitus’ dance) (Sydenham, 1686)
antineuronal antibodies - specificity for CNS targets may vary

Clinical features
obsessive-compulsive symptoms, anxiety, tics (Swedo, 1994)

Biologic risk factors
D8/17 marker on B lymphocytes (also in 78% of autism cases in absence of evidence of recent strep infection; Hollander et al., 1999)

Response to plasmapheresis, IVIg, antibiotics (Perlmutter et al., 1999)
ENLARGED BASAL GANGLIA IN CHILDREN WITH PANDAS OCD/TICS

OCD/TICS, n=34;  NL, n= 84

IMMUNITY TO CNS AND STEREOTYPIC BEHAVIOR IN PANDAS MOUSE

- Normal mouse brain
- PANDAS mouse brain
- Goat α mouse IgG
- Neonatal rat striatum culture
- Normal mouse serum
- PANDAS mouse serum
PANDAS MOUSE SERA BINDS TWO PROTEINS IN CEREBELLUM AND TWO IN STRIATUM

Non-denaturing Western blot
PANDAS Sera

Mouse ID#

15  16  17  13  6  Normal

Non-denaturing IP

Normal  GABHS #16  Marker

LC-Mass Spectrometry → complement C4, α2-macroglobulin (cblm)
→ complement C4, heat shock protein 70 (striatum)
HUMAN PANDAS SERA BINDS RECOMBINANT HUMAN HSC70 PROTEIN

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LESSONS FROM PANDAS MODEL

Phenotype depends on interaction of:

*genes x environment x timing*

Immune challenge in the absence of infection can
→ anti-CNS antibodies and behavior disturbances

Animal models need not replicate every aspect of human disease
to be useful in generating data in support of causal hypotheses
and may stimulate discovery of biomarkers and novel interventions

What classes of environmental agents can disrupt neural development via
an immune mechanism?

What is the window of vulnerability?
maturation of human limbic structures extends from third trimester through second
year of life, possibly beyond
WHAT GENETIC AND TEMPORAL FACTORS INFLUENCE MERCURY TOXICITY AND RISK OF NEURODEVELOPMENTAL DISORDERS SUCH AS AUTISM?

*Lessons from a mouse strain-dependent model of thimerosal (ethylmercury) neurotoxicity*

*Sensitivity to mercury-induced autoimmune disease predicts neurotoxic effects following postnatal thimerosal*
A MOUSE MODEL OF STRAIN-DEPENDENT THIMEROSAL TOXICITY

Hypothesis: Genes determine CNS outcomes after postnatal thimerosal

18% of population hypersensitive to thimerosal, 0.2% to inorganic mercury (Pink dis.)

Population differences in isoforms of susceptibility genes:
glutathione-S-transferase, HLA loci, metallothioneins

Thimerosal is associated with:
↓ intracellular glutathione  ↑ arachidonic acid levels
Ca^{2+} dysregulation (via IP3 rec.)  apoptosis of T cells, microglia, astrocytes, neurons
↑ conversion to iHg and brain/blood Hg ratios compared to MeHg

autoimmune renal syndrome after oral exposures of adult H-2^{s} mice (as with iHg):

Th1→Th2
↑ IgE and IgG1 (Th2-related isotypes) and IgG2a (Th1-related isotype)
↑ autoAb’s (antinucleolar/antifibrillarin) (Havarinasab et al., 2004)
# Rationale for Dosing Strategy and Schedule

## Human Age at Vaccine Administration

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<th>6 Months</th>
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<td>Day 7</td>
<td>Day 9</td>
<td>Day 11</td>
<td>Day 15</td>
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<td>Ethylmercury load (µg)</td>
<td>62.5</td>
<td>62.5</td>
<td>62.5</td>
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<td>10th percentile weight (boys, kg)</td>
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<td>5.8</td>
<td>6.8</td>
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<tr>
<td>Ethylmercury dose (µg/kg)</td>
<td>14.2</td>
<td>10.8</td>
<td>9.2</td>
<td>5.6</td>
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<tr>
<td>Vaccines administered to children</td>
<td>Hep B</td>
<td>Hep B</td>
<td>Hep B</td>
<td>DTaP</td>
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<tr>
<td></td>
<td>HiB</td>
<td>HiB</td>
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Others possible through age 12 mo (not included): pneumococcal, influenza
MOUSE STRAIN-DEPENDENT, IMMUNE-MEDIATED THIMEROSAL NEUROTOXICITY PROJECT

- Thimerosal
- Thimerosal + vax (DTaP, HiB)
- PBS

Gene-environment interactions in neurodevelopmental disorders

Brain anatomy & molecular biology

Functional analyses

Thimerosal

SJL H-2^s +++
C57 H-2^b -
BALB H-2^d +/-

Week 0
Week 7
Week 11
Week 14

Behavior

Tissue Collection
Week 5

Tissue Collection
Week 13

Days 8, 10

Week 4

Behavior

Week 10

Behavior

Week 12

Behavior
POSTNATAL THIMEROSAL ALTERS HIPPOCAMPUS IN SJL MICE

Timing of lateral > dorsal/ventral enlargement (eurycephaly, as noted by Deutsch et al. 2002 in autism) suggests failure to prune mossy fiber bundles.
POSTNATAL THIMEROSAL ALTERS EAAT3 RECEPTORS IN HIPPOCAMPUS OF SJL MICE
POSTNATAL THIMEROSAL IS ASSOCIATED WITH EXTREME COMPULSIVE BEHAVIORS IN SJL MICE

Self-grooming/mutilation

Social grooming and interaction
IgG1 DEPOSITS IN BRAINS OF SJL THIMEROSAL MICE

DENTATE GYRUS

STRIATUM

LVN

CBLM
LESSONS FROM THE THIMEROSAL MOUSE MODEL

Phenotype depends on interaction of:

\[ \text{genes} \times \text{environment} \times \text{timing} \]

Host response genes that increase risk for mercury-induced autoimmune disturbance predict behavioral and neuropathologic changes following postnatal challenge

What is the relevance of these animal models for host:environment interactions affecting human neural development?
MERCURY EXPOSURE

Sources of thimerosal exposure (ethylmercury)
Vaccinations - still in some influenza and meningococcal vaccines, Td boosters
Prescribed eye medications, nasal drops, Rho D immunoglobulin

Other sources of Hg
- methylmercury (fish)
  - industrial (coal-fired power plants)
  - amalgams

“Bio-accumulation”
- 4x ↑ mercury in white albacore vs. light tuna
  - highest: king mackerel, shark, swordfish, tilefish
  - lowest: clams, crab, haddock, hake, herring, ocean perch, pickerel, salmon, shrimp, freshwater trout

1 in 6 US women of childbearing age have unsafe blood levels of mercury, putting > 600,000 children at risk/yr

Mercury toxicity is ↑ where nutrition is poor - selenium deficiency
Mercury in Plasma-Derived Products

“The EPA has raised concerns regarding mercury exposure. These concerns have been in the context of chronic exposure to methyl mercury in milligram amounts. In contrast, blood plasma-derived products (except anti-venoms) containing ethyl mercury are usually given as one or two injections. Furthermore, the ethyl mercury content of these products is in the form of a preservative, thimerosal, which breaks down to form ethyl mercury in microgram amounts.”

*Rho (D) Immune Globulin (Human) products* -

RhoGAM, Ortho Clinical Diagnostics, Inc  
10.5 micrograms ethylHg/dose  
2001 - Ortho approved by FDA to produce RhoGAM without thimerosal for US market

BayRho, Bayer Corporation  
35 micrograms ethylHg/dose  
1996 - BayRho becomes thimerosal-free

WinRho SD, Cangene Corporation  
Never contained a preservative
# MERCURY IN DRUG AND BIOLOGIC PRODUCTS

**Date created:** August 5, 2003; updated September 14, 2004

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<th>Name of Product</th>
<th>Ingredient</th>
<th>%</th>
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<td>AK Spore Ophthalmic Solution</td>
<td>TM</td>
<td>.001</td>
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<td>TM</td>
<td>NS</td>
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<td>AK Spore HC Otic Suspension</td>
<td>TM</td>
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<tr>
<td>* Alcon Laboratories</td>
<td>Profenal 1% Ophthalmic Solution</td>
<td>TM</td>
<td>.005</td>
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<td>* Alcon Laboratories</td>
<td>Adsorbonac 2% Ophthalmic Solution</td>
<td>TM</td>
<td>.004</td>
</tr>
<tr>
<td>* Alcon Laboratories</td>
<td>Adsorbonac 5% Ophthalmic Solution</td>
<td>TM</td>
<td>.004</td>
</tr>
<tr>
<td>Allergan America</td>
<td>Ocufen Ophthalmic Solution</td>
<td>TM</td>
<td>.005</td>
</tr>
<tr>
<td>Allergan America</td>
<td>Poly Pred Ophthalmic Suspension</td>
<td>TM</td>
<td>.001</td>
</tr>
<tr>
<td>Allergan Inc.</td>
<td>Blephamide SOP Ophthalmic Ointment</td>
<td>PMA</td>
<td>.0008</td>
</tr>
<tr>
<td>Allergan Inc.</td>
<td>Bleph-10 Ophthalmic Ointment 10%</td>
<td>PMA</td>
<td>.0008</td>
</tr>
</tbody>
</table>

**Legend:**
- **TM** = thimerosal
- **PMN** = phenylmercuric nitrate
- **MN** = mercuric nitrate
- **MOY** = mercuric oxide yellow
- **PMA** = phenylmercuric acetate
- **MA** = mercuric acetate
- **MB** = merbromin
<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product Description</th>
<th>Agency</th>
<th>Mercury Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>K and B Distributors</td>
<td>Mercurochrome Aqueous Solution</td>
<td>MB</td>
<td>2</td>
</tr>
<tr>
<td>* King Pharmaceuticals</td>
<td>Cortisporin Ophthalmic Suspension</td>
<td>TM</td>
<td>.001</td>
</tr>
<tr>
<td>* King Pharmaceuticals</td>
<td>Neosporin Ophthalmic Suspension</td>
<td>TM</td>
<td>.001</td>
</tr>
<tr>
<td>* King Pharmaceuticals</td>
<td>Viroptic Ophthalmic Solution</td>
<td>TM</td>
<td>.001</td>
</tr>
<tr>
<td>King Pharmaceuticals</td>
<td>Neomycin Polymyxin B Sulfates Hydrocortisone Otic Suspension</td>
<td>TM</td>
<td>NS</td>
</tr>
<tr>
<td>* King Pharmaceuticals</td>
<td>Pediotic Suspension</td>
<td>TM</td>
<td>.001</td>
</tr>
<tr>
<td>* King Pharmaceuticals</td>
<td>Cortisporin Otic Suspension</td>
<td>TM</td>
<td>.01</td>
</tr>
<tr>
<td>Kinray</td>
<td>Oxymetazoline Nasal Spray</td>
<td>PMA</td>
<td>.002</td>
</tr>
<tr>
<td>Laboratori Derivati</td>
<td>Adrenal Cortex Injection</td>
<td>TM</td>
<td>.01</td>
</tr>
<tr>
<td>Leader</td>
<td>12 Hour Nasal Spray</td>
<td>PMA</td>
<td>NS</td>
</tr>
<tr>
<td>Leader</td>
<td>Nasal Pump Spray</td>
<td>PMA</td>
<td>NS</td>
</tr>
<tr>
<td>Longs Drug Stores</td>
<td>Nasal Spray Pump</td>
<td>PMA</td>
<td>NS</td>
</tr>
<tr>
<td>Martin Surgical Supply</td>
<td>Testosterone Injection Suspension 50 mg</td>
<td>TM</td>
<td>.008</td>
</tr>
<tr>
<td>Martin Surgical Supply</td>
<td>Testosterone Injection Suspension 100 mg</td>
<td>TM</td>
<td>NS</td>
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<tr>
<td>Mays Drug Stores</td>
<td>Hemorrhoid Relief Ointment</td>
<td>PMN</td>
<td>.01</td>
</tr>
<tr>
<td>Medalist Laboratories</td>
<td>Long Lasting Nasal Spray Pump</td>
<td>PMA</td>
<td>NS</td>
</tr>
<tr>
<td>Meyers Supply Inc.</td>
<td>Long Acting Nasal Spray</td>
<td>PMA</td>
<td>.002</td>
</tr>
<tr>
<td>Navresso</td>
<td>Long Acting Nasal Spray</td>
<td>PMA</td>
<td>NS</td>
</tr>
</tbody>
</table>
CONTRIBUTE TO THE DEVELOPMENTAL NEUROTOXICITY OF MERCURY?

Mercury induces activation of glia (astrocytes, microglia)

Microglia are macrophage lineage cells, role in ICAM/cytokine/chemokine pathways with influences on neuronal migration, express all Toll-like Receptors (TLRs), secrete proinflammatory cytokines, role in innate immune activation

Can developmental mercury exposure affect these signaling pathways and alter neuronal subsets, plasticity, or connectivity?
Mercury accentuates or initiates infection-induced autoimmunity

Coxsackie B3 virus autoimmune myocarditis

Exposures to inorganic mercury increase disease severity

Myocarditis is a major cause of heart failure and sudden death in young adults (athletes - testosterone exposure?); dilated cardiomyopathy in susceptible individuals
CAUSATION: IMPLICATION OF MICROBES IN DISEASE

Koch’s Postulate
10th International Congress of Medicine, Berlin, 1890

Microbe occurs in every case of a disease
Microbe must be specific for that disease
Microbe can be isolated, grown in the laboratory, and cause disease after inoculation into animals

Problems with invoking Koch’s Postulate
Some microbes cannot be grown in the laboratory
Suitable animal models may not exist
Host and environmental factors may influence expression of disease
Longterm or distant sequelae may obscure relationship between infection and disease
Assumes single agent, single disease
### Hill’s epidemiologic criteria for causal association

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strength of association</strong></td>
<td>What is the relative risk?</td>
</tr>
<tr>
<td><strong>Consistency of association</strong></td>
<td>Is there agreement among repeated observations in different places, at different times, using different methods, by different researchers, under different circumstances?</td>
</tr>
<tr>
<td><strong>Specificity of association</strong></td>
<td>Is the outcome unique to the exposure?</td>
</tr>
<tr>
<td><strong>Temporality</strong></td>
<td>Does exposure precede the outcome variable?</td>
</tr>
<tr>
<td><strong>Biological gradient</strong></td>
<td>Is there evidence of a dose-response relationship?</td>
</tr>
<tr>
<td><strong>Plausibility</strong></td>
<td>Does the causal relationship make biological sense?</td>
</tr>
<tr>
<td><strong>Coherence</strong></td>
<td>Is the causal association compatible with present knowledge of the disease?</td>
</tr>
<tr>
<td><strong>Experimentation</strong></td>
<td>Does controlled manipulation of the exposure variable change the outcome?</td>
</tr>
<tr>
<td><strong>Analogy</strong></td>
<td>Does the causal relationship conform to a previously described relationship?</td>
</tr>
</tbody>
</table>
Logical gaps in applying Hill’s criteria in the context of what is/isn’t known about autism, mercury, other toxins, and host susceptibility factors:

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strength of association</strong></td>
<td>What is the relative risk? [<strong>G*E interactions</strong> can alter results]</td>
</tr>
<tr>
<td><strong>Consistency of association</strong></td>
<td>Is there agreement among repeated observations? [methods largely the same, those that consider potential confounds yield different results, <strong>hypothesis framing</strong> of most epi studies makes little biologic or clinical sense]</td>
</tr>
<tr>
<td><strong>Specificity of association</strong></td>
<td>Is the outcome unique to the exposure? [need to consider possibilities other than <strong>single agent, single disease</strong>]</td>
</tr>
<tr>
<td><strong>Temporality</strong></td>
<td>Does exposure precede the outcome variable? [belys a ‘<strong>multiple hit’</strong> hypothesis]</td>
</tr>
<tr>
<td><strong>Biological gradient</strong></td>
<td>Is there a dose-response relationship? [<strong>env susceptibility genes</strong> may alter excretion/distribution, antioxidant or autoimmune responses, age/developmental status of host or other host factors; exposures may be misclassified if not directly measured (Hg bioaccumulation); effects may depend on <strong>Hg species</strong>, <strong>chronicity or route</strong>, or other toxic exposures (<strong>sensitization</strong>); low dose exposures may predispose to diff set of sequelae (immune/infectious) than high dose (neurotoxic, lethal)]</td>
</tr>
</tbody>
</table>
**Logical gaps in applying Hill’s criteria - II**

<table>
<thead>
<tr>
<th>Category</th>
<th>Question</th>
</tr>
</thead>
</table>
| **Plausibility** | Does the causal relationship make biological sense?  
[Broader conceptions of pathogenesis will give a different answer than single disease conceptions] |
| **Coherence**   | Is the causal association compatible with present knowledge of the disease?  
[Data gaps impair our capacity to determine coherence] |
| **Experimentation** | Does controlled manipulation of the exposure variable change the outcome?  
[There is resistance to accepting data from experimental models, and a lack of balance in considering the import of epidemiologic evidence] |
| **Analogy**     | Does the causal relationship conform to a previously described relationship?  
[Broader conceptions of pathogenesis will give a different answer than single disease conceptions] |
PROSPECTIVE APPROACH TO EXAMINING THE ROLE OF ENVIRONMENT IN CHRONIC DISEASES

MoBa Autism Birth Cohort
Norway - 100,000
U01-NS047537 (Hirtz; PI, Lipkin)

Clinical Database

Q’naire
Ultrasound

Maternal recruitment
17 wk

Blood, urine (maternal)

Paternal blood

Maternal ultrasound

30 wk

Mat. blood, urine

Blood, urine

Med. birth registry

Q’naire

Cord blood

Q’naire

Birth

6 mo

Q’naire

18 mo

Q’naire

36 - 40 mo

Blood

Core Specimen Bank

Ongoing
Clinic visits
Immunization, Pharmacy Registries
Referrals

X y
Large unselected cohort (100,000 mothers and children, fathers)

Questionnaires and diagnostic instruments focused on ASDs

Serial, prospective collection of clinical data and biologic specimens begins early in gestation

*Biological and clinical phenotypes*
*Longitudinal trajectory of disease*
*Functional genomics, proteomics, toxicology, as well as genetics*

High throughput assays and programs for computational biology

**Resources to be established**
Clinical databases (questionnaires, videography, clinical exams, outcomes)
Tools for early screening and diagnostic assessment
Biobanks of unique clinical materials
Pathogenesis may be *direct* or *indirect*, and manifest *locally* or *distally* with respect to the *site* or *timing* of an insult.

Animal models provide examples of interactions of *genes x environment x timing*, resulting in outcomes reminiscent of complex diseases: “three strikes model”

Clues from animal models can translate into promising leads for human biomarkers/diagnostics, interventions, and preventions; epidemiologic findings guide strategic animal model inquiries.
In the period that Einstein was active as a professor, one of his students came to him and said:

“The questions of this year’s exams are the same as last year!”

“True,” Einstein said, “but this year all the answers are different.”