



FETAL ALCOHOL SPECTRUM DISORDERS

Current Science & Research Trends

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www.fasds.org



- The FASDSG is a society of over 400 clinicians and scientists performing research on FASD.
- The binding interests of our members are
 - the desire to fully understand the etiology of FASD
 - describe the characteristics of FASD at multiple levels
 - devise ways to improve the lives of children, adolescents, and adults with FASD.

Current Science & Research Trends: An Update

❖ TERATOGENIC EFFECTS

- CONSEQUENCES OF DAMAGE
- UNDERLYING MECHANISMS OF DAMAGE

❖ DIAGNOSIS

- BEHAVIOR
- IMAGING
- BIOMARKERS

❖ PREVENTION, INTERVENTION & TREATMENT

TERATOGENIC EFFECTS OF PAE

- Growth and developmental retardation
- Craniofacial anomalies
- Skeletal defects
- Malformed organs including:
 - brain, heart, liver, kidneys, eyes, teeth and others
- Developmental brain deficits
- Cognitive and behavioral abnormalities



- **Growth delay or deficiency**

small head, small body size, slower development, failure to catch up

- **Facial dysmorphism**

small eye openings, drooping eyelids, nearsightedness, short upturned nose, sunken nasal bridge, flat philtrum, thin upper lip, opening in roof of mouth, small jaw

- **Skeletal deformity**

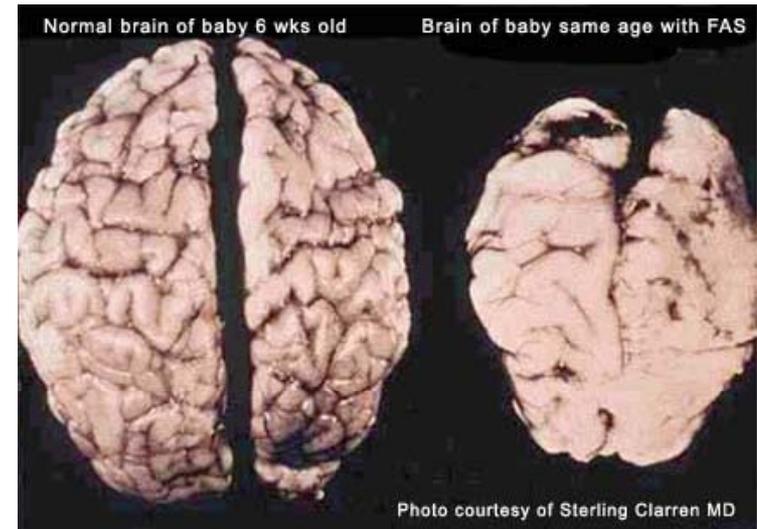
deformed ribs and sternum, curved spine; bent, fused, webbed or missing fingers or toes; limited joint movement

- **Organ deformity**

heart defects, heart murmurs, liver, kidney and urinary defects, genital malformations, vision and hearing problems

Neuro-Developmental Defects:

- small brain (microencephaly)
- abnormal arrangement of brain cells (pathology)
- cognitive deficits:
 - mental retardation
 - learning disabilities
 - Reduced high-level intellectual skills, such as executive function
 - many others
- behavioral problems: irritability, short attention span, hyperactivity, and many others
- poor muscle coordination

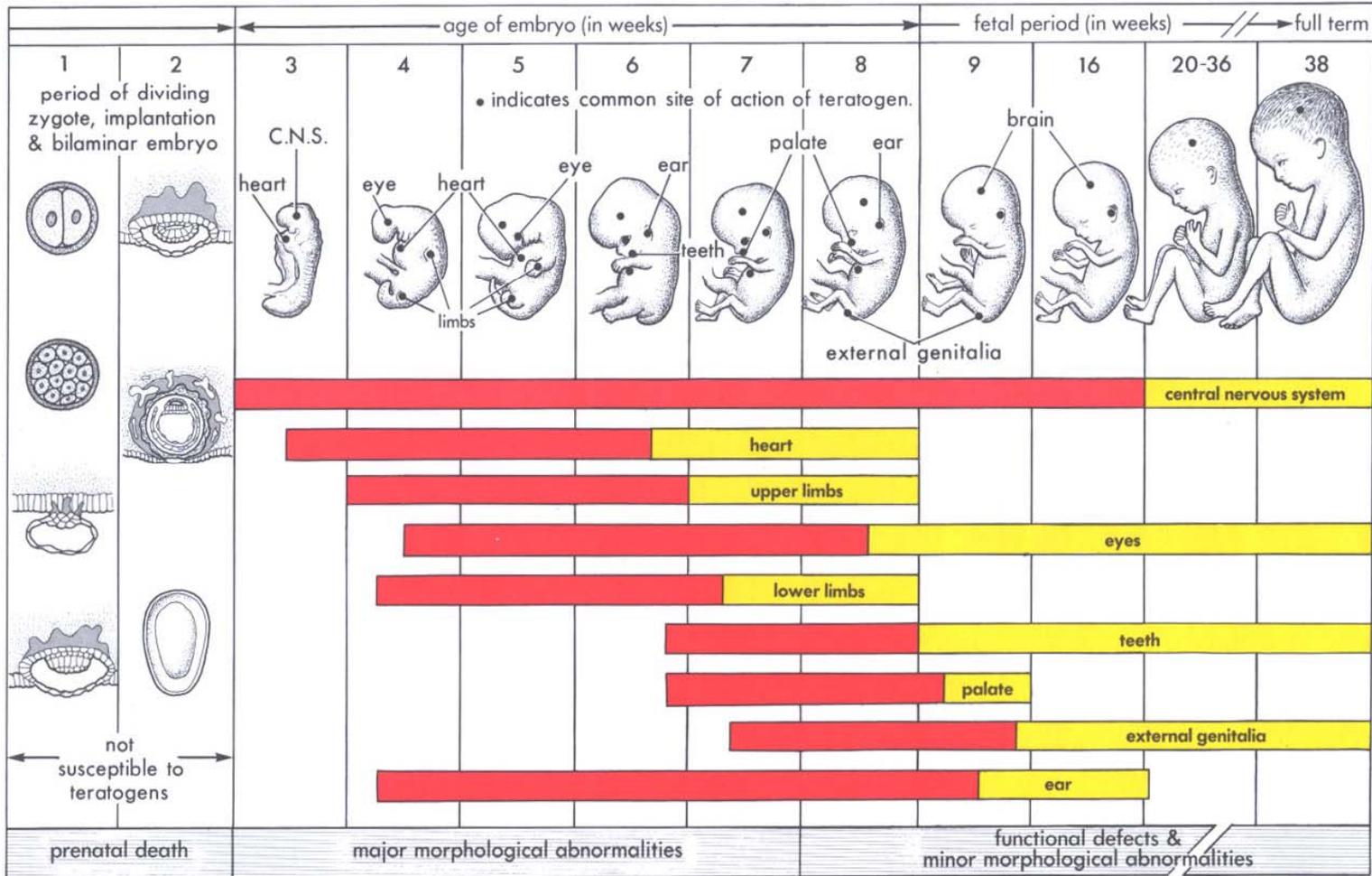


Periods of Human Development

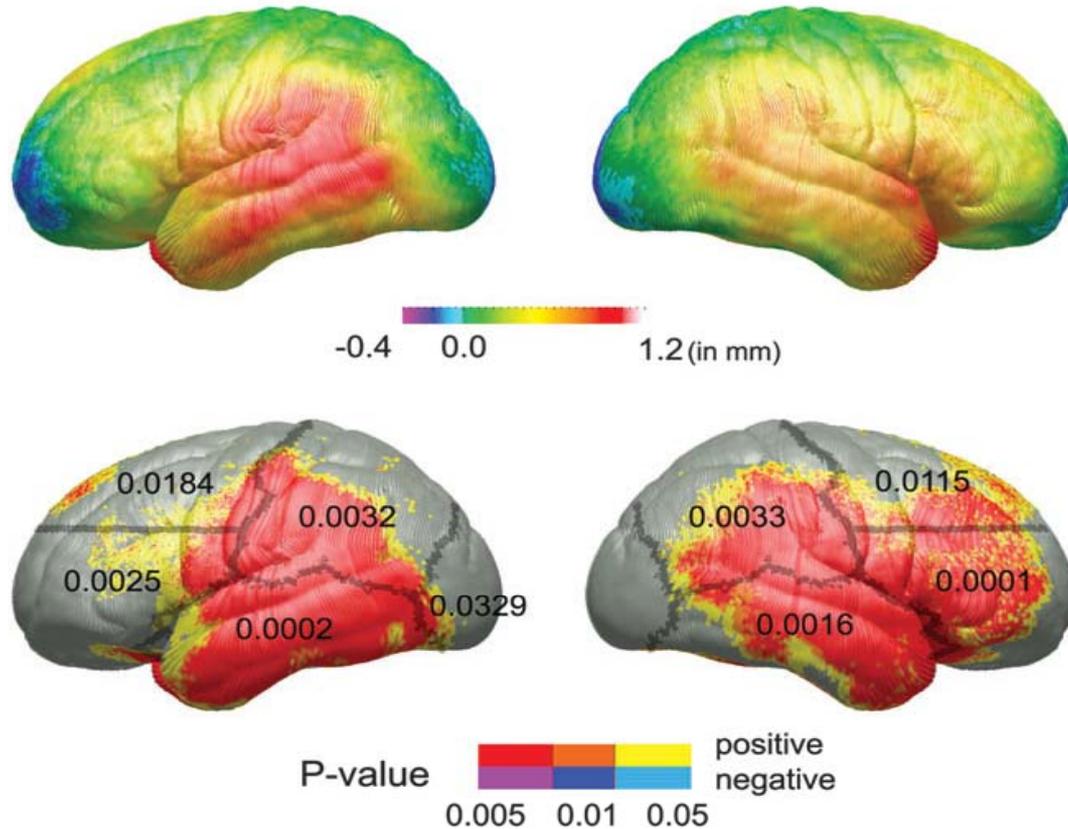
Pre-organogenic

Embryonic

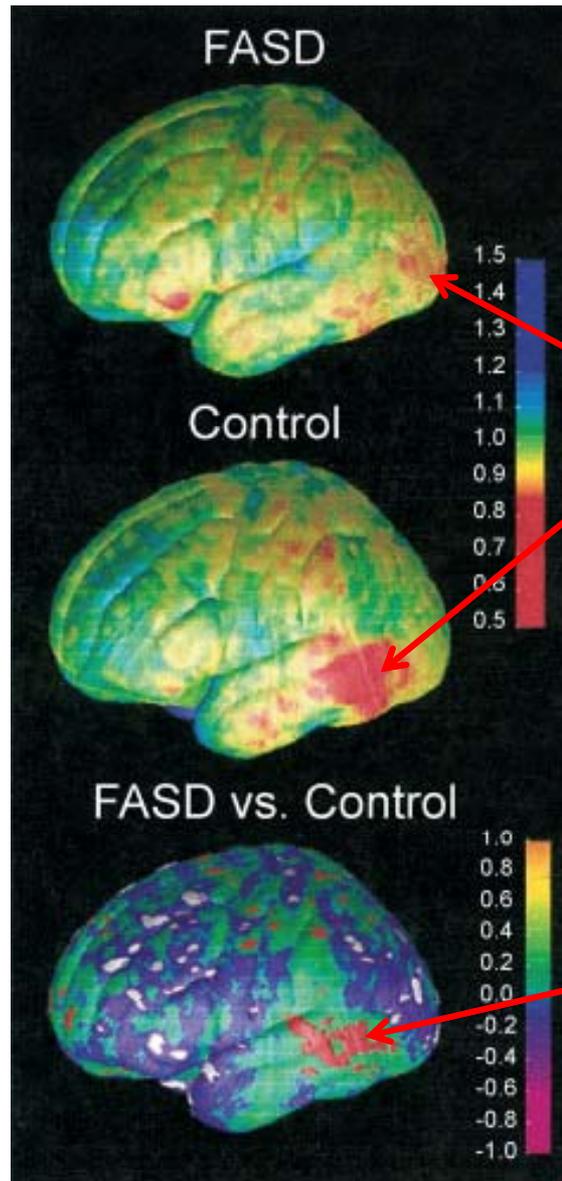
Fetal



Cortical Thickness Changes in FASD: Correlating qMRI with Cognitive Learning



Brain Gray Matter Asymmetry



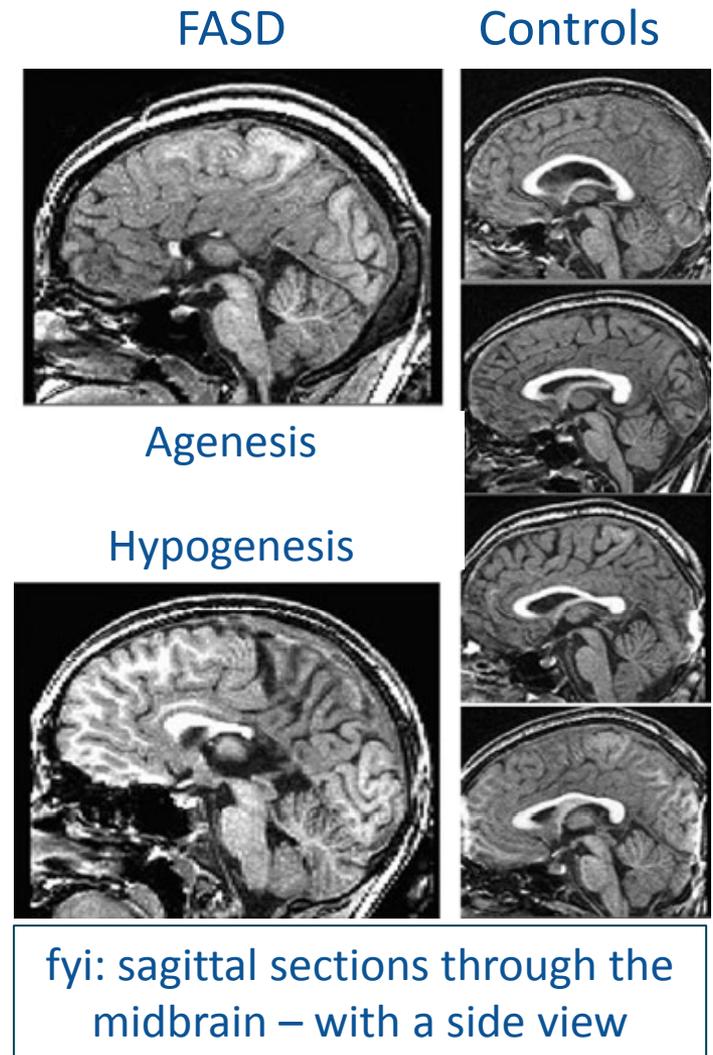
red = more gray matter in right hemisphere (side) of the brain

red = lower asymmetry in FASD compared to control

American Journal of Medical Genetics, Semin. Med. Genet. (2004) 127C:35

Magnetic Resonance Imaging (MRI)

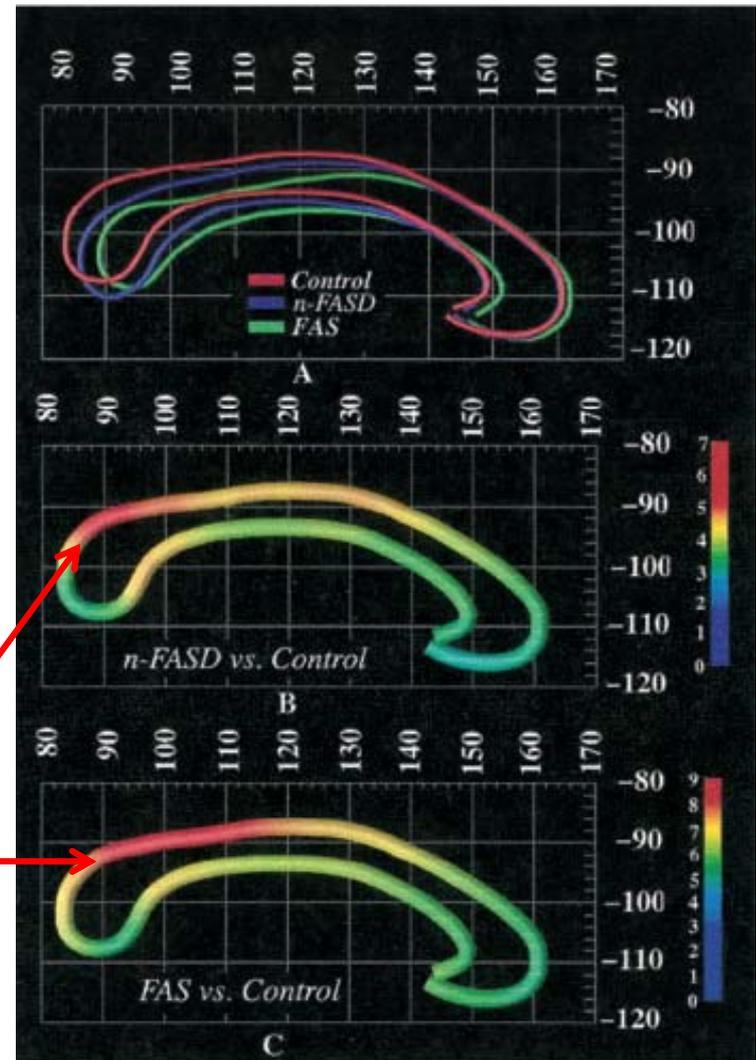
- Identification of abnormalities in the corpus callosum region
- Abnormalities in this region associated with deficits in intellectual function, learning, memory, executive function and attention
- FASD 4-digit code used to define 4 clinically-distinct subpopulations
- Severity of diagnostic coding correlated with the severity of brain damage quantified by:
 - qMRI, fMRI, and MRS imaging
 - a comprehensive battery of neuro-psychological/psychiatric tests



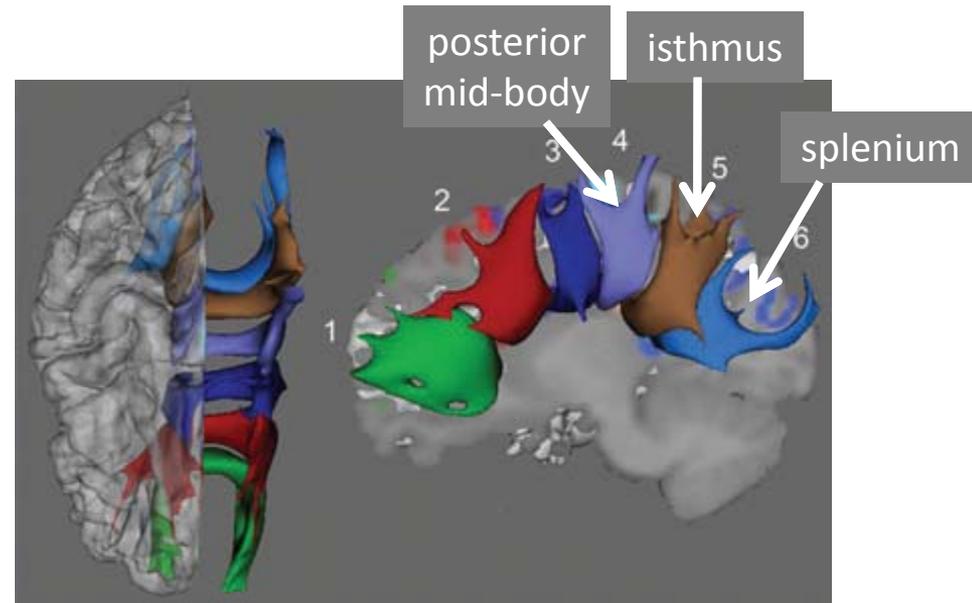
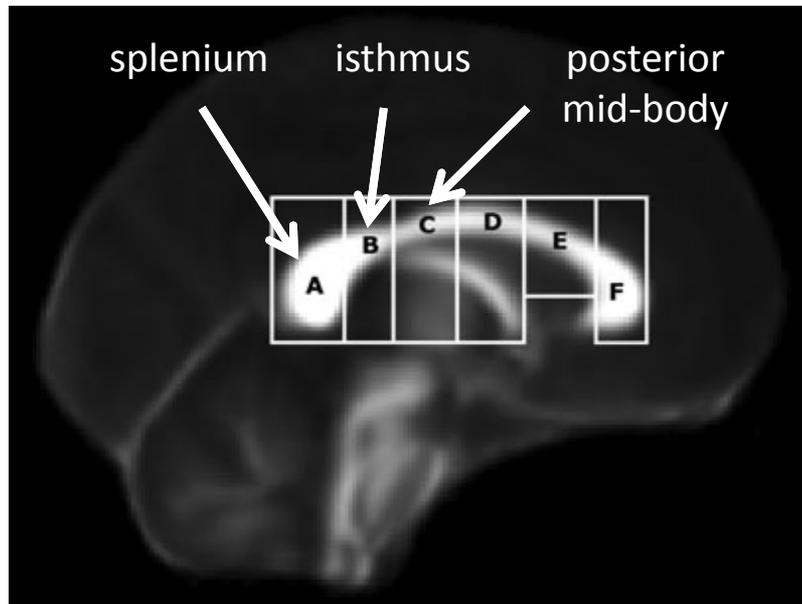
Corpus Callosum

- The abnormal shape can be measured in people w/o facial dysmorphology
- The difference in shape is greater with:
 - facial dysmorphology
 - cognitive deficits

Red = greater difference in FASD groups than in controls



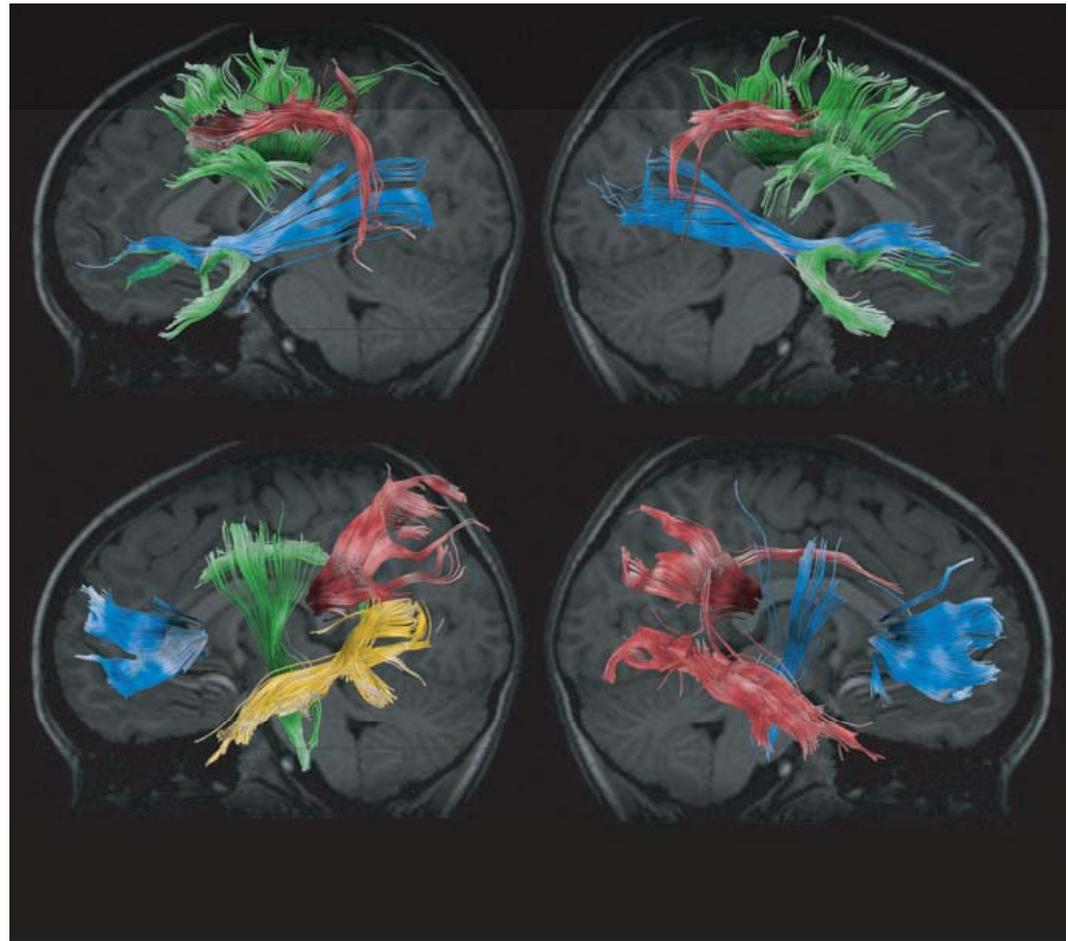
Microstructural Corpus Callosum Anomalies in Children with Prenatal Alcohol Exposure



Diffusion Tensor Imaging (DTI): FA and MD white matter tracts projecting through the CC and connecting the left and right side of the brain

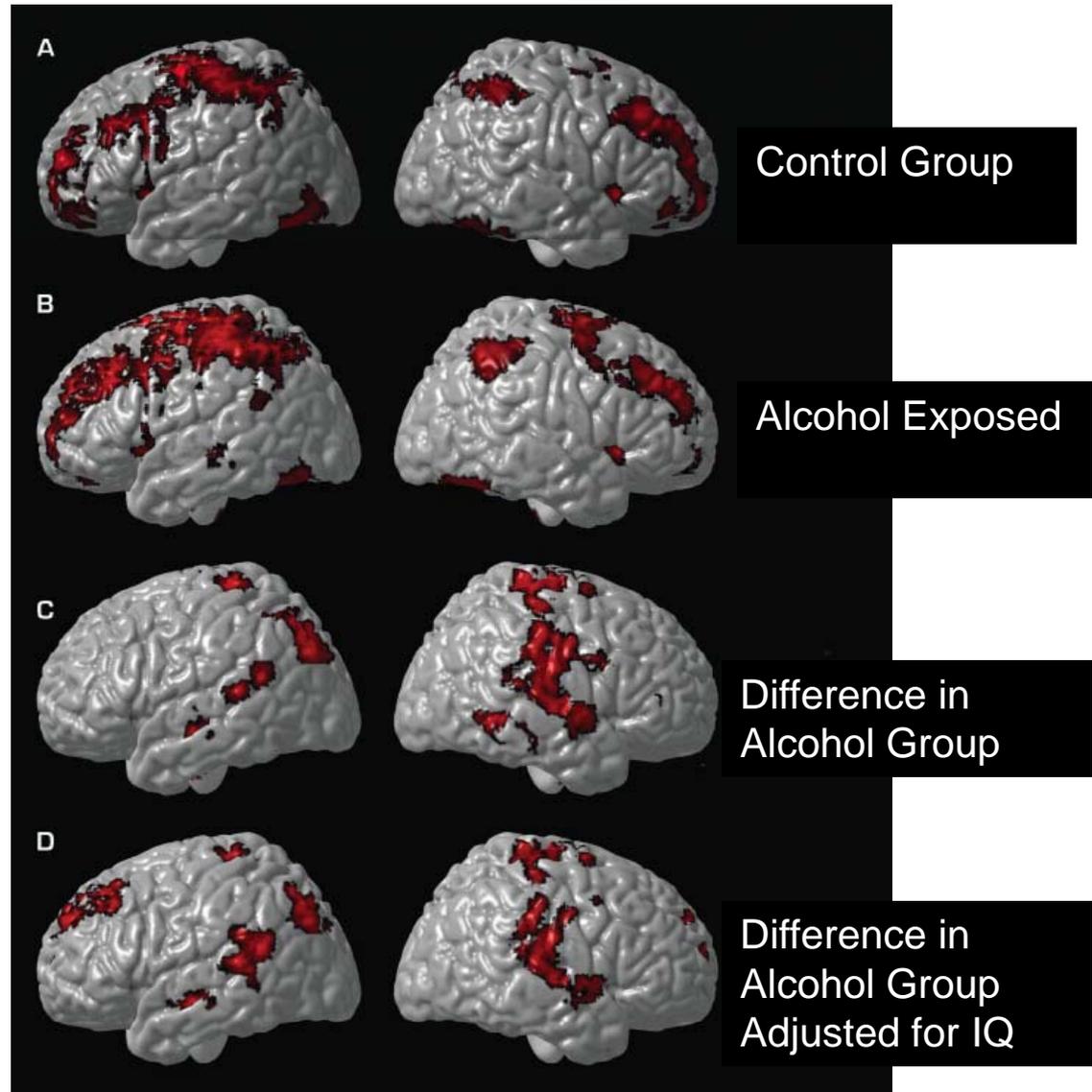
Diffusion Tensor Imaging (DTI)

Brain White Matter Tracts



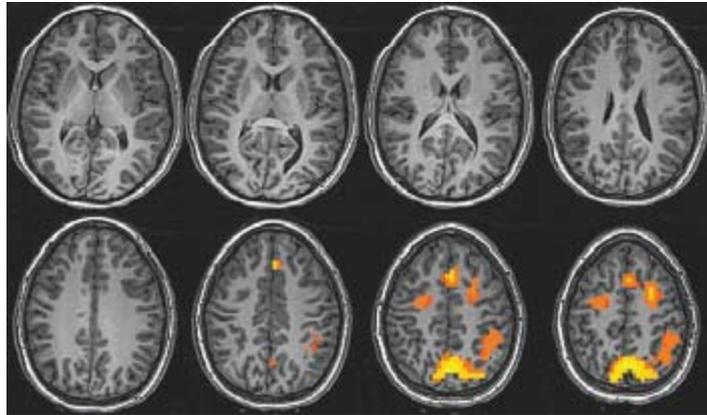
Verbal Working Memory Activation

- Greater activation in frontal and parietal regions in FASD
- Frontal-parietal processing during verbal working memory is less efficient in FASD

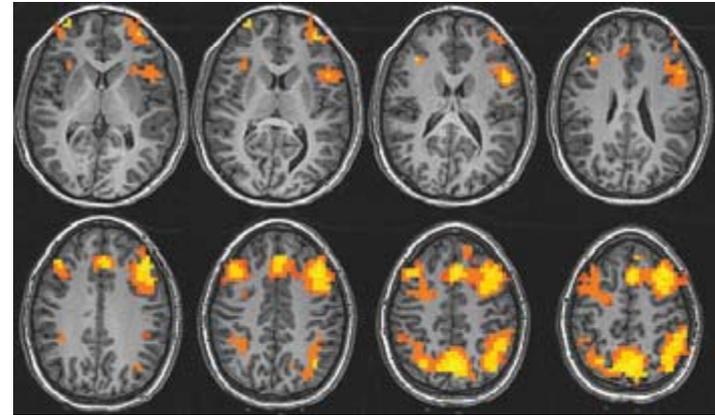


Functional MRI (fMRI) Assessment of Blood Oxygen Level Dependent (BOLD) Response in Spatial Working Memory Task

Control group



Heavy prenatal alcohol exposure



Heavy prenatal alcohol exposure

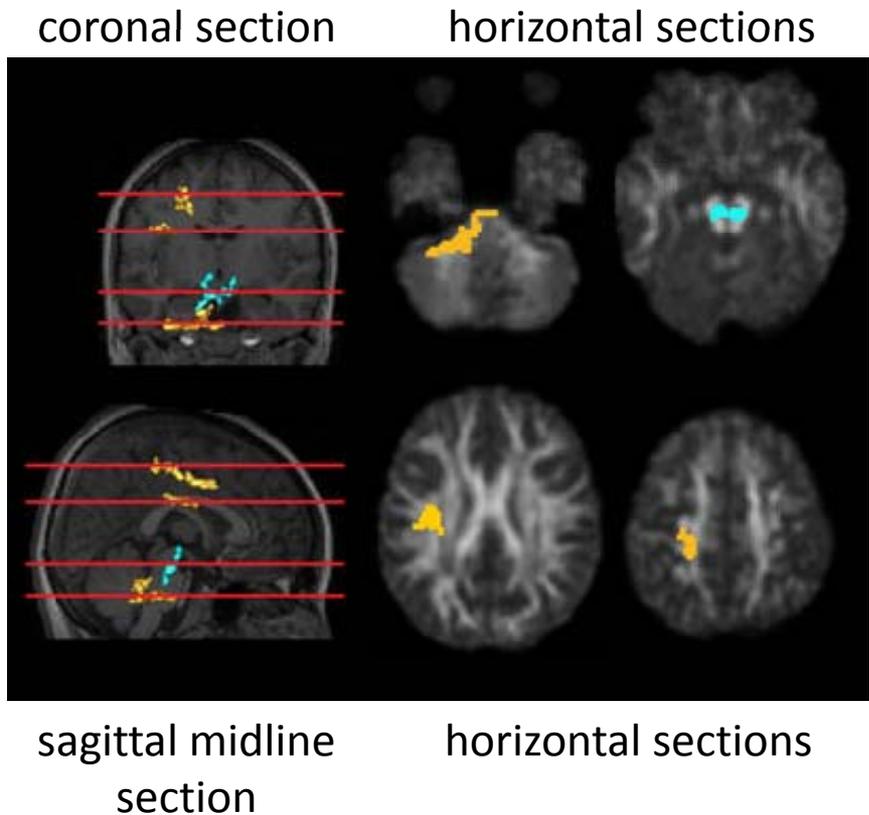


Yellow = greater activation in alcohol-exposed individual

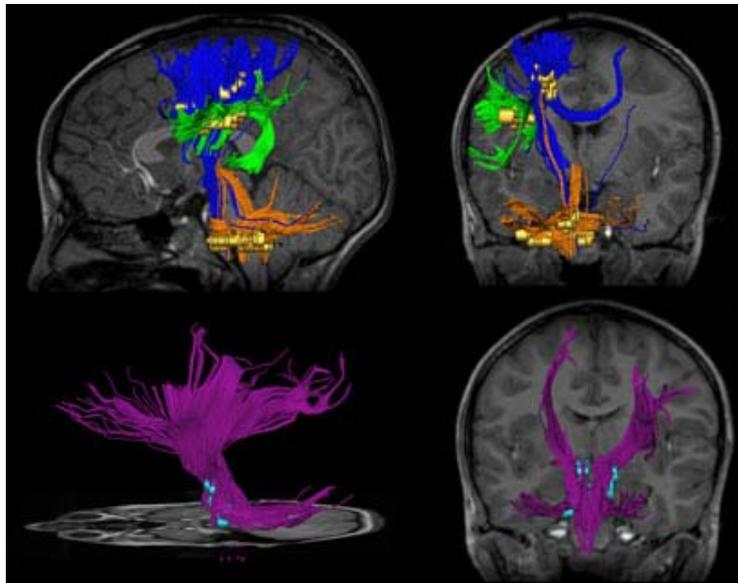
Math Ability and Brain Microstructure in FASD

Fractional Anisotropy (FA) scan of 21 FASD children

- ❖ Found relationship with brain microstructure and low math ability
 - Yellow = positive correlation in left parietal region and cerebellum
 - Blue = negative correlation in brain stem
- 4 key structural regions of brain identified
- Left parietal cortex region important

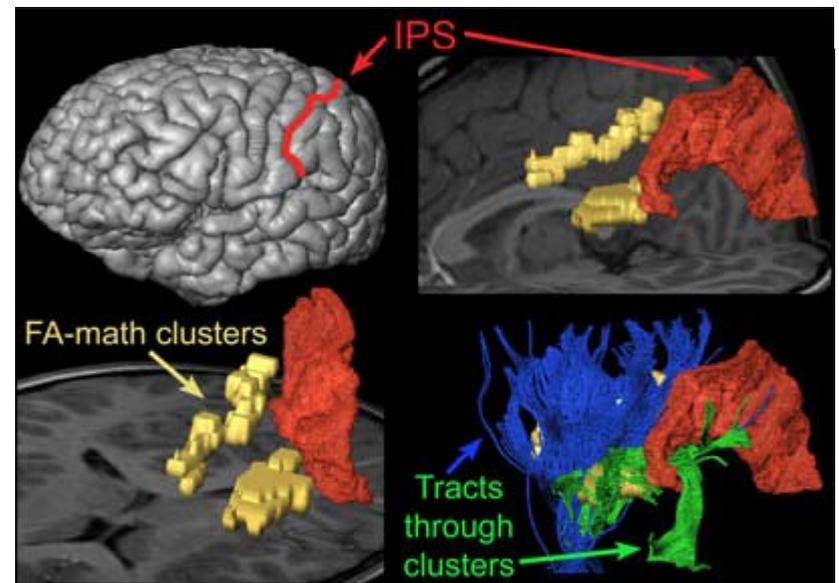


Math Ability and Brain Microstructure in FASD (DTI using FA)



White matter tracts passing through the correlated FA clusters (yellow):

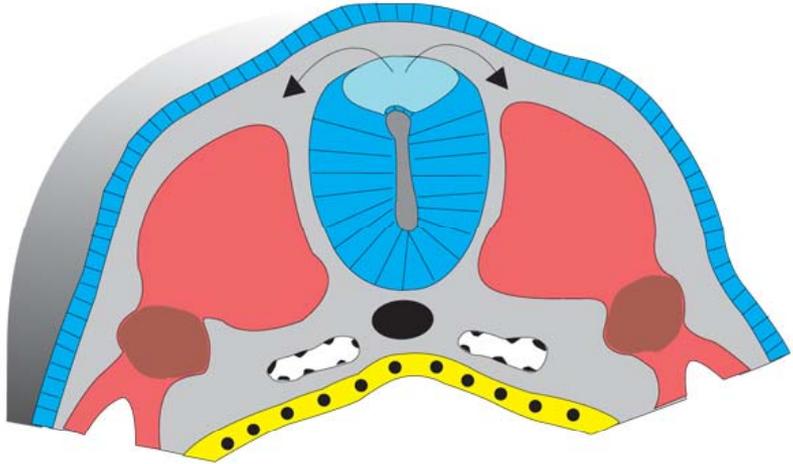
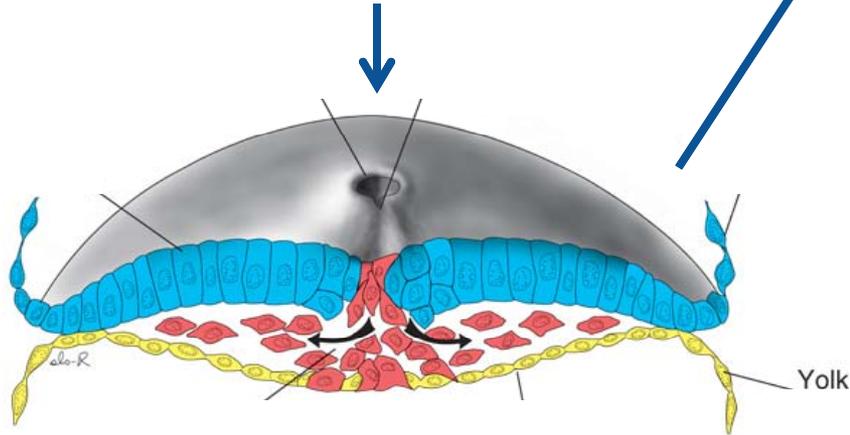
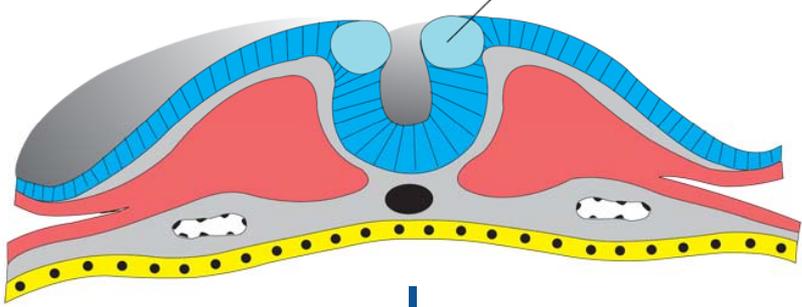
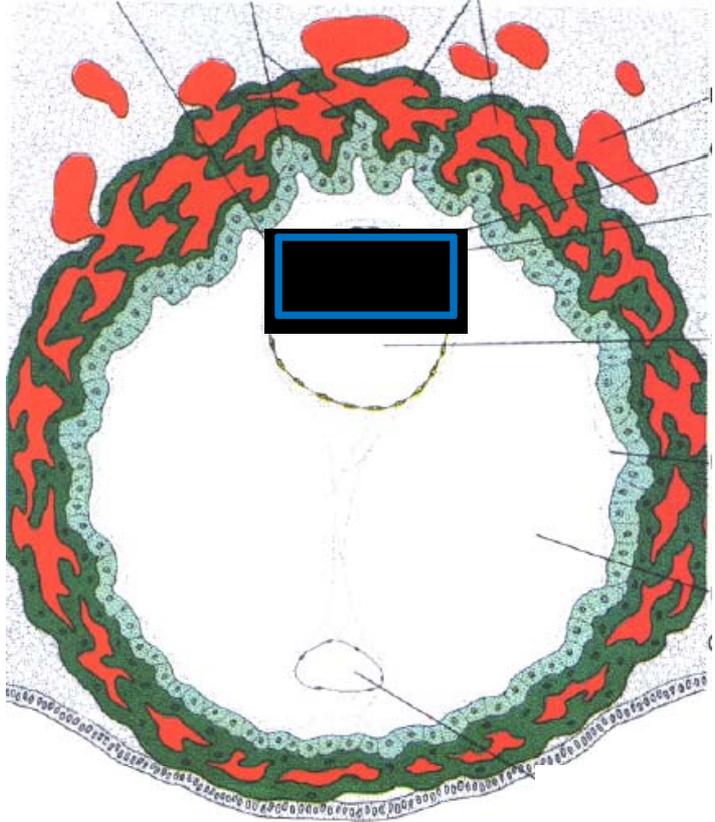
- Upper and lower parietal and corpus callosum (blue and green)
- Cerebellar (orange)



Parietal FA Clusters (yellow) in relation to:

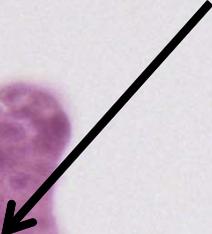
- Left intra-parietal sulcus (red)
- White matter tracts

Embryo: Week 2-3



Normal Embryo

Neural Plate Tissue



Midline Position
of the Body

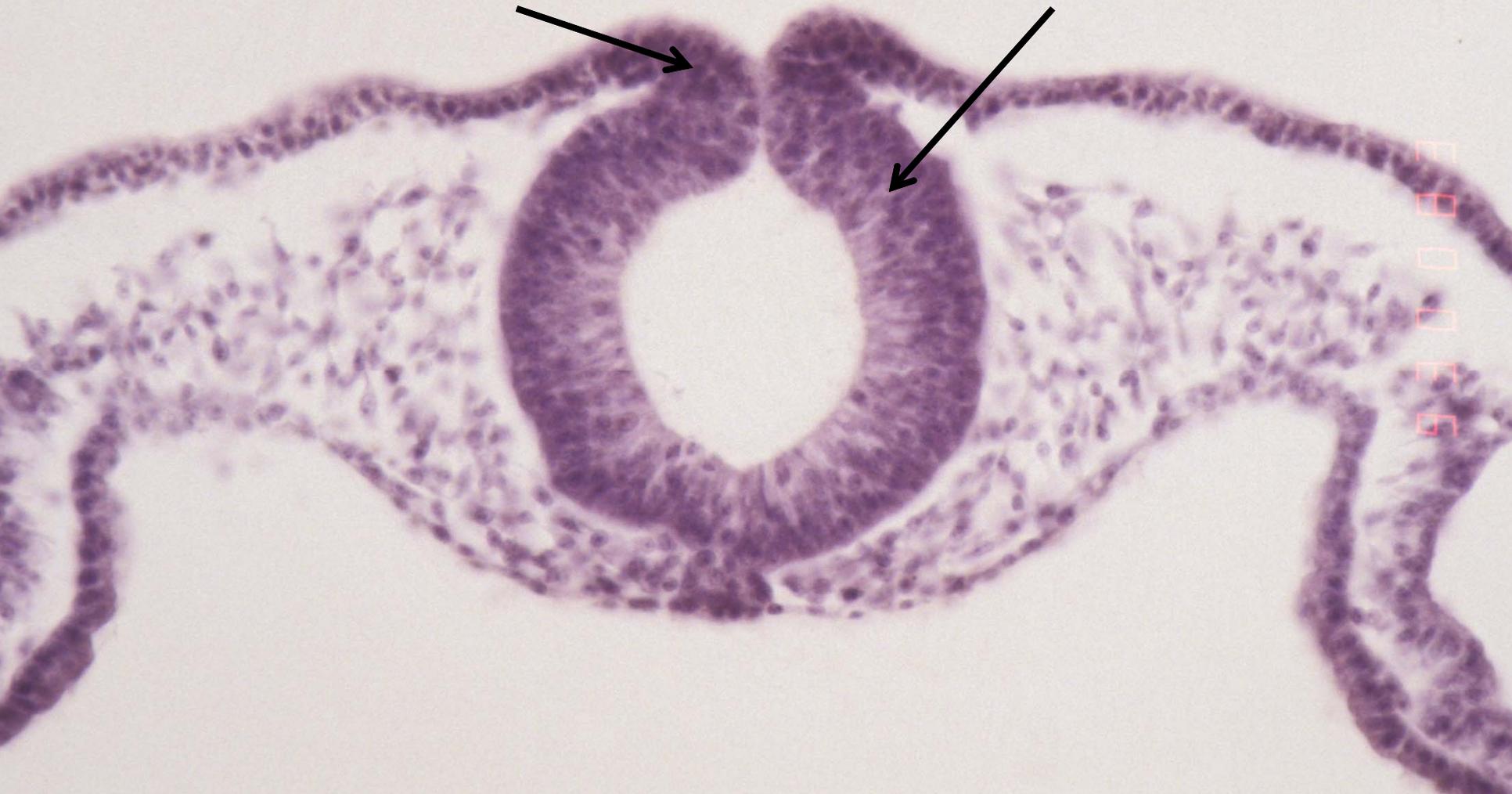


9100E6

Normal Embryo

Neural Crest Tissue

Neural Tube Tissue
(Fusing)



Normal Embryo

Neural Tube Tissue
(Fused)



990074

Normal Embryo

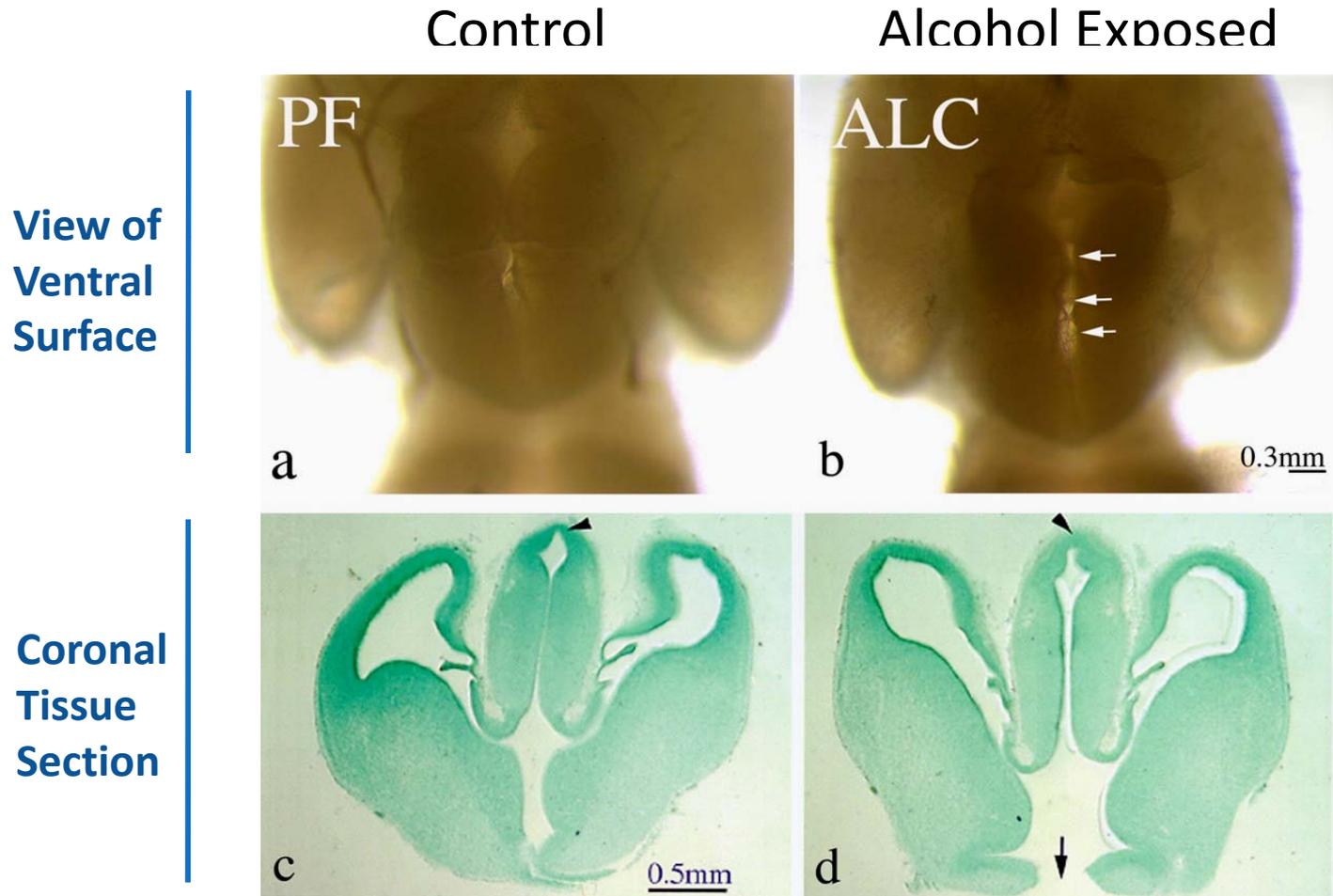
Neural Tube Tissue
(Growing and Maturing)



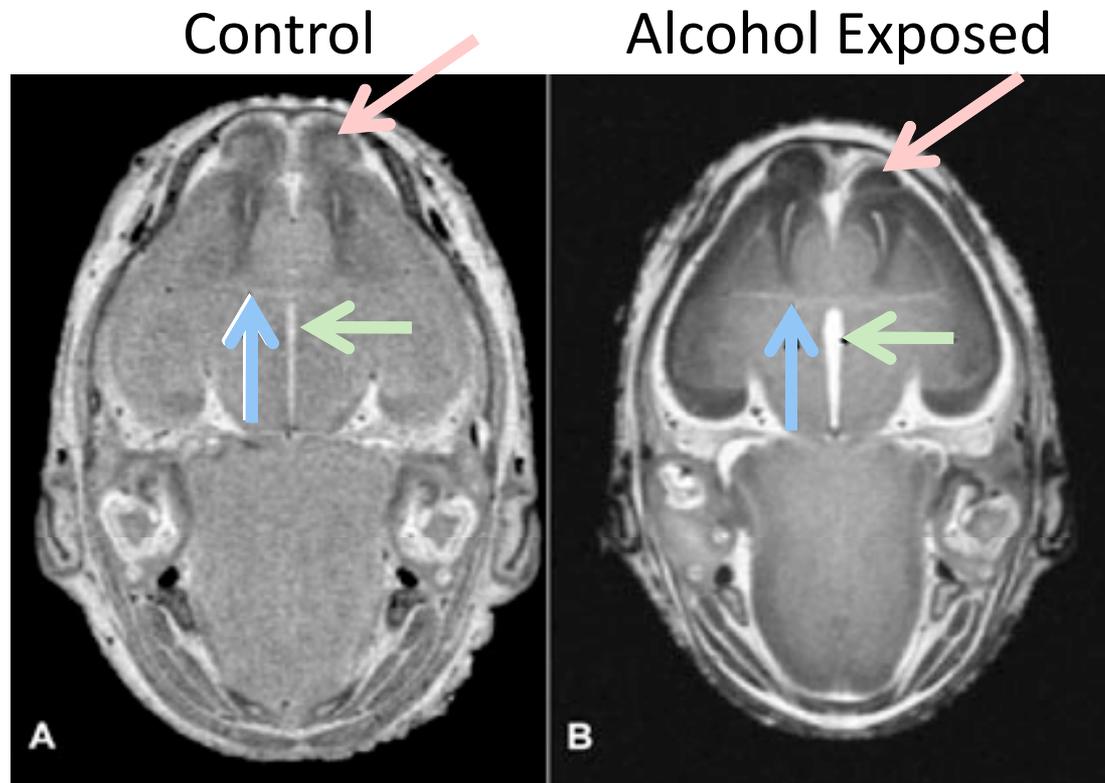
Midline Position
of the Body



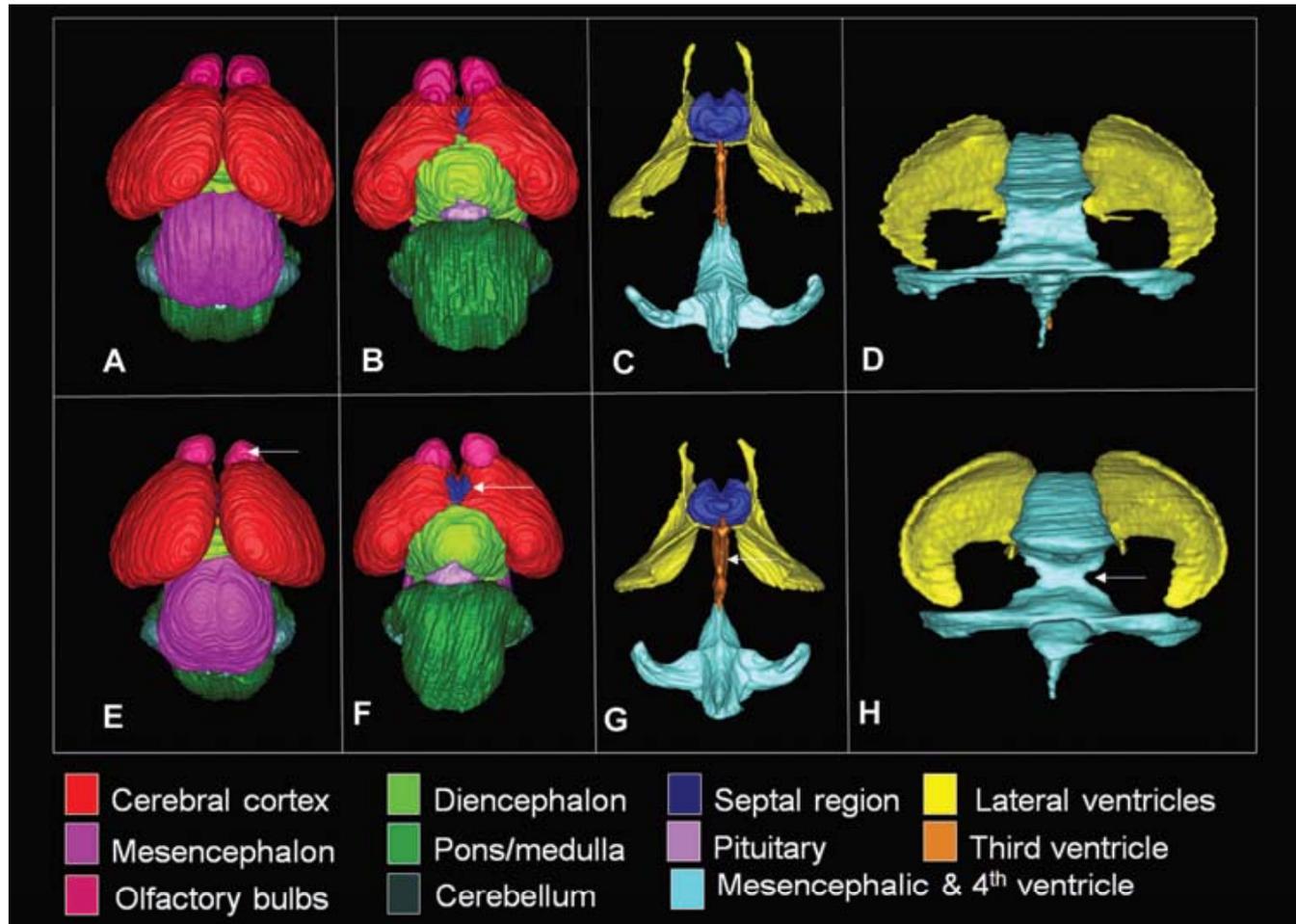
Animal Model of Incomplete Neural Tube Closure



Magnetic Resonance Microscopy (MRM) Reveals Defects in Brain Development in Mice Exposed to Alcohol during Gestation



3-Dimensional Reconstruction of MRI

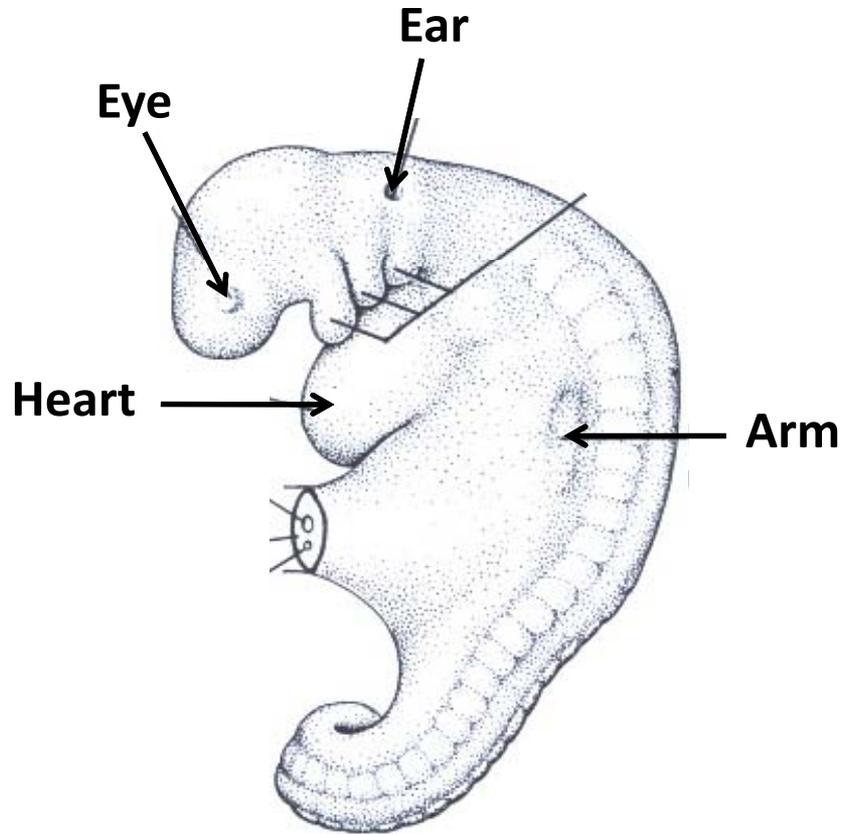


Control
Mouse

Alcohol
Exposed
Mouse

Normal Embryo

- Day 28

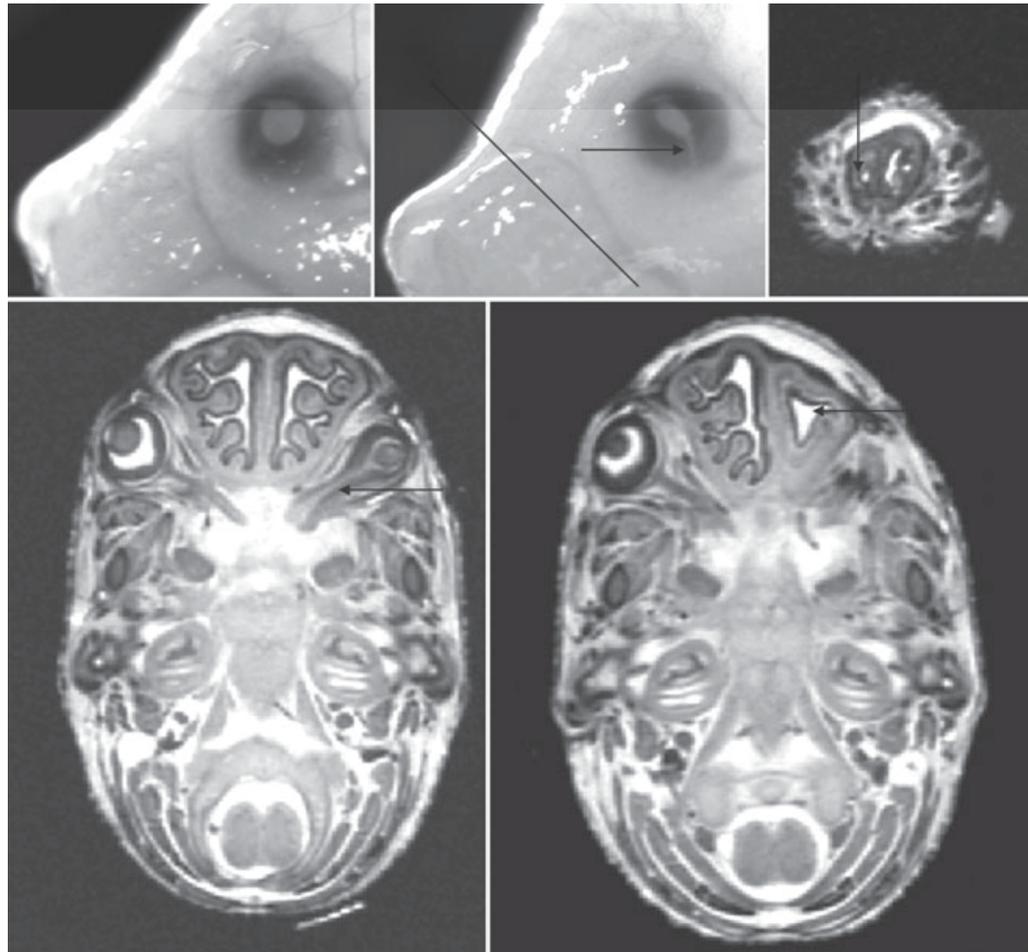


Modified from Sadler, ed.: *Langman's Medical Embryology*, Lippincott Williams & Wilkins

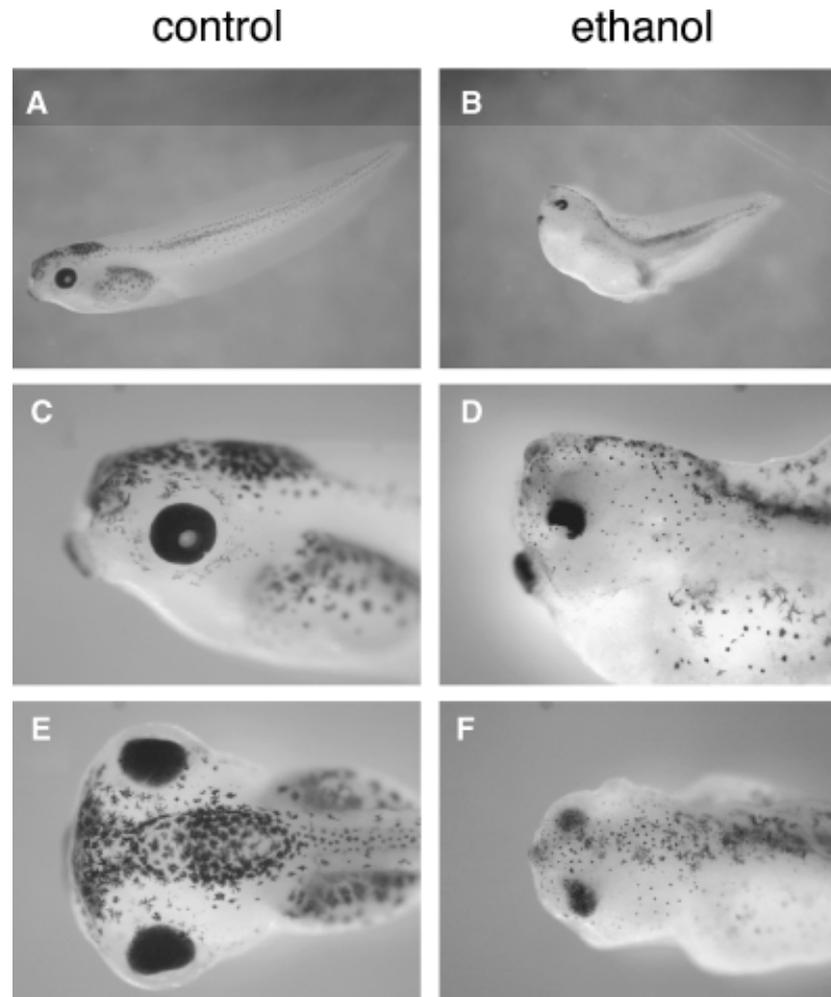
Visual Acuity Measured in FAS



Eye Defect in Animal Model: Mouse



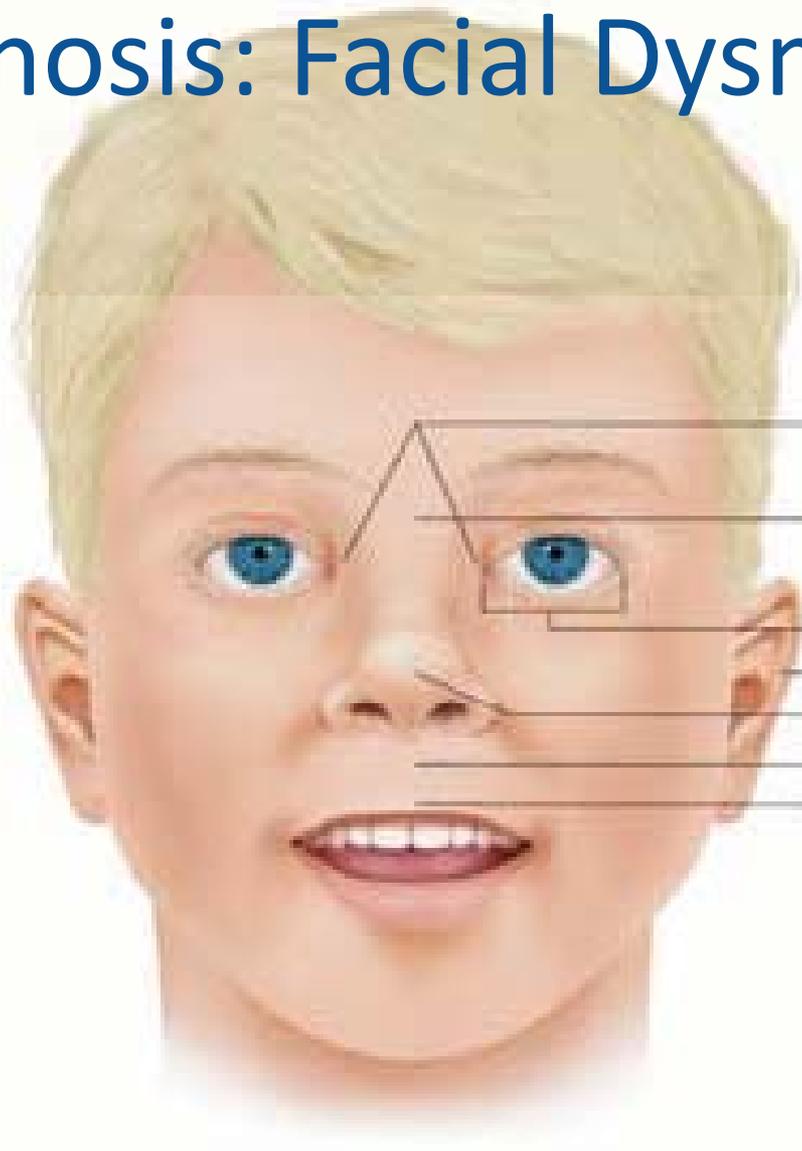
Eye Defect in Animal Model: Zebrafish



DIAGNOSIS

- Maternal: Drinking history, Biomarkers
- Prenatal: Ultrasound imaging
- Newborn: Brain imaging, Biomarkers
- Children: Facial dysmorphology, IQ, Learning disabilities, Behavior, Brain imaging

Diagnosis: Facial Dysmorphology



Epicanthal folds

Flat nasal bridge

Small palpebral fissures

"Railroad track" ears

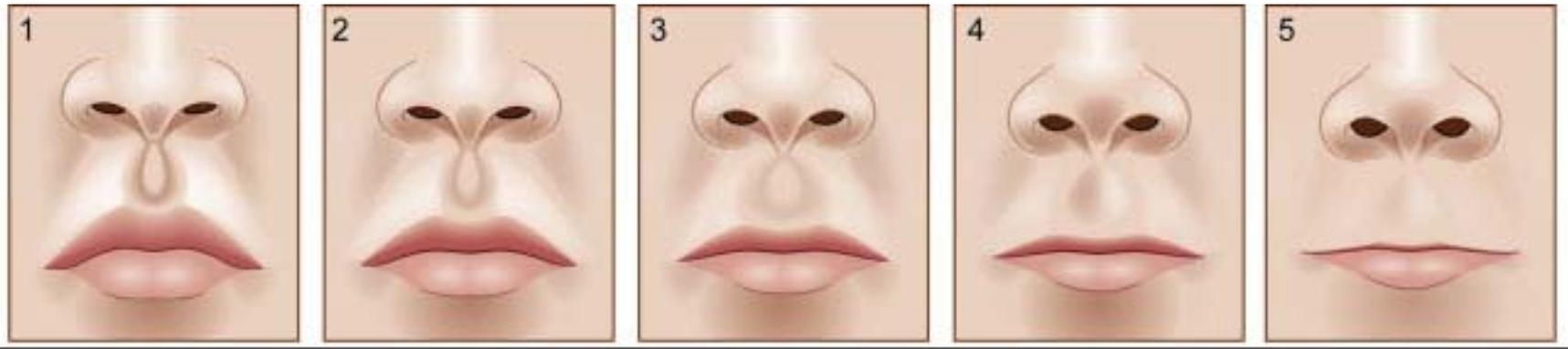
Upturned nose

Smooth philtrum

Thin upper lip

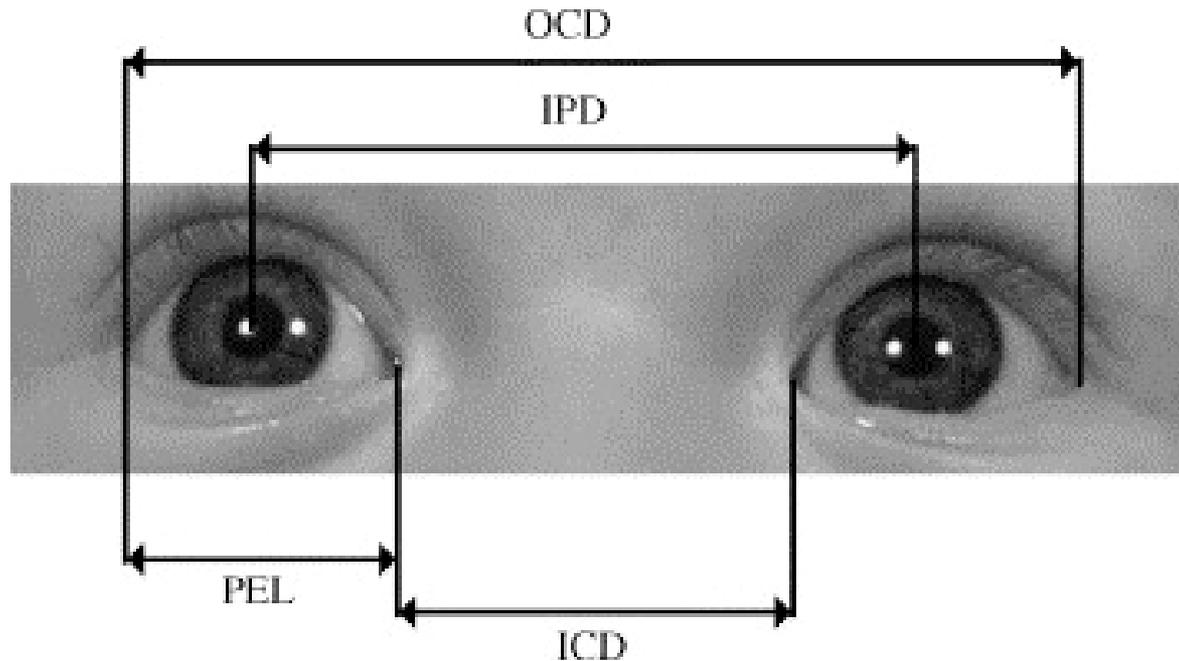
American Family Physician (2005) 72:279

The Lip/Philtrum Guide



American Journal of Medical Genetics 140A:137–143 (2006)

Diagnostic Facial Measurement

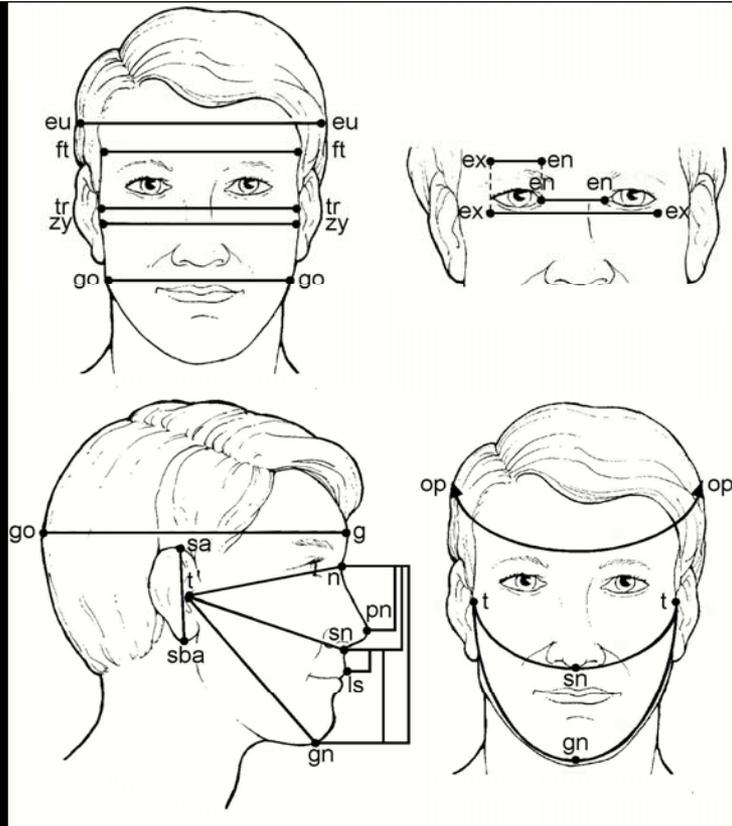


Characteristic Facial Features in Children of Different Ethnicities



American Family Physician (2005) 72:279

Anthropometric Analysis



Measurements	NAC	AA	FC	CC
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Widths



Depths



Lengths



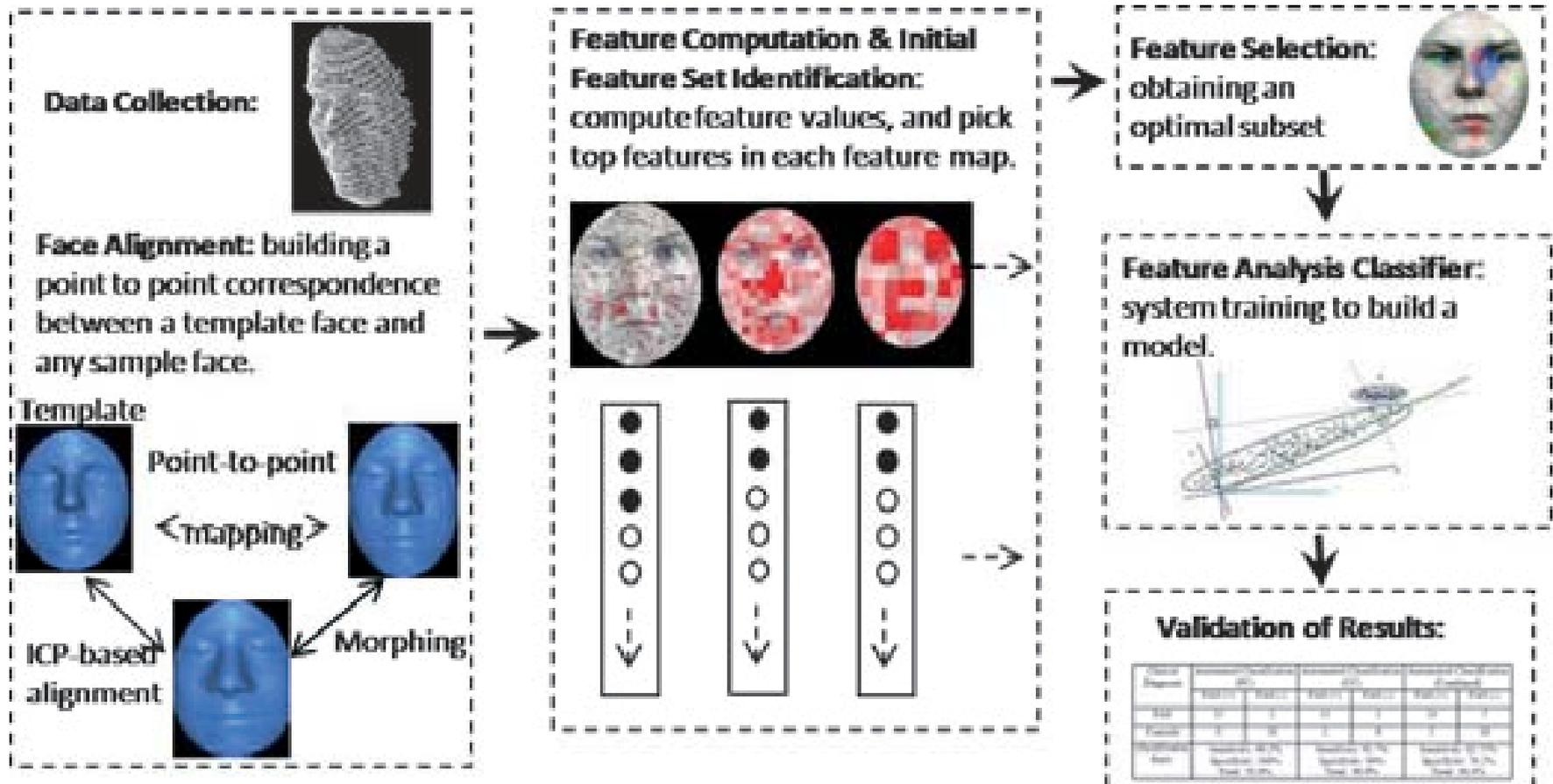
Sensitivity

% FAS correct	74	73	96	94
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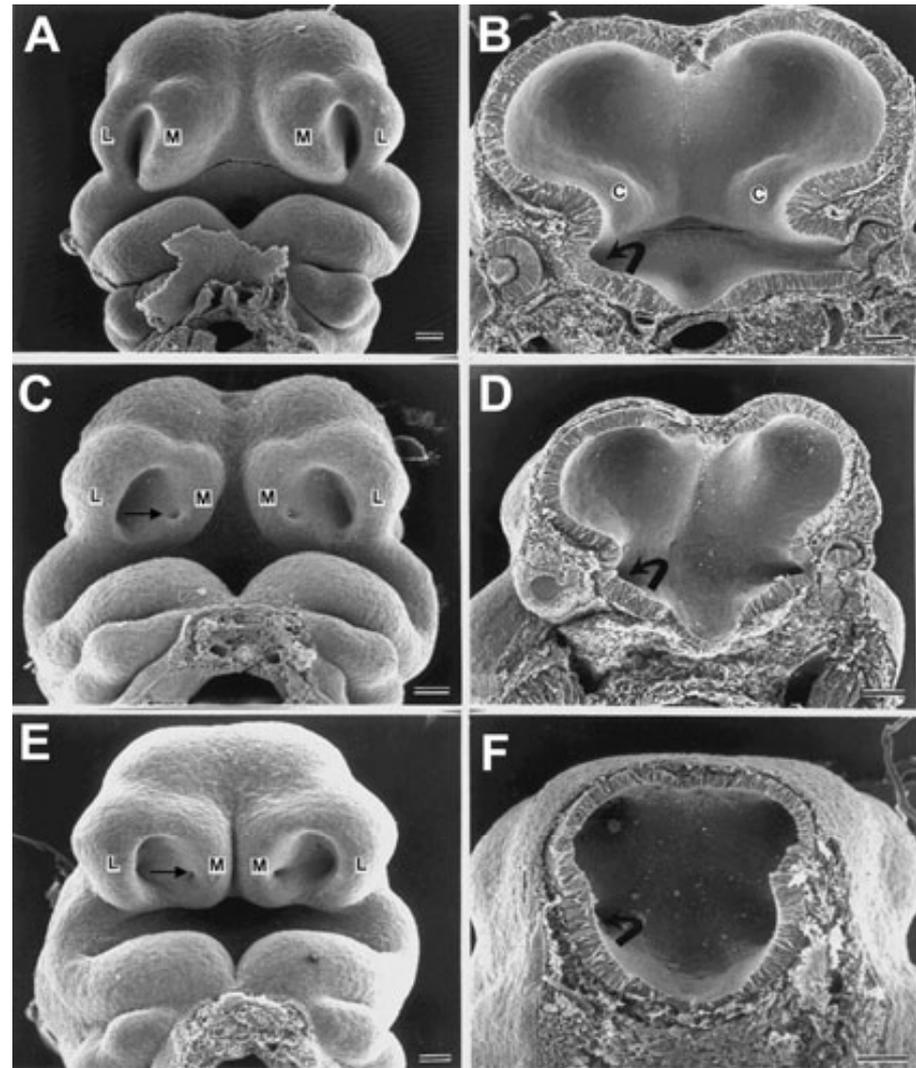
Specificity

% Control correct	81	85	91	91
% Overall correct	77	79	93	92

Computational Facial Diagnosis: Telemedicine



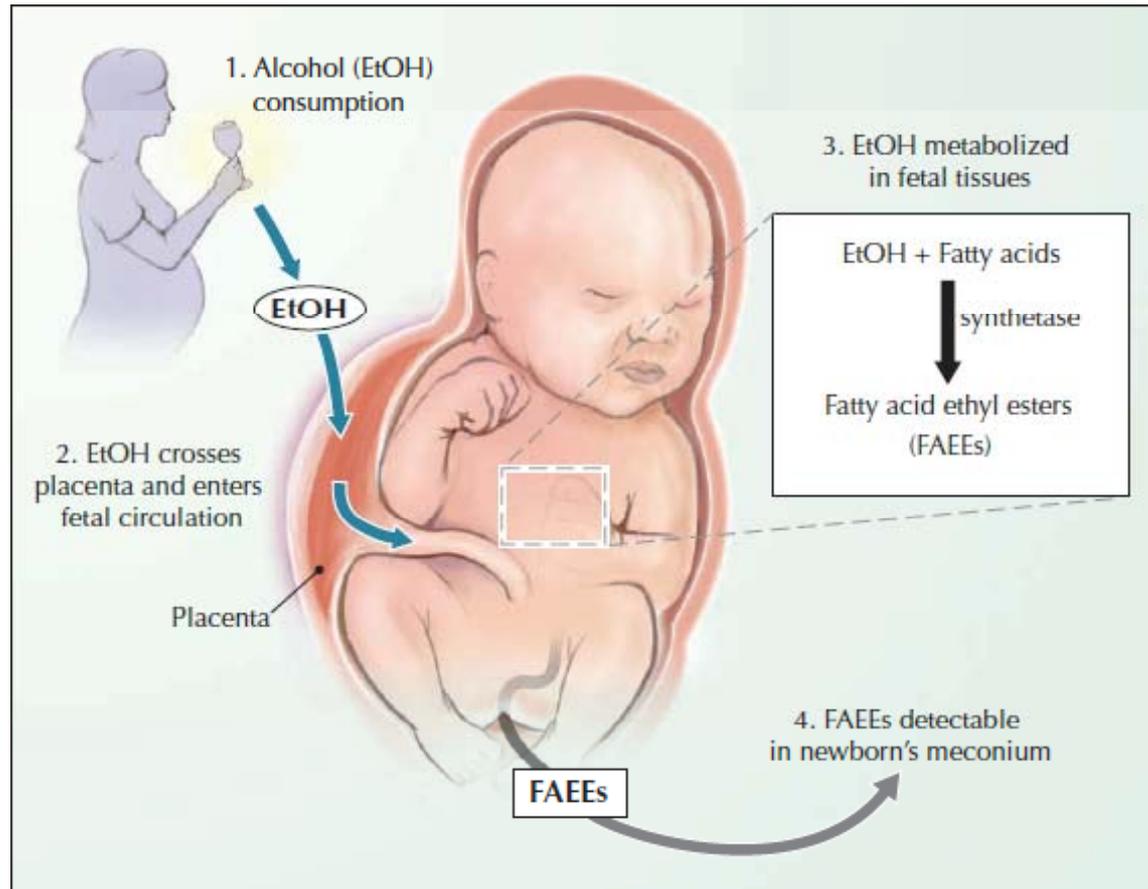
Facial Dysmorphology In Mouse



FAEE as a Biomarker for In Utero Alcohol Exposure

- **Fatty acid ethyl esters (FAEE)** are a product of non-oxidative metabolism of ethanol, persisting in blood for >24 hours after significant alcohol consumption.
- Any FAEE detected in neonatal biological matrices are likely produced by the fetus from ethanol transferred to and metabolized by the fetus.

Formation of FAEE



Meconium Screening

- **Meconium** is a matrix unique to the developing fetus that is commonly used in neonatal drug screening.
- FAEEs have the ability to accumulate in meconium.
- Substances begin to accumulate in meconium from the 13th week of human gestation, allowing for analysis in the 2nd and 3rd trimesters.
- FAEEs can be found independent of maternal alcohol consumption because ethanol is a common byproduct of metabolism.
- A total FAEE accumulation of 2 nmol/g of meconium is the cutoff for distinguishing neonates of non-drinking mothers from offspring of heavy drinkers

FAEE Hair Testing

- Neonatal hair collection can occur up to 3 months after birth; meconium is only available the first 2-3 days of life.
- Neonatal hair begins its growth at approximately the third or fourth week of fetal life. Consequently, any exposures within the last trimester of pregnancy may be theoretically found in neonatal hair after birth.
- FAEE have prolonged stability in the hair matrix.

High Throughput Screening of Biomarkers – the Future

- Gene array: Gene profile
- Proteomics: Protein profile
- Metabolomics: Metabolite profile
- Epigenomics: Epigenetic profile

Identify Alcohol-Induced Gene Alterations: Gene Chip and Informatics

From the global gene expression patterns to:

- Identify genes altered by alcohol exposure
- Identify transcription-factor binding sites (TFBS)
- Evaluate the functions of predicted TFBS in response to certain biological treatment

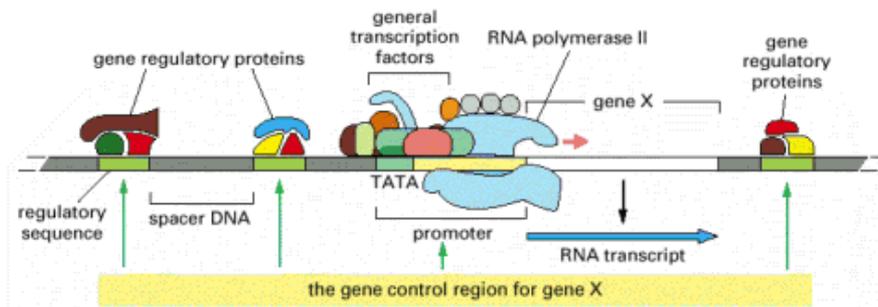
Global gene expression patterns



Gene chip
&
Informatics



Understand how transcription factors
work cooperatively



Transcriptional machinery

Search for Diagnostic Biomarkers by High-Throughput Analysis

- ❖ The effects of moderate drinking on gene expression in the rat placenta
- ❖ Daily voluntary drinking, 4 hr, 84 mg/dl BEC (chronic, binge drinking model)

Cytoplasm

Hemoglobin, epsilon 1
Hydroxysteroid (11b) dehydrogenase 2
Hemoglobin, gamma A
Deiodinase, iodothyronine type II
S100 calcium binding protein A4
S100 calcium binding protein G
S100 calcium binding protein B
Small muscle protein, X-linked
Heat shock 70 kDa protein 1B
Flavin-containing monooxygenase 3
Cytochrome P450 1A1
Cytochrome P450 2E1

Nucleus

Heart and neural crest derivatives expressed 2
Progesterone receptor

Extracellular space

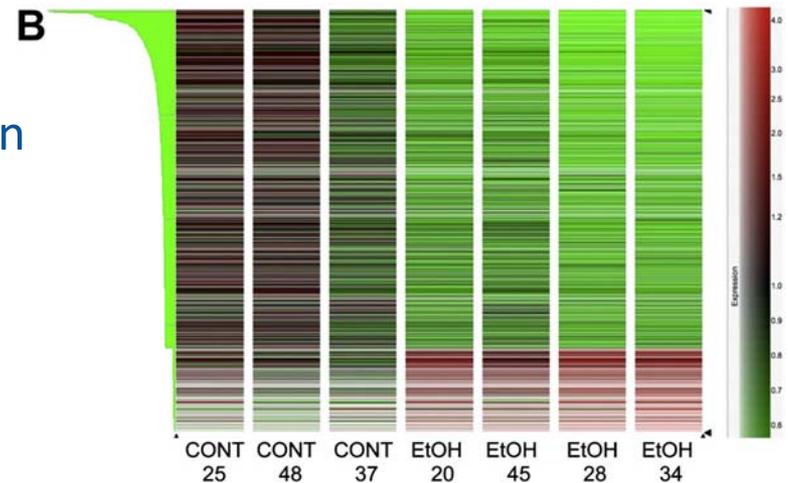
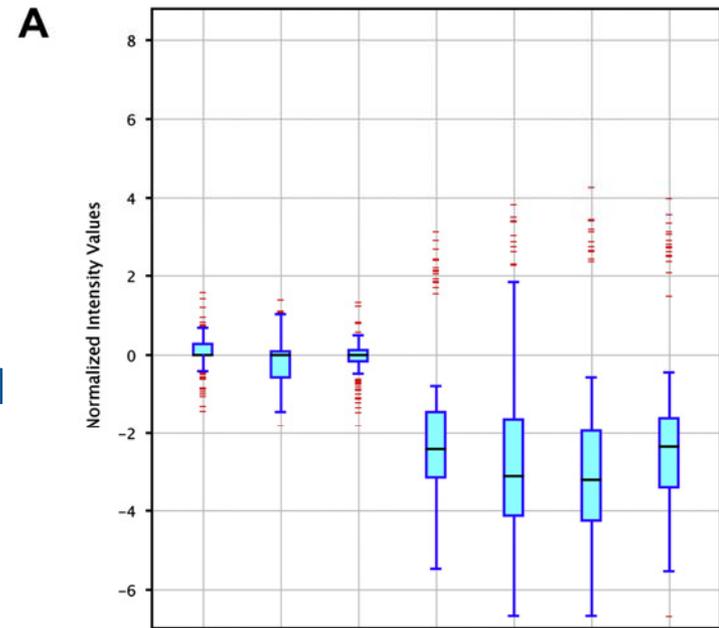
Gremlin 1
Matrix metalloproteinase
EGF-containing fibulin-like extracellular matrix protein 1
Galanin
Transforming growth factor alpha
Serine peptidase inhibitor
Placental growth factor
Thyrotropin-releasing hormone
Orosomucoid
Insulin-like growth factor binding protein 6
Matrix metalloproteinase
Matrix metalloproteinase 10
Insulin
Alpha-fetoprotein

Plasma membrane

Cannabinoid receptor
Galanin receptor
Toll-like receptor
Integrin a7
Secreted frizzled-related protein
Lipopolysaccharide binding protein
Transient receptor potential cation channel
Secreted frizzled-related protein
Nicotinic cholinergic receptor, a2 subunit 1
Nicotinic cholinergic receptor, a7 subunit

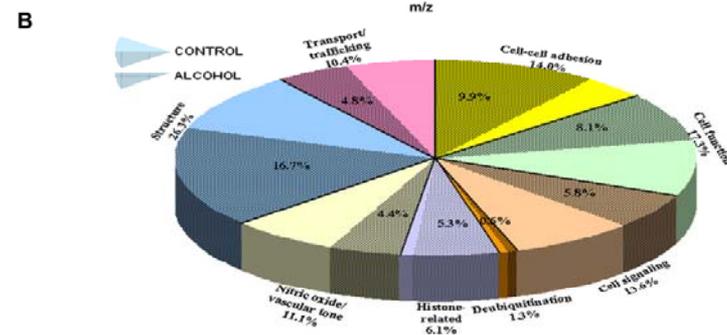
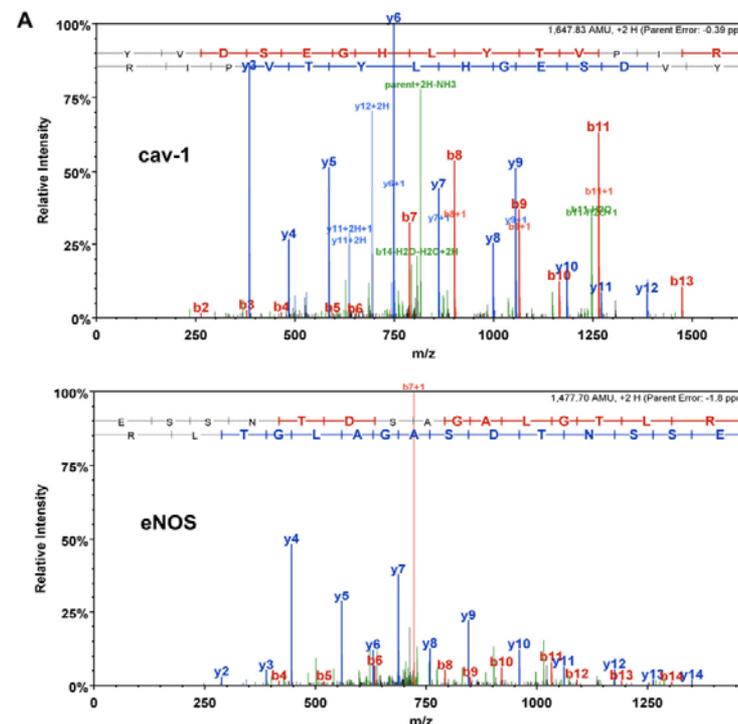
Search for Diagnostic Biomarkers by High-Throughput Analysis

- ❖ The effects of moderate drinking on gene expression in the rat placenta
- ❖ Daily voluntary drinking, 4 hr, 84 mg/dl BEC (chronic binge drinking model)
- ❖ 28,000 genes analyzed
- ❖ 304 had altered expression, 76% of those were repressed
- ❖ Type of genes: CNS development; organ morphogenesis; skeletal, cartilage, cardiovascular development; immunological responses; endocrine function; regulation of ions



Proteomic Profiles of Moderate Maternal Binge Drinking

- Uterine endothelial cells from placenta of pregnant sheep given alcohol in chronic binge drinking model
- Protein analysis by high-throughput techniques





- P REVENTION

- I NTERVENTION

- T REATMENT

Prevention of FASD

- **Best policy:**

- The only guaranteed prevention of FASD is abstinence from alcohol during pregnancy.

- **Education and Awareness:**

- Interventions aimed at ensuring that all community members understand the adverse consequences of drinking alcohol during pregnancy.

- Use of a brief intervention technique about alcohol use (10- to 15-minute sessions of counseling) among pregnant women or women of childbearing age has been shown to be effective.

- In nonpregnant women, a brief motivational intervention was shown to decrease the risk of exposure to alcohol during pregnancy through decreasing risky drinking



- **Targeting High Risk Individuals:**

- Selective prevention interventions targeting women at higher risk of having an infant with a FASD because they drink alcohol and are in the reproductive age range.

- Prevention interventions targeting women who drink heavily while pregnant or at risk for pregnancy, or women who have given birth to a child with FAS.

Early Intervention

- ❖ The optimizing impact of early intervention programs on children's development and behavior has been well documented in the literature.
- ❖ Compelling evidence for the promotion of early intervention services for infants and toddlers, particularly those at-risk for poor developmental outcomes.
- ❖ The basis for this advocacy includes:
 - Early experience, coupled with the influence of genes, literally shape the architecture of the brain
 - Early experiences take place in relationships
 - All domains of development are interdependent
 - Development is cumulative, so early experiences lay the foundation for all that follows
 - Because early experiences matter, we must intervene with young children who are at risk
 - Early experiences are a proven investment in our future



- P REVENTION

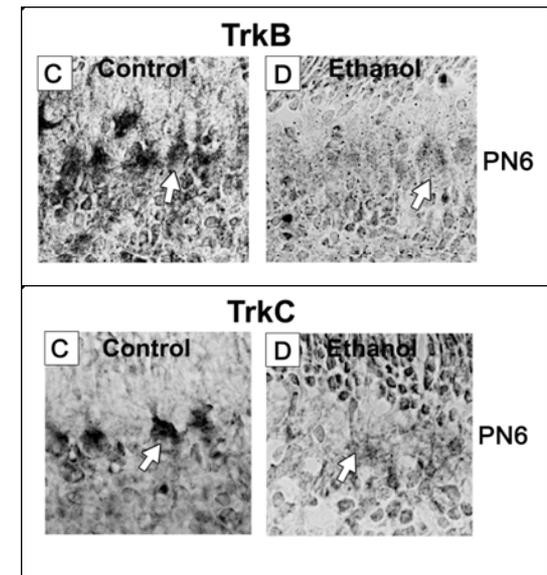
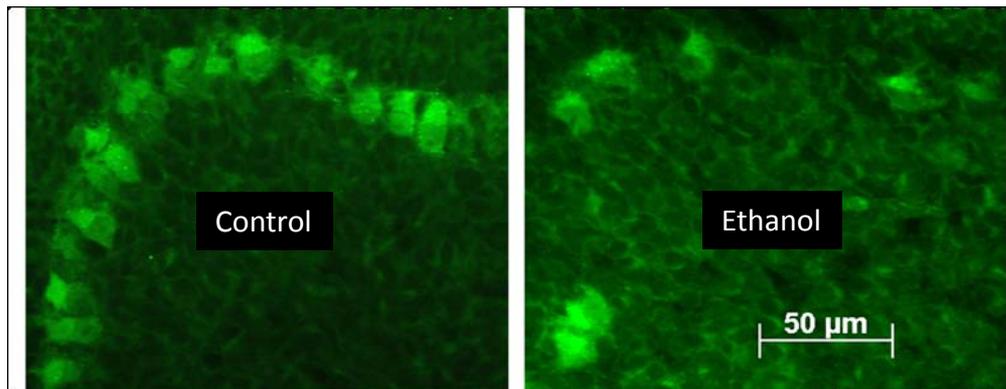
- I NTERVENTION

- T REATMENT

Mechanisms of Neuropathology

- Known from Animal Studies

- Apoptosis and neurodegeneration
- Restricted neurogenesis
- Decreased arborization and synaptogenesis
- Restriction of neurotrophic support
- Oxidative stress
- Imbalance in neurotransmitter synthesis
- Defective neuronal migration





Brain Chemistry

- Glutamate and its binding receptor NMDA
- GABA
- Serotonin (5-HT)

Neuronal Circuitry & Function

- Fronto-parietal circuitry: executive function, sensory motor function, IQ
- Hippocampal circuitry: learning and memory
- Cerebellum circuitry: reflex movement and motor coordination
- Limbic circuitry: emotion and EQ

Serotonergic (5-HT) Neurons in Neural Tube Rodent Embryonic Day 11

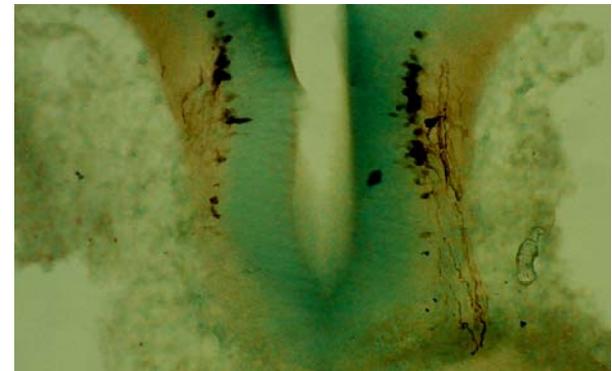
Neural Tube



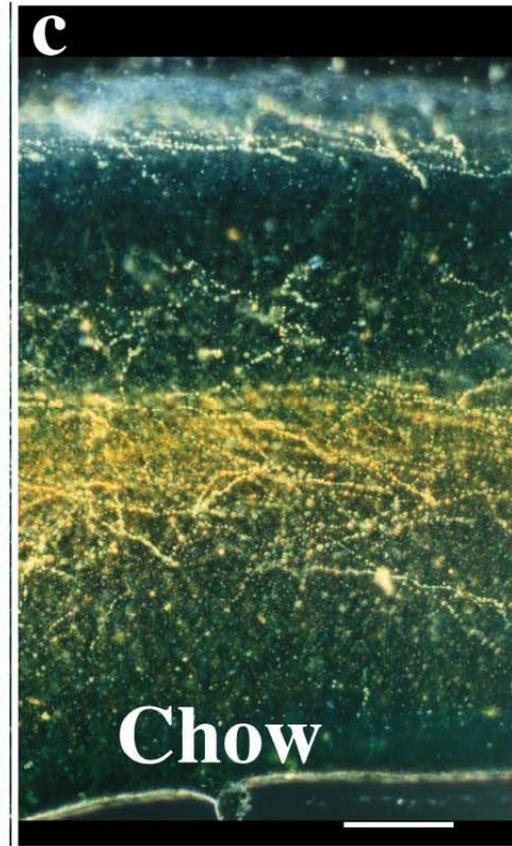
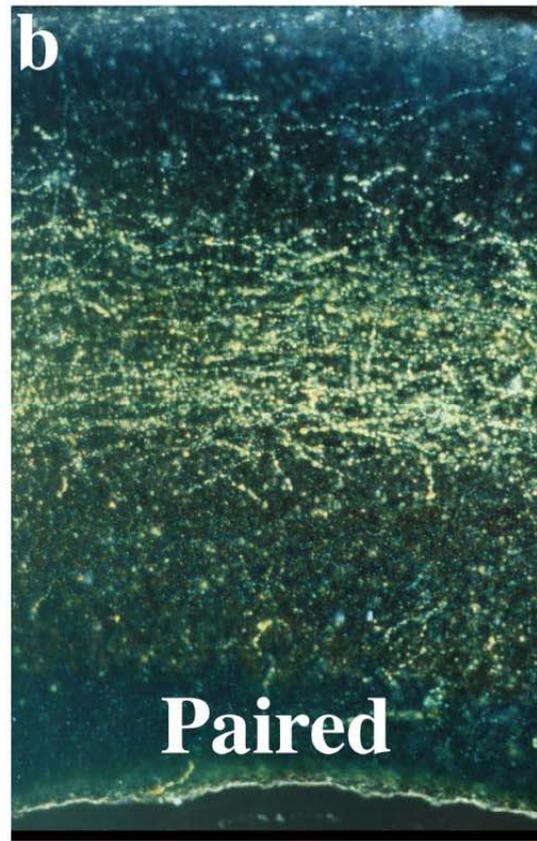
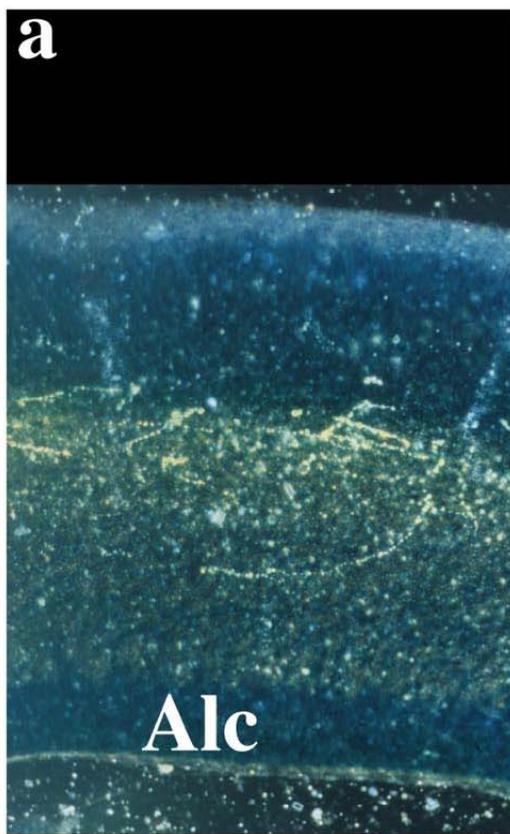
Control



Alcohol Exposed

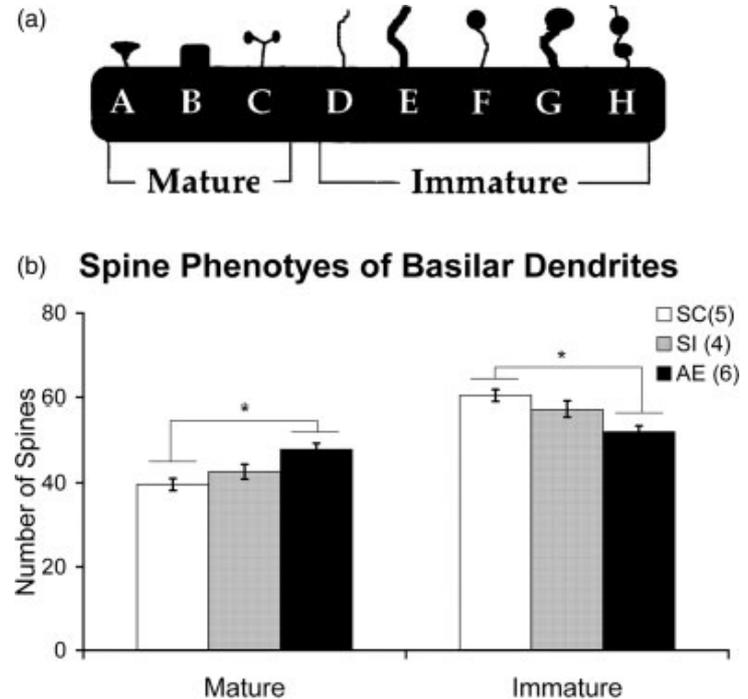
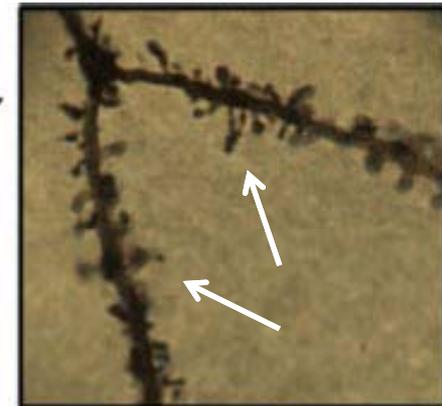
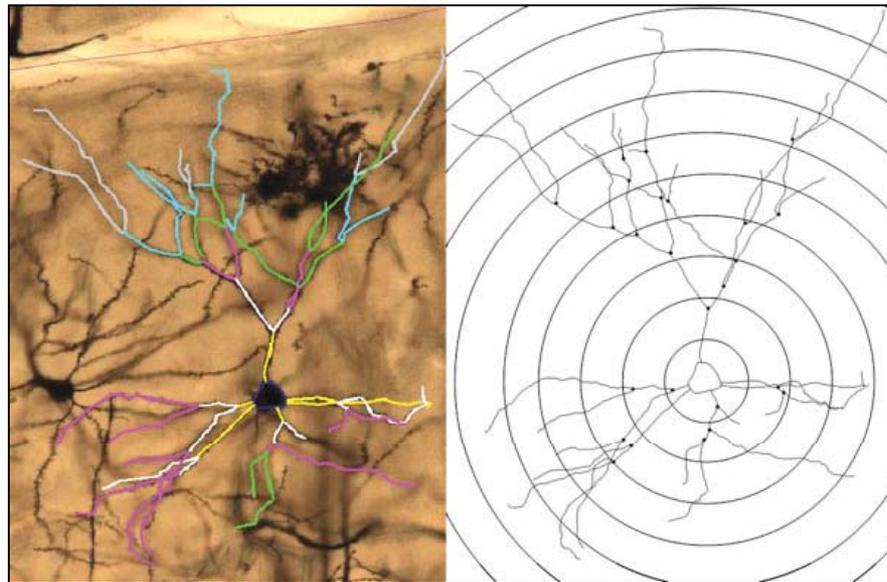


Serotonergic (5-HT) Neurons Critical to Maturation of Cerebral Cortex



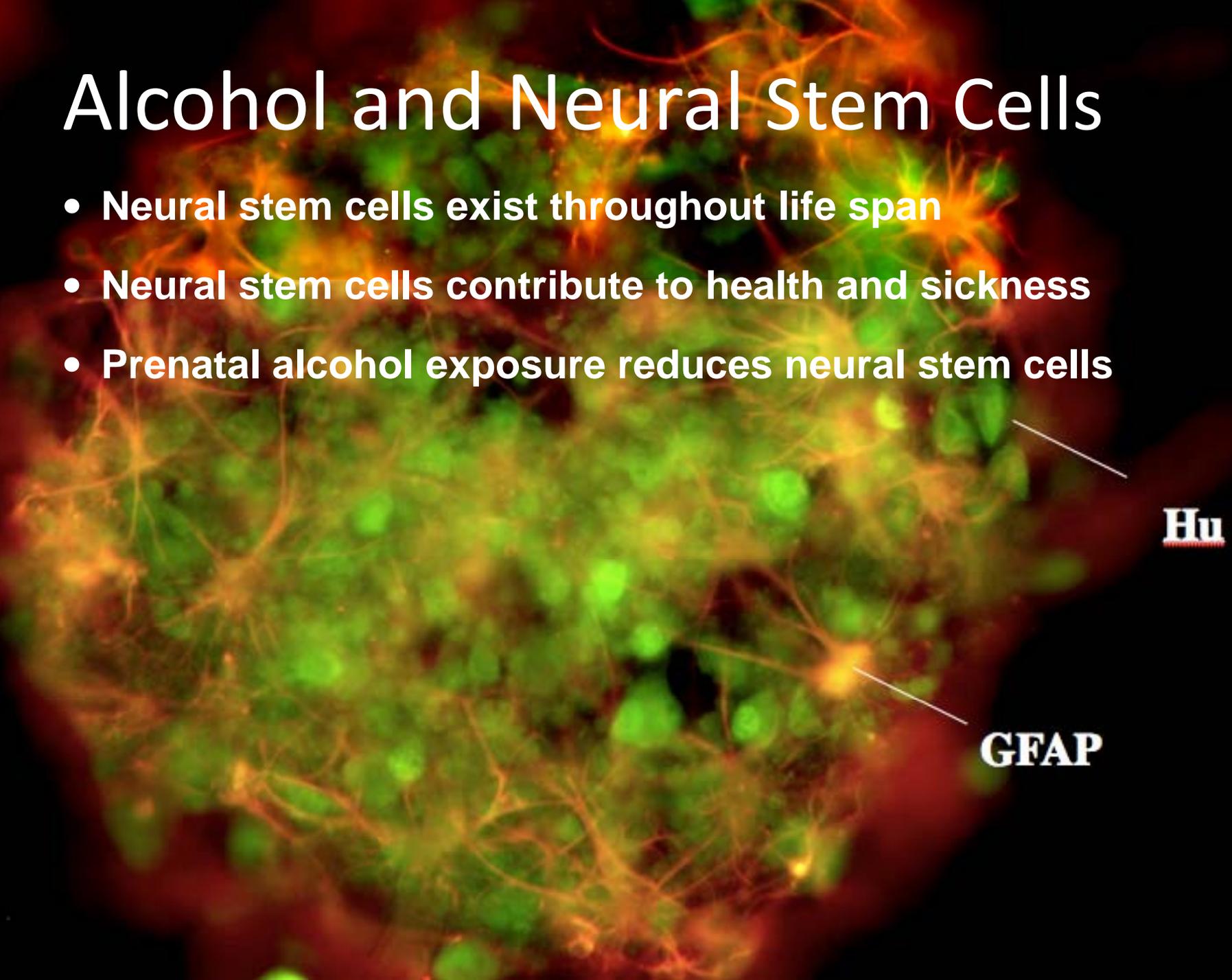
Alcohol Produces Defects in Neurons

- decreases dendrites
- increases synapses

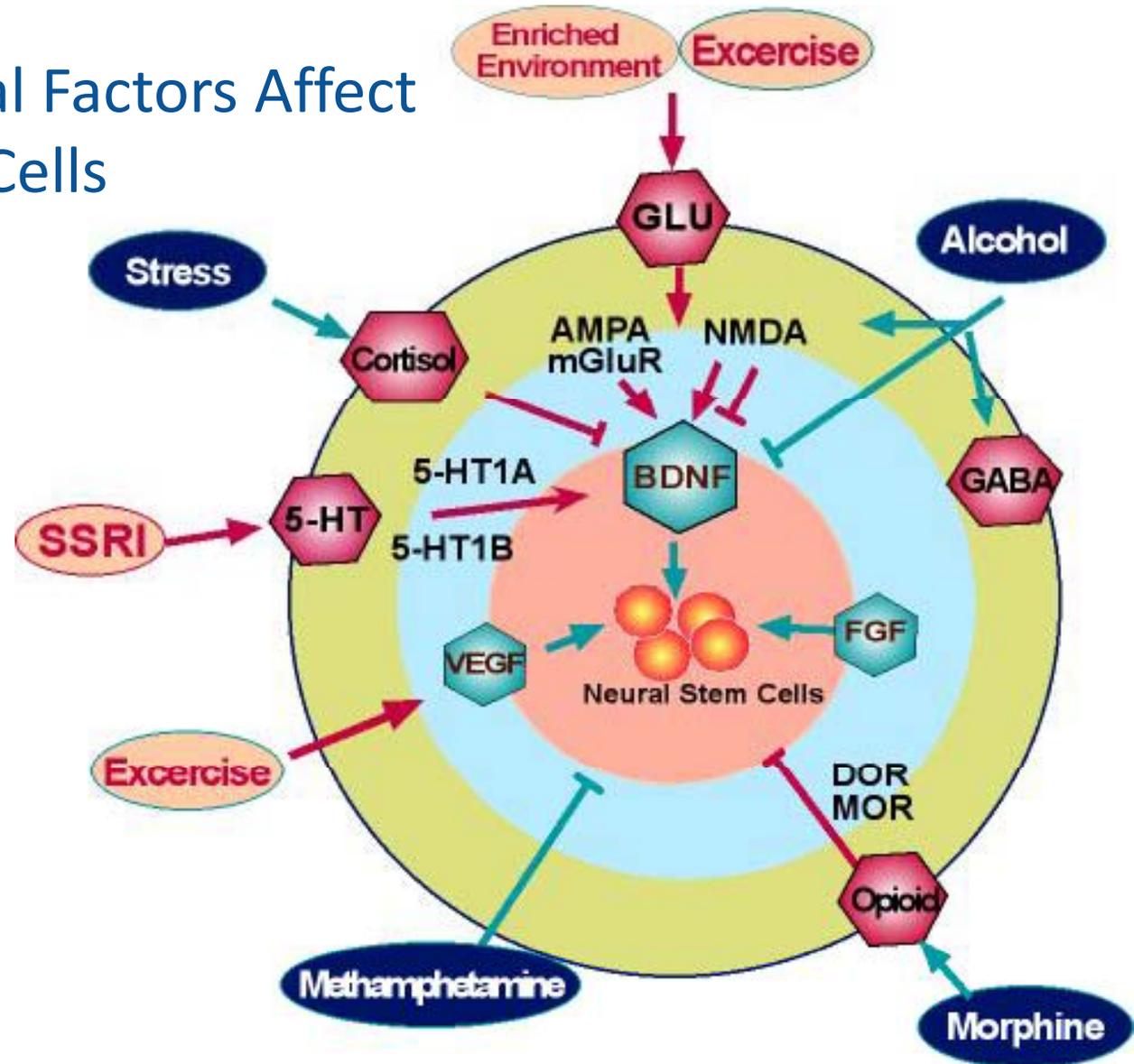


Alcohol and Neural Stem Cells

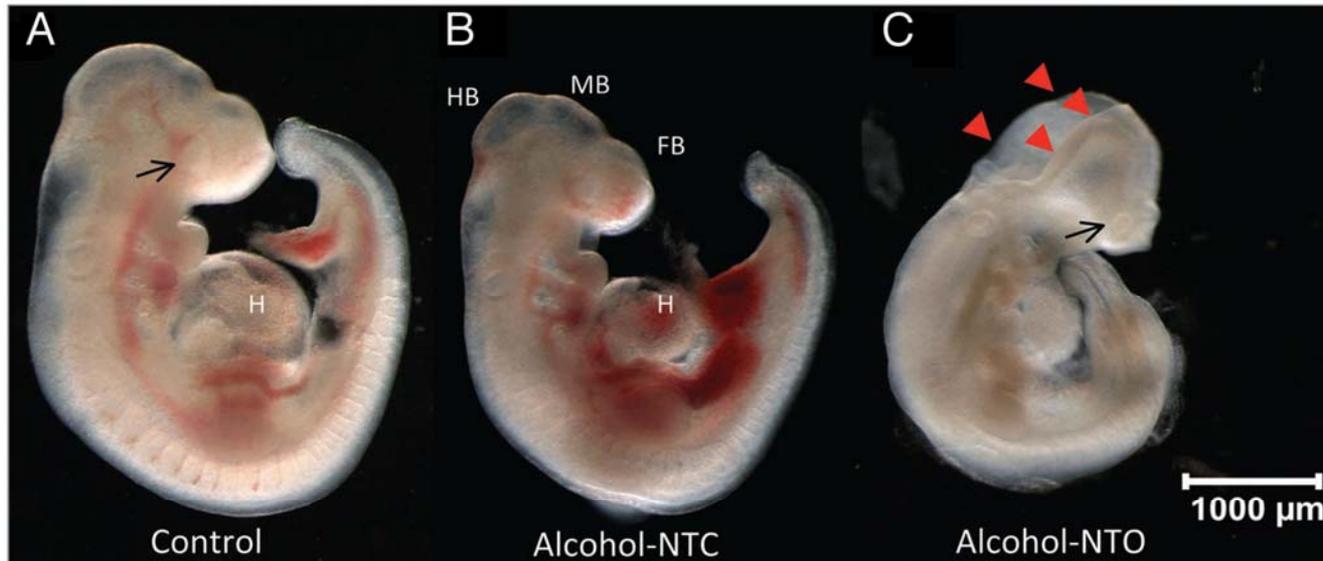
- Neural stem cells exist throughout life span
- Neural stem cells contribute to health and sickness
- Prenatal alcohol exposure reduces neural stem cells



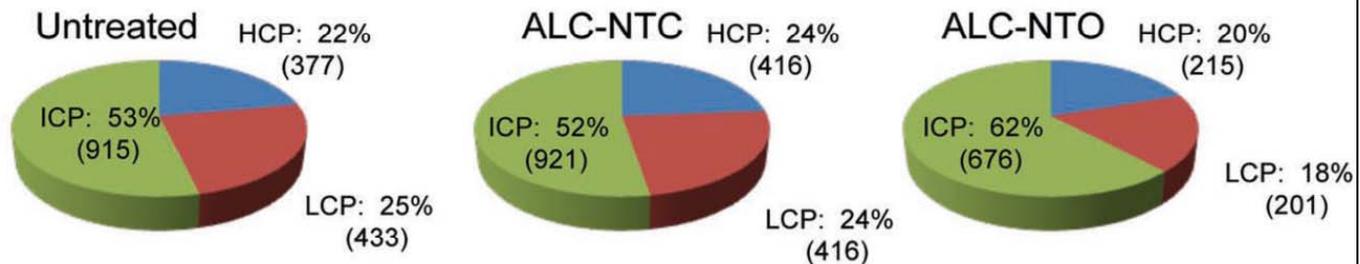
Environmental Factors Affect Neural Stem Cells



Patterns of DNA Methylation: Disrupted by Alcohol

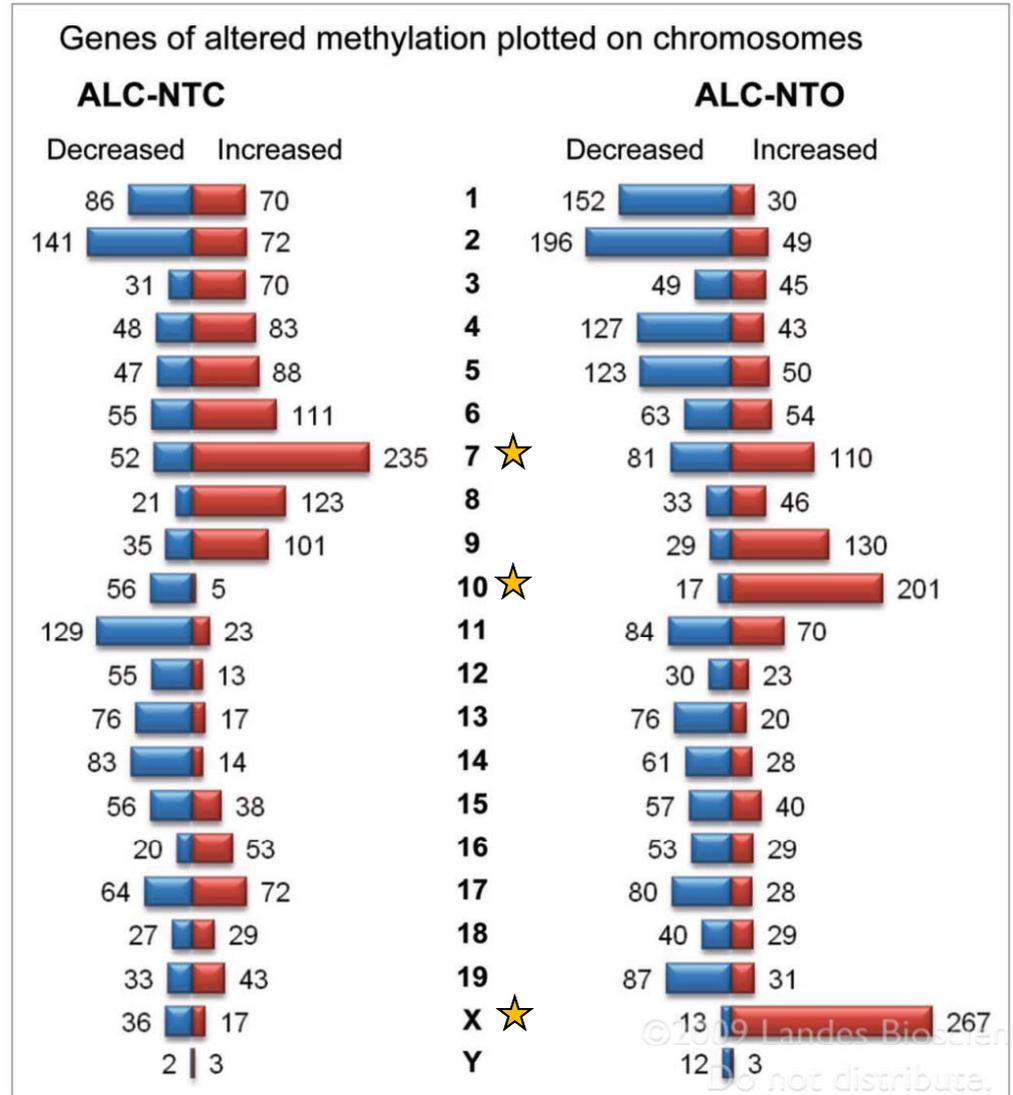


% of Hypermethylated promoters



DNA Methylation in Gene Promoters

- Alcohol-induced changes occur in all chromosomes
- Methylation in some promoters is increased (blue) and in other promoters is decreased (red)



Experimental Treatment To Block Damage

- **Folic acid:** prevention of neural tube defect
- **Minerals:** iron, zinc
- **Anti-oxidants:** N-acetyl cysteine, vitamin E
- **Nutritional metabolites:** choline
- **Neurotrophic peptides:** BDNF, NAP
- **Neurotransmitter or ion channel drugs:** glutamate antagonist, potassium channel inhibitor
- **Anti-inflammatory drugs:** just beginning
- **Enriched neonatal growth environment**

Animal Studies - examples

Survival and Body Weight of Mice



Vehicle + Ethanol
Survival: 3 of 8 *
Weight 2.47 ± 0.15 g *

15d-PGJ2 + Ethanol
Survival: 8 of 8
Weight 3.05 ± 0.56 g *

Control Animals
(not shown)

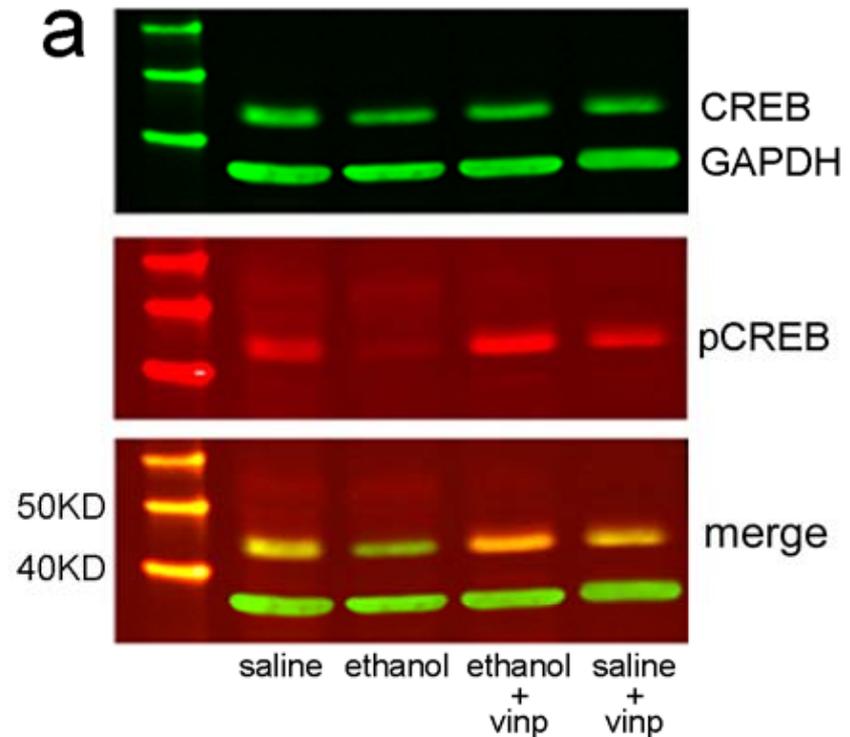
Vehicle + Vehicle
Survival: 8 of 8
Weight 3.97 ± 0.32 g

**Facial Dysmorphology
of Mice**

Regulation of Signaling Pathways within the Brain in Animal Models

Inhibition of phosphodiesterase protein activity using the experimental drug, vinpocetine:

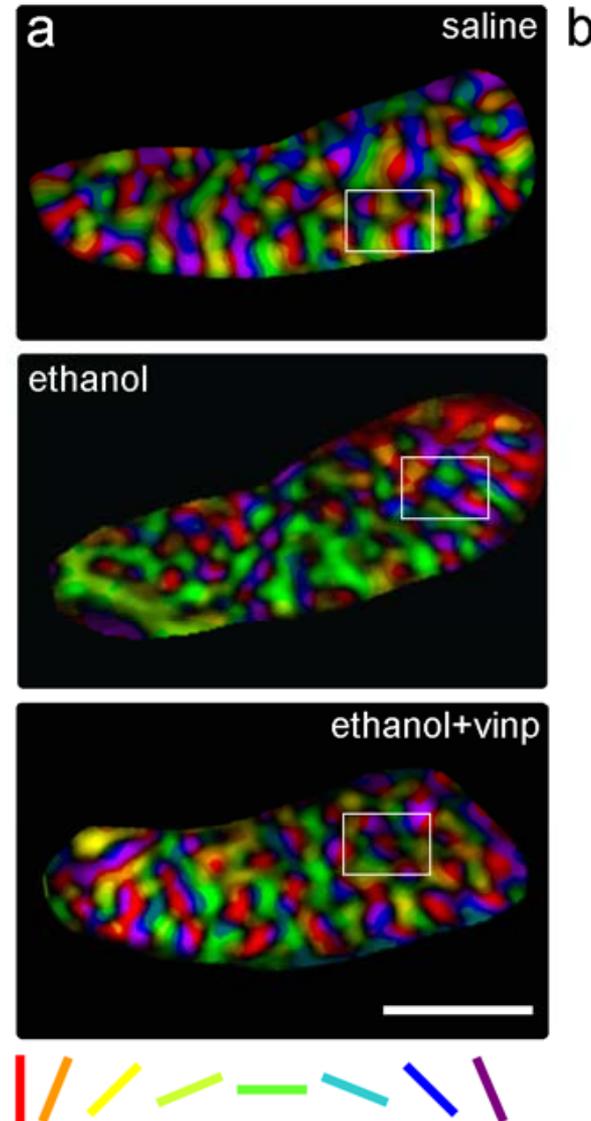
- Increases phosphorylation of CREB signaling protein in the visual cortex of the brain
- Restores normal orientation selectivity behavior



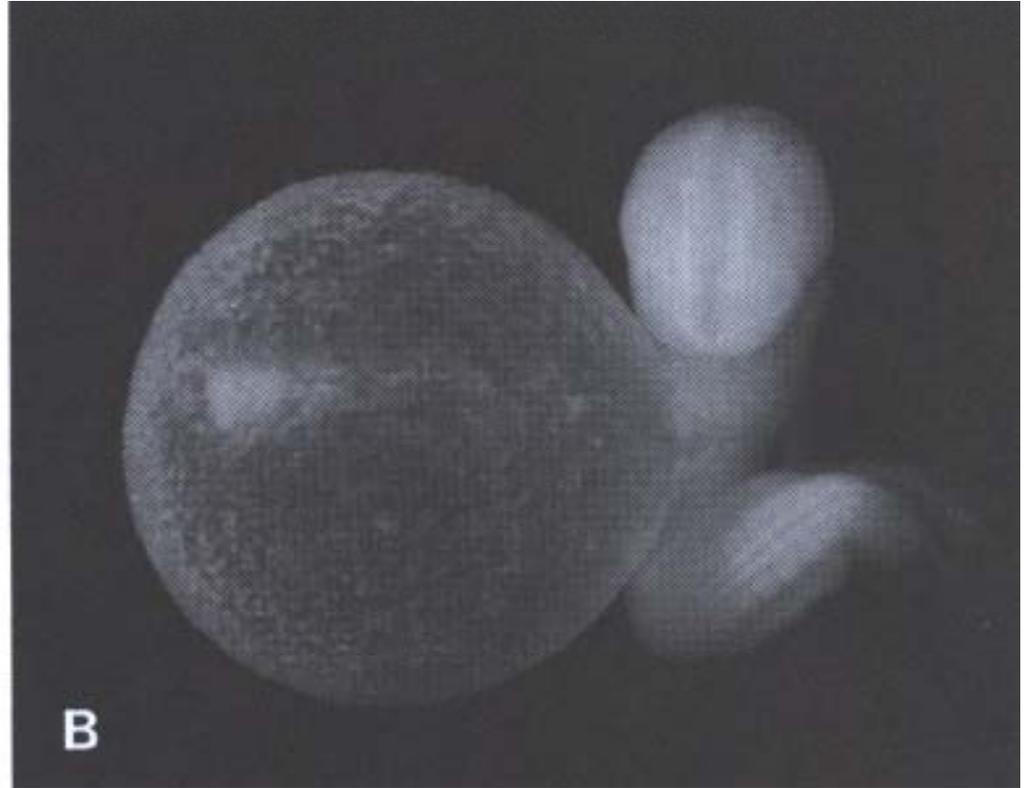
Regulation of Signaling Pathways within the Brain in Animal Models

Inhibition of phosphodiesterase protein activity using the experimental drug, vinpocetine:

- Functional analysis of visual cortex with magnetic polar maps shows return to normal structure and function
- Restores normal orientation selectivity behavior

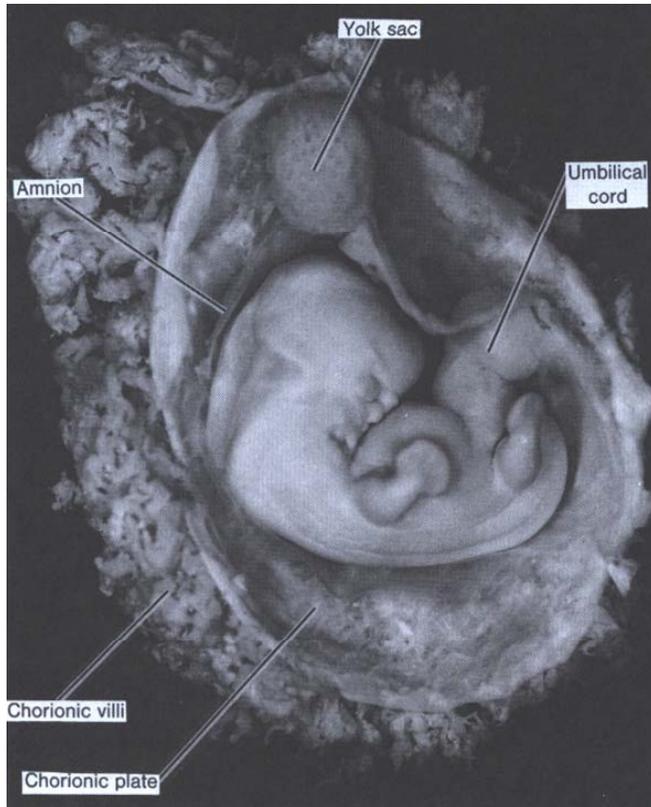


NORMAL DEVELOPMENT



Day 28

NORMAL DEVELOPMENT

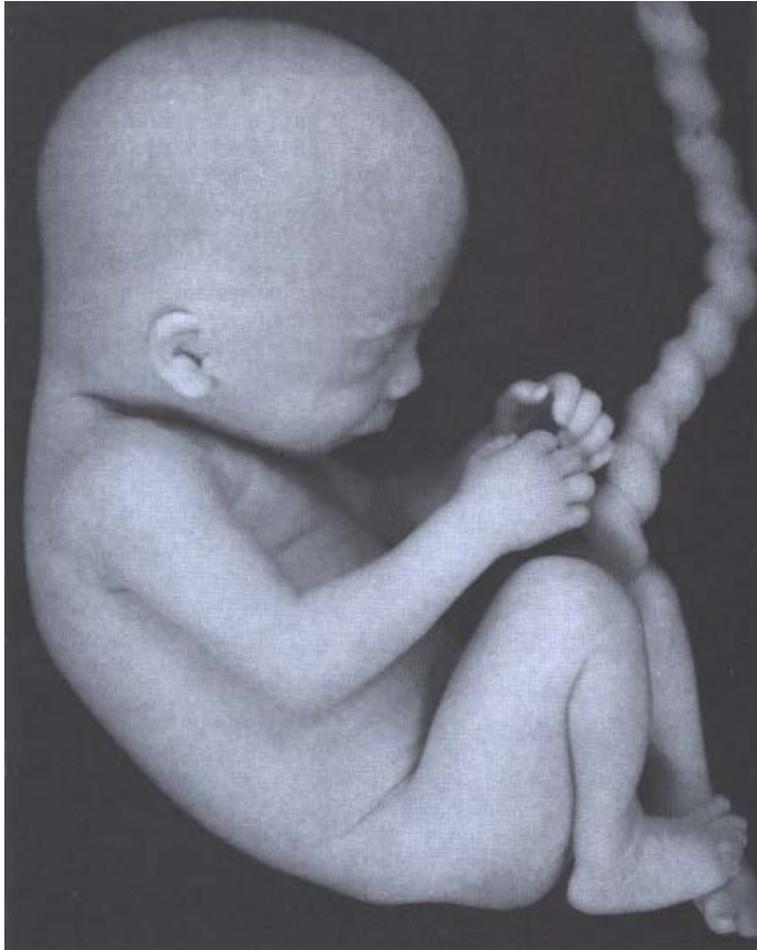


Week 6



Week 12

NORMAL DEVELOPMENT



Month 7

OUR GOAL

