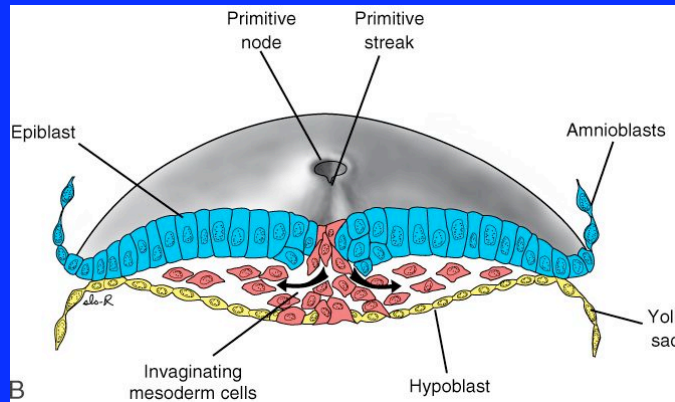
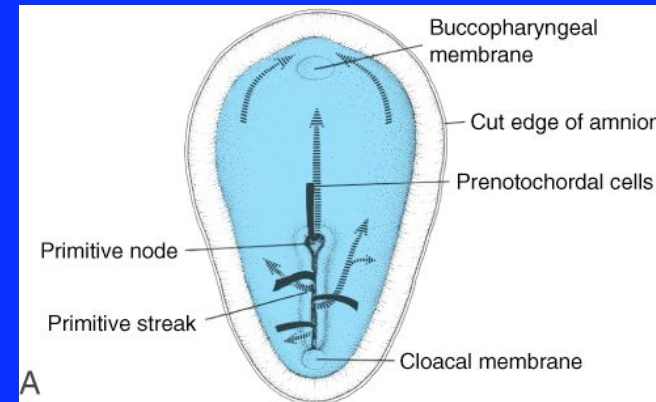


# Prenatal Brain Development and the Fetal Alcohol Syndrome

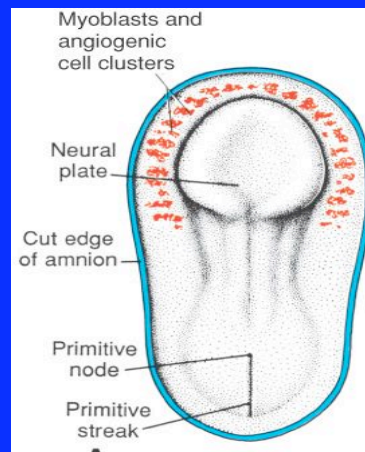
# Gastrulation



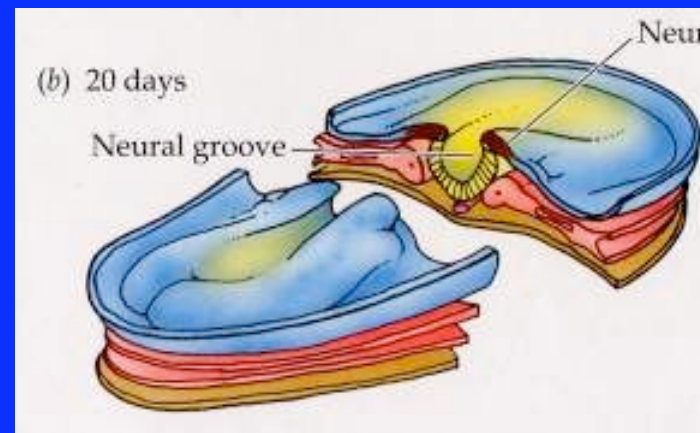
15-day embryo



16-day embryo



18-day embryo

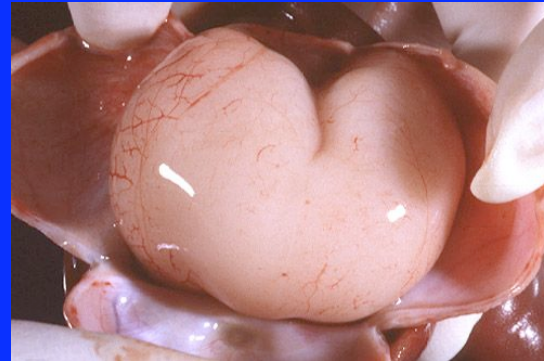


20-day embryo

# Holoprosencephaly



Cyclopia

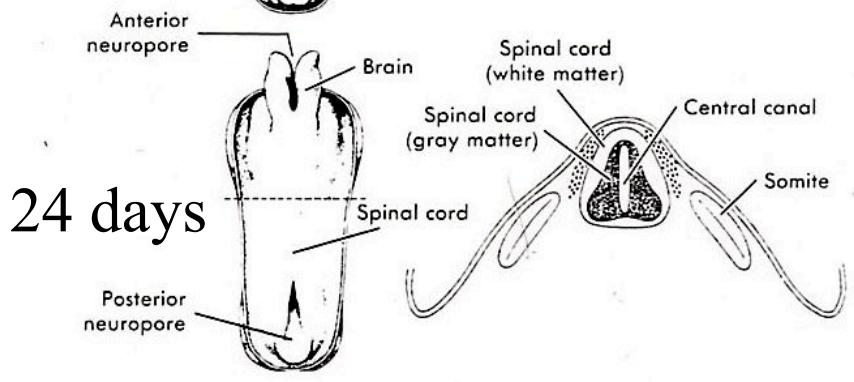
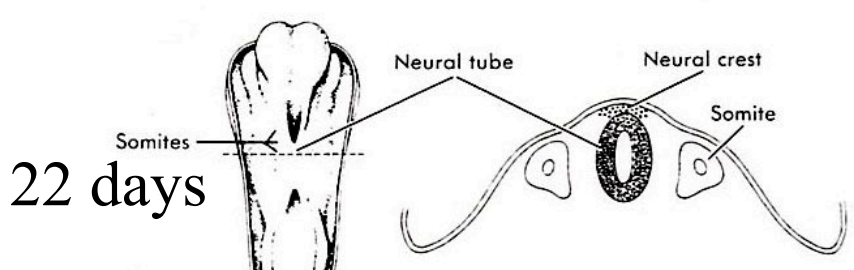
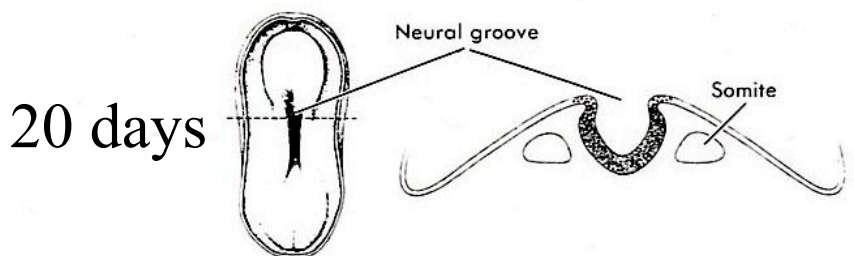
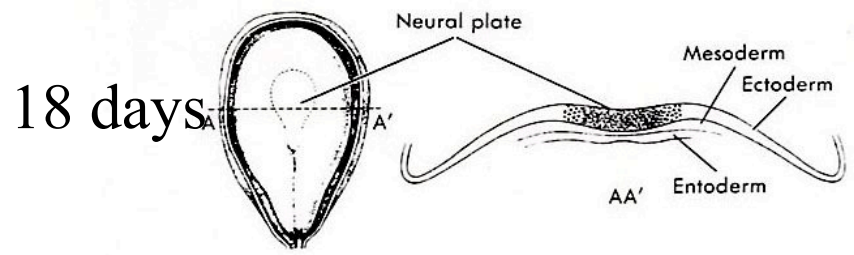


Partial holoprosencephaly brain



Normal 25-week human brain

Possible causes - trisomy 13, maternal diabetes



## Neurulation

Cowan WM The Development of the Brain Sci Am 1979; 241:112



## Anencephaly

[www.vh.org/.../FetalYoung](http://www.vh.org/.../FetalYoung)  
CNS/Images/fig03.gif



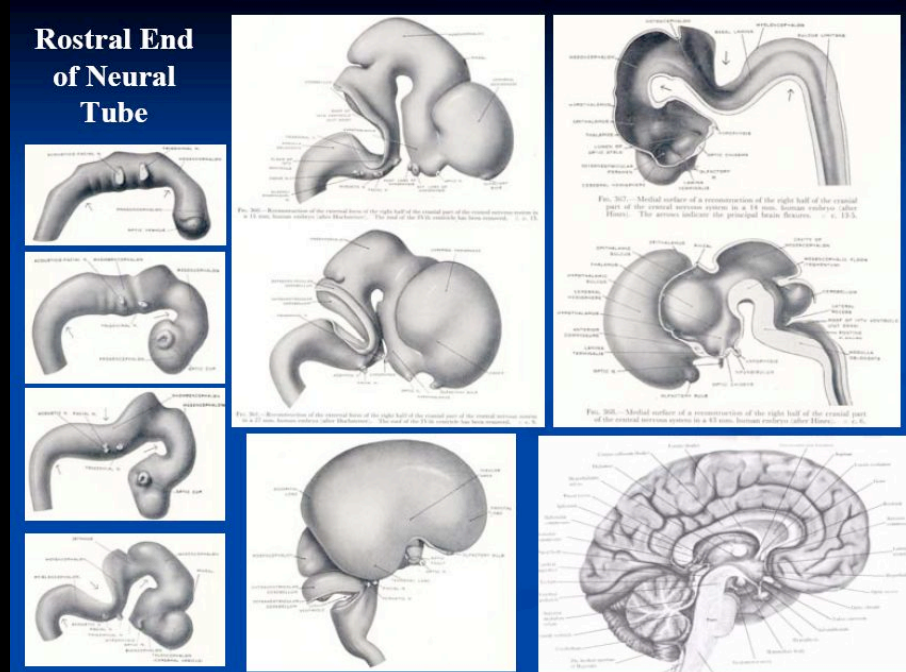
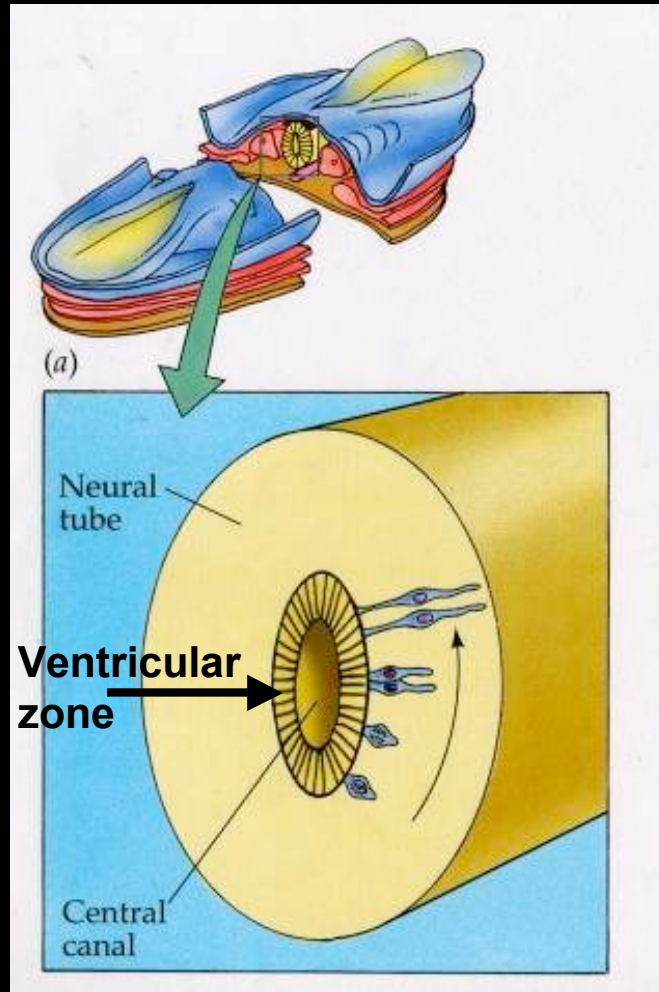
## Spina Bifida

Picture from Illustrated guide to malformations of the CNS at birth by N. C. Nevin and J. A. C. Weatherall 1983, Churchill Livingstone

# 1. Mitosis/Proliferation

The cells lining the neural tube (the ventricular zone) repeatedly divide to form the neurons of the various parts of the CNS. The neurons of the spinal cord and brainstem are the first to form. Most neurons of the cerebral cortex form between weeks 7 and 16.

At its peak the ventricular zone forms about 250,000 new cells per minute.

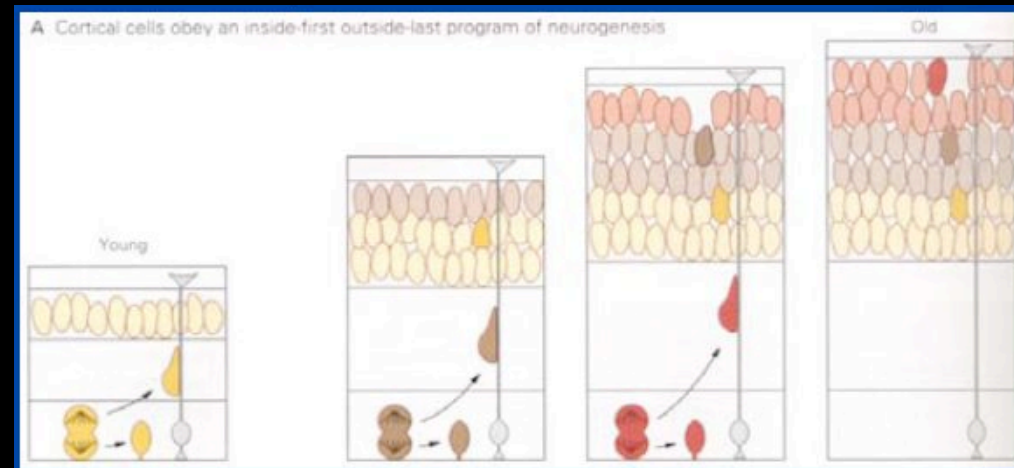
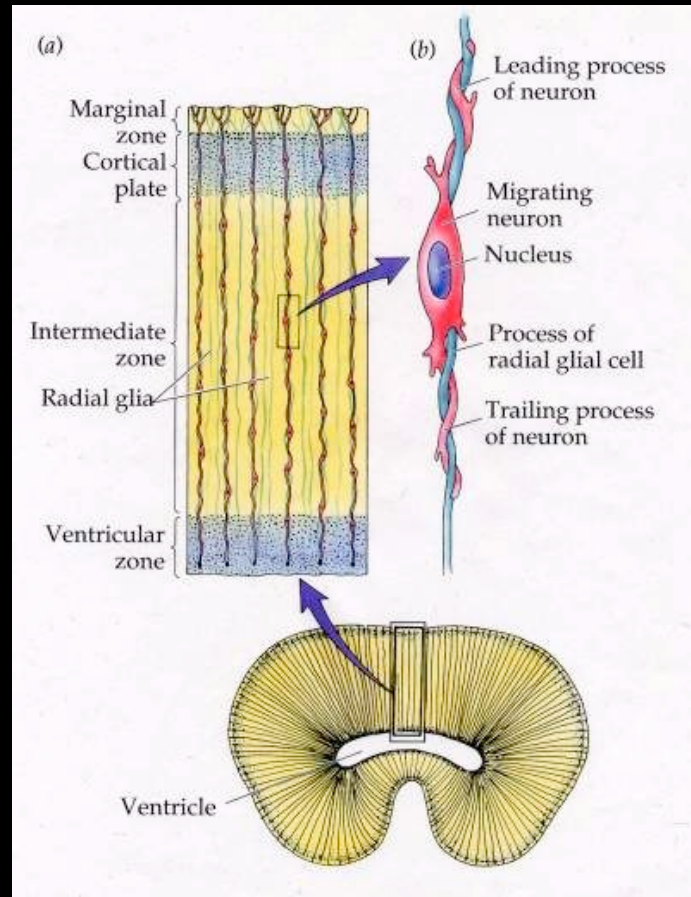




Interference with neuronal cell proliferation can lead to a small head (microcephaly) and a small brain (micrencephaly). Mental retardation can be severe.

Possible causes are exposure to excessive amounts of X-irradiation between weeks 7 and 16; maternal rubella in the first trimester; use of anti-cancer drugs in the first and second trimesters of pregnancy. There are also genetic causes.

## 2. Migration



The cerebral cortex forms in an inside-out sequence so that the last neurons to be formed have to migrate past all previously formed neurons. Migration in the cerebral cortex lasts to about 25 weeks gestation.

More than 25 syndromes resulting from abnormal neuronal migration have been described.

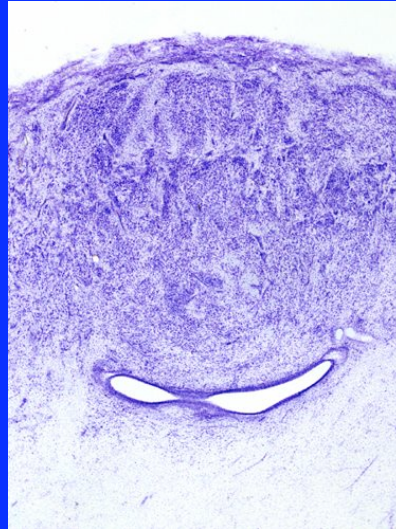
Radial glial cells act as guide wires for the migration of neurons.

<http://astro.temple.edu/~pak/development.ppt>

# Defective neuronal migration



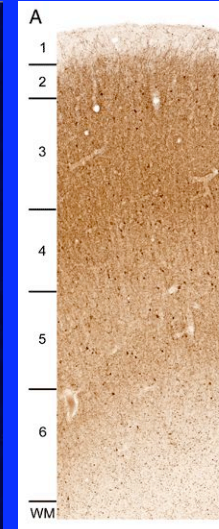
Cobblestone lissencephaly



Section of cerebral cortex - highly disordered



Normal cerebral cortex.



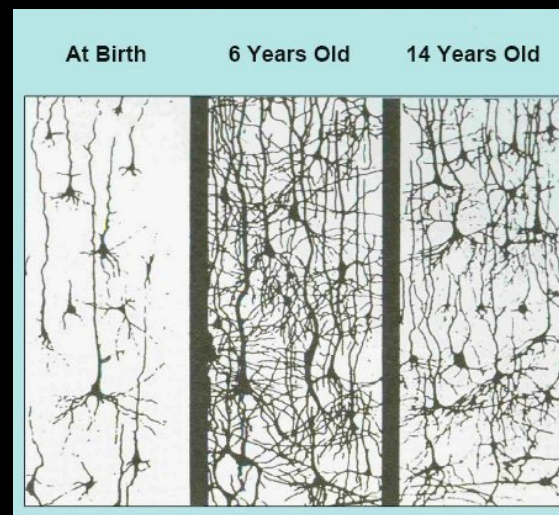
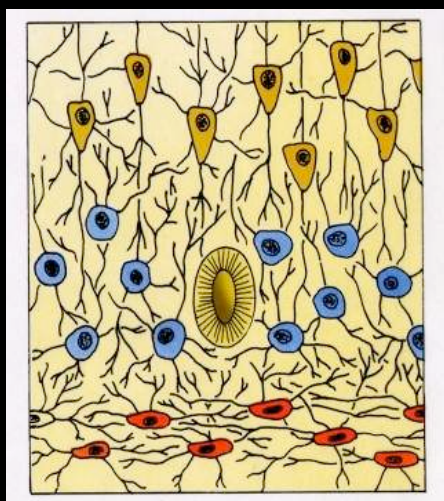
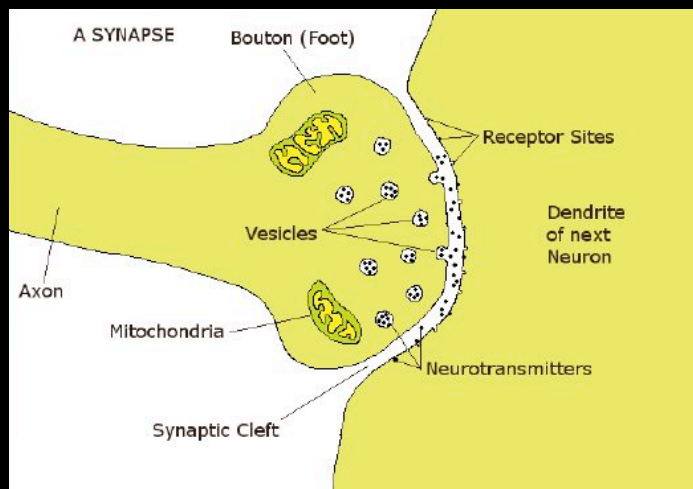
Section of normal cerebral cortex - 6 layers.

Cobblestone lissencephaly occurs in several genetic diseases, caused by mutations that affect protein glycosylation. Defective glycosylation of matrix proteins in the brain impairs the interaction of migrating neurons with matrix elements.

Patients with cobblestone cortex have severe psychomotor retardation, seizures, visual loss, and congenital muscular dystrophy.



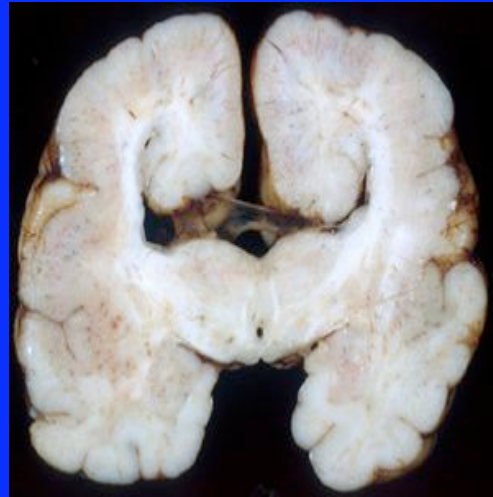
### 3. Differentiation and Synaptogenesis



Once neurons have completed their migrations they start to differentiate and form connections. Neurons need to connect with other neurons and with end organs such as muscle. These connections are called synapses. The first synapses in the cerebral cortex occur in the third trimester but most develop postnatally in the first two years of life. At the end of this period there is synapse elimination with almost 50% of synapses being eliminated.

In the visual cortex synapses reach their peak by 5<sup>th</sup> prenatal month but do not reach adult values until about age 6 years. In the frontal cortex peak is obtained by 1 year of age but adult numbers are not obtained until late adolescence.

# Abnormal differentiation and synaptogenesis



**Absent corpus callosum**



**Normal brain**

Between 10 and 20 weeks gestation axons cross between the two hemispheres of the brain to interconnect The two sides. This structure containing 300 million axons is the corpus callosum

Absent corpus callosum is one of the most common brain malformation. It occurs in many chromosomal abnormalities and malformation syndromes and in several inherited metabolic disorders. Among others, it is associated with mutations of the L1 cell adhesion molecule, a cell surface glycoprotein that is important for guidance of growing axons. It has also been associated with the fetal alcohol syndrome.

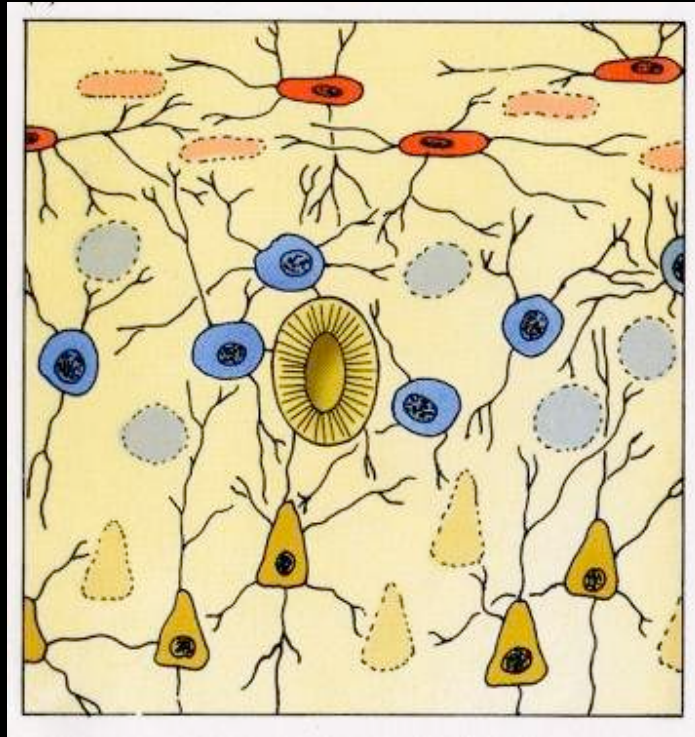
# Effect of testosterone

Between weeks 8 to 20 of gestation the male embryo is exposed to high levels of testosterone, peak levels are reached about week 15. The male embryo is exposed to testosterone levels 6-fold greater than the female as determined by levels in amniotic fluid.

As well as determining internal and external genital development the testosterone is thought to "sex" the brain giving the individual gender identity.



## 4. Neuronal Death

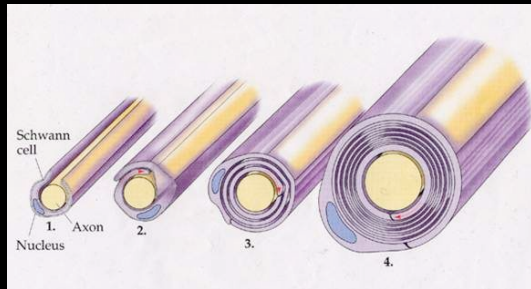


If neurons fail to make optimal synapses they die by the process of apoptosis.

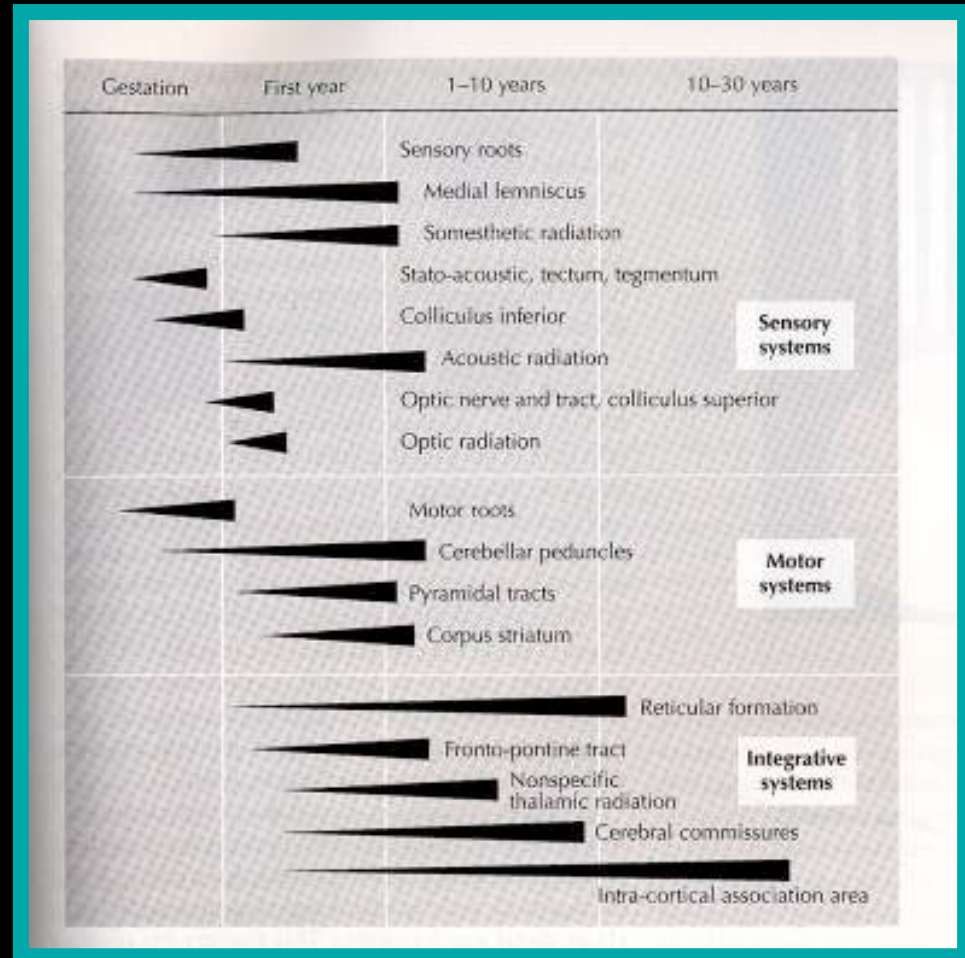
Between 40 and 75 percent of all neurons formed in embryonic and fetal development do not survive.

Excessive cell death in the developing brain may be a feature of the fetal alcohol syndrome.

# 5. Myelination



Myelination provides a fatty sheath around axons. This provides more rapid nerve impulse conduction.

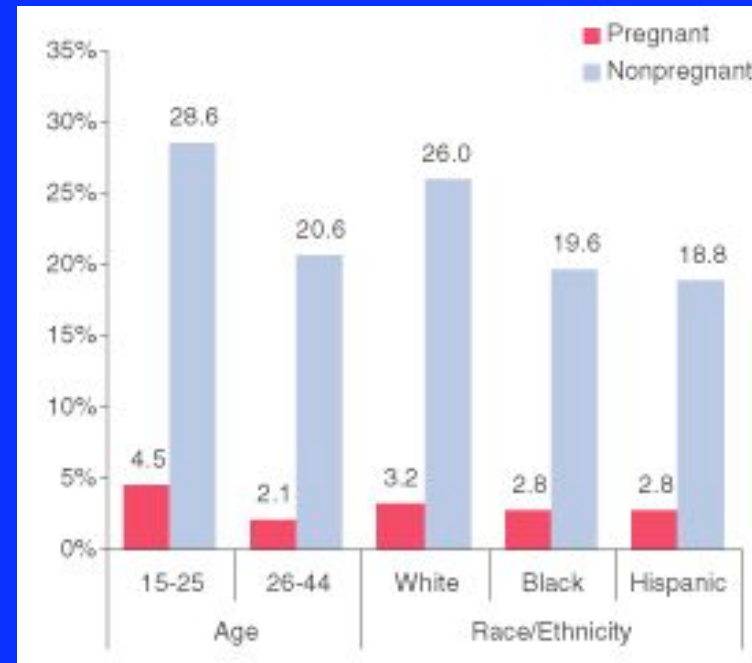


# Prenatal brain development

- **Days 16-18 - Gastrulation** - diabetes may cause holoprosencephaly
- **Days 18-26 - Neurulation** - spina bifida and anencephaly - increased risk with carbamazepine or valproic acid. Folic acid may prevent 70-80% non-drug induced neural tube defects.
- **Weeks 4-10** Proliferation of neurons for most of the brain excluding the cerebral cortex
- **Weeks 7-16** Proliferation of neurons for the cerebral cortex - May be damaged by radiation, alcohol, rubella, anticancer drugs, iodine deficiency - microcephaly
- **Weeks 16-postnatal years** - synaptogenesis - may be damaged by alcohol, methyl mercury, iodine deficiency, lead?
- **First 2 postnatal years** - formation of microneurons of the cerebellum - programming for movement

# The Fetal Alcohol Syndrome

## Percentages of Past Month Binge Drinking among Women Aged 15 to 44, by Pregnancy Status, Age, and Race/Ethnicity



Results from the 2002 National Survey on Drug Use and Health (NSDUH) in USA. Binge alcohol use is defined as drinking five or more drinks on the same occasion (i.e., at the same time or within a couple of hours of each other) on at least 1 day in the past 30 days. Heavy alcohol use is defined as drinking five or more drinks on the same occasion on each of 5 or more days in the past 30 days; all heavy alcohol users are also binge alcohol users.

Recent study reported 15% prevalence of risk use of alcohol during early pregnancy (>70g/week) in present day urban Sweden (J. Stud. Alcohol 66:157-164 2005).



# What is the Fetal Alcohol Syndrome?

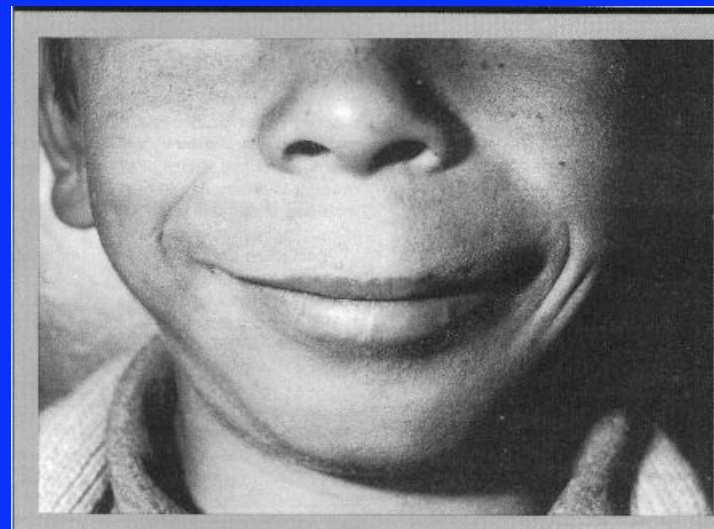
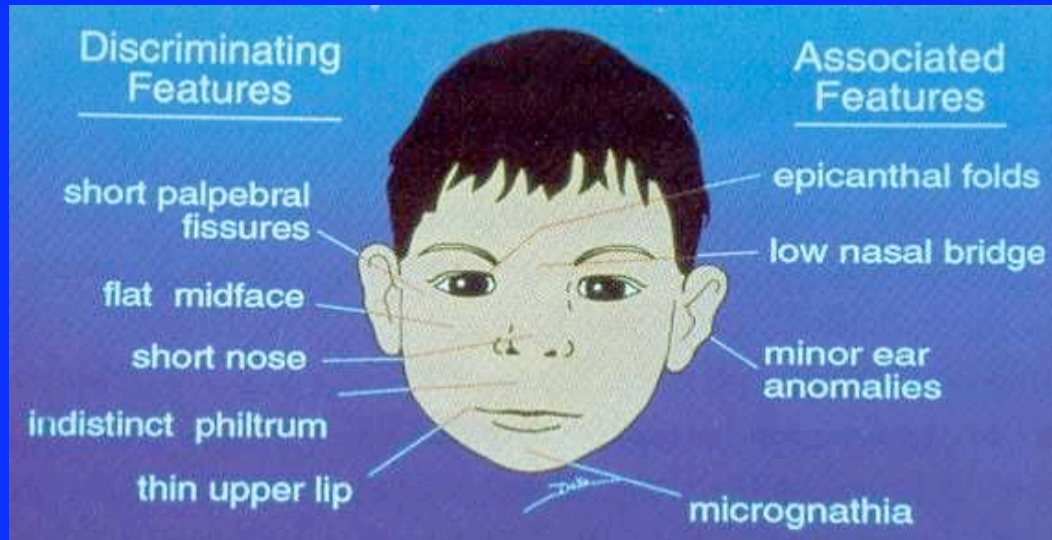
- In 1973, Drs. Kenneth Jones and David Smith noted unusual physical features and a failure to thrive in 8 children of alcoholic mothers at the Harborview Hospital in Seattle, Washington, brought to their attention by a pediatric resident, Dr. C. Ulleland.
- Later, after identifying other similar infants, they sought the assistance of a child psychologist who diagnosed various levels of mental anomalies in these infants.
- Jones KL, Smith DW, Ulleland CH, Streissguth AP. Pattern of malformation in offspring of chronic alcohol mothers. *Lancet*. 1973;1:1267-1271.
- Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. *Lancet*. 1973;2:999-1001.

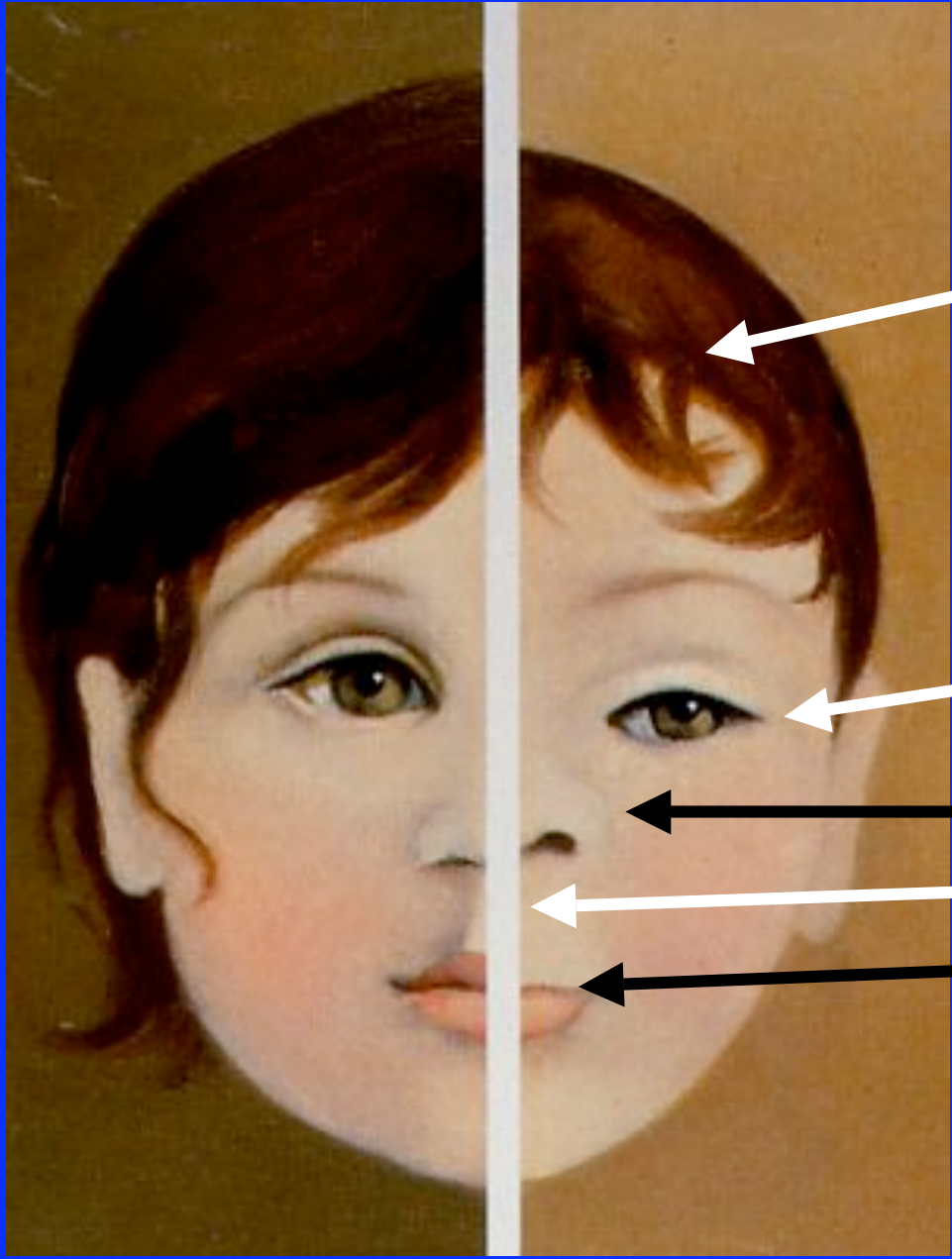
# What is the Fetal Alcohol Syndrome?

Spectrum of effects that can occur if a woman drinks alcohol during pregnancy:

1. **Fetal death**
2. **Fetal alcohol syndrome (FAS)**- abnormal facial features and retarded fetal growth and brain growth (microcephaly), mental retardation. Increased risk of major malformations particularly heart defects.
3. **Alcohol-related neurodevelopmental disorder (ARND)**. Children with ARND do not have full FAS but might demonstrate learning and behavioral problems.
4. **A normal baby!**
5. CDC studies show FAS rates ranging from 0.2 to 1.5 per 1,000 live births in different areas of the United States. Other FASDs are believed to occur approximately three times as often as FAS.

# Facies in FAS





Microcephaly

Short palpebral fissure

Short nose

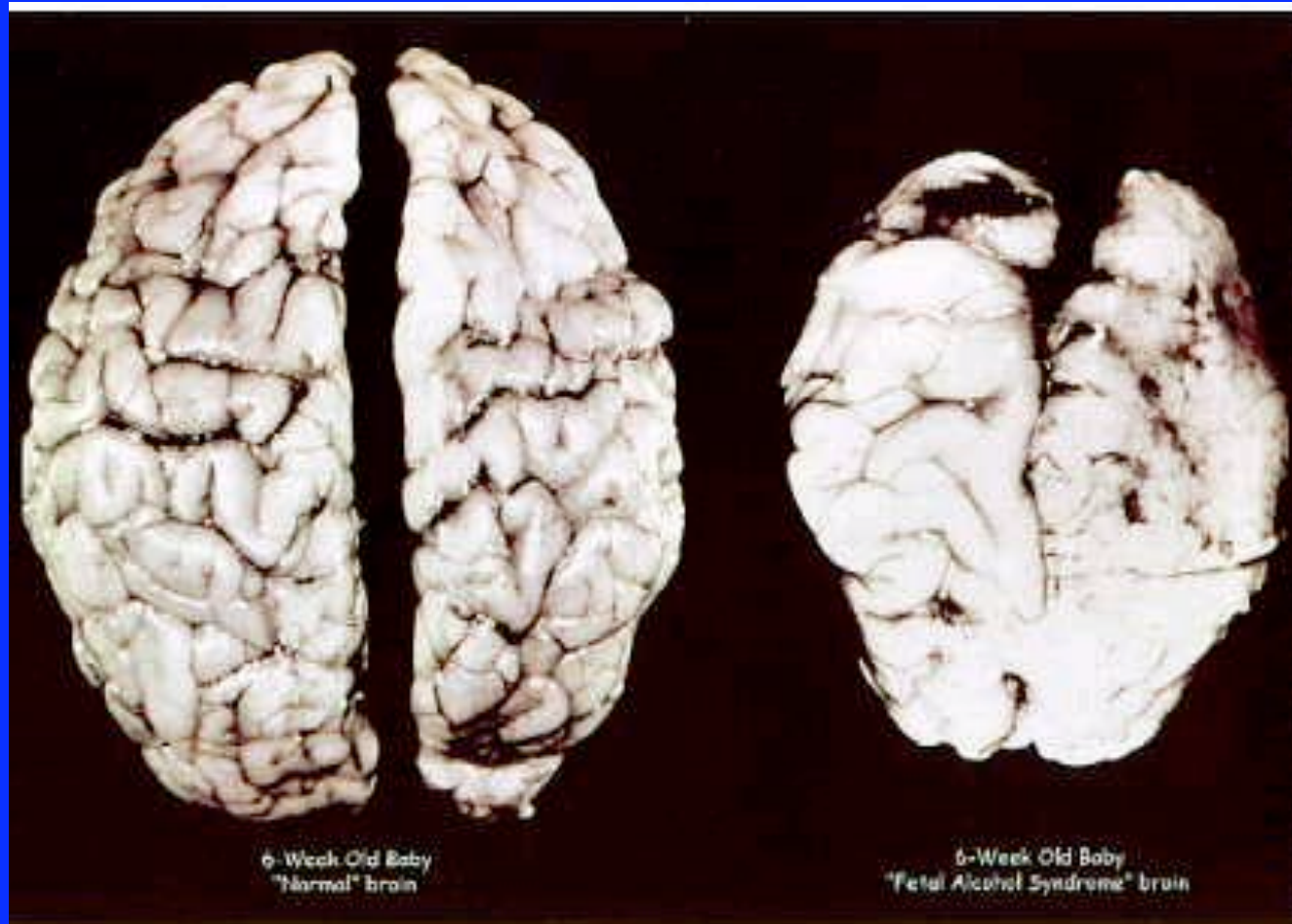
Long philtrum

Thin upper lip

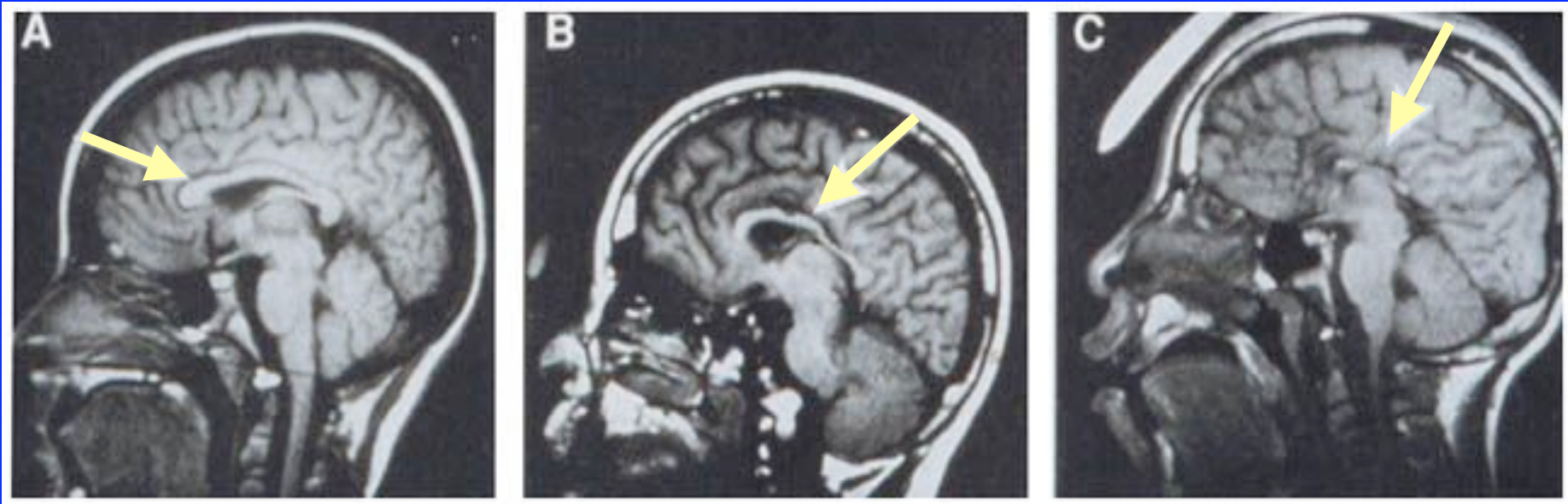
**NORMAL**

**FAS**

# Brain damage due to alcohol



**Visualization of the brain of a normal individual (A) and two with FAS (B,C) shows permanent loss of the tissue indicated by the arrows (portions of the corpus callosum).**



**Normal**

**FAS**

**FAS**

Images courtesy of Dr. S. Mattson

# Centre for Disease Control and Prevention

- When a pregnant woman drinks alcohol, so does her unborn baby. There is no known safe amount of alcohol to drink while pregnant and there also does not appear to be a safe time to drink during pregnancy either.
- Therefore, it is recommended that women abstain from drinking alcohol at any time during pregnancy. Women who are sexually active and do not use effective birth control should also refrain from drinking because they could become pregnant and not know for several weeks or more.



# Possible mechanisms for FAS

Alcohol acts as a typical teratogen affecting different organ systems as they pass through their critical periods. In pregnant mice alcohol can cause malformations of the brain, face, heart and limbs. For alcohol to have this effect in the pregnant mouse it needs to reach very high maternal blood alcohol levels unlikely to be achieved by non-alcoholic humans.

It is possible that the "typical" alcohol face is caused by alcohol exposure during the gastrulation period.

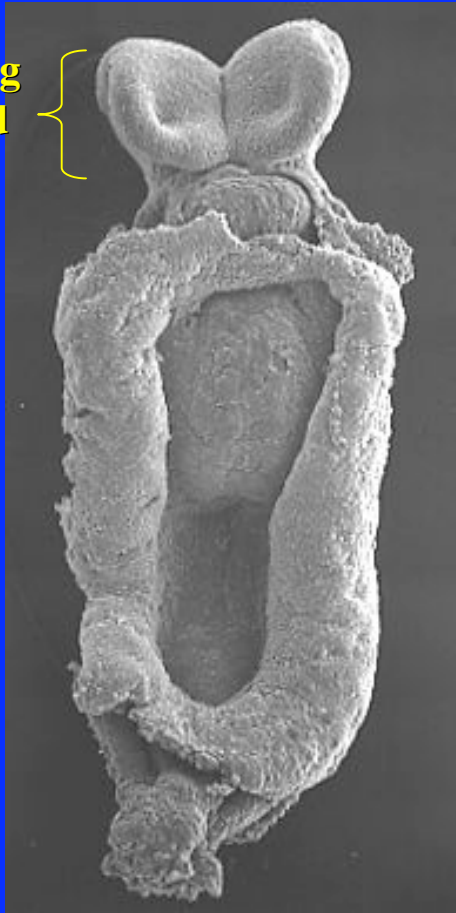
Webster WS et al Some teratogenic properties of ethanol and acetaldehyde in C57BL/6J mice: implications for the study of the fetal alcohol syndrome. *Teratology* 27:231-243, (1983)

Sulik KK Genesis of alcohol-induced craniofacial dysmorphism. *Exp Biol Med* 230: 366-375 (2005)

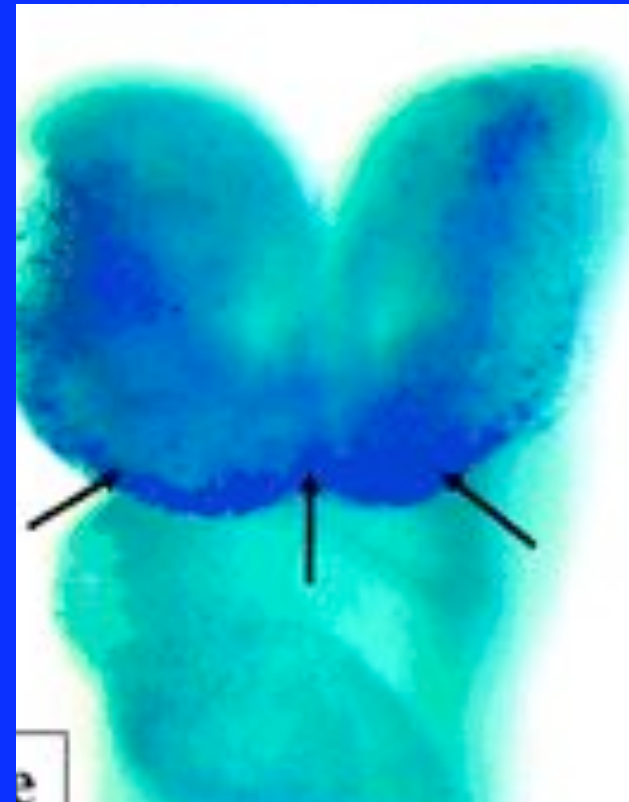


## CELLS THAT SHOULD FORM MIDLINE STRUCTURES OF THE BRAIN AND FACE ARE KILLED BY ALCOHOL

Developing  
brain and  
face



Mouse embryo (viewed from the front) at a stage corresponding to a 22-23 day old human.



A close-up view of an alcohol-exposed mouse embryo shows cells killed by alcohol that have taken up a dark blue stain.

The facial features of FAS can be seen in both a child and a mouse fetus that were exposed to alcohol during development.

child with FAS



Narrow forehead

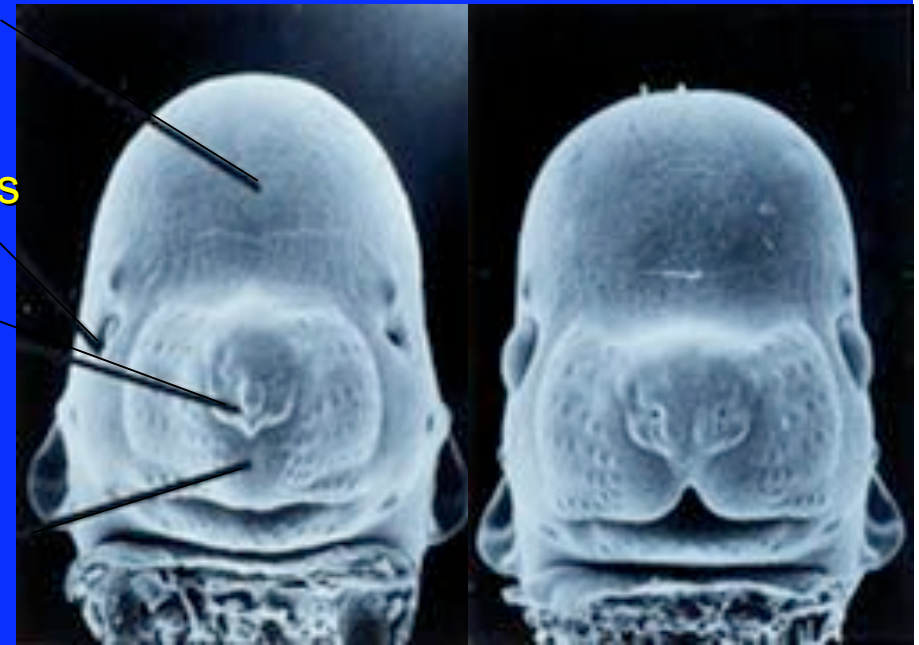
Short palpebral fissures

Small nose

Small midface

Long upper lip with  
deficient philtrum

mouse fetuses



alcohol-exposed

normal

# Possible mechanisms for FAS

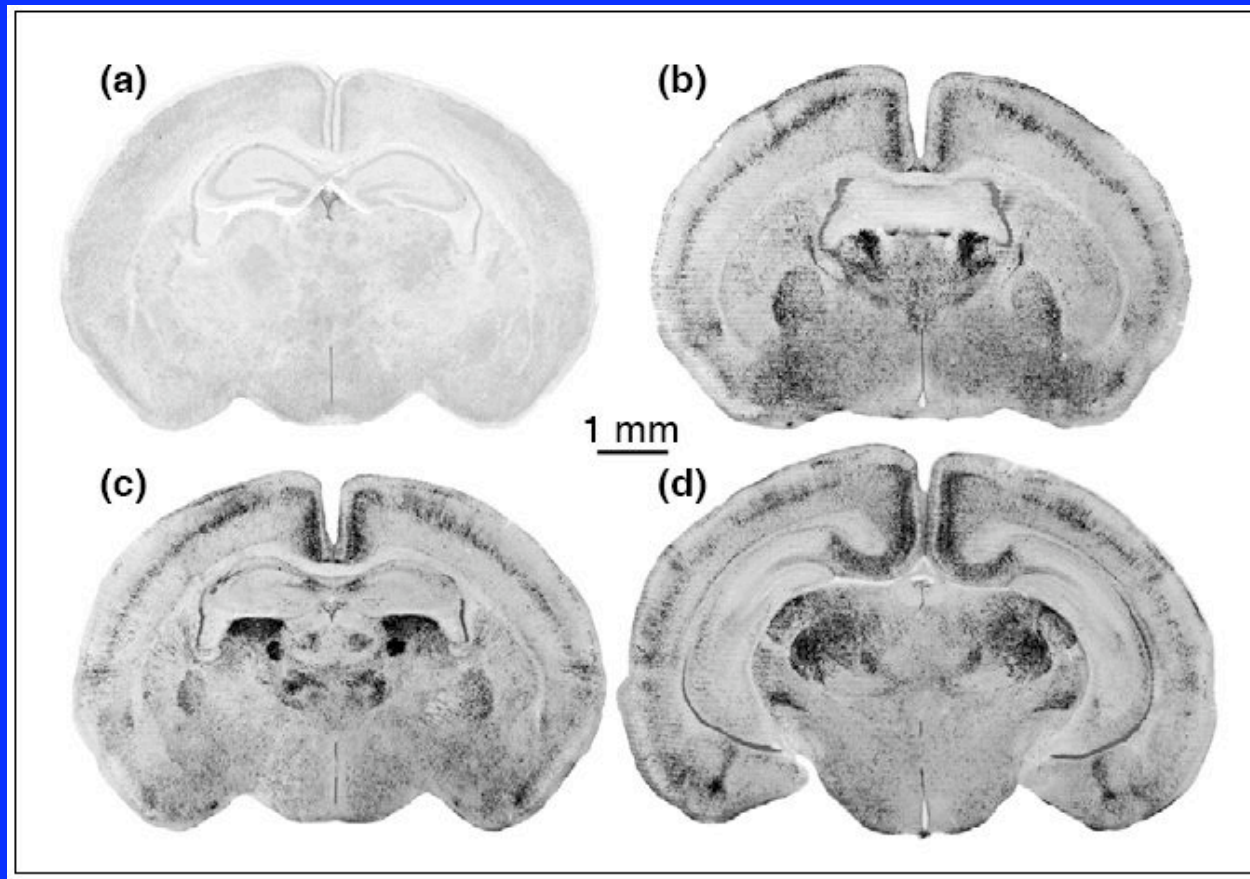
John Olney at Washington University School of Medicine has investigated the effect of drugs that interfere with neurotransmitters during the period of synaptogenesis (postnatal day 7) in the mouse.

They found that NMDA receptor antagonists and agents that mimic or potentiate the action of GABA at GABA<sub>A</sub> receptors triggered a massive wave of apoptosis throughout the brain.

Ethanol has both NMDA receptor antagonist properties and GABA-mimetic properties. Studies in mice showed that ethanol caused extensive apoptotic neuronal death when administered during the period of synaptogenesis.

Olney JW et al., Trends in Pharmacological Sciences 25:135-9 (2004)

# Effects of alcohol on the rat brain during the period of synaptogenesis



Sections are from brains of 8-day-old C57Bl/6 mice 24 h after subcutaneous treatment with (a) saline or b-d ethanol. Each black spot is a degenerating neuron stained by cupric silver method.

# How much alcohol is too much in pregnancy?

- Teratogenic effects in mice need maternal blood alcohol levels of 200 - 400 mg/dl (0.2-0.4%).
- Neuronal apoptosis described by Olney was initially associated with blood alcohol levels in the treated rats of 200 mg/dl (0.2%) for at least 4h.

In more recent studies they claim some neuroapoptosis at 80 mg/dl (0.08%) for 1 hour.

# Blood alcohol levels



<b>ALCOHOL EFFECTS</b>	
<b>LEVEL</b>	<b>EFFECTS</b>
<b>.03%</b>	<b>Mild euphoria</b>
<b>.05%</b>	<b>Coordination mildly impaired</b>
<b>.10%</b>	<b>Legally drunk in many U.S. states</b>
<b>.20%</b>	<b>Drowsy &amp; confused</b>
<b>.30%</b>	<b>Stuporous</b>
<b>.40%</b>	<b>Deep anesthesia, possibly fatal</b>

# How much alcohol is too much in pregnancy?

Epidemiology studies do not provide the answers at this stage. Results are inconsistent from study to study. An example of the problem is seen in a recent paper: Moderate prenatal alcohol exposure and cognitive status of children at age 10.

Willford et al., 2006 Alcoholism, Clinical and Experimental Research 30:1051-9

15-23% of the women were considered heavy drinkers in the first trimester dropping to 1-6% in the third trimester.

The results showed a significant negative effect of alcohol in the first or second trimesters (but not the third) on the child's IQ in African-American women.

## HOWEVER

No effect of maternal alcohol intake for any trimester was seen on the child's IQ for Caucasian women.

For recent Swedish review see Strömland (2004) Fetal Maternal Med Rev 15:59-71 and Magnusson et al., (2005) J Stud Alcohol 66:157-64.

THE END!