The NADIA is devoted to understanding the neurobiological consequences of underage drinking to inform parents, to guide public health decisions on drinking age, alcohol access and other alcohol policies as well as finding treatments to reverse or prevent pathology.
Adolescence is a unique period of development.

Adolescence is marked by characteristic behaviors: high risk taking, novelty-seeking and reward driven, socialization.

Defining Adolescence for pre-clinical studies.
Across species characteristic adolescent behaviors include increases in reward- and sensation-seeking, peer directed social interactions, risk-taking, and low responses to aversion. These characteristic behaviors along with hormonal changes in puberty define a developmental period (Review: Rewards, aversions and affect in adolescence: Emerging convergences across laboratory animal and human data (LP Spear, Dev Cogn Neurosci . 2011)

Maturation of brain parallels development of self-control, reflection on future consequences, planning and socialization.

Developing brain is particularly sensitive to alcohol toxicity.
Adolescence is a unique stage of development across species. Adolescents have a unique response to alcohol and a unique binge drinking pattern.

3rd Trimester

Postnatal day 1-7 by Brain Morphology

Adolescence

Postnatal Days 28-48
Defined by Adolescent Behaviors and Puberty

High levels of drinking

Adulthood

>P60 Adult
32 month lifespan

Hypothesis: Underage drinking alters adolescent brain development.
<table>
<thead>
<tr>
<th>Event</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>First drink</td>
<td>12-14</td>
</tr>
<tr>
<td>First intoxication</td>
<td>14-18</td>
</tr>
<tr>
<td>Minor alcohol problem</td>
<td>18-25</td>
</tr>
<tr>
<td>Age of onset (3+ DSM-IV criteria for dependence)</td>
<td>23-33</td>
</tr>
<tr>
<td>Age on entering treatment</td>
<td>40</td>
</tr>
<tr>
<td>Age of deaths (heart disease, cancer, accident, suicide)</td>
<td>55-60</td>
</tr>
</tbody>
</table>

*Chronic relapsing: In any one year abstinence alternates with active drinking in 1/4 to 1/3

Adolescents binge drink: 5-20 drinks max. per event.

Figure 1 Mean Maximum Drinks 6 Months Prior to Interview:
4 Classes, 4 Time Points

Maximum drinks per occasion in the six months prior to each follow up between mean age 18 and 24 for 833 COGA adolescent and young adult men and women. Class 1 = 571 subjects; Class 2 = 123; Class 3 = 86; and Class 4 = 53 subjects. Response to the SSAGA question: “In the past six months, what was the largest number of (standard) drinks you’ve drunk in a 24-hour period?”
Adolescents Binge Drink to Blackouts
Blackouts reflect high blood alcohol levels

Prevalence of blackouts in a sample of U.S. college students (n = 772)\(^1\)

12% of students who drank in a two-week period over summer between high school graduation and college\(^2\)

Adolescence have different responses to alcohol than adults.

Adolescents have a low sedative response to alcohol.

Adolescent

Adult
Adolescents are low responders to Ethanol Sedation. Sleep time vs Ethanol Dose:

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

Dose of Ethanol (g/kg)

Recovery Time (min)

Adolescents drink more ethanol than adults. Adolescent Low Response to ethanol is greater than adult tolerance.

CIE
AIE

Intoxication rating

BEC (mg\%)

1 = no signs of intoxication

2 = slightly intoxicated
(slight motor impairment)

3 = moderate intox.
(impaired, but able to walk)

4 = highly intoxicated,
(dragging abdomen)

>350mg\%

200mg\%

Gass et.al.
Neuropsychopharm.2014
Chandler cp. MUSC
Adolescent Cortical Development: A Critical Period for Vulnerability to Addiction

Humans (years): 12 to 25+; Rodents (days): 25 to 48+

- Normal cortical development
- More focal cortical excitation as executive functions improve
- Binge drinking disrupts cortical development

Age

- Critical period of frontal cortical development
- Uncontrolled drinking addiction

- ↑ drinking
- ↑ preoccupation and tolerance
- ↑ withdrawal negative affect
- Loss of other activities

Crews et al. Pharm. Biochem. Behav. 2007
Adolescent Cortical Development: A Critical Period for Vulnerability to Addiction

NADIA Adolescent Intermittent Ethanol (AIE) Exposure Model

Common procedures for breeding, housing and adolescent intermittent ethanol binge exposure (Oral or Vapor).

Adolescence

Adulthood

E E E E E E E E E

P28 P55 P56

No Ethanol

P80 P220

assessments adulthood

E E E E E E E E E

Adolescent Intermittent Ethanol (AIE) (Must be Binge BEC > 100 mg %)

puberty

Standardized NADIA procedures data collection, animal treatment, numbering, age, and other quantitative end points to establish consistency across components.
NADIA findings are that AIE results in the persistence of adolescent-typical phenotypes into adulthood.

- Low response to alcohol sedation.
- High sensitivity to alcohol cognitive disruption.
- High reward sensitivity and low aversion sensitivity.
- Adolescent-like EEG *insensitivity* to EtOH.
- High anxiety and low anxiolytic sensitivity to alcohol.
- High alcohol preference and drinking, increased reward.
- High LTP sensitivity and immature synaptic spines.
- High disinhibitory behavior and risky decisions.
- Enhanced sensitivity to ethanol induced social stimulation.
- Reduced ethanol social inhibition.

NADIA: Emerging Evidence for an Adolescent Alcohol Syndrome.

AIE results in decreased behavioral flexibility.

- **Deficits in Set-shifting tasks.** (Gass, Chandler et al, 2014)
- **Deficits in Reversal learning:** (Barnes maze; Morris water maze) (Vetreno & Crews, 2012; Coleman, Crews et al, 2011, 2014)
- **Resistance to Extinction of EtOH self-admin:** (Gass, Chandler et al. 2014)
- **Delayed context fear extinction** (Broadwater & Spear, 2013a)
- **Preseverative behavior:** modified H2O maze task (Acheson, Swartwelder, 2013).
- **Decreased spontaneous alternation:** (McClory & Spear)
- **Disinhibition of exploratory behavior:** (Elhers; Gass, Chandler et al, 2014)

AIE results in increased adult anxiety-like negative affect behavior

- **Light-dark box** (Pandey 2014; Slawecki, Ehlers et al; Vetreno, Crews, 2014)
- **Elevated Plus Maze** (Pandey 2014)
- **Open field test** (Coleman, Crews et al, 2011; 2014)
- **Social interaction test** (Varlinskiy, Spear, et al, 2014; 2015)
- **Porsolt swim test** (Slawecki and Ehlers, Ehlers et al, 2011)
AIE increases adult brain neuroimmune gene expression in orbital frontal cortex.

**Fig. 4 Crews-Vetreno Psychopharm.**
Neuroimmune Expression Correlates with HUMAN Age of Drinking Onset.

Earlier Age increased OFC HMGB1.


Grant et. al. NIAAA NESARC: 2001-2002. Earlier age of drinking onset is associated with increased risk of binge drinking, alcohol dependence, onset of dependence at a younger age, and adult injury after drinking.
Decreased Adult ChAT expression across brain following AIE (P80)
Acetylcholine inhibits microglia and is anti-inflammatory.

- Adolescent specific: Does not occur with adult binge ethanol
- Persists from P56 to at least P220
- Mimicked by LPS (1.0 mg/kg, i.p.) at P70

**Human Basal Forebrain**

**Medial Habenula**

**Basal Forebrain**

**Striatum**

**Acetylcholine inhibits microglia and is anti-inflammatory.**
Increased Risky decision making in adults after AIE. AIE exposure increased risky choice that correlated with decreased cholinergic function.

\[ r_{20} = -0.50, \ p < .05 \]

Wistar Rats

NADIA Components find AIE induces a persistent loss of adult hippocampal neurogenesis

Persistent loss of dorsal and ventral hippocampal neurogenesis

Vetreno and Crews, 2014

Ehlers CL, Liu W, Wills DN, Crews FT: Neuroscience, AIE induced Reductions in adult neurogenesis is significantly correlated with measures of disinhibition in the modified open-field conflict.

Vetreno, Crews et.al. 2015 AIE reduced adult neurogenesis associated with increased neuroimmune activation. -Prevention/Reversal by exercise (resilience!).

Pandey, Vetreno, Crews et.al., 2015, AIE reduced adult neurogenesis and decreased BDNF reversed by TSA (HDAC inhibitor).

Swartzwelder, Liu, Crews, AIE reduced neurogenesis and hippocampal function.

Occurs after AIE, but not CIE

Broadwater, Spear et.al. 2013
Epigenetic Drugs Reverse AIE-induced Adult Anxiety and Alcohol Drinking

**AIE increases adult alcohol-drinking**

* (p < 0.001)  
\( n = 6 \)

**TSA (HDAC inhibitor) reversal**

**Amygdala**
- DNMT 3b- increase
- GAD45 g-demethylation-decrease
- NPY and BDNF gene hypermethylated
- DNMT inhibitor- Reversal of anxiety and alcohol intake as NPY gene hypermethylation

**Amygdala/Hippocampus**
- HDAC2-Increase
- H3-K9 acetylation-Decrease
- BDNF –Arc expression-Decrease
- Neurogenesis-Decrease
- HDAC inhibitor-reversal of anxiety and alcohol intake as well as neurogenesis and histone acetylation of BDNF gene

Subhash Pandey
Persistent Alterations in Adult brain (MRI) following AIE:
Consistent with changes found in human alcoholics

MRI PUBLICATIONS
1. COLEMAN L., LIU W., OGUZ I., STYNER M., CREWS FT. Adolescent binge ethanol treatment alters adult brain and behavioral flexibility. Pharmacol Biochem Behav. 2014
2. EHLERS CL, OGUZ I, BUDIN F, WILLS DN, CREWS FT. Peri-adolescent ethanol vapor exposure produces reductions in hippocampal volume that are correlated with deficits in prepulse inhibition of the startle. Alcohol Clin Exp Res. 2013
Effects of Adolescent Ethanol on Adult Neuronal Activation (cFos) following ethanol challenge.

Liu and Crews, 2015
AIE supports evidence for an Adolescent Alcohol Syndrome: persistence of adolescent phenotypes in adulthood; e.g., low alcohol response, impulsivity, risky decisions, high anxiety, high alcohol drinking, as well as negative affect and deficits in behavioral flexibility.

AIE changes adult amygdala, hippocampus, frontal cortex, forebrain, nucleus accumbens, hypothalamus and other brain regions.

AIE increases neuroimmune gene expression across brain that are increased in human alcoholic brain and correlate with age of drinking onset.

AIE, but not identical adult treatment, causes a persistent decrease in ChAT neurons.

AIE, but not identical adult treatment, causes a persistent decrease in adult hippocampal neurogenesis.

AIE causes a persistent change in DNA methylation and histone acetylation as well as BDNF, ARC, NPY gene promoters in amygdala and hippocampus associated with increased ethanol drinking and anxiety.

AIE disrupts frontal cortical processing that contributes to loss of behavioral flexibility.

New Directions:

◆ Converge on robust translational quantitative Adolescent Alcohol Syndrome phenotypes through standardized procedures that replicate across components.

◆ Understand how AIE induced persistent changes in adult brain neuroimmune, ChAT, HDAC, etc. relate to changes in adult behavior.

◆ Determine AIE induced changes in neuronal networks (rsfMRI) and ethanol responses. AIE brain region specific vs whole brain changes. Synaptic spines, Excitatory/Inhibitory Balance.

◆ Prevention-Reversal experiments: Exercise, HDAC inhibitors, Anti-inflammatory, cholinesterase inhibitors, mGluR5, Adolescent Alcohol Syndrome. Therapeutic and Mechanistic studies.

◆ Female responses to AIE need to be fully determined.

◆ Early vs late AIE exposure phenotypes.
Increased HMGB1- TLRs and RAGE
Neuroimmune increases
Hyperglutamate –hyperexcitable

Activated glia release proinflammatory cytokines

Exercise + environment
CREB

Exercise
↑CREB-↓NFkB
↑ChAT
Growth-plasticity
BDNF-Arc-NPY
neuroimmune
happiness
↓H3-K9/DNMT
↑gene expression

Exercise

Alcohol
Stress
Endotoxin

TLR -neuroimmune
↓CREB-↑NFkB
↓ChAT-
↓BDNF-Arc-NPY
Anxiety

↑H3-K9/DNMT
down

gene expression

HMGB1

Neuronal-Glial
Signaling

HMGB1

IL1β-IL6

Cytokines

ChAT

BDNF

Arc

NPY

Anxiety

Devitalization-Malaize

Vigor

Infirmity

Endurance
Increased expression of HMGB1-TLR-RAGE in human alcoholic OFC (Crews, 2012)

Minocycline and naltrexone reduce ETOH neuroimmune activation and neurodegeneration. (Qin, 2012)


LPS, a TLR4, agonist induces long lasting increases in brain cytokines (Qin, 2007, 2011)

Chronic alcohol treatment of mice & rats increases expression of brain HMGB1, TLR4 and RAGE. (Crews, 2012)

Ethanol releases HMGB1 stimulating TLR4 receptors inducing neuroimmune genes-blocked by naltrexone. (Crews, et.al. 2012.)

Increased expression of HMGB1-TLR-RAGE in human alcoholic OFC (Crews, 2012)

Naltrexone blocks TLR4 receptors. (Hutchinson, 2008)

Minocycline reduces ethanol self-admin. (Syapin, 2011)

siRNA-TLR4 blocks ETOH operant responding (Liu, 2011)

Naltrexone treatment of mice increases alcohol drinking and preference for months. (Blednov et. al. 2011).

TLR4-KO mice do not show ethanol degeneration, neuroimmune gene induction, DA responses to ETOH. (Guerri et.al. 2006, 2010)

CD14-KO mice do not drink ethanol (Blednov et.al., 2011)

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Innate immune genes, cytokines, proteases, NADPH oxidases, iNOS, TLR, cytokine receptors, ECM

Rodent alcohol drinking transcriptome meta-analysis finds NFκB, TNF signaling genes. (Mulligan 2006)

Human alcoholic PFC increased immune (Liu, 2006)
### Neuroimmune Gene Expression Increased in Post-mortem Human Alcoholic Brain

#### Marker | Full Name | Brain Region and % of control | Citation
--- | --- | --- | ---
RAGE | Receptor for Advanced Glycation End-products | OFC: ↑ 198% | (Vetreno et al. 2013)
TLR2 | Toll-like receptor 2 | OFC: ↑ 208% | (Crews et al. 2013b)
TLR3 | Toll-like receptor 3 | OFC: ↑ 259% | (Crews et al. 2013b)
TLR4 | Toll-like receptor 4 | OFC: ↑ 356% | (Crews et al. 2013b)
HMGB1 | High-mobility group box 1 | OFC: ↑ 200% | (Crews et al. 2013b)
IL-1β | Interleukin-1β | HPC: ↑ 230% | (Zou and Crews 2012)
NALP1 | Inflammasome marker | HPC: ↑ 270% | (Zou and Crews 2012)
gp91phox | NADPH oxidase 2 | OFC: ↑ 305% | (Qin and Crews 2012b)
MCP-1 | CCL2 | VTA: ↑ 210%<br>SN: ↑ 300%<br>HPC: ↑ 250%<br>AMY: ↑ 300% | (He and Crews 2008)
MDK | Medkine 1 | PFC: ↑ 400% | (Flatscher-Bader et al. 2005)
Executive Dysfunction
- impulsivity
- compulsivity
- impaired decision making