Escalated aggression in rodent models: Novel brain mechanisms for alcohol

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Workshop on Mental Health and Violence
27 February 2014
1. Two thirds of all criminal violence is linked to alcohol

2. Discovery of alcohol action on ionotropic receptors in neuronal membranes (NMDA, GABA_A, glycine)

3. Basic pharmacology: biphasic dose-effect: alcohol-heightened aggression, alcohol withdrawal aggression

4. Individual differences for alcohol-heightened aggression

5. Alcohol-heightened aggression as a source of pleasure: brain dopamine

6. Alcohol-heightened aggression and the serotonin deficiency hypothesis

7. Alcohol-heightened aggression and GABA_A receptors

8. Alcohol-heightened aggression and glutamate receptors

9. Alcohol-heightened aggression and neuroendocrine factors: CRF, opioids, allopregnanolone
2/3 of all violent crime involves alcohol
• 86% of homicide offenders, 37% of assault offenders, 60% of sexual offenders, up to 57% of men and 27% of women involved in marital violence, and 13% of child abusers were drinking at the time of the offense. National Institute on Alcohol Abuse and Alcoholism, 1997

• Ca. 40% of violent crimes involve alcohol, according to the crime victim. Ca. 40% of criminal offenders report that they were using alcohol at the time of their offense. Bureau of Justice Statistics, US Department of Justice, 1998

• Among spousal abuse victims, 75% of the incidents were reported to have involved an offender who had been drinking. Bureau of Justice Statistics, US Department of Justice, 2006

• Almost one in four victims of violent crime report that the perpetrator had been drinking prior to committing the violence. Approximately 3 million crimes occur every year in which the offenders are perceived to have been under the influence of alcohol. Greenfeld, L. (1998) Alcohol and Crime: An analysis of national data on the prevalence of alcohol involvement in crime
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Theories of ethanol action at the cellular level

Evolution of concepts of ethanol action

Membrane theories of anesthesia

Meyer-Overton, 1896-1901

Franks & Lieb, 1984

Luciferase

Protein theories

Lovinger et al; Hoffman et al, 1989

NMDA R

Allan & Harris; Suzdak et al; Ticku et al, 1986

GABA A R

Mihic et al 1997

GABA A/glycine "site"
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Alcohol-Enhanced Aggression in Rats

Alcohol Dose (g/kg p.o.)

<table>
<thead>
<tr>
<th>Dose (g/kg)</th>
<th>0.1</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
</tr>
</thead>
</table>

- **AHA** (n=44)
- **ASA** (n=23)

* * *


Alcohol Withdrawal Aggression

Proportion of outbred mice showing aggression during withdrawal

Weeks of Intermittent Alcohol

1 4 8

0.0 0.2 0.4 0.6 0.8 1.0

(31) (24) (21)

H2O drinkers

Attack Bites

Memantine (mg/kg, i.p.)

VEH

3 5 10 30

* #

8 weeks EtOH
8 weeks H2O
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Effect of 1.0 g/kg Ethanol on Fighting in Individual Mice

Alcohol and Aggression: Individual Differences

Gavage
31% AHA, 59% ANA, 10% ASA

Operant Self-Administered
37% AHA, 62% ANA, <1% ASA

Median split
Upper/lower third
Outlier
Alcohol-heightened aggression (AHA)

H2O

EtOH

Hind 19.3%

Torso 72.3%

Head 53.7%

Tail 32.0%

Abdomen
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“Aggression not only produces dangers which must be avoided, but pleasures which can be enjoyed”

John Paul Scott
Aggression (1958)
Male aggression in rodent experimental model with real-time neurochemical assays
Experimental protocol for daily aggressive episodes

Assays in the absence of aggression
11th Day: No aggressive episode

Ferrari et al. 2003
Functional identification of an aggression locus in the mouse hypothalamus

Dayu Lin¹,², Maureen P. Boyle³, Piotr Dollar⁴, Hyosang Lee¹, E. S. Lein³, Pietro Perona⁴ & David J. Anderson¹,²

Electrical stimulation of certain hypothalamic regions in cats and rodents can elicit attack behaviour, but the exact location of relevant cells within these regions, their requirement for naturally occurring aggression and their relationship to mating circuits have not been clear. Genetic methods for neural circuit manipulation in mice provide a potentially powerful approach to this problem, but brain-stimulation-evoked aggression has never been demonstrated in this species. Here we show that optogenetic, but not electrical, stimulation of neurons in the ventromedial hypothalamus, ventrolateral subdivision (VMHvl) causes male mice to attack both females and inanimate objects, as well as males. Pharmacogenetic silencing of VMHvl reversibly inhibits inter–male aggression. Immediate early gene analysis and single unit recordings from VMHvl during social interactions reveal overlapping but distinct neuronal subpopulations involved in fighting and mating. Neurons activated during attack are inhibited during mating, suggesting a potential neural substrate for competition between these opponent social behaviours.
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3 major classes of molecules for in vivo neuron perturbation

**TOX-GENETICS**
(long-term switches)
- stable long-term
- potent

**CHEMICAL-GENETICS**
(medium-term switches)
- inducible
- reversible

**OPTO-GENETICS**
- rapidly inducible
- reversible

Kim, Dymecki, 2009
Armbruster, Roth, 2007
Ray, Dymecki, 2011
Boyden, Deisseroth, 2005
5-HT neurons are very heterogeneous

a) 5-HT neurons reside in the brainstem and project broadly

b) 5-HT neurons control many physiological, emotional and cognitive processes

breathing
thermoregulation
aggression
addiction
anxiety
mood
maternal care
learning and memory

relationship between anatomy and function?

Jensen, Dymecki and colleagues
Nat Neuro 2008

Niederkofer, Dymecki
Harvard Medical School

c) 5-HT neurons can be divided into subgroups based on gene expression

our approach:
link molecularly defined 5-HT subtypes to function
Intersectional genetic approach to ‘silence’ specific 5-HT neuron subtypes

A.  

RC::PFtox

\[
\begin{align*}
&\text{RC} \quad \text{loxP} \quad \text{STOP} \quad \text{loxP} \\
&\text{RC} \quad \text{loxP} \quad \text{STOP} \quad \text{loxP} \\
&\text{RC} \quad \text{loxP} \quad \text{FRT} \\
&\text{RC} \quad \text{loxP} \quad \text{FRT} \\
&\text{RC} \quad \text{loxP} \quad \text{FRT} \\
\end{align*}
\]

5-HT specific

\[
\begin{align*}
&\text{Pet1} \\
&\text{Flpe} \\
&\text{tissue specific} \\
&\text{Cre} \\
\end{align*}
\]

B.

C.  

neurotransmitter

\[
\begin{align*}
&\text{Tox cleaves Vamp2} \\
&\text{Syntaxin SNAP-25} \\
&\text{neurotransmitter release suppressed} \\
\end{align*}
\]

Kim, Dymecki and colleagues  
Neuron, 2009

Niederkofer, Dymecki  
Harvard Medical School
The Dymecki lab has identified molecular subtypes of 5-HT neurons involved in modulating aggression.

a) 5-HT neurons reside in the brainstem and project broadly.

b) 5-HT neurons control many physiological, emotional and cognitive processes.

- breathing
- thermoregulation
- aggression
- addiction
- anxiety
- mood
- maternal care
- learning and memory
- ....

C) 5-HT neurons can be divided into subgroups based on gene expression.

Our approach: link molecularly defined 5-HT subtypes to function.

Jensen, Dymecki and colleagues
Nat Neuro 2008

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Figure obtained from NIAAA website
Positive allosteric modulators of GABA_A receptors
Structure of a prototypical GABA\textsubscript{A} receptor
- heteropentamer
- 2 $\alpha$, 2 $\beta$, 1 $\gamma$
- ion pore: $\text{Cl}^-$
- Benzodiazepine binding site: $\alpha$ and $\gamma_2$

Immunohistochemical localization of diazepam-sensitive GABA\textsubscript{A} receptor subunits
Inactivation of dorsal raphe nucleus (DRN) by GABA agonists escalated aggression

- Mixture of GABA$_A$ and GABA$_B$ agonists (Bac-Mus): Baclofen 0.06 nmol + Muscimol 0.006 nmol
- Vehicle
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Alcohol-heightened Aggressors and NMDA Receptor

Baseline Attack Bites

Percent from Baseline Attack Bites

Memantine (mg/kg)

Ethanol

Water

Current Opinion in Pharmacology
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CRF-R1 antagonism and Alcohol-heightened Aggression in CFW Mice

**MTIP:**
- CRF-R1 antagonist
  - $K_i = 0.39$ nM

**CP-154526:**
- CRF-R1 antagonist
  - $K_i = 0.77$ nM

*From Schultz et al., 1996*

*From Gehlert et al., 2007*

**Attack Bites**

![Graph showing Frequency and % Baseline for Vehicle, Water, and 1 g/kg EtOH across different doses of CP-154,526 and MTIP.](chart)

**CP-154,526 dose (µg):**
- 0.3
- 0.6

**MTIP dose (µg):**
- veh
- 0.3

*Significant difference (*) compared to baseline and control groups.*
Excessive aggression as model of violence: a critical evaluation of current preclinical methods

Klaus A. Miczek, Sietse F. de Boer & Jozsef Haller

Psychopharmacology
ISSN 0033-3158
DOI 10.1007/s00213-013-3008-x
Characteristics of Escalated Aggressive Behavior

• Readily provoked (i.e. low threshold, short latency)
• High rate
• Intense and tissue-damaging
• Attacks lacking normal structure and context
• Failure to terminate “bursts”
• Oblivious to appeasement signals
• Insensitivity to long-term consequences

Miczek et al. 2013