Mechanistic underpinnings of cocaine addiction & the development of molecular therapeutics

Vidhya Kumaresan, PhD

Research Assistant Professor
Laboratory of Molecular Neurobiology

Laboratory of Molecular Neurobiology
Boston University
School of Medicine
Addiction represents a huge socio-economic and health burden on society

•~29 million adult users in the US (Koob and Volkow 2010, SAMHSA 2008)

•5.4 million transition to dependent users

•There is a lack of effective medications for the treatment of relapse

•Habitual and compulsive drug use occurs despite adverse consequences

•Chronic relapse is a major obstacle to developing a cure
Goal

- Understand molecular basis of persistent synaptic and intrinsic neuroplasticity
- Develop a platform for intervention
- Current focus:
  i) identification of cocaine-induced changes in regulation of AMPA glutamate receptor trafficking
  and
  ii) group1 mGluR mediated modulation of plasticity and its role in cocaine abuse
Repeated drug use generates neuropathological plasticity in the limbic circuit that persistently alters neurotransmission

Sesack and Grace, 2010

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The nucleus accumbens

- current focus is on the nucleus accumbens (NAc) shell and core
- translates motivation into action.
- critical nucleus for goal-directed behaviors
- medium spiny neurons (MSN) are projection neurons

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Cortico-striatal projection and glutamate

• Cortico-striatal projection is profoundly altered by repeated cocaine (Kalivas 2009)

• Extrasynaptic and synaptic glutamate levels decrease during abstinence (McFarland et al., 2003).

• mGluR2/3 activation reduced

• Cocaine re-exposure increases synaptic glutamate due (Baker et al. 2003)

• Disruption of glutamate homeostasis is critical for relapse (Kalivas 2009)
AMPAR receptor trafficking and PDZ domain proteins

- Classical pharmacological approaches: use of agonists and antagonists to alter entire population of glutamate receptors

- Novel molecular approach: use of cell-permeable molecular inhibitors of protein-protein interactions

Our Hypothesis:
Re-exposure to cocaine leads to glutamate-induced AMPA receptor internalization. This molecular process at least in part contributes to the precipitation of relapse of drug-seeking behavior

- receptor internalization – an LTD-like molecular mechanism described for hippocampal neurons in vitro (Malenka and Bear, 2004)
PDZ Domains

PDZ domains facilitate protein-protein interactions, specifically binding to the C-terminus of target proteins.

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PDZ trafficking proteins

Regulated endocytosis of GluR2-containing receptors

GluR2 containing AMPA receptor
PDZ domain
Acidic domain
BAR domain

Hanley 2008

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Cocaine-induced reinstatement

Animal model of drug-seeking behavior

- Lever Pressing for Cocaine
- Lever Pressing-Saline

Self-Administration

Addiction

Extinction

Rehab

Reinstatement

Craving/Relapse
Disrupting AMPA receptor subunit GluA2 interaction with PICK-1 with cell-permeable peptide *in vivo* attenuates primed reinstatement

Disrupting AMPA receptor subunit GluA2 interaction with PICK-1 with cell-permeable peptide in vivo minimizes reinstatement

Glutamate Receptor-PDZ domain interaction

Increased phosphorylation of GluA2 at the Ser 880 residue is observed in the shell after repeated cocaine.

Glutamate Receptor-PDZ domain interaction

Disrupting AMPA receptor subunit GluA2 interaction with GRIP in vivo minimizes cue-induced reinstatement behavior

Acute microinjections into NAc core attenuates cue-induced reinstatement
Glutamate Receptor-PDZ domain interaction

FITC- tagged cell permeable peptide is used to examine cell entry of peptide. Neuronal profiles filled with fluorescent peptide are seen


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Glutamate Receptor-PDZ domain interaction

Blocking GluA1 (old nomenclature is GluR1) insertion by over expression of C-terminus of GluA1 blunts primed reinstatement


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Group1 metabotropic glutamate receptors

- Gp1 mGluRs consist of mGluR 1 and mGluR5
- G-protein coupled receptors
- Regulate trafficking of AMPA receptors
- Play a critical role in learning and memory related plasticity in vitro. Role in vivo not well studied
Role of mGluR5 receptors

Primed-reinstatement is blunted by systemic MTEP, an mGluR5-specific negative modulator

Kumaresan, et al., Behav Brain Res (2009)
Role of mGluR5 receptors

Primed- and cued- reinstatement is blunted by systemic MPEP, an mGluR5-specific negative modulator

Kumaresan, et al., Behav Brain Res (2009)
Group1mGluRs in nucleus accumbens

Intra-accumbens (shell subregion) microinjections of MPEP attenuate cocaine-primed reinstatement

Kumaresan et al., Behav Brain Res (2009)

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Next, we examine protein-protein interactions in reinstatement of cocaine seeking. Our current focus is on Homer isoforms.
NMR of EVH1 Homer

$^{1}H,^{15}N$ HSQC spectrum validates ligand binding and determination of mode of binding.

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NMR of EVH1 Homer

A library of 1000 small molecules was screened against EVH1 of Homer. A number of hits were identified. Two small molecules disrupt the association of the C-terminal peptides.

These molecules are currently being examined for biological activity.

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Summary and Future directions

• Dysregulation of trafficking GluA2-containing receptors may be responsible for reinstatement

• Insertion of GluA1-containing receptors promotes reinstatement

• mGluR5 or mGluR1 antagonism blunts primed and cued reinstatement

• mGluR-Homer interaction is critical for reinstatement

• Further investigate role played by mGluR-Homer in extinction and relapse

Regulated endocytosis of GluR2-containing receptors

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Future Directions: Mechanistic & Translational Research

• screen small molecules/peptides that will serve both as probes and some to be developed as novel therapeutics.

• Explore gene association and linkage studies.

• Combine this approach with the ongoing drug discovery platform in the Laboratory of Molecular Neurobiology (LMN).
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