Identifying novel behavioral and protein biomarkers in addiction-related behaviors

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Addiction: a disorder of poor decision-making

- Taking drug in larger and longer amounts than intended
- Wanting to cut down or quit but not being able to
- Difficulty stopping or reducing drug use despite negative consequences

Ersche et al., 2008; Fillmore and Rush, 2003
Addiction: a disorder of poor decision-making

- Taking drug in larger and longer amounts than intended
- Wanting to cut down or quit but not being able to
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Jentsch et al., 2002; Schoenbaum et al., 2003
Decision-making: a risk-factor for addiction?

Cervantes et al. 2013
Decision-making as a biomarker of addiction?

Addiction

???

Poor decision-making

???
Decision-making in the rat: Probabilistic reversal learning

10%  70%  30%

p(Reward | NPx)

Trials

p(NPx)

Trial
Investigating decision-making in addiction pathophysiology

![Graph showing decision-making in addiction pathophysiology](image)
Investigating decision-making in addiction pathophysiology

**Graph Image**

- **Y-axis:** Number of infusions earned in a 6 h session
- **X-axis:** Self-administration session
- **Legend:**
  - Meth
  - Saline

**Diagram Details**

- The graph compares the number of infusions earned over time between self-administration with Meth and Saline.
- A significant increase is observed for Meth compared to Saline after the 8th session.

**Additional Text**

- **PRL assessments**
- **Self administration (saline or meth)**
Investigating decision-making in addiction pathophysiology

PRL assessments

Self administration (saline or meth)

PRL assessments
Decision-making as a biomarker of addiction?

Poor decision-making

???

Addiction
PRL performance predicts early-stages of meth use

**SALINE**

Total number of saline infusions earned vs. Number of reversals completed

$R^2 = 0.15; p = 0.27$

**METH**

Total number of meth infusions earned vs. Number of reversals completed

$R^2 = 0.67; p = 0.01$
Decision-making as a biomarker of addiction?
Self-administration of meth disrupts decision-making in PRL

**Time x group:** $F_{(1,16)}=4.81; p=0.04$

**Group:** $F_{(1,16)}=16.94; p=0.0008$

**Time:** $F_{(1,16)}=1.70; p=0.21$
Disruptions in PRL caused by experimenter administered meth

Groman et al., 2017
Decision-making: a biomarker of addiction

Poor decision-making

Addiction

Meth

**
Delineating decision-making processes

Reinforcement learning model (Barraclough et al., 2004):

If trial is rewarded:
\[ V(t) = \alpha \times V(t) + \Delta_1 \]

If trial is unrewarded:
\[ V(t) = \alpha \times V(t) + \Delta_2 \]

Unchosen actions:
\[ V(t) = \alpha \times V(t) \]

Three free parameters:
\[ \alpha = \text{forgetting rate} \]
- \(\uparrow\) retain action values longer

\[ \Delta_1 = \text{appetitive strength of rewards} \]
- \(\uparrow\) greater influence of rewards on choices

\[ \Delta_2 = \text{aversive strength of no reward} \]
- \(\downarrow\) greater influence of no rewards on choices
Decision-making processes that influence addiction vulnerability

???

Poor decision-making

Addiction
Appetitive strength of rewards predicts future drug use

\[ \alpha \] parameter

\[ \Delta_1 \] parameter

\[ \Delta_2 \] parameter

- \[ \alpha \] parameter estimate vs. total number of infusions earned: \( R^2 = 0.32; p = 0.14 \)
- \[ \Delta_1 \] parameter estimate vs. total number of infusions earned: \( R^2 = 0.43; p = 0.07 \)
- \[ \Delta_2 \] parameter estimate vs. total number of infusions earned: \( R^2 = 0.11; p = 0.4 \)
Decision-making process disrupted by drugs

Addiction

Poor decision-making

???
Effects of meth on decision-making

Saline

Meth

α parameter

Δ₁ parameter

Δ₂ parameter
Decision-making: a biomarker of addiction

Δ₁ parameter

Δ₂ parameter

Poor decision-making

Addiction
Decision-making and addiction interventions

**Prevention**
- Gene X
- Disruptions in Protein Y
  - Decision-making problems due to low $\Delta_1$ values
  - Heightened vulnerability to addiction

**Treatment**
- Chronic drug use
- Disruptions in Protein Z
  - Decision-making problems due to reductions in $\Delta_2$ values
  - Compulsive drug taking
Protein discovery with proteomics

**Sample collection**

**Sample preparation**

Protein digest (Trypsin) to create unique peptides for mass spec analysis

**Data analysis**

Compare protein measurements to decision-making phenotypes

**Data processing**

Matching peptides to protein sequences

**Processing protein digests**

Liquid chromatography for peptide separation
Tandem mass spec to identify and quantify each peptide
Identifying behavior-protein correlates

Behavior-protein correlates (N=16)
- PRL assessments
- Tissue collection (ventral striatum)
- Protein expression (LC-MS/MS)

Post-drug behavioral-protein correlates (N=18)
- PRL assessments
- Meth self-administration (6 h/day for 14 days)
- Tissue collection (ventral striatum)
- Protein expression (LC-MS/MS)
Data processing

Proteins must be detected in at least 25% of samples. Abundance lower threshold was set at $10^6$. 

2,833 proteins
Protein-behavior correlates

$p<0.05$; not corrected for multiple comparisons
Isolating protein targets involved in addiction vulnerability

Criterion:
- Correlates with $\Delta_1$ parameter in drug-naïve rats
- Correlates with $\Delta_1$ parameter in drug-exposed rats
- Is NOT disrupted in rats exposed to meth

[Diagram showing Venn diagram with sets Unaffected by meth, $\Delta_1$ drug naive, and $\Delta_1$ drug exposed, with numbers 2240, 300, 217, and 9]
# Protein targets involved in addiction vulnerability

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Function</th>
<th>Link to addiction?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ndufb10</td>
<td>NADH: ubiquinone oxidoreductase subunit B10</td>
<td>Subunit of mitochondrial membrane respiratory system</td>
<td>Altered in alcohol preferring rats (McClintick et al., 2017)</td>
</tr>
<tr>
<td>Dpp10</td>
<td>Inactive dipeptidyl peptidase 10</td>
<td>Promotes surface expression of KCND2</td>
<td></td>
</tr>
<tr>
<td>Setd7</td>
<td>Histone-lysine N-methyltransferase SETD7</td>
<td>Monomethylates Lys-4 of histone 3 (methylates nkkb and histones – wb hlk4); histone extraction; histone here repressive at lysine9</td>
<td>Genetic association with smoking behaviors (Thorgeirsson et al., 2010)</td>
</tr>
<tr>
<td>Sort1</td>
<td>Sortilin</td>
<td>Sorting receptor in the Golgi compartment</td>
<td>Low expression in high novelty seeking rats (Kabbaj et al., 2004)</td>
</tr>
<tr>
<td>Ryr2</td>
<td>Ryanodine receptor 2</td>
<td>Channel that mediates Ca2+ release from sarcoplasmic reticulum</td>
<td>Genetic association with impulsivity and gambling (Khadka et al., 2014; Lind et al., 2012)</td>
</tr>
<tr>
<td>Snx1</td>
<td>Sorting nexin-1</td>
<td>Intracellular trafficking</td>
<td>Reduced following meth CPP (Yang et al., 2008)</td>
</tr>
<tr>
<td>Gamt</td>
<td>Guanidinoacetate N-methyltransferase</td>
<td>Converts guanidoacetate to creatine</td>
<td>Reduced in alcohol dependent individuals (Sokolov et al., 2003)</td>
</tr>
<tr>
<td>Naa15</td>
<td>N(alpha)-acetyltransferase 15</td>
<td>Subunit of NatA complex; important for neuron growth</td>
<td>Gene expression disrupted in rats prenatally exposed to alcohol (Downing et al., 2012)</td>
</tr>
<tr>
<td>Atxn2l</td>
<td>Ataxin 2-like</td>
<td>Involved in stress granule and P-body formation</td>
<td>Genetic association with lifetime THC use (Pasman et al., 2018)</td>
</tr>
</tbody>
</table>
Addiction vulnerability proteins

- **Ryr2** (ryanonide receptor 2): forms channels that transport Ca2+
  - Target for heart disease (might be difficult to target systemically)

- **Snx1** (sorting nexin 1): involved in intracellular trafficking
  - Possible role in regulating GIRK channels

- **Atxn2l** (Ataxin-2 like): unknown function but part of the spinocerebellar ataxia family
  - Seems to be important in dopamine signaling (Atxn2 KO mice have lower D2 receptors)
Isolating behaviorally relevant protein targets altered by meth

Criterion:
• Correlates with the $\Delta_2$ parameter in drug-naïve rats
• Correlates with the $\Delta_2$ parameter in meth-exposed rats
• Disrupted in rats exposed to meth
Rab3B: a mechanism for drug-induced impairments?
Ras-related protein (Rab-3b)

- Monoameric GTPase protein enriched in synaptic vesicles
  - Involved in synaptic transmission and vesicle trafficking

- Knock down of Rab3B in the hippocampus impairs inhibitory LTD and improves reversal learning (Tsetsenis et al., 2011)

- Overexpression of Rab3B protects DA neurons from 6-OHDA insults (Chung et al., 2009)
Future studies

Reduce addiction vulnerability

• Increase expression of Ryr2, Snx1, or Atxn2l (via viral techniques)
  – Improve decision-making $\rightarrow$ reduce drug taking

Restore decision-making

• Reduce Rab3B expression
  – Improve decision-making $\rightarrow$ reduced relapse-related behaviors
Summary

Addiction

Poor decision-making

Rab3B

Ndufb10, Setd7, Dpp10, Ryr2, Sort1, Gamt, Naa15, Snx1, Atxn2l
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