Identification of Novel Regulators of Cocaine Associated Memories

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Why Memory?
Why Memory?
Stages of Memory

Consolidation:
Establishment of stable long term memory
What Happens in the Brain?

Menard and Quirion, 2012

O’Boyle et al., 2004
Memory Interference

Menard and Quirion, 2012

COCAINE

Memory Retrieval

"Reactivation"

= Memory Weakening

Reconsolidation

Craving

Relapse
Memory Extinction

[Image of a dollar bill and a screen]

COCAIN ≠ COCAINE

Craving Relapse
Extinction in the Brain

Consolidation of New Extinction Memory

Menard and Quirion, 2012
Craving

Inhibit
Reconsolidation

NMDAR
ERK
Protein Synthesis

Enhance
Extinction

NMDAR
ERK
Protein Synthesis

Relapse
What’s the Solution?

Reconsolidation Selective

Bidirectionally Regulated

Extinction Selective
Animal Model

TRAINING

0 CUES
3 CUES
120 CUES

“RELAPSE” TEST
Relapse-Like Behavior

![Bar graph showing active lever presses (+SEM) for different conditions.](image-url)
Where in the Brain?

[Diagram showing various brain regions including AMY]
Phosphoproteomics: Experimental Design

TRAINING

Homogenize
Trypsin Digestion

0 CUES
3 CUES
120 CUES
Experimental Design

Discovery Phase:
Label Free Analysis

Validation Phase:
MRM Analysis
Common Signaling Events

Protein Synthesis
Regulatory Proteins

ERK2

Estimated Fold Change from Controls

Phosphopeptide
Selective Signaling Events

Phosphopeptide

- STX1A (2)
- STMN1 (2)
- GIT1 (1)
- GABR1
- DGKB
- CTNA1 (1)
- CKD18
- AKA12

Estimated Fold Change from Controls

- VIAAT
- TNIK
- SYT1
- SYPH
- STX1A (1)
- SRCN1 (4)
- SRCN1 (2)
- RP3A
- NBEA (1)
- KCC2A (1)
- IPP2
- GIT1 (2)
- FGF12
- CXA1 (1)
- CTNA1 (2)
- ARHG7
- ADCY9

Estimated Fold Change from Controls

Legend:
- Green: Extinction
- Red: Reconsolidation
Bidirectional Signaling Events

- Phospholipase C beta
- c-Jun N-terminal kinase 3
- Calcium-calmodulin dependent protein kinase 2 alpha
- GABAB subunit 2
- Connexin 43

Rich et al., J Neurosci, 2016
What about other brain regions?
The Nucleus Accumbens and Drug Memory

• ERK signaling in the nucleus accumbens is required for reconsolidation of a cocaine CPP memory. *Miller and Marshall, 2005*

• NMDA receptor signaling in the nucleus accumbens mediates extinction of a cocaine cue memory. *Torregrossa et al., 2013*
What happens to the accumbens phosphoproteome?

Discovery Phase: Label Free Analysis

3 Take Home Messages

Torregrossa et al, Psychopharmacol, 2016
1. No Bidirectional Signaling in the NAc

Torregrossa et al, Psychopharmacol, 2016
2. Some Selective Signaling in NAc

Torregrossa et al, Psychopharmacol, 2016
3. Extinction Resulted in more Selective Phosphorylation Changes than Reconsolidation (12 vs. 4)

BLA Plasticity Regulates Cocaine Memory Strength

NAc Translates Memory into Behavior

![Graph showing Active Lever Presses (±SEM) for 0 CS, 3 CS, and 120 CS during Reactivation and Extinction.](chart.png)
# Common Signaling Across Brain Regions

<table>
<thead>
<tr>
<th>Protein (gene name)</th>
<th>Phosphopeptide NAc</th>
<th>Phosphopeptide BLA</th>
<th>NAc Fold Change from Con</th>
<th>BLA Fold Change from Con</th>
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<tbody>
<tr>
<td>Gamma-aminobutyric acid type B receptor subunit 2 (Gabbr2)</td>
<td>DPIEDINpSPEHIQR</td>
<td>DPIEDINpSPEHIQR</td>
<td>1.35</td>
<td>1.23</td>
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<tr>
<td>Syntaxin-1A (Stx1a)</td>
<td>TAKDpSDDDDDVTVTVDRDR</td>
<td>TAKDpSDDDDDVTVTVDRDR</td>
<td>1.61</td>
<td>1.19</td>
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<td>Caskin-1 (Caskin1)</td>
<td>KVPLPGPGpSPEVK</td>
<td>KVPLPGPGpSPEVK</td>
<td>1.24</td>
<td>0.77</td>
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<td>Receptor-type tyrosine-protein phosphatase-like N (Ptprn)</td>
<td>AEDpSSEGHEEEVLGGHGEK</td>
<td>LPEEGGSpSRAEDSpSEGHEEEVLGGHGEK</td>
<td>0.4</td>
<td>1.27</td>
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<tr>
<td>Sodium channel protein type 2 subunit alpha (Scn2a)</td>
<td>GKEDEGpTPIKEDIITDK</td>
<td>RFSpSPHqpsSLLSIR</td>
<td>1.16</td>
<td>1.12</td>
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<tr>
<td>SRC kinase signaling inhibitor 1 (Sric1)</td>
<td>RGpSDELTVPR</td>
<td>DSGSSSVFAEpSPGGK</td>
<td>1.52</td>
<td>1.14</td>
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<tr>
<td></td>
<td>RGpSDELTVPR</td>
<td>RFpSVGLVHTSER</td>
<td>1.52</td>
<td>0.77</td>
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<tr>
<td>Stathmin (Stmn1)</td>
<td>DLpSLEEIQK</td>
<td>ESVPEFPlpSPPK</td>
<td>1.34</td>
<td>0.92</td>
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<td>DLpSLEEIQK</td>
<td>RAspGQAFELILpSPR</td>
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<td>Protein bassoon (Bsn)</td>
<td>pSLSDPKPlpSPTAEESAK</td>
<td>SPQVLYpSPVpSPLSPHR</td>
<td>1.64</td>
<td>1.25</td>
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<td>Misshapen-like kinase 1 (Mink1)</td>
<td>LDSpSPVLSPGNK</td>
<td>SDSVLPASHGHPQAGpSLE</td>
<td>1.76</td>
<td>1.13</td>
</tr>
<tr>
<td>SH3 and multiple ankyrin repeat domains protein 3 (Shank3)</td>
<td>SAPSDINLK</td>
<td>SRpSPpSPLPSPLPSGSPSAGPR</td>
<td>2.49</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Torregrossa et al, Psychopharmacol, 2016
What can we achieve using phosphoproteomics?

• Identify novel protein regulators of a disease, state, or process.
• Gain insight into the differential function of brain regions to a disease, state, or process.
• Compare regulators of different diseases, states, or processes.
• Identify targets for developing novel treatments, potentially by looking for common regulatory events across brain regions.
Thank You!

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