UNIVERSITY OF CALIFORNIA

Santa Barbara

Self-Administration Results in Dynamic Changes in DNA Methylation of the Dorsal Medial Prefrontal Cortex Throughout Forced Abstinence, and After Re-exposure to Cues

A dissertation submitted in partial satisfaction of the requirements

of the degree Doctor of Philosophy in Psychology

by

Kyle Lawrence Ploense

Committee in charge:

Professor Tod E. Kippin, Chair

Professor Karen K. Szumlinski

Professor Aaron Ettenberg

Professor Skirmantas Janusonis

January 2018

The dissertation of Kyle Lawrence Ploense is approved.

Aaron Ettenberg

Skirmantas Janusonis

Karen Szumlinski

Tod Kippin

January 2018

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my wife Katie Bainbridge for her endless support: emotionally, scientifically, and in the many genres of scifi/ fantasy realms that help keep me sane. Also, I'd like to thank my (and Katie's) fetus/ soon to be infant "Raspberry", for providing the necessary motivation to finish my thesis before her birth. I want to give special thanks to my advisor, Dr. Tod Kippin, and co-advisor Dr. Karen Szumlinski for their constant mentorship and assistance throughout the many years I've been in their labs and through the various projects I have worked on. They have continued to challenge me and aid my development as a student, researcher, and academic over the past few years, but have also been warm and accepting of me, making me feel like part of a big scientific family. Furthermore, I'd like to thank Drs. Osnat Ben-Shahar and Ben Reese for sparking my interest in research, teaching me techniques I've needed to succeed in research, and believing in me at the very beginning, before I even believed in myself or knew what I wanted out of life.

Also, thank you to my dissertation committee, Drs. Aaron Ettenberg, Skirmantas Janusonis, Karen Szumlinski, and Tod Kippin, for your reviews and advice on my research, availability, mentorship, and instruction in the various classes I have taken with all of you. I'd also like to thank you for the shared laboratory equipment and collaborative atmosphere that is so special at UCSB. It's been a long road, but I appreciate all the instruction each of you has passed on to me over the years.

Last, but certainly not least, I would like to thank my collaborators, fellow graduate students, technicians, and undergraduate research assistants who contributed significantly to my dissertation. Thank you Xiang Li, Wei Wei, Danay Baker-Andresen

and Professor Tim Bredy for teaching me about epigenetics and PCR. Thank you to Amanda Carr, Nick Woodward, Lana Bubalo, Gema Olivarria, Julia Adams, Rachel Bozadjian, and Serina Williams for helping me run all of the operant behavior crucial to my thesis. Thank you to Kaziya Lee, Kevin Lominac, Andrew Thompson, Chris Knight, Sierra Webb, Christina Shin, Chelsea Brown, and Mari Purpura for all the fun nights out and about that helped make grad school special.

Curriculum Vita of Kyle Lawrence Ploense

December 2017

Education:

B.S. Biopsychology, University of California, Santa Barbara, CA, 2010

Doctoral Candidate, University of California, Santa Barbara, CA, 2011-Present

Professional Organizations:

Society for Neuroscience

College on Problems of Drug Dependence

American Anatomical Association

American Society for Pharmacology and Experimental Therapeutics

International Behavioral Neuroscience Society

Research Positions:

Laboratory Technician, 2010-2011

University of California, Santa Barbara

Teaching Assistant, 2011-Present

University of California, Santa Barbara

Courses:

Neuroanatomy

Neuroendocrinology

Intro to Biopsychology Laboratory

Laboratory in Advanced Research Methods

Concepts in Biopsychology

Biological Basis of Psychology

Statistics

Teaching Associate, 2014-Present

University of California, Santa Barbara

Courses:

The Biological Basis of Psychology

Motivation

Neuroanatomy and Psychopharmacology

Publications:

1. Ben-Shahar, O. M., Szumlinski, K. K., Lominac, K. D., Cohen, A., Gordon, E., Ploense, K. L., Woodward, N. (2012). Extended access to cocaine self-administration results in reduced glutamate function within the medial prefrontal cortex. *Addiction Biology*, 17(4), 746-757.
2. Ploense, K. L., Kerstetter, K. A., Wade, M. A., Woodward, N. C., Maliniak, D., Reyes, M., … Kippin, T. E. (2013). Exposure to histone deacetylase inhibitors during Pavlovian conditioning enhances subsequent cue-induced reinstatement of operant behavior. *Behavioural Pharmacology*, 24(3), 164-171.

3. Ferguson, B. S., Hoggarth, D. A., Maliniak, D., Ploense, K., White, R. J., Woodward, N., ... & Soh, H. T. (2013). Real-Time, Aptamer-Based Tracking of Circulating Therapeutic Agents in Living Animals. *Science Translational Medicine*, 5(213), 213ra165-213ra165.
4. Ben-Shahar, O., Sacramento, A. D., Miller, B. W., Webb, S. M., Wroten, M. G., Silva, H. E., ... & Szumlinski, K. K. (2013). Deficits in ventromedial prefrontal cortex group 1 metabotropic glutamate receptor function mediate resistance to extinction during protracted withdrawal from an extensive history of cocaine self-administration. *The Journal of Neuroscience*, 33(2), 495-506a.
5. Kippin, T. E., Campbell, J. C., Ploense, K., Knight, C. P., & Bagley, J. (2015). Prenatal Stress and Adult Drug-Seeking Behavior: Interactions with Genes and Relation to Nondrug-Related Behavior. In *Perinatal Programming of Neurodevelopment* (pp. 75-100). Springer New York.
6. Ploense, K.L., Carr, A.E., Baker-Andresen, D., Li, X., Woodward, N., Kerstetter, K.A., Sun, Y.E., Bredy, T.W., Kippin, T.E. (2015). Prolonged-Access to Cocaine Induces Distinct Homer2 DNA Methylation and Transcriptional Profiles in the Dorsomedial Prefrontal Cortex. *Brain and Behavior* (Under Review)
7. Arroyo-Currás, N., Somerson J., Vieira P., Ploense K.L., Kippin T.E., Plaxco KW (2017). Real-time measurement of small molecules directly in awake, ambulatory animals. Proc Natl Acad Sci U S A.
8. Mage, P.L., Ferguson, B.S, Maliniak, D., Ploense, K.L., Eisenstein, M., Kippin, T.E., Soh, H.T. (2017). Closed-loop Control of Therapeutic Molecules in Live Animals. *Nature Bioengineering.*

9. Arroyo-Currás, N., Scida, K., Ploense, K.L., Kippin, T.E., Plaxco, K.W. (2017). High Surface Area Electrodes Generated via Electrochemical Roughening Improve the Signaling of Electrochemical Aptamer-Based Biosensors. *Analytical Chemistry.*
10. Bagley J.R., Adams J., Bozadjian R.V., Bubalo L., Ploense K.L., Kippin T.E. (2017). Estradiol increases choice of cocaine over food in male rats. *Physiol Behav.* [Epub ahead of print]
11. Ploense, K.L., Vieira, P., Bubalo, L., Olivarria, G., Carr, A.E., Szumlinski, K.K., Kippin, T.E. (In press). Contributions of prolonged contingent and non-contingent cocaine exposure to escalation of cocaine intake and glutamatergic gene expression. *Psychopharmacology.*
12. Arroyo-Curras, N., Dauphine-Ducharme, P., Ortega, G., Ploense, K.L., Kippin, T.E., Plaxco, K.W. (In press). Sub-second-resolved molecular measurements in the living body using chronoamperometrically interrogated aptamer-based sensors. *ACS Sensors.*

Presentations:

1. Ben-Shahar, O., Szumlinski, K.K., Lominac, K., Cohen, A., Gordon, E., Ploense, K.L., Rudy, N., Sacramento, A., Pagano, K., Nabhan, A. (2010). Escalated cocaine self-administration is paralleled by "normalization" of basal extracellular dopamine levels within the medial prefrontal cortex. Poster presented at the Society for Neuroscience in San Diego, CA.
2. Ben-Shahar, O., Sacramento, A., Caruana, A., Webb, S., Gordon, E., Ploense, K.L., Rudy, N., Kippin, T.E., Szumlinski, K.K. (2011). Manipulations of Ventral Prefrontal Cortex Group1 mGluRs Do Not Affect Incubation of Cue-Induced Reinstatement of

Cocaine-seeking Behavior. Poster presented at the College of Problems on Drug Dependence in Hollywood, FL.

3. Ploense, K.L., Campbell, J.C., Knight, C.P., Woodward, N., Szumlinski, K.K., Kippin, T.E. (2011). Dose response and gene-early environment interactions determine cocaine-seeking behavior in mice. Poster presented at the Society for Neuroscience in Washington, D.C.
4. Kippin, T.E., Campbell, J.C., Szumlinski, K.K., Knight, C.P., Ploense, K.L., Woodward, N. (2011). Prenatal Stress and Genetic Background Interact to Determine Cocaine-Seeking in Mice. Poster presented at the College of Problems on Drug Dependence in Hollywood, FL.
5. Ploense, K.L., Carr, A.E., Bredy, T.W., Sun, Y.E., Kippin, T.E. (2012). Prolonged- and limited-access to cocaine self-administration at various periods of abstinence induces distinct patterns of DNA methylation. Poster presented at the Society for Neuroscience in New Oreleans, LA.
6. Ploense, K.L., Carr, A.E., Maliniak, D., Campbell, J.C., Knight, C.P., Szumlinski, K.K., Kippin, T.E. (2012) Gene-early Environment Interactions Determine Cocaine-seeking Behavior in Mice: A Dose-response Analysis. Oral presentation at the College of Problems on Drug Dependence in Palm Springs, CA.
7. Ploense, K.L., Bredy, T.W., Kippin, T.E. (2013). Cocaine Self-Administration and Abstinence Dynamically Regulates DNA Methylation in the Prefrontal Cortex. Poster presentation at the Society of Biological Psychiatry in San Francisco, CA.

8. Ploense, K.L., Carr, A.E., Bredy, T.W., Sun, Y.E., Kippin, T.E. (2013) DNA Methylation is Altered With Extended Access to Cocaine During Abstinence. Oral Presentation at the College of Problems on Drug Dependence in San Diego, CA.
9. Ploense, K.L., Carr, A.E., Baker-Andresen, D., Woodward, N., Sun, Y.E., Bredy, T.W., Kippin, T.E. (2013). Dynamic changes in DNA methylation and RNA transcription after cocaine abstinence. Poster presentation at the Society for Neuroscience in San Diego, CA.
10. Ploense, K.L. Carr, A.E., Baker-Andresen, D., Woodward, N., Sun, Y.E., Bredy, T.W., Kippin, T.E. (2014). Cocaine self-administration results in a distinct epigenetic state in the dorsal medial prefrontal cortex. Poster presentation at the Society for Neuroscience in Washington, D.C.
11. Mage, P.L., Ferguson, B.S., Maliniak, D., Ploense, K.L., Kippin, T.E., Soh, H.T. "Real-time control of in vivo drug concentrations in live animals." Poster presented at: Biosensors 2014. 24th World Anniversary Congress on Biosensors; 2014 May 27-31; Melbourne, Australia.
12. Mage, P.L., Ferguson, B.S., Maliniak, D., Ploense, K.L., Kippin, T.E., Soh, H.T. "Real-time closed-loop control of circulating drug levels in live animals." Oral presentation at: 2014 Materials Research Society Fall Meeting; 2014 December 1-5; Boston, MA.
13. Ploense, K.L., Carr, A.E., Baker-Andresen, D., Woodward, N., Sun, Y.E., Bredy, T.W., Kippin, T.E. (2015). Extended Cocaine Access Results in a Distinct Epigenetic Alterations to the Homer2 Gene in the Dorsal Medial Prefrontal Cortex. Poster presentation at the Experimental Biology conference in Boston, MA.

14. Ploense, K.L., Vieira, P., Kippin, T.E. (2016) “Extended-Access and Yoked-Access to Cocaine Self-Administration Have Distinct Behavioral and Molecular Profiles.” Poster presentation at the Experimental Biology meeting in San Diego, CA.
15. Vieira, P., Arroyo-Curras, N., Ploense, K.L., Somerson, J., Plaxco, K.W., Kippin, T.E. (2016) Real-Time, Continuous Electrochemical Monitoring of Therapeutic Drugs In Vivo. Poster presentation at the Experimental Biology meeting in San Diego, CA.
16. Ploense, K.L., Kippin, T.E., Hammond, S., Stucky, G., Kudela, D. (2016) Polyphosphate-Conjugated Silica Nanoparticles Attenuate Bleeding after Tail Amputation. Poster presentation at the Experimental Biology meeting in San Diego, CA.
17. Ploense, K.L., Vieira, P., Bubalo, L., Olivarria, G., Carr, A.E., Kippin, T.E. (2016) “The role of behavioral contingency in excessive cocaine-induced escalation of intake.” Poster presentation at the International Behavioral Neuroscience Society in Budapest, Hungary.

Abstract

Similar to the pattern observed in people with substance abuse disorders, laboratory animals will exhibit escalation of cocaine intake when the drug is readily available and will exhibit increased drug-seeking behaviors after long periods of abstinence. Additionally, there are long term changes in neuron structure, receptor function, and neurotransmission associated with abstinence from cocaine in humans and animals. DNA methylation is an epigenetic modification to the DNA structure that mediates mRNA expression to confer different cell types, but has recently been implicated in learning and memory mechanisms. The long-term control that DNA methylation has over gene expression in animals makes it a prime candidate for controlling gene expression over the course of abstinence in animals with previous drug experience. Therefore, here, I investigated the contribution of behavioral contingency of cocaine administration on escalation of cocaine intake and re-exposure to cocaine cues as well as DNA methylation and gene expression within the dorsal medial prefrontal cortex (dmPFC) in adult male Sprague-Dawley rats. I exposed rats to daily training for saline (1 h/ day) or cocaine (0.25 mg/kg/inf) in limited- (1 h access per day), prolonged- (6 h access per day), or limited + yoked-access (1 h contingent + 5 h non-contingent access per day) for 15 days. Rats were then put through forced abstinence for 1, 14, or 60 days, and then the dmPFC was dissected out. Saline- and prolonged-access rats were additionally separated into cue- and no cue- conditions after 60 days of abstinence, where cue rats were re-exposed to the operant chamber without cocaine delivery for 2 h. These studies led to 4 main findings. 1) cocaine contingency affects mRNA expression for glutamatergic genes, 2) DNA methylation changes dynamically throughout abstinence, 3) re-exposure to cocaine cues

rapidly alters DNA methylation and mRNA expression, and 4) DNA methylation, hydroxymethylation, and transcription factor binding all contribute to altered mRNA expression.

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BAI, G., & HOFFMAN, P. W. (2009). FRONTIERS IN NEUROSCIENCE__ ERROR! BOOKMARK NOT DEFINED.

TRANSCRIPTIONAL REGULATION OF NMDA RECEPTOR EXPRESSION. IN A. M. VAN DONGEN (ED.), BIOLOGY OF THE NMDA RECEPTOR. BOCA RATON (FL): CRC PRESS/TAYLOR & FRANCIS _____ ERROR! BOOKMARK NOT DEFINED.

TAYLOR & FRANCIS GROUP, LLC. _____ ERROR! BOOKMARK NOT DEFINED.

BAKER-ANDRESEN, D., FLAVELL, C. R., LI, X., & BREDY, T. W. (2013). ACTIVATION OF BDNF SIGNALING PREVENTS THE RETURN OF FEAR IN FEMALE MICE. LEARN MEM, 20(5), 237-240. DOI:10.1101/LM.029520.112 _____ ERROR! BOOKMARK NOT DEFINED.

BAKER-ANDRESEN, D., ZHAO, Q., LI, X., JUPP, B., CHESWORTH, R., LAWRENCE, A. J., & BREDY, T. (2015). PERSISTENT VARIATIONS IN NEURONAL DNA METHYLATION FOLLOWING COCAINE SELF-ADMINISTRATION AND PROTRACTED ABSTINENCE IN MICE. NEUROEPIGENETICS, 4, 1-11. DOI:10.1016/J.NEPIG.2015.10.001 ERROR! BOOKMARK NOT DEFINED.

BASTLE, R. M., KUFAHL, P. R., TURK, M. N., WEBER, S. M., PENTKOWSKI, N. S., THIEL, K. J., & NEISEWANDER, J. L. (2012). NOVEL CUES REINSTATE COCAINE-SEEKING BEHAVIOR AND INDUCE FOS PROTEIN EXPRESSION AS EFFECTIVELY AS CONDITIONED CUES. NEUROPSYCHOPHARMACOLOGY, 37(9), 2109-2120. DOI:10.1038/NPP.2012.60 _____ ERROR! BOOKMARK NOT DEFINED.

BEAR, M. F. (1996). A SYNAPTIC BASIS FOR MEMORY STORAGE IN THE CEREBRAL CORTEX. PROC NATL ACAD SCI U S A, 93(24), 13453-13459. _ ERROR! BOOKMARK NOT DEFINED.

BELIN, D., BALADO, E., PIAZZA, P. V., & DEROCHE-GAMONET, V. (2009). PATTERN OF INTAKE AND DRUG CRAVING PREDICT THE DEVELOPMENT OF COCAINE ADDICTION-LIKE BEHAVIOR IN RATS. BIOL PSYCHIATRY, 65(10), 863-868. DOI:10.1016/J.BIOPSYCH.2008.05.031 _____ ERROR! BOOKMARK NOT DEFINED.

BEN-SHAHAR, O., AHMED, S. H., KOOB, G. F., & ETTENBERG, A. (2004). THE TRANSITION FROM CONTROLLED TO COMPULSIVE DRUG USE IS ASSOCIATED WITH A LOSS OF SENSITIZATION. BRAIN RES, 995(1), 46-54. _____ ERROR! BOOKMARK NOT DEFINED.

BEN-SHAHAR, O., MOSCARELLO, J., JACOB, B., ROARTY, M., & ETTENBERG, A. (2005). PROLONGED DAILY EXPOSURE TO IV COCAINE RESULTS IN TOLERANCE TO ITS STIMULANT EFFECTS. PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR, 82(2), 411-416. DOI:10.1016/J.PBB.2005.09.013 _____ ERROR! BOOKMARK NOT DEFINED.

BEN-SHAHAR, O., POSTHUMUS, E., WALDROUP, S., & ETTENBERG, A. (2008). HEIGHTENED DRUG-SEEKING MOTIVATION FOLLOWING EXTENDED DAILY ACCESS TO SELF-ADMINISTERED COCAINE. PROG NEUROPSYCHOPHARMACOL BIOL PSYCHIATRY., 32(3), 7. _____ ERROR! BOOKMARK NOT DEFINED.

BEN-SHAHAR, O., SACRAMENTO, A., MILLER, B., WEBB, S., WROTEN, M., SILVA, H., . . . SZUMLINSKI, K. (2013). DEFICITS IN VENTROMEDIAL PREFRONTAL CORTEX GROUP 1 METABOTROPIC GLUTAMATE RECEPTOR FUNCTION MEDIATE RESISTANCE TO EXTINCTION DURING PROTRACTED WITHDRAWAL FROM AN EXTENSIVE HISTORY OF COCAINE SELF-ADMINISTRATION. J NEUROSCI, 33(2), 495-506A. DOI:10.1523/JNEUROSCI.3710-12.2013 _____ ERROR! BOOKMARK NOT DEFINED.

BEN-SHAHAR, O., SZUMLINSKI, K., LOMINAC, K., COHEN, A., GORDON, E., PLOENSE, K., . . . WOODWARD, N. (2012). EXTENDED ACCESS TO COCAINE SELF-ADMINISTRATION RESULTS IN REDUCED GLUTAMATE FUNCTION WITHIN THE MEDIAL PREFRONTAL CORTEX. ADDICT BIOL, 17(4), 746-757. DOI:10.1111/J.1369-1600.2011.00428.X_____ ERROR! BOOKMARK NOT DEFINED.

BEN SHAHAR, O., OBARA, I., ARY, A., MA, N., MANGIARDI, M., MEDINA, R., & SZUMLINSKI, K. (2009). EXTENDED DAILY ACCESS TO COCAINE RESULTS IN DISTINCT ALTERATIONS IN HOMER 1B/C AND NMDA RECEPTOR SUBUNIT EXPRESSION WITHIN THE MEDIAL PREFRONTAL CORTEX. SYNAPSE, 63, 10. DOI:10.1002/SYN.20640_____ ERROR! BOOKMARK NOT DEFINED.

BERGER, S. L. (2007). THE COMPLEX LANGUAGE OF CHROMATIN REGULATION DURING TRANSCRIPTION. NATURE, 447(7143), 407-412. DOI:10.1038/NATURE05915 _ ERROR! BOOKMARK NOT DEFINED.

BERGLIND, W. J., WHITFIELD, T. W., JR., LALUMIERE, R. T., KALIVAS, P. W., & MCGINTY, J. F. (2009). A SINGLE INTRA-PFC INFUSION OF BDNF PREVENTS COCAINE-INDUCED ALTERATIONS IN EXTRACELLULAR GLUTAMATE WITHIN THE NUCLEUS ACCUMBENS. J NEUROSCI, 29(12), 3715-3719. DOI:10.1523/JNEUROSCI.5457-08.2009 _ ERROR! BOOKMARK NOT DEFINED.

BERSTEN, D. C., WRIGHT, J. A., MCCARTHY, P. J., & WHITELAW, M. L. (2014). REGULATION OF THE NEURONAL TRANSCRIPTION FACTOR NPAS4 BY REST AND MICRORNAS. BIOCHIM BIOPHYS ACTA, 1839(1), 13-24. DOI:10.1016/J.BBAGRM.2013.11.004 _____ ERROR! BOOKMARK NOT DEFINED.

BIERGANS, S. D., CLAUDIANOS, C., REINHARD, J., & GALIZIA, C. G. (2016). DNA METHYLATION ADJUSTS THE SPECIFICITY OF MEMORIES DEPENDING ON THE LEARNING CONTEXT AND PROMOTES RELEARNING IN HONEYBEES. FRONT MOL NEUROSCI, 9, 82. DOI:10.3389/FNMOL.2016.00082 _____ ERROR! BOOKMARK NOT DEFINED.

BLANCO, E., PAVON, F. J., PALOMINO, A., LUQUE-ROJAS, M. J., SERRANO, A., RIVERA, P., . . . RODRIGUEZ DE FONSECA, F. (2014). COCAINE-INDUCED BEHAVIORAL SENSITIZATION IS ASSOCIATED WITH CHANGES IN THE EXPRESSION OF ENDOCANNABINOID AND GLUTAMATERGIC SIGNALING SYSTEMS IN THE MOUSE PREFRONTAL CORTEX. INT J NEUROPSYCHOPHARMACOL, 18(1). DOI:10.1093/IJNP/PYU024 _____ ERROR! BOOKMARK NOT DEFINED.

BOLLA, K., ERNST, M., KIEHL, K., MOURATIDIS, M., ELDRETH, D., CONTOREGGI, C., . . . LONDON, E. (2004). PREFRONTAL CORTICAL DYSFUNCTION IN ABSTINENT COCAINE ABUSERS. J NEUROPSYCHIATRY CLIN NEUROSCI, 16(4), 456-464. DOI:10.1176/APPI.NEUROPSYCH.16.4.456 _____ ERROR! BOOKMARK NOT DEFINED.

BORRELLI, E., NESTLER, E. J., ALLIS, C. D., & SASSONE-CORSI, P. (2008). DECODING THE EPIGENETIC LANGUAGE OF NEURONAL PLASTICITY. NEURON, 60(6), 961-974. DOI:10.1016/J.NEURON.2008.10.012_____ ERROR! BOOKMARK NOT DEFINED.

BREDY, T. W., & BARAD, M. (2008). THE HISTONE DEACETYLASE INHIBITOR VALPROIC ACID ENHANCES ACQUISITION, EXTINCTION, AND RECONSOLIDATION OF CONDITIONED FEAR. LEARN MEM, 15(1), 39-45. DOI:10.1101/LM.801108 __ ERROR! BOOKMARK NOT DEFINED.

BREDY, T. W., LIN, Q., WEI, W., BAKER-ANDRESEN, D., & MATTICK, J. S. (2011). MICRORNA REGULATION OF NEURAL PLASTICITY AND MEMORY. NEUROBIOL LEARN MEM, 96(1), 89-94. DOI:10.1016/J.NLM.2011.04.004 ERROR! BOOKMARK NOT DEFINED.

BREDY, T. W., WU, H., CREGO, C., ZELLHOEFER, J., SUN, Y. E., & BARAD, M. (2007). HISTONE MODIFICATIONS AROUND INDIVIDUAL BDNF GENE PROMOTERS IN PREFRONTAL CORTEX ARE ASSOCIATED WITH EXTINCTION OF CONDITIONED FEAR. LEARN MEM, 14(4), 268-276. DOI:10.1101/LM.500907 ERROR! BOOKMARK NOT DEFINED.

CARPENTER-HYLAND, E. P., & CHANDLER, L. J. (2006). HOMEOSTATIC PLASTICITY DURING ALCOHOL EXPOSURE PROMOTES ENLARGEMENT OF DENDRITIC SPINES. EUR J NEUROSCI, 24(12), 3496-3506. DOI:10.1111/J.1460-9568.2006.05247.X ERROR! BOOKMARK NOT DEFINED.

CASSEL, S., CAROUGE, D., GENSBURGER, C., ANGLARD, P., BURGUN, C., DIETRICH, J. B., . . . ZWILLER, J. (2006). FLUOXETINE AND COCAINE INDUCE THE EPIGENETIC FACTORS MECP2 AND MBD1 IN ADULT RAT BRAIN. MOL PHARMACOL, 70(2), 487-492. DOI:10.1124/MOL.106.022301 ERROR! BOOKMARK NOT DEFINED.

CATTS, V. S., DERMINIO, D. S., HAHN, C. G., & WEICKERT, C. S. (2015). POSTSYNAPTIC DENSITY LEVELS OF THE NMDA RECEPTOR NR1 SUBUNIT AND PSD-95 PROTEIN IN PREFRONTAL CORTEX FROM PEOPLE WITH SCHIZOPHRENIA. NPJ SCHIZOPHR, 1, 15037. DOI:10.1038/NPJSCHZ.2015.37 ERROR! BOOKMARK NOT DEFINED.

CERVO, L., & SAMANIN, R. (1995). EFFECTS OF DOPAMINERGIC AND GLUTAMATERGIC RECEPTOR ANTAGONISTS ON THE ACQUISITION AND EXPRESSION OF COCAINE CONDITIONING PLACE PREFERENCE. BRAIN RES, 673(2), 242-250. ERROR! BOOKMARK NOT DEFINED.

CHANDRASEKAR, V., & DREYER, J. L. (2009). MICRORNAS MIR-124, LET-7D AND MIR-181A REGULATE COCAINE-INDUCED PLASTICITY. MOL CELL NEUROSCI, 42(4), 350-362. DOI:10.1016/J.MCN.2009.08.009 ERROR! BOOKMARK NOT DEFINED.

CHANDRASEKAR, V., & DREYER, J. L. (2011). REGULATION OF MIR-124, LET-7D, AND MIR-181A IN THE ACCUMBENS AFFECTS THE EXPRESSION, EXTINCTION, AND REINSTATEMENT OF COCAINE-INDUCED CONDITIONED PLACE PREFERENCE. NEUROPSYCHOPHARMACOLOGY, 36(6), 1149-1164. DOI:10.1038/NPP.2010.250 ERROR! BOOKMARK NOT DEFINED.

COOVER, G. D., GOLDMAN, L., & LEVINE, S. (1971). PLASMA CORTICOSTERONE INCREASES PRODUCED BY EXTINCTION OF OPERANT BEHAVIOR IN RATS. PHYSIOL BEHAV, 6(3), 261-263. ERROR! BOOKMARK NOT DEFINED.

CRESPO, J. A., OLIVA, J. M., GHASEMZADEH, M. B., KALIVAS, P. W., & AMBROSIO, E. (2002). NEUROADAPTIVE CHANGES IN NMDAR1 GENE EXPRESSION AFTER EXTINCTION OF COCAINE SELF-ADMINISTRATION. ANN N Y ACAD SCI, 965, 78-91. ERROR! BOOKMARK NOT DEFINED.

DACKIS, C. A., & O'BRIEN, C. P. (2001). COCAINE DEPENDENCE: A DISEASE OF THE BRAIN'S REWARD CENTERS. J SUBST ABUSE TREAT, 21(3), 111-117. ERROR! BOOKMARK NOT DEFINED.

DAY, J. J., & SWEATT, J. D. (2010). DNA METHYLATION AND MEMORY FORMATION. NAT NEUROSCI, 13(11), 1319-1323. DOI:10.1038/NN.2666 ERROR! BOOKMARK NOT DEFINED.

DE BOER, S. F., DE BEUN, R., SLANGEN, J. L., & VAN DER GUGTEN, J. (1990). DYNAMICS OF PLASMA CATECHOLAMINE AND CORTICOSTERONE CONCENTRATIONS DURING REINFORCED AND EXTINGUISHED OPERANT BEHAVIOR IN RATS. PHYSIOL BEHAV, 47(4), 691-698. ERROR! BOOKMARK NOT DEFINED.

DEROCHE-GAMONET, V., BELIN, D., & PIAZZA, P. V. (2004). EVIDENCE FOR ADDICTION-LIKE BEHAVIOR IN THE RAT. SCIENCE, 305(5686), 1014-1017. DOI:10.1126/SCIENCE.1099020 ERROR! BOOKMARK NOT DEFINED.

DEROCHE-GAMONET, V., PIAT, F., LE MOAL, M., & PIAZZA, P. V. (2002). INFLUENCE OF CUE-CONDITIONING ON ACQUISITION, MAINTENANCE AND RELAPSE OF COCAINE INTRAVENOUS SELF-ADMINISTRATION. EUR J NEUROSCI, 15(8), 1363-1370. ERROR! BOOKMARK NOT DEFINED.

DOBI, A., SEABOLD, G. K., CHRISTENSEN, C. H., BOCK, R., & ALVAREZ, V. A. (2011). COCAINE-INDUCED PLASTICITY IN THE NUCLEUS ACCUMBENS IS CELL SPECIFIC AND DEVELOPS WITHOUT PROLONGED WITHDRAWAL. J NEUROSCI, 31(5), 1895-1904. DOI:10.1523/JNEUROSCI.5375-10.2011 ERROR! BOOKMARK NOT DEFINED.

DROUET, J. B., FAUVELLE, F., MAUNOIR-REGIMBAL, S., FIDIER, N., MAURY, R., PEINNEQUIN, A., . . . CANINI, F. (2015). DIFFERENCES IN PREFRONTAL CORTEX GABA/GLUTAMATE RATIO AFTER ACUTE RESTRAINT STRESS IN RATS ARE ASSOCIATED WITH SPECIFIC BEHAVIORAL AND NEUROBIOLOGICAL PATTERNS. NEUROSCIENCE, 285, 155-165. DOI:10.1016/J.NEUROSCIENCE.2014.10.058 ERROR! BOOKMARK NOT DEFINED.

DUCRET, E., PUAUD, M., LACOSTE, J., BELIN-RAUSCENT, A., FOUYSSAC, M., DUGAST, E., . . . BELIN, D. (2016). N-ACETYLCYSTEINE FACILITATES SELF-IMPOSED ABSTINENCE AFTER ESCALATION OF COCAINE INTAKE. BIOL PSYCHIATRY, 80(3), 226-234. DOI:10.1016/J.BIOPSYCH.2015.09.019 ERROR! BOOKMARK NOT DEFINED.

DUMITRIU, D., LAPLANT, Q., GROSSMAN, Y. S., DIAS, C., JANSSEN, W. G., RUSSO, S. J., . . . NESTLER, E. J. (2012). SUBREGIONAL, DENDRITIC COMPARTMENT, AND SPINE SUBTYPE SPECIFICITY IN COCAINE REGULATION OF DENDRITIC SPINES IN THE NUCLEUS ACCUMBENS. J NEUROSCI, 32(20), 6957-6966. DOI:10.1523/JNEUROSCI.5718-11.2012 ERROR! BOOKMARK NOT DEFINED.

DWORKIN, S. I., MIRKIS, S., & SMITH, J. E. (1995). RESPONSE-DEPENDENT VERSUS RESPONSE-INDEPENDENT PRESENTATION OF COCAINE: DIFFERENCES IN THE LETHAL EFFECTS OF THE DRUG. PSYCHOPHARMACOLOGY (BERL), 117(3), 262-266. ERROR! BOOKMARK NOT DEFINED.

EDWARDS, G. (1986). THE ALCOHOL DEPENDENCE SYNDROME: A CONCEPT AS STIMULUS TO ENQUIRY★. BRITISH JOURNAL OF ADDICTION, 81(2), 171-183. ERROR! BOOKMARK NOT DEFINED.

EDWARDS, S., & KOOB, G. F. (2013). ESCALATION OF DRUG SELF-ADMINISTRATION AS A HALLMARK OF PERSISTENT ADDICTION LIABILITY. BEHAV PHARMACOL, 24(5-6), 356-362. DOI:10.1097/FBP.0B013E3283644D15 ERROR! BOOKMARK NOT DEFINED.

EHRLICH, M., GAMA-SOSA, M. A., HUANG, L. H., MIDGETT, R. M., KUO, K. C., MCCUNE, R. A., & GEHRKE, C. (1982). AMOUNT AND DISTRIBUTION OF 5-METHYLCYTOSINE IN

HUMAN DNA FROM DIFFERENT TYPES OF TISSUES OF CELLS. NUCLEIC ACIDS RES, 10(8), 2709-2721. _____ ERROR! BOOKMARK NOT DEFINED.

EL-HUSSEINI, A. E., SCHNELL, E., CHETKOVICH, D. M., NICOLL, R. A., & BREDT, D. S. (2000). PSD-95 INVOLVEMENT IN MATURATION OF EXCITATORY SYNAPSES. SCIENCE, 290(5495), 1364-1368. _____ ERROR! BOOKMARK NOT DEFINED.

ELIAS, G. M., ELIAS, L. A., APOSTOLIDES, P. F., KRIEGSTEIN, A. R., & NICOLL, R. A. (2008). DIFFERENTIAL TRAFFICKING OF AMPA AND NMDA RECEPTORS BY SAP102 AND PSD-95 UNDERLIES SYNAPSE DEVELOPMENT. PROC NATL ACAD SCI U S A, 105(52), 20953-20958. DOI:10.1073/PNAS.0811025106_____ ERROR! BOOKMARK NOT DEFINED.

ELVIR, L., DUCLOT, F., WANG, Z., & KABBAJ, M. (2017). EPIGENETIC REGULATION OF MOTIVATED BEHAVIORS BY HISTONE DEACETYLASE INHIBITORS. NEUROSCI BIOBEHAV REV. DOI:10.1016/J.NEUBIOREV.2017.09.030 ____ ERROR! BOOKMARK NOT DEFINED.

ETTENBERG, A., RAVEN, M. A., DANLUCK, D. A., & NECESSARY, B. D. (1999). EVIDENCE FOR OPPONENT-PROCESS ACTIONS OF INTRAVENOUS COCAINE. PHARMACOL BIOCHEM BEHAV, 64(3), 507-512._____ ERROR! BOOKMARK NOT DEFINED.

EVERITT, B. J. (2017). DRUG CUES, CONDITIONED REINFORCEMENT, AND DRUG SEEKING: THE SEQUELAE OF A COLLABORATIVE VENTURE WITH ATHINA MARKOU. BIOL PSYCHIATRY. DOI:10.1016/J.BIOPSYCH.2017.09.013___ ERROR! BOOKMARK NOT DEFINED.

EVERITT, B. J., MORRIS, K. A., O'BRIEN, A., & ROBBINS, T. W. (1991). THE BASOLATERAL AMYGDALA-VENTRAL STRIATAL SYSTEM AND CONDITIONED PLACE PREFERENCE: FURTHER EVIDENCE OF LIMBIC-STRIATAL INTERACTIONS UNDERLYING REWARD-RELATED PROCESSES. NEUROSCIENCE, 42(1), 1-18. _____ ERROR! BOOKMARK NOT DEFINED.

FARTHING, C. R., FICZ, G., NG, R. K., CHAN, C. F., ANDREWS, S., DEAN, W., . . . REIK, W. (2008). GLOBAL MAPPING OF DNA METHYLATION IN MOUSE PROMOTERS REVEALS EPIGENETIC REPROGRAMMING OF PLURIPOTENCY GENES. PLOS GENET, 4(6), E1000116. DOI:10.1371/JOURNAL.PGEN.1000116 _____ ERROR! BOOKMARK NOT DEFINED.

FELTENSTEIN, M. W., & SEE, R. E. (2008). THE NEUROCIRCUITRY OF ADDICTION: AN OVERVIEW. BR J PHARMACOL, 154(2), 261-274. DOI:10.1038/BJP.2008.51__ ERROR! BOOKMARK NOT DEFINED.

FENG, J., SHAO, N., SZULWACH, K. E., VIALOU, V., HUYNH, J., ZHONG, C., . . . NESTLER, E. J. (2015). ROLE OF TET1 AND 5-HYDROXYMETHYLCYTOSINE IN COCAINE ACTION. NAT NEUROSCI, 18(4), 536-544. DOI:10.1038/NN.3976 _____ ERROR! BOOKMARK NOT DEFINED.

FERRARIO, C. R., GORNY, G., CROMBAG, H. S., LI, Y., KOLB, B., & ROBINSON, T. E. (2005). NEURAL AND BEHAVIORAL PLASTICITY ASSOCIATED WITH THE TRANSITION FROM CONTROLLED TO ESCALATED COCAINE USE. BIOLOGICAL PSYCHIATRY, 58(9), 751-759. DOI:10.1016/J.BIOPSYCH.2005.04.046_____ ERROR! BOOKMARK NOT DEFINED.

FEYISSA, A. M., CHANDRAN, A., STOCKMEIER, C. A., & KAROLEWICZ, B. (2009). REDUCED LEVELS OF NR2A AND NR2B SUBUNITS OF NMDA RECEPTOR AND PSD-95 IN THE PREFRONTAL CORTEX IN MAJOR DEPRESSION. PROG NEUROPSYCHOPHARMACOL BIOL PSYCHIATRY, 33(1), 70-75. DOI:10.1016/J.PNPBP.2008.10.005_____ ERROR! BOOKMARK NOT DEFINED.

FONTENEAU, M., FILLIOL, D., ANGLARD, P., BEFORT, K., ROMIEU, P., & ZWILLER, J. (2017). INHIBITION OF DNA METHYLTRANSFERASES REGULATES COCAINE SELF-ADMINISTRATION BY RATS: A GENOME-WIDE DNA METHYLATION STUDY. GENES BRAIN BEHAV, 16(3), 313-327. DOI:10.1111/GBB.12354 _____ ERROR! BOOKMARK NOT DEFINED.

FRANKFURT, M., SALAS-RAMIREZ, K., FRIEDMAN, E., & LUINE, V. (2011). COCAINE ALTERS DENDRITIC SPINE DENSITY IN CORTICAL AND SUBCORTICAL BRAIN REGIONS OF THE POSTPARTUM AND VIRGIN FEMALE RAT. SYNAPSE, 65(9), 955-961. DOI:10.1002/SYN.20918 _____ ERROR! BOOKMARK NOT DEFINED.

FREEMAN, W., PATEL, K., BRUCKLACHER, R., LULL, M., ERWIN, M., MORGAN, D., . . . VRANA, K. (2008). PERSISTENT ALTERATIONS IN MESOLIMBIC GENE EXPRESSION WITH ABSTINENCE FROM COCAINE SELF-ADMINISTRATION. NEUROPSYCHOPHARMACOLOGY, 33(8), 11. _____ ERROR! BOOKMARK NOT DEFINED.

FREEMAN, W. M., LULL, M. E., PATEL, K. M., BRUCKLACHER, R. M., MORGAN, D., ROBERTS, D. C., & VRANA, K. E. (2010). GENE EXPRESSION CHANGES IN THE MEDIAL PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS FOLLOWING ABSTINENCE FROM COCAINE SELF-ADMINISTRATION. BMC NEUROSCI, 11, 29. DOI:10.1186/1471-2202-11-29 _____ ERROR! BOOKMARK NOT DEFINED.

FUCHS, R. A., EVANS, K. A., LEDFORD, C. C., PARKER, M. P., CASE, J. M., MEHTA, R. H., & SEE, R. E. (2005). THE ROLE OF THE DORSOMEDIAL PREFRONTAL CORTEX, BASOLATERAL AMYGDALA, AND DORSAL HIPPOCAMPUS IN CONTEXTUAL REINSTATEMENT OF COCAINE SEEKING IN RATS. NEUROPSYCHOPHARMACOLOGY, 30(2), 296-309. DOI:10.1038/SJ.NPP.1300579 _____ ERROR! BOOKMARK NOT DEFINED.

GALICI, R., PECHNICK, R. N., POLAND, R. E., & FRANCE, C. P. (2000). COMPARISON OF NONCONTINGENT VERSUS CONTINGENT COCAINE ADMINISTRATION ON PLASMA CORTICOSTERONE LEVELS IN RATS. EUR J PHARMACOL, 387(1), 59-62. ERROR! BOOKMARK NOT DEFINED.

GANCARZ, A. M., ROBBLE, M. A., KAUSCH, M. A., LLOYD, D. R., & RICHARDS, J. B. (2012). ASSOCIATION BETWEEN LOCOMOTOR RESPONSE TO NOVELTY AND LIGHT REINFORCEMENT: SENSORY REINFORCEMENT AS A RODENT MODEL OF SENSATION SEEKING. BEHAV BRAIN RES, 230(2), 380-388. _____ ERROR! BOOKMARK NOT DEFINED.

GANCARZ, A. M., ROBBLE, M. A., KAUSCH, M. A., LLOYD, D. R., & RICHARDS, J. B. (2013). SENSORY REINFORCEMENT AS A PREDICTOR OF COCAINE AND WATER SELF-ADMINISTRATION IN RATS. PSYCHOPHARMACOLOGY (BERL), 226(2), 335-346. DOI:10.1007/S00213-012-2907-6 _____ ERROR! BOOKMARK NOT DEFINED.

GAO, X., JIA, M., ZHANG, Y., BREITLING, L. P., & BRENNER, H. (2015). DNA METHYLATION CHANGES OF WHOLE BLOOD CELLS IN RESPONSE TO ACTIVE SMOKING EXPOSURE IN ADULTS: A SYSTEMATIC REVIEW OF DNA METHYLATION STUDIES. CLIN EPIGENETICS, 7. DOI:10.1186/S13148-015-0148-3 _____ ERROR! BOOKMARK NOT DEFINED.

GAWIN, F., & KLEBER, H. (1986). ABSTINENCE SYMPTOMATOLOGY AND PSYCHIATRIC DIAGNOSIS IN COCAINE ABUSERS. CLINICAL OBSERVATIONS. ARCH GEN PSYCHIATRY, 43(2), 7. _____ ERROR! BOOKMARK NOT DEFINED.

GAWIN, F. H., KLEBER, H. D., BYCK, R., ROUNSAVILLE, B. J., KOSTEN, T. R., JATLOW, P. I., & MORGAN, C. (1989). DESIPRAMINE FACILITATION OF INITIAL COCAINE ABSTINENCE. ARCH GEN PSYCHIATRY, 46(2), 117-121. ____ ERROR! BOOKMARK NOT DEFINED.

GEORGE, O., & KOOB, G. F. (2010). INDIVIDUAL DIFFERENCES IN PREFRONTAL CORTEX FUNCTION AND THE TRANSITION FROM DRUG USE TO DRUG DEPENDENCE. NEUROSCI BIOBEHAV REV, 35(2), 232-247. DOI:10.1016/J.NEUBIOREV.2010.05.002 ERROR! BOOKMARK NOT DEFINED.

GEORGE, O., MANDYAM, C. D., WEE, S., & KOOB, G. F. (2008). EXTENDED ACCESS TO COCAINE SELF-ADMINISTRATION PRODUCES LONG-LASTING PREFRONTAL CORTEX-DEPENDENT WORKING MEMORY IMPAIRMENTS. NEUROPSYCHOPHARMACOLOGY, 33(10), 2474-2482. DOI:10.1038/SJ.NPP.1301626 _____ ERROR! BOOKMARK NOT DEFINED.

GERBER, G. J., & WISE, R. A. (1989). PHARMACOLOGICAL REGULATION OF INTRAVENOUS COCAINE AND HEROIN SELF-ADMINISTRATION IN RATS: A VARIABLE DOSE PARADIGM. PHARMACOL BIOCHEM BEHAV, 32(2), 527-531. __ ERROR! BOOKMARK NOT DEFINED.

GHASEMZADEH, M. B., MUELLER, C., & VASUDEVAN, P. (2009). BEHAVIORAL SENSITIZATION TO COCAINE IS ASSOCIATED WITH INCREASED GLUTAMATE RECEPTOR TRAFFICKING TO THE POSTSYNAPTIC DENSITY AFTER EXTENDED WITHDRAWAL PERIOD. NEUROSCIENCE, 159(1), 414-426. DOI:10.1016/J.NEUROSCIENCE.2008.10.027 _____ ERROR! BOOKMARK NOT DEFINED.

GHASEMZADEH, M. B., VASUDEVAN, P., GILES, C., PURGIANTO, A., SEUBERT, C., & MANTSCH, J. R. (2011). GLUTAMATERGIC PLASTICITY IN MEDIAL PREFRONTAL CORTEX AND VENTRAL TEGMENTAL AREA FOLLOWING EXTENDED-ACCESS COCAINE SELF-ADMINISTRATION. BRAIN RES, 1413, 60-71. DOI:10.1016/J.BRAINRES.2011.06.041 _____ ERROR! BOOKMARK NOT DEFINED.

GHASEMZADEH, M. B., VASUDEVAN, P., & MUELLER, C. (2009). LOCOMOTOR SENSITIZATION TO COCAINE IS ASSOCIATED WITH DISTINCT PATTERN OF GLUTAMATE RECEPTOR TRAFFICKING TO THE POSTSYNAPTIC DENSITY IN PREFRONTAL CORTEX: EARLY VERSUS LATE WITHDRAWAL EFFECTS. PHARMACOL BIOCHEM BEHAV, 92(3), 383-392. DOI:10.1016/J.PBB.2008.12.004 _____ ERROR! BOOKMARK NOT DEFINED.

GHASEMZADEH, M. B., VASUDEVAN, P., MUELLER, C., SEUBERT, C., & MANTSCH, J. R. (2009). REGION SPECIFIC ALTERATIONS IN GLUTAMATE RECEPTOR EXPRESSION AND SUBCELLULAR DISTRIBUTION FOLLOWING EXTINCTION OF COCAINE SELF-ADMINISTRATION. BRAIN RES. DOI:10.1016/J.BRAINRES.2009.01.047 ERROR! BOOKMARK NOT DEFINED.

GIPSON, C. D., KUPCHIK, Y. M., SHEN, H., REISSNER, K. J., THOMAS, C. A., & KALIVAS, P. W. (2013). RELAPSE INDUCED BY CUES PREDICTING COCAINE DEPENDS ON RAPID, TRANSIENT SYNAPTIC POTENTIATION. NEURON, 77(5), 867-872. DOI:10.1016/J.NEURON.2013.01.005_____ ERROR! BOOKMARK NOT DEFINED.

GLOBISCH, D., MÜNZEL, M., MÜLLER, M., MICHALAKIS, S., WAGNER, M., KOCH, S., . . . CARELL, T. (2010). TISSUE DISTRIBUTION OF 5-HYDROXYMETHYLCYTOSINE AND SEARCH FOR ACTIVE DEMETHYLATION INTERMEDIATES. PLOS ONE, 5(12), E15367. _____ ERROR! BOOKMARK NOT DEFINED.

GOEDERS, J. E., MURNANE, K. S., BANKS, M. L., & FANTEGROSSI, W. E. (2009). ESCALATION OF FOOD-MAINTAINED RESPONDING AND SENSITIVITY TO THE LOCOMOTOR STIMULANT EFFECTS OF COCAINE IN MICE. PHARMACOL BIOCHEM BEHAV, 93(1), 67-74. DOI:10.1016/J.PBB.2009.04.008 ERROR! BOOKMARK NOT DEFINED.

GONG, W., NEILL, D., & JUSTICE, J. B., JR. (1997). 6-HYDROXYDOPAMINE LESION OF VENTRAL PALLIDUM BLOCKS ACQUISITION OF PLACE PREFERENCE CONDITIONING TO COCAINE. BRAIN RES, 754(1-2), 103-112. ERROR! BOOKMARK NOT DEFINED.

GONG, Z., WANG, C., NIEH, J. C., & TAN, K. (2016). INHIBITING DNA METHYLATION ALTERS OLFACTORY EXTINCTION BUT NOT ACQUISITION LEARNING IN APIS CERANA AND APIS MELLIFERA. J INSECT PHYSIOL, 90, 43-48. DOI:10.1016/J.JINSPHYS.2016.05.007 ERROR! BOOKMARK NOT DEFINED.

GOULD, A. T., SACRAMENTO, A. D., WROTEN, M. G., MILLER, B. W., VON JONQUIERES, G., KLUGMANN, M., . . . SZUMLINSKI, K. K. (2015). COCAINE-ELICITED IMBALANCES IN VENTROMEDIAL PREFRONTAL CORTEX HOMER1 VERSUS HOMER2 EXPRESSION: IMPLICATIONS FOR RELAPSE. ADDICT BIOL, 20(1), 148-157. DOI:10.1111/ADB.12088 ERROR! BOOKMARK NOT DEFINED.

GRIMM, J. W., HOPE, B. T., WISE, R. A., & SHAHAM, Y. (2001). NEUROADAPTATION. INCUBATION OF COCAINE CRAVING AFTER WITHDRAWAL. NATURE, 412(6843), 141-142. DOI:10.1038/35084134 ERROR! BOOKMARK NOT DEFINED.

GRIMM, J. W., LU, L., HAYASHI, T., HOPE, B. T., SU, T. P., & SHAHAM, Y. (2003). TIME-DEPENDENT INCREASES IN BRAIN-DERIVED NEUROTROPHIC FACTOR PROTEIN LEVELS WITHIN THE MESOLIMBIC DOPAMINE SYSTEM AFTER WITHDRAWAL FROM COCAINE: IMPLICATIONS FOR INCUBATION OF COCAINE CRAVING. J NEUROSCI, 23(3), 742-747. ERROR! BOOKMARK NOT DEFINED.

GUO, M. L., XUE, B., JIN, D. Z., LIU, Z. G., FIBUCH, E. E., MAO, L. M., & WANG, J. Q. (2012). UPREGULATION OF NPAS4 PROTEIN EXPRESSION BY CHRONIC ADMINISTRATION OF AMPHETAMINE IN RAT NUCLEUS ACCUMBENS IN VIVO. NEUROSCI LETT, 528(2), 210-214. DOI:10.1016/J.NEULET.2012.07.048 ERROR! BOOKMARK NOT DEFINED.

HALDER, R., HENNION, M., VIDAL, R. O., SHOMRONI, O., RAHMAN, R. U., RAJPUT, A., . . . BONN, S. (2016). DNA METHYLATION CHANGES IN PLASTICITY GENES ACCOMPANY THE FORMATION AND MAINTENANCE OF MEMORY. NAT NEUROSCI, 19(1), 102-110. DOI:10.1038/NN.4194 ERROR! BOOKMARK NOT DEFINED.

HAO, Y., MARTIN-FARDON, R., & WEISS, F. (2010). BEHAVIORAL AND FUNCTIONAL EVIDENCE OF METABOTROPIC GLUTAMATE RECEPTOR 2/3 AND METABOTROPIC GLUTAMATE RECEPTOR 5 DYSREGULATION IN COCAINE-ESCALATED RATS: FACTOR IN THE TRANSITION TO DEPENDENCE. BIOLOGICAL PSYCHIATRY, 68(0350767C-96A0-5F8B-34FE-F9051C7B4CEB), 240-248. DOI:10.1016/J.BIOPSYCH.2010.02.011 ERROR! BOOKMARK NOT DEFINED.

HELLEMANS, J., MORTIER, G., DE PAEPE, A., SPELEMAN, F., & VANDESOMPELE, J. (2007). QBASE RELATIVE QUANTIFICATION FRAMEWORK AND SOFTWARE FOR MANAGEMENT AND AUTOMATED ANALYSIS OF REAL-TIME QUANTITATIVE PCR DATA. GENOME BIOL, 8(2), R19. DOI:10.1186/GB-2007-8-2-R19 ERROR! BOOKMARK NOT DEFINED.

HELLMAN, A., & CHESS, A. (2007). GENE BODY-SPECIFIC METHYLATION ON THE ACTIVE X CHROMOSOME. SCIENCE, 315(5815), 1141-1143. DOI:10.1126/SCIENCE.1136352 ERROR! BOOKMARK NOT DEFINED.

HEMBY, S. E., CO, C., KOVES, T. R., SMITH, J. E., & DWORKIN, S. I. (1997). DIFFERENCES IN EXTRACELLULAR DOPAMINE CONCENTRATIONS IN THE NUCLEUS ACCUMBENS DURING RESPONSE-DEPENDENT AND RESPONSE-INDEPENDENT COCAINE ADMINISTRATION IN THE RAT. PSYCHOPHARMACOLOGY (BERL), 133(1), 7-16. ERROR! BOOKMARK NOT DEFINED.

HEMBY, S. E., HORMAN, B., & TANG, W. (2005). DIFFERENTIAL REGULATION OF IONOTROPIC GLUTAMATE RECEPTOR SUBUNITS FOLLOWING COCAINE SELF-ADMINISTRATION. BRAIN RES, 1064(1-2), 75-82. DOI:10.1016/J.BRAINRES.2005.09.051 ERROR! BOOKMARK NOT DEFINED.

HEMBY, S. E., TANG, W., MULY, E. C., KUHAR, M. J., HOWELL, L., & MASH, D. C. (2005). COCAINE-INDUCED ALTERATIONS IN NUCLEUS ACCUMBENS IONOTROPIC GLUTAMATE RECEPTOR SUBUNITS IN HUMAN AND NON-HUMAN PRIMATES. J NEUROCHEM, 95(6), 1785-1793. DOI:10.1111/J.1471-4159.2005.03517.X ERROR! BOOKMARK NOT DEFINED.

HEUSNER, C. L., & PALMITER, R. D. (2005). EXPRESSION OF MUTANT NMDA RECEPTORS IN DOPAMINE D1 RECEPTOR-CONTAINING CELLS PREVENTS COCAINE SENSITIZATION AND DECREASES COCAINE PREFERENCE. J NEUROSCI, 25(28), 6651-6657. DOI:10.1523/JNEUROSCI.1474-05.2005 ERROR! BOOKMARK NOT DEFINED.

HOPF, F. W. (2017). DO SPECIFIC NMDA RECEPTOR SUBUNITS ACT AS GATEWAYS FOR ADDICTIVE BEHAVIORS? GENES BRAIN BEHAV, 16(1), 118-138. DOI:10.1111/GBB.12348 ERROR! BOOKMARK NOT DEFINED.

HUANG, C.-C., LIN, H.-J., & HSU, K.-S. (2006). REPEATED COCAINE ADMINISTRATION PROMOTES LONG-TERM POTENTIATION INDUCTION IN RAT MEDIAL PREFRONTAL CORTEX. CEREBRAL CORTEX, 17(8), 1877-1888. ERROR! BOOKMARK NOT DEFINED.

IACUC. (2011). GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS: EIGHTH EDITION: THE NATIONAL ACADEMIES PRESS. ERROR! BOOKMARK NOT DEFINED.

ISAAC, W. L., NONNEMAN, A. J., NEISEWANDER, J., LANDERS, T., & BARDO, M. T. (1989). PREFRONTAL CORTEX LESIONS DIFFERENTIALLY DISRUPT COCAINE-REINFORCED CONDITIONED PLACE PREFERENCE BUT NOT CONDITIONED TASTE AVERSION. BEHAV NEUROSCI, 103(2), 345-355. ERROR! BOOKMARK NOT DEFINED.

ITO, R., ROBBINS, T. W., & EVERITT, B. J. (2004). DIFFERENTIAL CONTROL OVER COCAINE-SEEKING BEHAVIOR BY NUCLEUS ACCUMBENS CORE AND SHELL. NAT NEUROSCI, 7(4), 389-397. DOI:10.1038/NN1217 ERROR! BOOKMARK NOT DEFINED.

ITO, S., D'ALESSIO, A. C., TARANOVA, O. V., HONG, K., SOWERS, L. C., & ZHANG, Y. (2010). ROLE OF TET PROTEINS IN 5MC TO 5HMC CONVERSION, ES-CELL SELF-RENEWAL AND INNER CELL MASS SPECIFICATION. NATURE, 466(7310), 1129-1133. DOI:10.1038/NATURE09303 ERROR! BOOKMARK NOT DEFINED.

IURLARO, M., FICZ, G., OXLEY, D., RAIBER, E. A., BACHMAN, M., BOOTH, M. J., . . . REIK, W. (2013). A SCREEN FOR HYDROXYMETHYLCYTOSINE AND FORMYLCYTOSINE BINDING PROTEINS SUGGESTS FUNCTIONS IN TRANSCRIPTION AND CHROMATIN

REGULATION. GENOME BIOL, 14(10), R119. DOI:10.1186/GB-2013-14-10-R119 _____ ERROR! BOOKMARK NOT DEFINED.

JAENISCH, R., & BIRD, A. (2003). EPIGENETIC REGULATION OF GENE EXPRESSION: HOW THE GENOME INTEGRATES INTRINSIC AND ENVIRONMENTAL SIGNALS. NAT GENET, 33 SUPPL, 245-254. DOI:10.1038/NG1089 _____ ERROR! BOOKMARK NOT DEFINED.

JEZIORSKA, D. M., MURRAY, R. J. S., DE GOBBI, M., GAENTZSCH, R., GARRICK, D., AYYUB, H., . . . TUFARELLI, C. (2017). DNA METHYLATION OF INTRAGENIC CPG ISLANDS DEPENDS ON THEIR TRANSCRIPTIONAL ACTIVITY DURING DIFFERENTIATION AND DISEASE. PROC NATL ACAD SCI U S A, 114(36), E7526-E7535. DOI:10.1073/PNAS.1703087114_____ ERROR! BOOKMARK NOT DEFINED.

JONES, P. A. (2012). FUNCTIONS OF DNA METHYLATION: ISLANDS, START SITES, GENE BODIES AND BEYOND. NAT REV GENET, 13(7), 484-492. DOI:10.1038/NRG3230 _____ ERROR! BOOKMARK NOT DEFINED.

JONES, P. L., VEENSTRA, G. J., WADE, P. A., VERMAAK, D., KASS, S. U., LANDSBERGER, N., . . . WOLFFE, A. P. (1998). METHYLATED DNA AND MECP2 RECRUIT HISTONE DEACETYLASE TO REPRESS TRANSCRIPTION. NAT GENET, 19(2), 187-191. DOI:10.1038/561 _____ ERROR! BOOKMARK NOT DEFINED.

KALIVAS, P. (2007). COCAINE AND AMPHETAMINE-LIKE PSYCHOSTIMULANTS: NEUROCIRCUITRY AND GLUTAMATE NEUROPLASTICITY. DIALOGUES IN CLINICAL NEUROSCIENCE, 9(E540C0C0-FB52-97CF-1A4D-FC8EC1F4BBBC), 389-486. ERROR! BOOKMARK NOT DEFINED.

KALIVAS, P. W. (2008). ADDICTION AS A PATHOLOGY IN PREFRONTAL CORTICAL REGULATION OF CORTICOSTRIATAL HABIT CIRCUITRY. NEUROTOX RES, 14(2-3), 185-189. DOI:10.1007/BF03033809_____ ERROR! BOOKMARK NOT DEFINED.

KALIVAS, P. W., MCFARLAND, K., BOWERS, S., SZUMLINSKI, K., XI, Z. X., & BAKER, D. (2003). GLUTAMATE TRANSMISSION AND ADDICTION TO COCAINE. ANN N Y ACAD SCI, 1003, 169-175. _____ ERROR! BOOKMARK NOT DEFINED.

KALIVAS, P. W., SZUMLINSKI, K. K., & WORLEY, P. (2004). HOMER2 GENE DELETION IN MICE PRODUCES A PHENOTYPE SIMILAR TO CHRONIC COCAINE TREATED RATS. NEUROTOX RES, 6(5), 385-387. _____ ERROR! BOOKMARK NOT DEFINED.

KALIVAS, P. W., VOLKOW, N., & SEAMANS, J. (2005). UNMANAGEABLE MOTIVATION IN ADDICTION: A PATHOLOGY IN PREFRONTAL-ACCUMBENS GLUTAMATE TRANSMISSION. NEURON, 45(5), 647-650. DOI:10.1016/J.NEURON.2005.02.005 _____ ERROR! BOOKMARK NOT DEFINED.

KAWAI, J., HIROSE, K., FUSHIKI, S., HIROTSUNE, S., OZAWA, N., HARA, A., . . . WATANABE, S. (1994). COMPARISON OF DNA METHYLATION PATTERNS AMONG MOUSE CELL LINES BY RESTRICTION LANDMARK GENOMIC SCANNING. MOL CELL BIOL, 14(11), 7421-7427. _____ ERROR! BOOKMARK NOT DEFINED.

KERSTETTER, K. A., AGUILAR, V. R., PARRISH, A. B., & KIPPIN, T. E. (2008). PROTRACTED TIME-DEPENDENT INCREASES IN COCAINE-SEEKING BEHAVIOR DURING COCAINE WITHDRAWAL IN FEMALE RELATIVE TO MALE RATS. PSYCHOPHARMACOLOGY (BERL), 198(1), 63-75. DOI:10.1007/S00213-008-1089-8 _____ ERROR! BOOKMARK NOT DEFINED.

KIM, E., & SHENG, M. (2004). PDZ DOMAIN PROTEINS OF SYNAPSES. NAT REV NEUROSCI, 5(10), 771-781. DOI:10.1038/NRN1517 _____ ERROR! BOOKMARK NOT DEFINED.

KIPPIN, T. E., FUCHS, R. A., & SEE, R. E. (2006). CONTRIBUTIONS OF PROLONGED CONTINGENT AND NONCONTINGENT COCAINE EXPOSURE TO ENHANCED REINSTATEMENT OF COCAINE SEEKING IN RATS. PSYCHOPHARMACOLOGY (BERL), 187(1), 60-67. DOI:10.1007/S00213-006-0386-3 _____ ERROR! BOOKMARK NOT DEFINED.

KIRKLAND HENRY, P., DAVIS, M., & HOWELL, L. L. (2009). EFFECTS OF COCAINE SELF-ADMINISTRATION HISTORY UNDER LIMITED AND EXTENDED ACCESS CONDITIONS ON IN VIVO STRIATAL DOPAMINE NEUROCHEMISTRY AND ACOUSTIC STARTLE IN RHESUS MONKEYS. PSYCHOPHARMACOLOGY (BERL), 205(2), 237-247. DOI:10.1007/S00213-009-1534-3 _____ ERROR! BOOKMARK NOT DEFINED.

KNACKSTEDT, L., & KALIVAS, P. (2007). EXTENDED ACCESS TO COCAINE SELF-ADMINISTRATION ENHANCES DRUG-PRIMED REINSTATEMENT BUT NOT BEHAVIORAL SENSITIZATION. J PHARMACOL EXP THER., 322(3), 7.ERROR! BOOKMARK NOT DEFINED.

KNACKSTEDT, L. A., MOUSSAWI, K., LALUMIERE, R., SCHWENDT, M., KLUGMANN, M., & KALIVAS, P. W. (2010). EXTINCTION TRAINING AFTER COCAINE SELF-ADMINISTRATION INDUCES GLUTAMATERGIC PLASTICITY TO INHIBIT COCAINE SEEKING. J NEUROSCI, 30(23), 7984-7992. DOI:10.1523/JNEUROSCI.1244-10.2010 _____ ERROR! BOOKMARK NOT DEFINED.

KOOB, G., & VOLKOW, N. (2009). NEUROCIRCUITRY OF ADDICTION. NEUROPSYCHOPHARMACOLOGY, 35(1), 217-238. DOI:10.1038/NPP.2009.110 _____ ERROR! BOOKMARK NOT DEFINED.

KOOB, G. F. (2004). ALLOSTATIC VIEW OF MOTIVATION: IMPLICATIONS FOR PSYCHOPATHOLOGY. NEBR SYMP MOTIV, 50, 1-18._____ ERROR! BOOKMARK NOT DEFINED.

KOOB, G. F., & VOLKOW, N. D. (2010). NEUROCIRCUITRY OF ADDICTION. NEUROPSYCHOPHARMACOLOGY, 35(1), 217-238. DOI:10.1038/NPP.2009.110 _____ ERROR! BOOKMARK NOT DEFINED.

KOSIK, K. S., & KRICHEVSKY, A. M. (2005). THE ELEGANCE OF THE MICRORNAS: A NEURONAL PERSPECTIVE. NEURON, 47(6), 779-782. DOI:10.1016/J.NEURON.2005.08.019 _____ ERROR! BOOKMARK NOT DEFINED.

KOVACS, K., LAJTHA, A., & SERSHEN, H. (2010). EFFECT OF NICOTINE AND COCAINE ON NEUROFILAMENTS AND RECEPTORS IN WHOLE BRAIN TISSUE AND SYNAPTONEUROSOME PREPARATIONS. BRAIN RES BULL, 82(1-2), 109-117. DOI:10.1016/J.BRAINRESBULL.2010.02.008 _____ ERROR! BOOKMARK NOT DEFINED.

KRAWCZYK, M., MASON, X., DEBACKER, J., SHARMA, R., NORMANDEAU, C. P., HAWKEN, E. R., . . . DUMONT, E. C. (2013). D1 DOPAMINE RECEPTOR-MEDIATED LTP AT GABA SYNAPSES ENCODES MOTIVATION TO SELF-ADMINISTER COCAINE IN RATS. J NEUROSCI, 33(29), 11960-11971. DOI:10.1523/JNEUROSCI.1784-13.2013 _ ERROR! BOOKMARK NOT DEFINED.

KRIAUCIONIS, S., & HEINTZ, N. (2009). THE NUCLEAR DNA BASE 5-HYDROXYMETHYLCYTOSINE IS PRESENT IN PURKINJE NEURONS AND THE BRAIN. SCIENCE, 324(5929), 929-930. DOI:10.1126/SCIENCE.1169786__ ERROR! BOOKMARK NOT DEFINED.

KUMAR, A., CHOI, K. H., RENTHAL, W., TSANKOVA, N. M., THEOBALD, D. E., TRUONG, H. T., . . . NESTLER, E. J. (2005). CHROMATIN REMODELING IS A KEY MECHANISM UNDERLYING COCAINE-INDUCED PLASTICITY IN STRIATUM. NEURON, 48(2), 303-314. DOI:10.1016/J.NEURON.2005.09.023_____ ERROR! BOOKMARK NOT DEFINED.

LAUNAY, J. M., DEL PINO, M., CHIRONI, G., CALLEBERT, J., PEOC'H, K., MEGNIEN, J. L., . . . RENDU, F. (2009). SMOKING INDUCES LONG-LASTING EFFECTS THROUGH A MONOAMINE-OXIDASE EPIGENETIC REGULATION. PLOS ONE, 4(11), E7959. DOI:10.1371/JOURNAL.PONE.0007959 _____ ERROR! BOOKMARK NOT DEFINED.

LAVIOLETTE, S., & VAN DER KOOY, D. (2004). THE NEUROBIOLOGY OF NICOTINE ADDICTION: BRIDGING THE GAP FROM MOLECULES TO BEHAVIOUR. NATURE REVIEWS. NEUROSCIENCE, 5(D5F9F78F-AB8A-DBB5-DA17-1BA091F0D853), 55-120. DOI:10.1038/NRN1298 _____ ERROR! BOOKMARK NOT DEFINED.

LECCA, D., CACCIAPAGLIA, F., VALENTINI, V., ACQUAS, E., & DI CHIARA, G. (2007). DIFFERENTIAL NEUROCHEMICAL AND BEHAVIORAL ADAPTATION TO COCAINE AFTER RESPONSE CONTINGENT AND NONCONTINGENT EXPOSURE IN THE RAT. PSYCHOPHARMACOLOGY (BERL), 191(3), 653-667. DOI:10.1007/S00213-006-0496-Y __ ERROR! BOOKMARK NOT DEFINED.

LEVINE, A. A., GUAN, Z., BARCO, A., XU, S., KANDEL, E. R., & SCHWARTZ, J. H. (2005). CREB-BINDING PROTEIN CONTROLS RESPONSE TO COCAINE BY ACETYLATING HISTONES AT THE FOSB PROMOTER IN THE MOUSE STRIATUM. PROC NATL ACAD SCI U S A, 102(52), 19186-19191. DOI:10.1073/PNAS.0509735102 ____ ERROR! BOOKMARK NOT DEFINED.

LI, X., BAKER-ANDRESEN, D., ZHAO, Q., MARSHALL, V., & BREDY, T. W. (2014). METHYL CPG BINDING DOMAIN ULTRA-SEQUENCING: A NOVEL METHOD FOR IDENTIFYING INTER-INDIVIDUAL AND CELL-TYPE-SPECIFIC VARIATION IN DNA METHYLATION. GENES BRAIN BEHAV, 13(7), 721-731. DOI:10.1111/GBB.12150 ERROR! BOOKMARK NOT DEFINED.

LI, X., WEI, W., ZHAO, Q. Y., WIDAGDO, J., BAKER-ANDRESEN, D., FLAVELL, C. R., . . . BREDY, T. W. (2014). NEOCORTICAL TET3-MEDIATED ACCUMULATION OF 5-HYDROXYMETHYLCYTOSINE PROMOTES RAPID BEHAVIORAL ADAPTATION. PROC NATL ACAD SCI U S A, 111(19), 7120-7125. DOI:10.1073/PNAS.1318906111 ___ ERROR! BOOKMARK NOT DEFINED.

LIN, Y., BLOODGOOD, B. L., HAUSER, J. L., LAPAN, A. D., KOON, A. C., KIM, T. K., . . . GREENBERG, M. E. (2008). ACTIVITY-DEPENDENT REGULATION OF INHIBITORY SYNAPSE DEVELOPMENT BY NPAS4. NATURE, 455(7217), 1198-1204. DOI:10.1038/NATURE07319 _____ ERROR! BOOKMARK NOT DEFINED.

LIU, C., SUN, X., WANG, Z., LE, Q., LIU, P., JIANG, C., . . . MA, L. (2017). RETRIEVAL-INDUCED UPREGULATION OF TET3 IN PYRAMIDAL NEURONS OF THE DORSAL HIPPOCAMPUS MEDIATES COCAINE-ASSOCIATED MEMORY RECONSOLIDATION. INT J NEUROPSYCHOPHARMACOL. DOI:10.1093/IJNP/PYX099 ___ ERROR! BOOKMARK NOT DEFINED.

LUIS, C., CANNELLA, N., SPANAGEL, R., & KOHR, G. (2017). PERSISTENT STRENGTHENING OF THE PREFRONTAL CORTEX - NUCLEUS ACCUMBENS PATHWAY DURING INCUBATION OF COCAINE-SEEKING BEHAVIOR. NEUROBIOL LEARN MEM, 138, 281-290. DOI:10.1016/J.NLM.2016.10.003_____ ERROR! BOOKMARK NOT DEFINED.

MA, Y. Y., HENLEY, S. M., TOLL, J., JENTSCH, J. D., EVANS, C. J., LEVINE, M. S., & CEPEDA, C. (2013). DRUG-PRIMED REINSTATEMENT OF COCAINE SEEKING IN MICE: INCREASED EXCITABILITY OF MEDIUM-SIZED SPINY NEURONS IN THE NUCLEUS ACCUMBENS. ASN NEURO, 5(4), 257-271. DOI:10.1042/AN20130015 ____ ERROR! BOOKMARK NOT DEFINED.

MADDOX, S. A., & SCHAFE, G. E. (2011). EPIGENETIC ALTERATIONS IN THE LATERAL AMYGDALA ARE REQUIRED FOR RECONSOLIDATION OF A PAVLOVIAN FEAR MEMORY. LEARN MEM, 18(9), 579-593. DOI:10.1101/LM.2243411 _____ ERROR! BOOKMARK NOT DEFINED.

MALOUSI, A., & KOUIDOU, S. (2012). DNA HYPERMETHYLATION OF ALTERNATIVELY SPLICED AND REPEAT SEQUENCES IN HUMANS. MOL GENET GENOMICS, 287(8), 631-642. DOI:10.1007/S00438-012-0703-Y _____ ERROR! BOOKMARK NOT DEFINED.

MANDT, B. H., COPENHAGEN, L. I., ZAHNISER, N. R., & ALLEN, R. M. (2015). ESCALATION OF COCAINE CONSUMPTION IN SHORT AND LONG ACCESS SELF-ADMINISTRATION PROCEDURES. DRUG ALCOHOL DEPEND, 149, 166-172. DOI:10.1016/J.DRUGALCDEP.2015.01.039 _____ ERROR! BOOKMARK NOT DEFINED.

MANTSCH, J., YUFEROV, V., MATHIEU-KIA, A., HO, A., & KREEK, M. (2004). EFFECTS OF EXTENDED ACCESS TO HIGH VERSUS LOW COCAINE DOSES ON SELF-ADMINISTRATION, COCAINE-INDUCED REINSTATEMENT AND BRAIN MRNA LEVELS IN RATS. PSYCHOPHARMACOLOGY (BERL), 175(1), 11. _____ ERROR! BOOKMARK NOT DEFINED.

MARKOU, A., & KOOB, G. F. (1991). POSTCOCAINE ANHEDONIA. AN ANIMAL MODEL OF COCAINE WITHDRAWAL. NEUROPSYCHOPHARMACOLOGY, 4(1), 17-26. ERROR! BOOKMARK NOT DEFINED.

MARKOU, A., & KOOB, G. F. (1992). BROMOCRIPTINE REVERSES THE ELEVATION IN INTRACRANIAL SELF-STIMULATION THRESHOLDS OBSERVED IN A RAT MODEL OF COCAINE WITHDRAWAL. NEUROPSYCHOPHARMACOLOGY, 7(3), 213-224. _____ ERROR! BOOKMARK NOT DEFINED.

MARTIN, T. A., JAYANTHI, S., MCCOY, M. T., BRANNOCK, C., LADENHEIM, B., GARRETT, T., . . . CADET, J. L. (2012). METHAMPHETAMINE CAUSES DIFFERENTIAL ALTERATIONS IN GENE EXPRESSION AND PATTERNS OF HISTONE ACETYLATION/HYPOACETYLATION IN THE RAT NUCLEUS ACCUMBENS. PLOS ONE, 7(3), E34236. DOI:10.1371/JOURNAL.PONE.0034236 _____ ERROR! BOOKMARK NOT DEFINED.

MASSART, R., BARNEA, R., DIKSHTEIN, Y., SUDERMAN, M., MEIR, O., HALLETT, M., . . . YADID, G. (2015). ROLE OF DNA METHYLATION IN THE NUCLEUS ACCUMBENS IN INCUBATION OF COCAINE CRAVING. J NEUROSCI, 35(21), 8042-8058. DOI:10.1523/JNEUROSCI.3053-14.2015 _____ ERROR! BOOKMARK NOT DEFINED.

MCFARLAND, K., LAPISH, C. C., & KALIVAS, P. W. (2003). PREFRONTAL GLUTAMATE RELEASE INTO THE CORE OF THE NUCLEUS ACCUMBENS MEDIATES COCAINE-INDUCED REINSTATEMENT OF DRUG-SEEKING BEHAVIOR. J NEUROSCI, 23(8), 3531-3537._____ ERROR! BOOKMARK NOT DEFINED.

MCGINTY, J. F., ZELEK-MOLIK, A., & SUN, W. L. (2014). COCAINE SELF-ADMINISTRATION CAUSES SIGNALING DEFICITS IN CORTICOSTRIATAL CIRCUITRY

THAT ARE REVERSED BY BDNF IN EARLY WITHDRAWAL. BRAIN RES. DOI:10.1016/J.BRAINRES.2014.09.050 ERROR! BOOKMARK NOT DEFINED.

MCGLINCHEY, E. M., JAMES, M. H., MAHLER, S. V., PANTAZIS, C., & ASTON-JONES, G. (2016). PRELIMBIC TO ACCUMBENS CORE PATHWAY IS RECRUITED IN A DOPAMINE-DEPENDENT MANNER TO DRIVE CUED REINSTATEMENT OF COCAINE SEEKING. J NEUROSCI, 36(33), 8700-8711. DOI:10.1523/JNEUROSCI.1291-15.2016 ERROR! BOOKMARK NOT DEFINED.

MCINTOSH, S., HOWELL, L., & HEMBY, S. E. (2013). DOPAMINERGIC DYSREGULATION IN PREFRONTAL CORTEX OF RHESUS MONKEYS FOLLOWING COCAINE SELF-ADMINISTRATION. FRONT PSYCHIATRY, 4, 88. DOI:10.3389/FPSYT.2013.00088 ERROR! BOOKMARK NOT DEFINED.

MEIL, W. M., & SEE, R. E. (1996). CONDITIONED CUED RECOVERY OF RESPONDING FOLLOWING PROLONGED WITHDRAWAL FROM SELF-ADMINISTERED COCAINE IN RATS: AN ANIMAL MODEL OF RELAPSE. BEHAV PHARMACOL, 7(8), 754-763. ERROR! BOOKMARK NOT DEFINED.

MIGUENS, M., DEL OLMO, N., HIGUERA-MATAS, A., TORRES, I., GARCIA-LECUMBERRI, C., & AMBROSIO, E. (2008). GLUTAMATE AND ASPARTATE LEVELS IN THE NUCLEUS ACCUMBENS DURING COCAINE SELF-ADMINISTRATION AND EXTINCTION: A TIME COURSE MICRODIALYSIS STUDY. PSYCHOPHARMACOLOGY (BERL), 196(2), 303-313. DOI:10.1007/S00213-007-0958-X ERROR! BOOKMARK NOT DEFINED.

MILLER, C. A., & SWEATT, J. D. (2007). COVALENT MODIFICATION OF DNA REGULATES MEMORY FORMATION. NEURON, 53(6), 857-869. DOI:10.1016/J.NEURON.2007.02.022 ERROR! BOOKMARK NOT DEFINED.

MOST, D., WORKMAN, E., & HARRIS, R. A. (2014). SYNAPTIC ADAPTATIONS BY ALCOHOL AND DRUGS OF ABUSE: CHANGES IN MICRORNA EXPRESSION AND MRNA REGULATION. FRONT MOL NEUROSCI, 7, 85. DOI:10.3389/FNMOL.2014.00085 ERROR! BOOKMARK NOT DEFINED.

MUTSCHLER, N. H., & MICZEK, K. A. (1998). WITHDRAWAL FROM A SELF-ADMINISTERED OR NON-CONTINGENT COCAINE BINGE: DIFFERENCES IN ULTRASONIC DISTRESS VOCALIZATIONS IN RATS. PSYCHOPHARMACOLOGY (BERL), 136(4), 402-408. ERROR! BOOKMARK NOT DEFINED.

NADER, M. A. (2016). ANIMAL MODELS FOR ADDICTION MEDICINE: FROM VULNERABLE PHENOTYPES TO ADDICTED INDIVIDUALS. PROG BRAIN RES, 224, 3-24. DOI:10.1016/BS.PBR.2015.07.012 ERROR! BOOKMARK NOT DEFINED.

NAKAMURA, M., GAO, S., OKAMURA, H., & NAKAHARA, D. (2011). INTRATHECAL COCAINE DELIVERY ENABLES LONG-ACCESS SELF-ADMINISTRATION WITH BINGE-LIKE BEHAVIOR IN MICE. PSYCHOPHARMACOLOGY (BERL), 213(1), 119-129. DOI:10.1007/S00213-010-2021-6 ERROR! BOOKMARK NOT DEFINED.

NERI, F., RAPELLI, S., KREPELOVA, A., INCARNATO, D., PARLATO, C., BASILE, G., . . . OLIVIERO, S. (2017). INTRAGENIC DNA METHYLATION PREVENTS SPURIOUS TRANSCRIPTION INITIATION. NATURE, 543(7643), 72-77. DOI:10.1038/NATURE21373 ERROR! BOOKMARK NOT DEFINED.

NESTLER E.J., H. S. E., & MALENKA R.C. (2009). MOLECULAR NEUROPHARMACOLOGY: A FOUNDATION FOR CLINICAL NEUROSCIENCE (2 ED.): MCGRAW HILL. _____ ERROR! BOOKMARK NOT DEFINED.

NESTLER, E. J. (2013). CELLULAR BASIS OF MEMORY FOR ADDICTION. DIALOGUES CLIN NEUROSCI, 15(4), 431-443. _____ ERROR! BOOKMARK NOT DEFINED.

NIDA. (2016). COCAINE. RETRIEVED FROM HTTPS://WWW.DRUGABUSE.GOV/PUBLICATIONS/RESEARCH-REPORTS/COCAINE ERROR! BOOKMARK NOT DEFINED.

NIELSEN, D. A., HUANG, W., HAMON, S. C., MAILI, L., WITKIN, B. M., FOX, R. G., . . . MOELLER, F. G. (2012). FORCED ABSTINENCE FROM COCAINE SELF-ADMINISTRATION IS ASSOCIATED WITH DNA METHYLATION CHANGES IN MYELIN GENES IN THE CORPUS CALLOSUM: A PRELIMINARY STUDY. FRONT PSYCHIATRY, 3, 60. DOI:10.3389/FPSYT.2012.00060 _____ ERROR! BOOKMARK NOT DEFINED.

NUMACHI, Y., YOSHIDA, S., YAMASHITA, M., FUJIYAMA, K., NAKA, M., MATSUOKA, H., . . . SORA, I. (2004). PSYCHOSTIMULANT ALTERS EXPRESSION OF DNA METHYLTRANSFERASE MRNA IN THE RAT BRAIN. ANN N Y ACAD SCI, 1025, 102-109. DOI:10.1196/ANNALS.1316.013 _____ ERROR! BOOKMARK NOT DEFINED.

OLSEN, C. M., CHILDS, D. S., STANWOOD, G. D., & WINDER, D. G. (2010). OPERANT SENSATION SEEKING REQUIRES METABOTROPIC GLUTAMATE RECEPTOR 5 (MGLUR5). PLOS ONE, 5(11), E15085. DOI:10.1371/JOURNAL.PONE.0015085 ERROR! BOOKMARK NOT DEFINED.

OLSEN, C. M., & WINDER, D. G. (2009). OPERANT SENSATION SEEKING ENGAGES SIMILAR NEURAL SUBSTRATES TO OPERANT DRUG SEEKING IN C57 MICE. NEUROPSYCHOPHARMACOLOGY, 34(7), 1685-1694. DOI:10.1038/NPP.2008.226 _____ ERROR! BOOKMARK NOT DEFINED.

OLSEN, C. M., & WINDER, D. G. (2010). OPERANT SENSATION SEEKING IN THE MOUSE. J VIS EXP(45). DOI:10.3791/2292 _____ ERROR! BOOKMARK NOT DEFINED.

OOE, N., SAITO, K., MIKAMI, N., NAKATUKA, I., & KANEKO, H. (2004). IDENTIFICATION OF A NOVEL BASIC HELIX-LOOP-HELIX-PAS FACTOR, NXF, REVEALS A SIM2 COMPETITIVE, POSITIVE REGULATORY ROLE IN DENDRITIC-CYTOSKELETON MODULATOR DREBRIN GENE EXPRESSION. MOL CELL BIOL, 24(2), 608-616._____ ERROR! BOOKMARK NOT DEFINED.

OTTO, S. J., MCCORKLE, S. R., HOVER, J., CONACO, C., HAN, J. J., IMPEY, S., . . . MANDEL, G. (2007). A NEW BINDING MOTIF FOR THE TRANSCRIPTIONAL REPRESSOR REST UNCOVERS LARGE GENE NETWORKS DEVOTED TO NEURONAL FUNCTIONS. J NEUROSCI, 27(25), 6729-6739. DOI:10.1523/JNEUROSCI.0091-07.2007 ___ ERROR! BOOKMARK NOT DEFINED.

OUACHIKH, O., DIEB, W., DURIF, F., & HAFIDI, A. (2014). ANTERIOR VENTRAL TEGMENTAL AREA DOPAMINERGIC NEURONS ARE NOT INVOLVED IN THE MOTIVATIONAL EFFECTS OF BROMOCRIPTINE, PRAMIPEXOLE AND COCAINE IN DRUG-FREE RATS. BEHAV BRAIN RES, 262, 1-7. DOI:10.1016/J.BBR.2013.12.021_____ ERROR! BOOKMARK NOT DEFINED.

PALAMARCHOUK, V., SMAGIN, G., & GOEDERS, N. E. (2009). SELF-ADMINISTERED AND PASSIVE COCAINE INFUSIONS PRODUCE DIFFERENT EFFECTS ON CORTICOSTERONE CONCENTRATIONS IN THE MEDIAL PREFRONTAL CORTEX (MPC) OF RATS. PHARMACOL BIOCHEM BEHAV, 94(1), 163-168. DOI:10.1016/J.PBB.2009.08.003 _____ ERROR! BOOKMARK NOT DEFINED.

PARVAZ, M. A., MOELLER, S. J., & GOLDSTEIN, R. Z. (2016). INCUBATION OF CUE-INDUCED CRAVING IN ADULTS ADDICTED TO COCAINE MEASURED BY ELECTROENCEPHALOGRAPHY. JAMA PSYCHIATRY, 73(11), 1127-1134. DOI:10.1001/JAMAPSYCHIATRY.2016.2181 _____ ERROR! BOOKMARK NOT DEFINED.

PASCOLI, V., TERRIER, J., ESPALLERGUES, J., VALJENT, E., O'CONNOR, E. C., & LÜSCHER, C. (2014). CONTRASTING FORMS OF COCAINE-EVOKED PLASTICITY CONTROL COMPONENTS OF RELAPSE. NATURE, 509(7501), 459-464. ERROR! BOOKMARK NOT DEFINED.

PATERSON, N. E., & MARKOU, A. (2003). INCREASED MOTIVATION FOR SELF-ADMINISTERED COCAINE AFTER ESCALATED COCAINE INTAKE. NEUROREPORT, 14(17), 2229-2232. DOI:10.1097/01.WNR.0000091685.94870.BA __ ERROR! BOOKMARK NOT DEFINED.

PATERSON, N. E., & MARKOU, A. (2004). PROLONGED NICOTINE DEPENDENCE ASSOCIATED WITH EXTENDED ACCESS TO NICOTINE SELF-ADMINISTRATION IN RATS. PSYCHOPHARMACOLOGY, 173(1), 64-72. DOI:10.1007/S00213-003-1692-7 _____ ERROR! BOOKMARK NOT DEFINED.

PAULSON, P. E., CAMP, D. M., & ROBINSON, T. E. (1991). TIME COURSE OF TRANSIENT BEHAVIORAL DEPRESSION AND PERSISTENT BEHAVIORAL SENSITIZATION IN RELATION TO REGIONAL BRAIN MONOAMINE CONCENTRATIONS DURING AMPHETAMINE WITHDRAWAL IN RATS. PSYCHOPHARMACOLOGY (BERL), 103(4), 480-492._____ ERROR! BOOKMARK NOT DEFINED.

PELTIER, R. L., GUERIN, G. F., DORAIRAJ, N., & GOEDERS, N. E. (2001). EFFECTS OF SALINE SUBSTITUTION ON RESPONDING AND PLASMA CORTICOSTERONE IN RATS TRAINED TO SELF-ADMINISTER DIFFERENT DOSES OF COCAINE. J PHARMACOL EXP THER, 299(1), 114-120._____ ERROR! BOOKMARK NOT DEFINED.

PEOPLES, L. L., KRAVITZ, A. V., & KARINE, G. (2010). FRONTIERS IN NEUROSCIENCE _____ ERROR! BOOKMARK NOT DEFINED.

APPLICATION OF CHRONIC EXTRACELLULAR RECORDING METHOD TO STUDIES OF COCAINE SELF-ADMINISTRATION: METHOD AND PROGRESS. IN C. M. KUHN & G. F. KOOB (EDS.), ADVANCES IN THE NEUROSCIENCE OF ADDICTION. BOCA RATON (FL): CRC PRESS/TAYLOR & FRANCIS _____ ERROR! BOOKMARK NOT DEFINED.

LLC._____ ERROR! BOOKMARK NOT DEFINED.

PERRY, J. L., MORGAN, A. D., ANKER, J. J., DESS, N. K., & CARROLL, M. E. (2006). ESCALATION OF I.V. COCAINE SELF-ADMINISTRATION AND REINSTATEMENT OF COCAINE-SEEKING BEHAVIOR IN RATS BRED FOR HIGH AND LOW SACCHARIN INTAKE. PSYCHOPHARMACOLOGY (BERL), 186(2), 235-245. DOI:10.1007/S00213-006-0371-X _____ ERROR! BOOKMARK NOT DEFINED.

PETERS, J., KALIVAS, P. W., & QUIRK, G. J. (2009). EXTINCTION CIRCUITS FOR FEAR AND ADDICTION OVERLAP IN PREFRONTAL CORTEX. LEARN MEM, 16(5), 279-288. DOI:10.1101/LM.1041309 ERROR! BOOKMARK NOT DEFINED.

PIECHOTA, M., KOROSTYNSKI, M., SOLECKI, W., GIERYK, A., SLEZAK, M., BILECKI, W., . . . PRZEWLOCKI, R. (2010). THE DISSECTION OF TRANSCRIPTIONAL MODULES REGULATED BY VARIOUS DRUGS OF ABUSE IN THE MOUSE STRIATUM. GENOME BIOL, 11(5), R48. DOI:10.1186/GB-2010-11-5-R48 ERROR! BOOKMARK NOT DEFINED.

PLOENSE, K. L., KERSTETTER, K. A., WADE, M. A., WOODWARD, N. C., MALINIAK, D., REYES, M., … KIPPIN, T. E. (2013). EXPOSURE TO HISTONE DEACETYLASE INHIBITORS DURING PAVLOVIAN CONDITIONING ENHANCES SUBSEQUENT CUE-INDUCED REINSTATEMENT OF OPERANT BEHAVIOR. BEHAVIOURAL PHARMACOLOGY, 24(3), 164-171. ERROR! BOOKMARK NOT DEFINED.

PLOENSE, K. L., VIEIRA, P. A., BUBALO, L., OLIVARIA, G., CARR, A. E., SZUMLINSKI, K. K., & KIPPIN, T. E. (IN PRESS). CONTRIBUTIONS OF PROLONGED CONTINGENT AND NON-CONTINGENT COCAINE EXPOSURE TO ESCALATION OF COCAINE INTAKE AND GLUTAMATERGIC GENE EXPRESSION. PSYCHOPHARMACOLOGY. ERROR! BOOKMARK NOT DEFINED.

PLOSKI, J., MONSEY, M., NGUYEN, T., DILEONE, R., & SCHAFE, G. (2011). THE NEURONAL PAS DOMAIN PROTEIN 4 (NPAS4) IS REQUIRED FOR NEW AND REACTIVATED FEAR MEMORIES. PLOS ONE, 6(8). ERROR! BOOKMARK NOT DEFINED.

PLOSKI, J. E., MONSEY, M. S., NGUYEN, T., DILEONE, R. J., & SCHAFE, G. E. (2011). THE NEURONAL PAS DOMAIN PROTEIN 4 (NPAS4) IS REQUIRED FOR NEW AND REACTIVATED FEAR MEMORIES. PLOS ONE, 6(8), E23760. DOI:10.1371/JOURNAL.PONE.0023760 ERROR! BOOKMARK NOT DEFINED.

POL BODETTO, S., CAROUGE, D., FONTENEAU, M., DIETRICH, J. B., ZWILLER, J., & ANGLARD, P. (2013). COCAINE REPRESSES PROTEIN PHOSPHATASE-1CBETA THROUGH DNA METHYLATION AND METHYL-CPG BINDING PROTEIN-2 RECRUITMENT IN ADULT RAT BRAIN. NEUROPHARMACOLOGY, 73, 31-40. DOI:10.1016/J.NEUROPHARM.2013.05.005 ERROR! BOOKMARK NOT DEFINED.

POMIERNY-CHAMIOLO, L., MISZKIEL, J., FRANKOWSKA, M., BYSTROWSKA, B., & FILIP, M. (2017). COCAINE SELF-ADMINISTRATION, EXTINCTION TRAINING AND DRUG-INDUCED RELAPSE CHANGE METABOTROPIC GLUTAMATE MGLU5 RECEPTORS EXPRESSION: EVIDENCE FROM RADIOLIGAND BINDING AND IMMUNOHISTOCHEMISTRY ASSAYS. BRAIN RES, 1655, 66-76. DOI:10.1016/J.BRAINRES.2016.11.014 ERROR! BOOKMARK NOT DEFINED.

PRAST, J. M., SCHARDL, A., SCHWARZER, C., DECHANT, G., SARIA, A., & ZERNIG, G. (2014). REACQUISITION OF COCAINE CONDITIONED PLACE PREFERENCE AND ITS INHIBITION BY PREVIOUS SOCIAL INTERACTION PREFERENTIALLY AFFECT D1-MEDIUM SPINY NEURONS IN THE ACCUMBENS CORRIDOR. FRONT BEHAV NEUROSCI, 8, 317. DOI:10.3389/FNBEH.2014.00317 ERROR! BOOKMARK NOT DEFINED.

RADLEY, J. J., ANDERSON, R. M., COSME, C. V., GLANZ, R. M., MILLER, M. C., ROMIG-MARTIN, S. A., & LALUMIERE, R. T. (2015). THE CONTINGENCY OF COCAINE ADMINISTRATION ACCOUNTS FOR STRUCTURAL AND FUNCTIONAL MEDIAL PREFRONTAL DEFICITS AND INCREASED ADRENOCORTICAL ACTIVATION. J

NEUROSCI, 35(34), 11897-11910. DOI:10.1523/JNEUROSCI.4961-14.2015 _ ERROR! BOOKMARK NOT DEFINED.

RAMAMOORTHI, K., FROPF, R., BELFORT, G. M., FITZMAURICE, H. L., MCKINNEY, R. M., NEVE, R. L., . . . LIN, Y. (2011). NPAS4 REGULATES A TRANSCRIPTIONAL PROGRAM IN CA3 REQUIRED FOR CONTEXTUAL MEMORY FORMATION. SCIENCE, 334(6063), 1669-1675. DOI:10.1126/SCIENCE.1208049 _____ ERROR! BOOKMARK NOT DEFINED.

RENTHAL, W., MAZE, I., KRISHNAN, V., COVINGTON, H. E., 3RD, XIAO, G., KUMAR, A., . . . NESTLER, E. J. (2007). HISTONE DEACETYLASE 5 EPIGENETICALLY CONTROLS BEHAVIORAL ADAPTATIONS TO CHRONIC EMOTIONAL STIMULI. NEURON, 56(3), 517-529. DOI:10.1016/J.NEURON.2007.09.032 _____ ERROR! BOOKMARK NOT DEFINED.

RENTHAL, W., & NESTLER, E. J. (2009). CHROMATIN REGULATION IN DRUG ADDICTION AND DEPRESSION. DIALOGUES CLIN NEUROSCI, 11(3), 257-268._____ ERROR! BOOKMARK NOT DEFINED.

ROBERTS, D. C. S., MORGAN, D., & LIU, Y. (2007). HOW TO MAKE A RAT ADDICTED TO COCAINE. PROGRESS IN NEURO-PSYCHOPHARMACOLOGY AND BIOLOGICAL PSYCHIATRY, 31(8), 1614-1624. DOI:HTTP://DX.DOI.ORG/10.1016/J.PNPBP.2007.08.028 ERROR! BOOKMARK NOT DEFINED.

ROBINSON, T. E., & KOLB, B. (2004). STRUCTURAL PLASTICITY ASSOCIATED WITH EXPOSURE TO DRUGS OF ABUSE. NEUROPHARMACOLOGY, 47, 33-46. DOI:10.1016/J.NEUROPHARM.2004.06.025 _____ ERROR! BOOKMARK NOT DEFINED.

ROCHE, K. W., STANDLEY, S., MCCALLUM, J., DUNE LY, C., EHLERS, M. D., & WENTHOLD, R. J. (2001). MOLECULAR DETERMINANTS OF NMDA RECEPTOR INTERNALIZATION. NAT NEUROSCI, 4(8), 794-802. DOI:10.1038/90498 ERROR! BOOKMARK NOT DEFINED.

ROMIEU, P., HOST, L., GOBAILLE, S., SANDNER, G., AUNIS, D., & ZWILLER, J. (2008). HISTONE DEACETYLASE INHIBITORS DECREASE COCAINE BUT NOT SUCROSE SELF-ADMINISTRATION IN RATS. J NEUROSCI, 28(38), 9342-9348. DOI:10.1523/JNEUROSCI.0379-08.2008_____ ERROR! BOOKMARK NOT DEFINED.

RUAN, H., & YAO, W.-D. (2017). COCAINE PROMOTES COINCIDENCE DETECTION AND LOWERS INDUCTION THRESHOLD DURING HEBBIAN ASSOCIATIVE SYNAPTIC POTENTIATION IN PREFRONTAL CORTEX. JOURNAL OF NEUROSCIENCE, 37(4), 986-997. _____ ERROR! BOOKMARK NOT DEFINED.

RUDENKO, A., DAWLATY, M. M., SEO, J., CHENG, A. W., MENG, J., LE, T., . . . TSAI, L. H. (2013). TET1 IS CRITICAL FOR NEURONAL ACTIVITY-REGULATED GENE EXPRESSION AND MEMORY EXTINCTION. NEURON, 79(6), 1109-1122. DOI:10.1016/J.NEURON.2013.08.003 _____ ERROR! BOOKMARK NOT DEFINED.

SADAKIERSKA-CHUDY, A., FRANKOWSKA, M., WYDRA, K., JASTRZEBSKA, J., MISZKIEL, J., & FILIP, M. (2017). INCREASED 5-HYDROXYMETHYLATION LEVELS IN THE HIPPOCAMPUS OF RAT EXTINGUISHED FROM COCAINE SELF-ADMINISTRATION. HIPPOCAMPUS, 27(7), 811-821. DOI:10.1002/HIPO.22733 _____ ERROR! BOOKMARK NOT DEFINED.

SCHILTZ, C. A., BREMER, Q. Z., LANDRY, C. F., & KELLEY, A. E. (2007). FOOD-ASSOCIATED CUES ALTER FOREBRAIN FUNCTIONAL CONNECTIVITY AS ASSESSED

WITH IMMEDIATE EARLY GENE AND PROENKEPHALIN EXPRESSION. BMC BIOL, 5, 16. DOI:10.1186/1741-7007-5-16 _____ ERROR! BOOKMARK NOT DEFINED.

SHEN, H. W., TODA, S., MOUSSAWI, K., BOUKNIGHT, A., ZAHM, D. S., & KALIVAS, P. W. (2009). ALTERED DENDRITIC SPINE PLASTICITY IN COCAINE-WITHDRAWN RATS. J NEUROSCI, 29(9), 2876-2884. DOI:10.1523/JNEUROSCI.5638-08.2009 ____ ERROR! BOOKMARK NOT DEFINED.

SHIN, C. B., SERCHIA, M. M., SHAHIN, J. R., RUPPERT-MAJER, M. A., KIPPIN, T. E., & SZUMLINSKI, K. K. (2016). INCUBATION OF COCAINE-CRAVING RELATES TO GLUTAMATE OVER-FLOW WITHIN VENTROMEDIAL PREFRONTAL CORTEX. NEUROPHARMACOLOGY, 102, 103-110. DOI:10.1016/J.NEUROPHARM.2015.10.038 ___ ERROR! BOOKMARK NOT DEFINED.

SHIN, C. B., TEMPLETON, T. J., CHIU, A. S., KIM, J., GABLE, E. S., VIEIRA, P. A., . . . SZUMLINSKI, K. K. (2017). ENDOGENOUS GLUTAMATE WITHIN THE PRELIMBIC AND INFRALIMBIC CORTICES REGULATES THE INCUBATION OF COCAINE-SEEKING IN RATS. NEUROPHARMACOLOGY, 128, 293-300. DOI:10.1016/J.NEUROPHARM.2017.10.024 _____ ERROR! BOOKMARK NOT DEFINED.

SHIRAISHI, Y., MIZUTANI, A., MIKOSHIBA, K., & FURUICHI, T. (2003). COINCIDENCE IN DENDRITIC CLUSTERING AND SYNAPTIC TARGETING OF HOMER PROTEINS AND NMDA RECEPTOR COMPLEX PROTEINS NR2B AND PSD95 DURING DEVELOPMENT OF CULTURED HIPPOCAMPAL NEURONS. MOL CELL NEUROSCI, 22(2), 188-201._____ ERROR! BOOKMARK NOT DEFINED.

SIEGEL, R. K. (1984). CHANGING PATTERNS OF COCAINE USE: LONGITUDINAL OBSERVATIONS, CONSEQUENCES, AND TREATMENT. NIDA RES MONOGR, 50, 92-110. _____ ERROR! BOOKMARK NOT DEFINED.

SMITH, G. A., ROCHA, E. M., MCLEAN, J. R., HAYES, M. A., IZEN, S. C., ISACSON, O., & HALLETT, P. J. (2014). PROGRESSIVE AXONAL TRANSPORT AND SYNAPTIC PROTEIN CHANGES CORRELATE WITH BEHAVIORAL AND NEUROPATHOLOGICAL ABNORMALITIES IN THE HETEROZYGOUS Q175 KI MOUSE MODEL OF HUNTINGTON'S DISEASE. HUM MOL GENET, 23(17), 4510-4527. DOI:10.1093/HMG/DDU166 ERROR! BOOKMARK NOT DEFINED.

SMITH, M. A., WARD, S. J., & ROBERTS, D. C. (2008). LESIONS OF THE DORSOMEDIAL FRONTAL CORTEX BLOCK SENSITIZATION TO THE POSITIVE-REINFORCING EFFECTS OF COCAINE. PHARMACOL BIOCHEM BEHAV, 88(3), 238-246. DOI:10.1016/J.PBB.2007.08.007 _____ ERROR! BOOKMARK NOT DEFINED.

SPANAGEL, R., & WEISS, F. (1999). THE DOPAMINE HYPOTHESIS OF REWARD: PAST AND CURRENT STATUS. TRENDS NEUROSCI, 1999(22), 7. _____ ERROR! BOOKMARK NOT DEFINED.

SPIEGEL, I., MARDINLY, A. R., GABEL, H. W., BAZINET, J. E., COUCH, C. H., TZENG, C. P., . . . GREENBERG, M. E. (2014). NPAS4 REGULATES EXCITATORY-INHIBITORY BALANCE WITHIN NEURAL CIRCUITS THROUGH CELL-TYPE-SPECIFIC GENE PROGRAMS. CELL, 157(5), 1216-1229. DOI:10.1016/J.CELL.2014.03.058_____ ERROR! BOOKMARK NOT DEFINED.

SPYRAKI, C., NOMIKOS, G. G., GALANOPOULOU, P., & DAIFOTIS, Z. (1988). DRUG-INDUCED PLACE PREFERENCE IN RATS WITH 5,7-DIHYDROXYTRYPTAMINE LESIONS

OF THE NUCLEUS ACCUMBENS. BEHAV BRAIN RES, 29(1-2), 127-134. ERROR! BOOKMARK NOT DEFINED.

STEWART, J. (1983). CONDITIONED AND UNCONDITIONED DRUG EFFECTS IN RELAPSE TO OPIATE AND STIMULANT DRUG SELF-ADMINSTRATION. PROG NEUROPSYCHOPHARMACOL BIOL PSYCHIATRY, 7(4-6), 591-597. ___ ERROR! BOOKMARK NOT DEFINED.

STROUD, H., FENG, S., MOREY KINNEY, S., PRADHAN, S., & JACOBSEN, S. E. (2011). 5-HYDROXYMETHYLCYTOSINE IS ASSOCIATED WITH ENHANCERS AND GENE BODIES IN HUMAN EMBRYONIC STEM CELLS. GENOME BIOL, 12(6), R54. DOI:10.1186/GB-2011-12-6-R54 _____ ERROR! BOOKMARK NOT DEFINED.

SU, Z. I., WENZEL, J., ETTENBERG, A., & BEN-SHAHAR, O. (2014). PRIOR EXTENDED DAILY ACCESS TO COCAINE ELEVATES THE REWARD THRESHOLD IN A CONDITIONED PLACE PREFERENCE TEST. ADDICT BIOL, 19(5), 826-837. DOI:10.1111/ADB.12053_____ ERROR! BOOKMARK NOT DEFINED.

SUN, J., WANG, L., JIANG, B., HUI, B., LV, Z., & MA, L. (2008). THE EFFECTS OF SODIUM BUTYRATE, AN INHIBITOR OF HISTONE DEACETYLASE, ON THE COCAINE- AND SUCROSE-MAINTAINED SELF-ADMINISTRATION IN RATS. NEUROSCI LETT, 441(1), 72-76. DOI:10.1016/J.NEULET.2008.05.010 _____ ERROR! BOOKMARK NOT DEFINED.

SUN, X., & LIN, Y. (2016). NPAS4: LINKING NEURONAL ACTIVITY TO MEMORY. TRENDS NEUROSCI, 39(4), 264-275. DOI:10.1016/J.TINS.2016.02.003____ ERROR! BOOKMARK NOT DEFINED.

SWANSON, C. J., BAKER, D. A., CARSON, D., WORLEY, P. F., & KALIVAS, P. W. (2001). REPEATED COCAINE ADMINISTRATION ATTENUATES GROUP I METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED GLUTAMATE RELEASE AND BEHAVIORAL ACTIVATION: A POTENTIAL ROLE FOR HOMER. J NEUROSCI, 21(22), 9043-9052. __ ERROR! BOOKMARK NOT DEFINED.

SWEATT, J. D. (2016). NEURAL PLASTICITY AND BEHAVIOR - SIXTY YEARS OF CONCEPTUAL ADVANCES. J NEUROCHEM, 139 SUPPL 2, 179-199. DOI:10.1111/JNC.13580 _____ ERROR! BOOKMARK NOT DEFINED.

SWEATT, J. D., MEANEY, M. J., NESTLER, E. J., & AKBARIAN, S. (2013). EPIGENETIC REGULATION IN THE NERVOUS SYSTEM (1 ED.). OXFORD, UK: ELSEVIER INC. .__ ERROR! BOOKMARK NOT DEFINED.

SWINFORD-JACKSON, S. E., ANASTASIO, N. C., FOX, R. G., STUTZ, S. J., & CUNNINGHAM, K. A. (2016). INCUBATION OF COCAINE CUE REACTIVITY ASSOCIATES WITH NEUROADAPTATIONS IN THE CORTICAL SEROTONIN (5-HT) 5-HT2C RECEPTOR (5-HT2CR) SYSTEM. NEUROSCIENCE, 324, 50-61. DOI:10.1016/J.NEUROSCIENCE.2016.02.052 _____ ERROR! BOOKMARK NOT DEFINED.

SZUMLINSKI, K. K., ABERNATHY, K. E., OLESON, E. B., KLUGMANN, M., LOMINAC, K. D., HE, D. Y., . . . KALIVAS, P. W. (2006). HOMER ISOFORMS DIFFERENTIALLY REGULATE COCAINE-INDUCED NEUROPLASTICITY. NEUROPSYCHOPHARMACOLOGY, 31(4), 768-777. DOI:10.1038/SJ.NPP.1300890 _____ ERROR! BOOKMARK NOT DEFINED.

SZUMLINSKI, K. K., ARY, A. W., & LOMINAC, K. D. (2008). HOMERS REGULATE DRUG-INDUCED NEUROPLASTICITY: IMPLICATIONS FOR ADDICTION. BIOCHEM PHARMACOL, 75(1), 112-133. DOI:10.1016/J.BCP.2007.07.031 _ ERROR! BOOKMARK NOT DEFINED.

SZUMLINSKI, K. K., DEHOFF, M. H., KANG, S. H., FRYS, K. A., LOMINAC, K. D., KLUGMANN, M., . . . KALIVAS, P. W. (2004). HOMER PROTEINS REGULATE SENSITIVITY TO COCAINE. NEURON, 43(3), 401-413. DOI:10.1016/J.NEURON.2004.07.019 ERROR! BOOKMARK NOT DEFINED.

SZUMLINSKI, K. K., TODA, S., MIDDAUGH, L. D., WORLEY, P. F., & KALIVAS, P. W. (2003). EVIDENCE FOR A RELATIONSHIP BETWEEN GROUP 1 MGLUR HYPOFUNCTION AND INCREASED COCAINE AND ETHANOL SENSITIVITY IN HOMER2 NULL MUTANT MICE. ANN N Y ACAD SCI, 1003, 468-471. _____ ERROR! BOOKMARK NOT DEFINED.

SZUMLINSKI, K. K., WROTEN, M. G., MILLER, B. W., SACRAMENTO, A. D., COHEN, M., BEN-SHAHAR, O., & KIPPIN, T. E. (2016). COCAINE SELF-ADMINISTRATION ELEVATES GLUN2B WITHIN DMPFC MEDIATING HEIGHTENED CUE-ELICITED OPERANT RESPONDING. J DRUG ABUSE, 2(2). _____ ERROR! BOOKMARK NOT DEFINED.

SZWAGIERCZAK, A., BULTMANN, S., SCHMIDT, C. S., SPADA, F., & LEONHARDT, H. (2010). SENSITIVE ENZYMATIC QUANTIFICATION OF 5-HYDROXYMETHYLCYTOSINE IN GENOMIC DNA. NUCLEIC ACIDS RES, 38(19), E181. DOI:10.1093/NAR/GKQ684 _____ ERROR! BOOKMARK NOT DEFINED.

TANG, W., WESLEY, M., FREEMAN, W. M., LIANG, B., & HEMBY, S. E. (2004). ALTERATIONS IN IONOTROPIC GLUTAMATE RECEPTOR SUBUNITS DURING BINGE COCAINE SELF-ADMINISTRATION AND WITHDRAWAL IN RATS. J NEUROCHEM, 89(4), 1021-1033. DOI:10.1111/J.1471-4159.2004.02392.X _____ ERROR! BOOKMARK NOT DEFINED.

TANG, W. X., FASULO, W. H., MASH, D. C., & HEMBY, S. E. (2003). MOLECULAR PROFILING OF MIDBRAIN DOPAMINE REGIONS IN COCAINE OVERDOSE VICTIMS. J NEUROCHEM, 85(4), 911-924. _____ ERROR! BOOKMARK NOT DEFINED.

TANIGUCHI, M., CARREIRA, M. B., COOPER, Y. A., BOBADILLA, A. C., HEINSBROEK, J. A., KOIKE, N., . . . COWAN, C. W. (2017). HDAC5 AND ITS TARGET GENE, NPAS4, FUNCTION IN THE NUCLEUS ACCUMBENS TO REGULATE COCAINE-CONDITIONED BEHAVIORS. NEURON, 96(1), 130-144.E136. DOI:10.1016/J.NEURON.2017.09.015_____ERROR! BOOKMARK NOT DEFINED.

THIELS, E., XIE, X., YECKEL, M. F., BARRIONUEVO, G., & BERGER, T. W. (1996). NMDA RECEPTOR-DEPENDENT LTD IN DIFFERENT SUBFIELDS OF HIPPOCAMPUS IN VIVO AND IN VITRO. HIPPOCAMPUS, 6(1), 43-51. DOI:10.1002/(SICI)1098-1063(1996)6:1<43::AID-HIPO8>3.0.CO;2-8 _____ ERROR! BOOKMARK NOT DEFINED.

TIAN, W., WANG, J., ZHANG, K., TENG, H., LI, C., SZYF, M., . . . ZHAO, M. (2016). DEMETHYLATION OF C-MYB BINDING SITE MEDIATES UPREGULATION OF BDNF IV IN COCAINE-CONDITIONED PLACE PREFERENCE. SCI REP, 6, 22087. DOI:10.1038/SREP22087 _____ ERROR! BOOKMARK NOT DEFINED.

TIAN, W., ZHAO, M., LI, M., SONG, T., ZHANG, M., QUAN, L., . . . SUN, Z. S. (2012). REVERSAL OF COCAINE-CONDITIONED PLACE PREFERENCE THROUGH METHYL SUPPLEMENTATION IN MICE: ALTERING GLOBAL DNA METHYLATION IN THE PREFRONTAL CORTEX. PLOS ONE, 7(3), E33435. DOI:10.1371/JOURNAL.PONE.0033435 _____ ERROR! BOOKMARK NOT DEFINED.

TRAN-NGUYEN, L. T., FUCHS, R. A., COFFEY, G. P., BAKER, D. A., O'DELL, L. E., & NEISEWANDER, J. L. (1998). TIME-DEPENDENT CHANGES IN COCAINE-SEEKING

BEHAVIOR AND EXTRACELLULAR DOPAMINE LEVELS IN THE AMYGDALA DURING COCAINE WITHDRAWAL. NEUROPSYCHOPHARMACOLOGY, 19(1), 48-59. DOI:10.1016/S0893-133X(97)00205-4 ERROR! BOOKMARK NOT DEFINED.

TRONSON, N. C., & TAYLOR, J. R. (2013). ADDICTION: A DRUG-INDUCED DISORDER OF MEMORY RECONSOLIDATION. CURR OPIN NEUROBIOL, 23(4), 573-580. DOI:10.1016/J.CONB.2013.01.022 ERROR! BOOKMARK NOT DEFINED.

TWINING, R. C., BOLAN, M., & GRIGSON, P. S. (2009). YOKED DELIVERY OF COCAINE IS AVERSIVE AND PROTECTS AGAINST THE MOTIVATION FOR DRUG IN RATS. BEHAV NEUROSCI, 123(4), 913-925. DOI:10.1037/A0016498 ERROR! BOOKMARK NOT DEFINED.

TZSCHENTKE, T. M. (2000). THE MEDIAL PREFRONTAL CORTEX AS A PART OF THE BRAIN REWARD SYSTEM. AMINO ACIDS, 19(1), 211-219. ERROR! BOOKMARK NOT DEFINED.

VERDEJO-GARCIA, A., CLARK, L., VERDEJO-ROMAN, J., ALBEIN-URIOS, N., MARTINEZ-GONZALEZ, J. M., GUTIERREZ, B., & SORIANO-MAS, C. (2015). NEURAL SUBSTRATES OF COGNITIVE FLEXIBILITY IN COCAINE AND GAMBLING ADDICTIONS. BR J PSYCHIATRY, 207(2), 158-164. DOI:10.1192/BJP.BP.114.152223 ERROR! BOOKMARK NOT DEFINED.

VOORN, P., VANDERSCHUREN, L. J., GROENEWEGEN, H. J., ROBBINS, T. W., & PENNARTZ, C. M. (2004). PUTTING A SPIN ON THE DORSAL-VENTRAL DIVIDE OF THE STRIATUM. TRENDS NEUROSCI, 27(8), 468-474. DOI:10.1016/J.TINS.2004.06.006 ERROR! BOOKMARK NOT DEFINED.

WADDINGTON, C. (1939). DEVELOPMENT AS AN EPIGENETIC PROCESS. AN INTRODUCTION TO MODERN GENETICS. ERROR! BOOKMARK NOT DEFINED.

WANG, H., & PENG, R. Y. (2016). BASIC ROLES OF KEY MOLECULES CONNECTED WITH NMDAR SIGNALING PATHWAY ON REGULATING LEARNING AND MEMORY AND SYNAPTIC PLASTICITY. MIL MED RES, 3(1). DOI:10.1186/S40779-016-0095-0 ERROR! BOOKMARK NOT DEFINED.

WANG, Z. J., MARTIN, J. A., MUELLER, L. E., CACCAMISE, A., WERNER, C. T., NEVE, R. L., . . . DIETZ, D. M. (2016). BRG1 IN THE NUCLEUS ACCUMBENS REGULATES COCAINE-SEEKING BEHAVIOR. BIOL PSYCHIATRY, 80(9), 652-660. DOI:10.1016/J.BIOPSYCH.2016.04.020 ERROR! BOOKMARK NOT DEFINED.

WEE, S., ORIO, L., GHIRMAI, S., CASHMAN, J. R., & KOOB, G. F. (2009). INHIBITION OF KAPPA OPIOID RECEPTORS ATTENUATED INCREASED COCAINE INTAKE IN RATS WITH EXTENDED ACCESS TO COCAINE. PSYCHOPHARMACOLOGY, 205(4), 565-575. DOI:10.1007/S00213-009-1563-Y ERROR! BOOKMARK NOT DEFINED.

WEE, S., SPECIO, S. E., & KOOB, G. F. (2007). EFFECTS OF DOSE AND SESSION DURATION ON COCAINE SELF-ADMINISTRATION IN RATS. J PHARMACOL EXP THER, 320(3), 1134-1143. DOI:10.1124/JPET.106.113340 ERROR! BOOKMARK NOT DEFINED.

WEI, W., COELHO, C. M., LI, X., MAREK, R., YAN, S., ANDERSON, S., . . . BREDY, T. W. (2012). P300/CBP-ASSOCIATED FACTOR SELECTIVELY REGULATES THE EXTINCTION OF CONDITIONED FEAR. J NEUROSCI, 32(35), 11930-11941. DOI:10.1523/JNEUROSCI.0178-12.2012 ERROR! BOOKMARK NOT DEFINED.

WEISSENBORN, R., DEROCHE, V., KOOB, G. F., & WEISS, F. (1996). EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS ON COCAINE-INDUCED OPERANT

RESPONDING FOR A COCAINE-ASSOCIATED STIMULUS. PSYCHOPHARMACOLOGY (BERL), 126(4), 311-322. ERROR! BOOKMARK NOT DEFINED.

WILL, M. J., DER-AVAKIAN, A., BLAND, S. T., GRAHN, R. E., HAMMACK, S. E., SPARKS, P. D., . . . MAIER, S. F. (2004). ELECTROLYTIC LESIONS AND PHARMACOLOGICAL INHIBITION OF THE DORSAL RAPHE NUCLEUS PREVENT STRESSOR POTENTIATION OF MORPHINE CONDITIONED PLACE PREFERENCE IN RATS. PSYCHOPHARMACOLOGY (BERL), 171(2), 191-198. DOI:10.1007/S00213-003-1572-1 ERROR! BOOKMARK NOT DEFINED.

WILLIAMS, J. M., GUEVREMONT, D., KENNARD, J. T., MASON-PARKER, S. E., TATE, W. P., & ABRAHAM, W. C. (2003). LONG-TERM REGULATION OF N-METHYL-D-ASPARTATE RECEPTOR SUBUNITS AND ASSOCIATED SYNAPTIC PROTEINS FOLLOWING HIPPOCAMPAL SYNAPTIC PLASTICITY. NEUROSCIENCE, 118(4), 1003-1013. ERROR! BOOKMARK NOT DEFINED.

WILLUHN, I., BURGENO, L. M., EVERITT, B. J., & PHILLIPS, P. E. (2012). HIERARCHICAL RECRUITMENT OF PHASIC DOPAMINE SIGNALING IN THE STRIATUM DURING THE PROGRESSION OF COCAINE USE. PROC NATL ACAD SCI U S A, 109(50), 20703-20708. DOI:10.1073/PNAS.1213460109 ERROR! BOOKMARK NOT DEFINED.

WISE, R. (2004). DOPAMINE, LEARNING AND MOTIVATION. NATURE REVIEWS. NEUROSCIENCE, 5(EC5AFFBA-3D9E-4CBA-ED20-D274F2382E41), 483-577. DOI:10.1038/NRN1406 ERROR! BOOKMARK NOT DEFINED.

WITTEN, I. B., LIN, S. C., BRODSKY, M., PRAKASH, R., DIESTER, I., ANIKEEVA, P., . . . DEISSEROTH, K. (2010). CHOLINERGIC INTERNEURONS CONTROL LOCAL CIRCUIT ACTIVITY AND COCAINE CONDITIONING. SCIENCE, 330(6011), 1677-1681. DOI:10.1126/SCIENCE.1193771 ERROR! BOOKMARK NOT DEFINED.

WOLFFE, A. P., & MATZKE, M. A. (1999). EPIGENETICS: REGULATION THROUGH REPRESSION. SCIENCE, 286(5439), 481-486. ERROR! BOOKMARK NOT DEFINED.

WOOLVERTON, W. L. (1992). DETERMINANTS OF COCAINE SELF-ADMINISTRATION BY LABORATORY ANIMALS. CIBA FOUND SYMP, 166, 149-161; DISCUSSION 161-144. ERROR! BOOKMARK NOT DEFINED.

WRIGHT, K. N., HOLLIS, F., DUCLOT, F., DOSSAT, A. M., STRONG, C. E., FRANCIS, T. C., . . . KABBAJ, M. (2015). METHYL SUPPLEMENTATION ATTENUATES COCAINE-SEEKING BEHAVIORS AND COCAINE-INDUCED C-FOS ACTIVATION IN A DNA METHYLATION-DEPENDENT MANNER. J NEUROSCI, 35(23), 8948-8958. DOI:10.1523/JNEUROSCI.5227-14.2015 ERROR! BOOKMARK NOT DEFINED.

WU, H., & ZHANG, Y. (2011). MECHANISMS AND FUNCTIONS OF TET PROTEIN-MEDIATED 5-METHYLCYTOSINE OXIDATION. GENES & DEVELOPMENT, 25(23), 2436-2452. ERROR! BOOKMARK NOT DEFINED.

WU, X., & ZHANG, Y. (2017). TET-MEDIATED ACTIVE DNA DEMETHYLATION: MECHANISM, FUNCTION AND BEYOND. NAT REV GENET, 18(9), 517-534. DOI:10.1038/NRG.2017.33 ERROR! BOOKMARK NOT DEFINED.

XIAO, B., TU, J. C., PETRALIA, R. S., YUAN, J. P., DOAN, A., BREDER, C. D., . . . WORLEY, P. F. (1998). HOMER REGULATES THE ASSOCIATION OF GROUP 1 METABOTROPIC

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List of Abbreviations

5-HT	Serotonin
5caC	5-carboxyl cytosine
5fC	5-formyl cytosine
5hmC	5-hydroxymethyl cytosine
5mC	5-methyl cytosine
ACC	Anterior cingulate cortex
Actβ	Beta Actin
AMPA	□-amino-3-dydroxy-5-methyl-isoxazol-4-propionic acid
BDNF	Brain derived neurotrophic factor
BLA	Basolateral amygdala
CC	Corpus callosum
cDNA	Complimentary DNA
CNS	Central nervous system
CP	Caudate-putamen
CPA	Conditioned place aversion
CPP	Conditioned place preference
CS	Conditioned stimulus
DA	Dopamine
ddPCR	Digital droplet polymerase chain reaction
Dlg4	Disks large homolog 4 (gene encoding for PSD-95)

dlPFC	Dorsolateral prefrontal cortex
dmPFC	Dorsal prefrontal cortex
DNMT	DNA methyltransferase
FI	Fixed-interval
FR	Fixed ratio
GABA	□-Aminobutyric acid
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Grin1	Gene for N-methyl-D-Aspartate receptor subunit 1
Homer2	Homer 2 gene
HPA	Hypothalamic pituitary axis
HPC	Hippocampus
hMeDIP	Hydroxymethylated DNA immunoprecipitation
IL	Infralimbic
IM	Intramuscular
inf	Infusion
IP	Intraperitoneal
IV	Intravenous
LTD	Long term depression
MD	Mediodorsal
MeCP2	Methyl-CpG-binding protein 2
MeDIP	Methylated DNA immunoprecipitation
MET	i-Methionine
mGluR	Metabotropic glutamate receptor

mPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
NAC	Nucleus accumbens
NE	Norepinephrine
NMDA	N-methyl-d-aspartate
Npas4	Neuronal PAS domain protein 4
NR1	N-methyl-D-aspartate receptor 1
NRT	No reverse transcriptase (negative control)
NTC	No template control (reaction mixture without cDNA)
OFC	Orbitofrontal cortex
PET	Positron emission tomography
PFC	Prefrontal cortex
PL	Prelimbic
PSD-95	Postsynaptic density protein 95
qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative real-time polymerase chain reaction
UCS	Unconditioned stimulus
RT	Reverse transcriptase
SA	Self-administration
SAM	S-adenosylmethionine
Tet1	Ten-eleven translocation protein 1
Tet3	Ten-eleven translocation protein 3

vmPFC	Ventromedial prefrontal cortex
VP	Ventral pallidum
VS	Ventral striatum
VTA	Ventral tegmental area
ZFP	Zinc Finger Protein

Chapter 1: General Introduction

"I've been clean for seven years but still think about using [drugs] every day. Sometimes the thought is fleeting, but sometimes it scares me how long I think about using. Whenever it gets to be too much, I also think about the hopelessness of that time in my life. Recovery can be a struggle, but it's a struggle that gives me my life today." **https://drugabuse.com/9-memorable-quotes-from-former-addicts/**

Introduction

Cocaine abuse is a debilitating psychological disorder that is characterized by recurrent substance use despite negative and recurrent social, interpersonal, legal, or domestic problems (APA, 2013). According to the National Institute on Drug Abuse (NIDA), annual drug related costs equate to $485 billion per year with 161 billion dollars directly related to elicit drug use. The drug related costs include 60% of all incarcerated adults, $23 million per year for specialized child care, 460,000 American deaths, and a whopping 40 million debilitating diseases and injuries associated with drug use. In 2008, 15% of all Americans had used cocaine. Of the 15% that had used cocaine, 6% had used it by their senior year in high school. In 2004, NIDA reported 2 million current cocaine users and 1.5 million users dependent upon cocaine.(NIDA., 2016). Currently, there are no generally accepted (or FDA-approved) treatments for cocaine addiction which makes research into this disease critical.

Despite the numbers of individuals afflicted with cocaine dependence or addiction, the majority of cocaine users will administer the drug recreationally and do not become addicted to cocaine (Edwards, 1986). One major reason for this is that cocaine addiction requires a variety of factors including: route of administration, accessibility of the drug, duration of use, stress, etc. Specifically, cocaine addiction is characterized by compulsive, uncontrollable, binging of cocaine during moments when cocaine is readily available (Ahmed & Koob, 1998; Edwards, 1986). However, cocaine binging is only one phase of an addiction cycle. Cocaine addicts have been theorized to cycle through three distinct behavioral/ cognitive stages during the transition from recreational drug use to addicted drug use, and they progressively spiral further and further into addiction as the cycle continues. The three stages of the addiction cycle are referred to as binge/

intoxication, withdrawal/ negative affect, and preoccupation/ anticipation (i.e. craving in humans). Each stage is associated with the activation of unique neuroanatomical substrates, and patients will cycle through each stage progressively until addiction worsens. Briefly, binging on a drug (such as cocaine) activates innate reinforcement and habit circuitry within the VTA, SN, NAC, DS, and thalamus. Once the initial "hedonic" effects of drug have worn off, withdrawal/ negative affect kicks in and is mediated via brain stem nuclei, the CEA, BNST, hypothalamus, and NAC; activation of these circuits creates a negative affective state in humans that is opposite of the binge/ intoxication phase. Lastly, during drug abstinence, the preoccupation/ anticipation stage sets in and humans experience "craving" for the previously administered drug. The experience of craving for a drug is associated with activation of the PFC, hippocampus, BLA, and insula in humans (Koob & Volkow, 2010). Animal studies of addiction attempt to model the three stages of addiction through various procedure such as repeated drug injection, conditioned place preference (CPP), or self-administration (SA). Although human cognitive processes such as "craving" cannot be assessed in animal studies, certain behaviors, such as drug-seeking after prolonged abstinence, can be used to model the human condition.

Experimental Models of Addiction

Similar to the findings that high drug use is associated with worse consequences in human drug users, rats allowed differential access to cocaine exhibit very different behavioral profiles; experimenter-administered cocaine produces sensitization to the locomotor effects of cocaine, whereas prolonged cocaine self-administration produces tolerance to the effects of cocaine (Ahmed & Koob, 1998; Ben-Shahar, Ahmed, Koob, &

Ettenberg, 2004; Ben-Shahar et al. 2005). Additionally, the length of cocaine administration (Belin, Balado, Piazza, & Deroche-Gamonet, 2009; Perry, Morgan, Anker, Dess, & Carroll, 2006) and contingency of cocaine administration affect subsequent drug-seeking tests (Kippin, Fuchs, & See, 2006; Twining, Bolan, & Grigson, 2009). Since there are such a large number of differences in behavioral models for cocaine addiction, I briefly summarize them prior to justifying the behavioral model employed in my thesis.

Place conditioning

Place conditioning is a useful tool to evaluate the subjective nature of drug exposure based on associative processes that impact subsequent approach as well as avoidance behaviors (Tzschentke, 1998; Tzshentke, 2007; Ettenberg, 2004). Place conditioning is relatively quick and simple to perform as it tests the effects of cocaine in rats and mice with daily repeated injections. Most studies involve an apparatus with 2 or 3 chambers that have distinct environmental cues. Rats or mice are then injected with a drug (i.e. cocaine) and placed in one chamber or injected with a control solution (i.e. saline) and placed in a separate chamber; they are not allowed to move between chambers during training. This methodology is a form of Pavlovian training that allows the rats to associate internal states (i.e. the UCS, aka subjective effects of cocaine) with external environmental cues (CS). The animals are then tested by allowing access to both chambers, and quantifying the amount of time spent in each chamber. A conditioned place preference (CPP) is achieved if the animal spends more time in the "drug-associated" chamber, whereas a conditioned place aversion (CPA) is achieved if the animal spends more time in the "control-associated" chamber. Based on these simple

principles, a vast amount of information has been gathered on the behavioral, neuroanatomical, and molecular effects of cocaine.

Place conditioning has been powerful in demonstrating evidence for theories on the subjective effects of drugs, such as for the opponent-process theory posited by Solomon & Corbit in 1974. Briefly, they hypothesized that after a drug is administered, there is an initial positive affective experience that occurs through the drugs actions (State A). However, after the initial onset of positive affect, State A begins to decline until it has reached a stable baseline, and an opposing process, or negative affective state (aka State B), is experienced. In 1999, Ettenberg et al. was able to test the affective properties of cocaine by administering cocaine intravenously into rats, and staggering the time with which they were introduced to the CPP chambers. Rats allowed no delay, or 5 minutes of delay after cocaine injection spent more time in the cocaine-associated chamber (CPP was observed), whereas rats delayed for 15 minutes before training and testing spent more time in the control chamber (CPA was observed) (Ettenberg, Raven, Danluck, & Necessary, 1999). Additionally, CPP testing has been instrumental in addiction research because it can test the reward properties of cocaine after cocaine self-administration procedures. In 2014, Su et al. demonstrated a shift in the rewarding properties of cocaine after prolonged-access to cocaine self-administration (SA). Specifically, the authors trained rats to self-administer cocaine (0.25 mg/mg/ 0.1 mL infusion) for 6 hours per day or saline (0.1 mL infusion) for 1 hour per day, then they trained them for CPP with an immediate or delayed (15 minutes) exposure to the CPP chamber; cocaine SA rats exhibited CPA whereas saline SA rats exhibited CPP when immediately placed in the chamber. Additionally, Su et al. trained cocaine SA rats for

CPP with a 0.25 mg/ kg injection of cocaine, whereupon they did observe CPP when rats were immediately placed in the CPP chamber (Su, Wenzel, Ettenberg, & Ben-Shahar, 2014). Their results reflect the power of CPP tests to determine the affective properties of cocaine, and established that prior access to cocaine training diminishes the reward value of cocaine.

CPP studies have also been instrumental when assessing the function of neural substrates and neurotransmitter systems implicated in addiction. For example, CPP combined with selective lesions of brain nuclei has helped to elucidate the roles of the prefrontal cortex (PFC) (Isaac, Nonneman, Neisewander, Landers, & Bardo, 1989; Tzschentke, 2000; Zavala, Weber, Rice, Alleweireldt, & Neisewander, 2003), nucleus accumbens (NAC) (Spyraki, Nomikos, Galanopoulou, & Daifotis, 1988), ventral tegmental area (VTA) (Ouachikh, Dieb, Durif, & Hafidi, 2014), basolateral amygdala (Everitt, Morris, O'Brien, & Robbins, 1991), ventral pallidum (VP) (Gong, Neill, & Justice, 1997), and dorsal raphe nucleus (DRN) (Will et al., 2004). Recently, more selective studies have been performed to investigate the roles of specific neurotransmitters and receptors in cocaine place preference. For instance, pharmacological antagonism of D1 or AMPA/Kainate receptors (Cervo & Samanin, 1995) as well as mGluR1 receptors (Yu et al., 2013), impairs cocaine CPP. Furthermore, selective overexpression of the *Homer2* gene within the PFC and NAC of mice elevated basal levels of glutamate, and potentiated cocaine-seeking during CPP testing (Ary et al., 2013). Lastly, recent advances in optogenetic technology has allowed direct manipulation of specific neuron populations, and has demonstrated how cocaine CPP can be influenced by specific cell populations. For example, optogenetic stimulation of cholinergic

interneurons within the NAC accompanied with cocaine injection, enhances cocaine CPP over cocaine-only and non-cocaine paired controls (Witten et al., 2010).

Although CPP is a powerful tool for investigating addiction, it has two major limitations, particularly for understanding the behavioral neurobiological consequences of drug exposure relevant to addiction. First, several studies have demonstrated that non-contingent as opposed to contingent (i.e. self-administration) exposure to drugs produce distinct neurobiological consequences (e.g. Lecca, 2007; McFarland et al., 2003; Radley et al., 2015). Second, animals in CPP studies generally receive very limited amounts of cocaine and only receive them when an experimenter delivers the cocaine. Lastly, non-contingent access to cocaine (as with CPP) increases the stress response to cocaine (Palamarchouk, Smagin, & Goeders, 2009), and increases the lethality of cocaine (Dworkin, Mirkis, & Smith, 1995), thus, non-contingent models may confound the behavioral and neurobiological of drug administration via stress and the hypothalamic-pituitary-adrenal axis (HPA axis). Therefore, if the goal of studying addiction is to model the human condition in order to understand the neurological basis of addiction or to develop potential treatments for addiction, then a study should model the behavioral contingency and pattern of use that humans engage in, such as via intravenous cocaine self-administration.

Cocaine Self-Administration Studies

Investigation of the long-term, addiction rates of humans has previously indicated that increased access to cocaine, possession of cocaine, and use of fast routes of cocaine administration (i.e. free base smoking) results in more patients that exhibit “intensified cocaine use” and “compulsive cocaine use” (Siegel, 1984). Similarly, repeated,

prolonged-access to intravenous cocaine self-administration in rats has been shown to produce an addiction-like phenotype across a number of different studies (Ahmed & Koob, 1998; Ben-Shahar et al., 2005; Su et al., 2014). Therefore, as previously argued (e.g. recent reviews Feltenstein & See, 2008; Nader, 2016; Peoples et al 2010), the best model to replicate the behavioral and neurochemical effects of cocaine use in humans, is through cocaine self-administration studies in laboratory animals such as mice, rats, and primates. Adjusting parameters such as dose, injection rate, cue presentation, reinforcement schedule, and length of drug access, experimenters are able to test various aspects of addiction-like behaviors in animals (Woolverton, 1992). For instance, increasing cocaine dose also increases the time between operant responses for cocaine (Gerber & Wise, 1989). Additionally, the presentation of an initially neutral cue (i.e the CS) paired with drug delivery (i.e. the UCS) during cocaine self-administration allows subsequent conditioned reinforcement (Everitt, 2017), as well as reinstatement of responding on the previously reinforced operandum after extinction (Deroche-Gamonet, Piat, Le Moal, & Piazza, 2002; Meil & See, 1996; Stewart, 1983) .

More recently, differential amounts of access to cocaine self-administration has become a major focus in the study the behavioral and neurobiological aspects of drug-taking. Ahmed and Koob (1998) demonstrated that "limited" daily-access (1 h/ day) and "prolonged" daily-access conditions (6 h/ day) are distinct in their ability to model aspects of drug abuse/addiction in two major ways: 1) rats with prolonged cocaine-access escalate their cocaine intake across daily sessions, whereas the limited-access rats exhibit stable intake for several weeks and 2) rats with daily "prolonged" cocaine-access dramatically increase intake of cocaine for the 1st 10 minutes of self-administration while

"limited" access rats do not escalate cocaine intake within the 1st 10 minutes of self-administration (Ahmed & Koob, 1998). This differential pattern of responding has since been replicated with a number of procedural variations to determine the underlying changes in drug-taking behavior (Ahmed & Koob, 1999), neurocircuitry (Ahmed, Kenny, Koob, & Markou, 2002; Ben-Shahar et al., 2012; Robinson & Kolb, 2004), and cellular/ molecular function in rats (Ben-Shahar et al., 2009; Ben-Shahar et al., 2013) as well as in other species (Kirkland Henry, Davis, & Howell, 2009; Nakamura, Gao, Okamura, & Nakahara, 2011).

Despite substantial study, it remains to be determined whether escalated cocaine intake (and the neurobiological changes associated there with) is dependent upon the amount of cocaine exposure or the act of self-administering the cocaine. Indeed, escalated responding and intake of non-drug reinforcers can be achieved by prolonged-access to liquid food, suggesting that the escalation phenomenon may be mediated by behavioral processes underlying the self-administration of appetitive stimuli (Goeders, Murnane, Banks, & Fantegrossi, 2009). One way to dissociate the relative contribution of total drug exposure from the behavioral contingency of that exposure is to employ yoked-access procedures (i.e. the administration of cocaine under the control of a separate self-administering rat). For instance, Hemby et al. (1997) investigated the effects of cocaine under response-dependent and –independent conditions in a yoked-triad. Both self-administering and yoked-access rats exhibited elevated levels of dopamine in the nucleus accumbens during the first hour of self-administration, but the self-administering animals exhibited greater dopamine levels than their yoked- counterparts (Hemby, Co, Koves, Smith, & Dworkin, 1997). Additionally, relative to rats self-administering cocaine, yoked

rats exhibit differential corticosterone levels both in systemic plasma (Galici, Pechnick, Poland, & France, 2000) and brain (Palamarchouk et al. 2009), have a higher morbidity rate (Dworkin et al., 1995) and exhibit higher indices of distress (measured by ultrasonic vocalization) (Mutschler & Miczek, 1998). These studies demonstrate that, despite equivalent cocaine dosing, animals given contingent-access to cocaine exhibit distinct behavioral and neurobiological effects compared to animals with non-contingent access.

To expand upon the role of behavioral contingency in the behavioral sequelae of cocaine exposure, Kippin et al. (2006) employed a novel mixed self-administration/yoked cocaine exposure procedure in which rats received 1-h access to cocaine self-administration before receiving non-contingent cocaine infusions via yoking procedures during its last 5 hours of cocaine self-administration. Although escalated cocaine intake was not observed in this earlier study, both the contingent and non-contingent excessive cocaine exposure groups exhibited greater cue-induced reinstatement than rats with a history of limited cocaine-access only. However, only the prolonged contingent-access rats exhibited greater cocaine-primed reinstatement of responding. In the present study, we employ the prolonged-access and limited-access + yoked procedures to determine the impact of contingent and non-contingent "excessive" cocaine exposure on the escalation of cocaine intake and operant responding for cocaine to determine how behavioral contingency of cocaine delivery influences these aspects of cocaine addiction-related behavior.

Given the chronic nature of addiction, another major theme in models of addiction behavior and neurobiology has been the persistent, and even intensifying, nature of drug-seeking through protracted periods of drug abstinence. Several studies have indicated that

cocaine self-administration, followed by differential lengths of abstinence produce an "incubation of cocaine craving", whereby animals will engage in increasing cocaine-seeking behavior over the course of a few months of abstinence (Grimm, Hope, Wise, & Shaham, 2001; Tran-Nguyen et al., 1998). The observed increases in cocaine-seeking behavior as abstinence continues are also accompanied by time-dependent changes in neuron/synapse structure (Ben-Shahar et al., 2013; Swinford-Jackson, Anastasio, Fox, Stutz, & Cunningham, 2016; Szumlinski et al., 2016), neurotransmitter levels (Ben-Shahar et al., 2012; Grimm et al., 2003), and neuronal activity (Luis, Cannella, Spanagel, & Kohr, 2017).

Neural Bases for Addiction.

The use of psychostimulants such as cocaine or amphetamine facilitates the release and prevents the reuptake of dopamine, as well as other monoamine transmitters, in the synaptic cleft. Under natural circumstances, the presence of dopamine in the synaptic cleft is controlled through dopaminergic binding of the D2 auto-receptor to initiate reuptake through the dopamine transporter (DAT), as well as breakdown within the cleft via monoamine oxidase (MAO). However, under the influence of cocaine, the reuptake system is impeded, which results in a pronounced increase of dopamine within the synaptic cleft that is free to bind to the postsynaptic density (Kalivas, 2007; Nestler, 2009).

The dopaminergic projections implicated in cocaine self-administration originate from either midbrain SN or VTA nuclei and extend throughout limbic regions of the telencephalon into the PFC, dorsal striatum, NAC, amygdalar nuclei, and the hippocampus (Laviolette & van der Kooy, 2004). Release of dopamine from the VTA

into the NAC serves to cue an organism of novel and motivationally important events (Kalivas, 2007). NAC dopamine mediates the incentive reward for natural reinforcers, such as food and water in deprived animals as well as novelty induced reinforcers, such as palatable food over standard chow in rats. (Hao, Martin-Fardon, & Weiss, 2010; Wise, 2004). The incentive reward placed upon the unconditioned natural stimuli (UCS) is also placed upon the motivationally irrelevant cues (CS) that are presented concurrently with the UCS. In this manner, mesolimbic dopamine release mediates learned behavioral responses towards motivationally significant events as well as environmental cues associated with them (Kalivas, 2007).

Critically, several lines of evidence indicate that reliance of drug-taking and –seeking behavior on subregions of the striatum changes over the course of drug experience. Decades of work from Everitt, Robbins, and others have emphasized the necessity of the ventral striatum (aka nucleus accumbens) in acquisition of self-administration followed by the necessity of more dorsal regions after extensive experience with self-administration (reviewed in e.g. Everitt & Robbins, 2013; 2016) which have lead this group to conclude that there is a "shift over time and experience of control over drug seeking from a limbic cortical-ventral striatal circuit underlying goal-directed drug seeking to a dorsal striatal system mediating habitual drug seeking" (Everitt, 2016). One biological marker of this transition appears to the shift of dopamine systems with the nucleus accumbens showing robust responses during initial self-administration followed by blunting across weeks of self-administration whereas dorsolateral striatum exhibited negligible responses during initial self-administration followed by intensification during weeks of self-administration.

Therefore, drugs of abuse that potentiate dopaminergic action in the mesocorticolimbic circuit (i.e. cocaine), appear able to hijack the natural reward pathway through a massive potentiation of dopamine within the synaptic cleft. Thus, animals that have received experimenter-administered or self-administered cocaine, amphetamine, heroin, cannabinoids, nicotine, and ethanol all exhibit increased levels of dopamine within the NAC, PFC, amygdala, and dorsal striatum that originate in the midbrain VTA (and SN for dorsal striatum inputs) (Kalivas, 2007; Spanagel & Weiss, 1999; Willuhn, Burgeno, Everitt, & Phillips, 2012) (Willuhn, Burgeno, Everitt, & Phillips, 2012). Arguably, the influx of DA into the dmPFC is of particular importance because dmPFC activation is critical for drug-seeking behavior, as the dmPFC has a large number of glutamatergic pyramidal neurons that project into the NAC core (see Figure 1.1; Voorn et al., 2004; Peters, Kalivas, & Quirk, 2009). Moreover, the dmPFC, but not vmPFC, also projects to the more dorsal regions of the striatum (McGeorge et al 1989; Berendse et al., 1992) that are critical for the development and expression of habitual responding and strongly implicated in late stage addiction processes (e.g. Voorn et al., 2004; Willuhn et al., 2012). To support this claim, the dmPFC is known to be necessary for the increase in the positive-reinforcing effects of cocaine observed during prolonged access (Smith, Ward, & Roberts, 2008). Additionally, dmPFC NMDA receptor activity is implicated in cocaine-seeking behavior (Szumlinski et al., 2016), and increased dmPFC activation and glutamate transmission into the NAC mediates the reinstatement of cocaine-seeking behavior (McFarland et al., 2003).

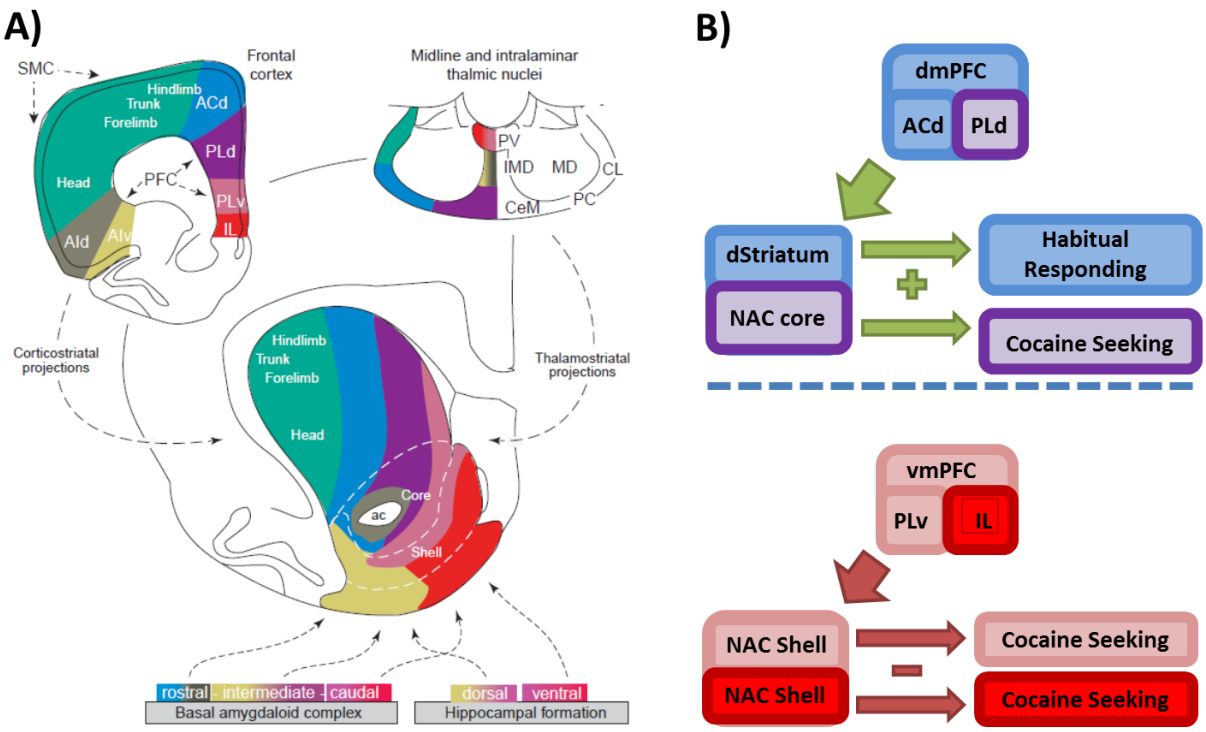

Figure 1.1: A) Circuit diagram of PFC and Striatal Connections. The dmPFC (Acd & PLd) projects to the dorsal striatum and NAC core, whereas the vmPFC (PLv & IL) projects to the NAC shell and ventral striatum. (Voorn et al., 2004). **B) Schematic outlining PFC function.** The dmPFC has glutamatergic projections to both the dStriatum and NAC core. Activation of the dmPFC drives cocaine-induced locomotion and cocaine seeking behavior. In contrast, the vmPFC projects to GABAergic neurons within the NAC shell and inhibits cocaine-seeking behavior when activated (Peters, Kalivas, & Quirk, 2009). Ac, anterior commissure; Acd, dorsal anterior cingulate; dmPFC, dorsal medial prefrontal cortex; dStriatum, dorsal striatum; IL, infralimbic cortex; PLd, dorsal prelimbic cortex; PLv, ventral prelimbic cortex; NAC core, nucleus accumbens core; NAC shell, nucleus accumbens shell; vmPFC, ventral medial prefrontal cortex.

The Role of the Prefrontal Cortex in Addiction-related Behaviors.

Human feelings of craving are hard to quantify behaviorally, but neuroimaging studies have identified a variety of neural substrates involved in the subjective experience of craving in humans. Studies of human cocaine addicts reveal a general reduction in baseline activity in the anterior cingulate cortex (ACC) and the orbital PFC (oPFC), as well as a reduction in cortical neuron populations (Goldstein & Volkow, 2002; Franklin et al., 2002). Interestingly, when human subjects are presented with drug-associated cues,

there is an increase in activation of the ACC and oPFC (Goldstein & Volkow, 2002). The necessity of these areas in drug-seeking behavior in animals is reflected in rodent models whereby localized inactivation of the VTA to dorsal PFC circuit, PFC to accumbens circuit, or accumbens to ventral pallidal circuit blocks drug-seeking behavior in animals trained to extinguish drug-related cues (McFarland & Kalivas, 2001).

As such, the PFC is widely implicated in several aspects of the addiction process and is specifically linked to neurobiological changes produced by prolonged-access to cocaine. For instance, extensive neuroimaging and neuropsychological evidence from clinical studies indicate that chronic drug abusers show deficits in PFC-dependent cognitive function (Bolla et al., 2004) with similar deficits observed in PFC function following prolonged access to cocaine in animal models (George, Mandyam, Wee, & Koob, 2008). The mPFC has also been implicated in facilitating cocaine reinforcement and is widely implicated in reinstatement of cocaine-seeking behavior (reviewed in e.g. Kalivas et al., 2005). Additionally, mPFC lesions enhance acquisition of cocaine reinforcement as well as cocaine responding under a second-order schedule of cocaine reinforcement (Weissenborn, Deroche, Koob, & Weiss, 1996). Such PFC alterations also contribute to impairment of executive control over behavior, including behavioral inhibition processes, which may contribute to drug relapse and addiction by aiding the transition from voluntary to habitual responding (Ito, Robbins, & Everitt, 2004).

Sub-regions of the dmPFC (comprised of the ACC and PL) are associated with addiction-like alterations in glutamate signaling, which are linked to escalated drug taking, in rats allowed prolonged-access to cocaine. In fact, the dmPFC is critical for the sensitization of cocaine reinforcement (as measured by increased breakpoints under a

progressive ratio schedule of reinforcement) that occurs during prolonged-access to cocaine in rats (Smith el al., 2008). Further, basal glutamate levels in the mPFC are diminished after 17 days of prolonged access to cocaine, but are not altered in limited-access rats (Ben-Shahar et al., 2012). Moreover, prolonged-access, but not limited-access to cocaine, increases mRNA expression of several glutamate-related signaling molecules within the dmPFC (Ploense et al, in press) and increases Homer 1b/c, GluNR2b and GluNR2a protein in a time-dependent fashion (Ben Shahar et al., 2009). Interestingly, increases in GluNR2b within the mPFC persist for up to 30 days following prolonged-access to cocaine, and pharmacological inhibition of GluNR2b decreases elevated cocaine-seeking during extinction conditions (Szumlinski et al., 2016). Additionally, the dmPFC projects to several areas crucial to drug-seeking behavior that the vmPFC does not project to, such as the NAC core, dorsal regions of the striatum, SN, dlVP, dHPC, and central BLA (see Figure 1.2 from Scofield et al., 2016). For example, prelimbic (PL) dmPFC neurons that project to the NAC core are activated in response to cocaine-cue reinstatement after extinction training, whereas infralimbic (IL) projections from the vmPFC to the NAC shell are not differentially activated during cued reinstatement (McGlinchey, James, Mahler, Pantazis, & Aston-Jones, 2016). Since changes in ACC and PL functionality and composition underlie craving behavior in humans and drug-seeking behavior in rodents, unravelling the molecular mechanisms underlying long term changes in the dmPFC are crucial to understanding cocaine addiction.

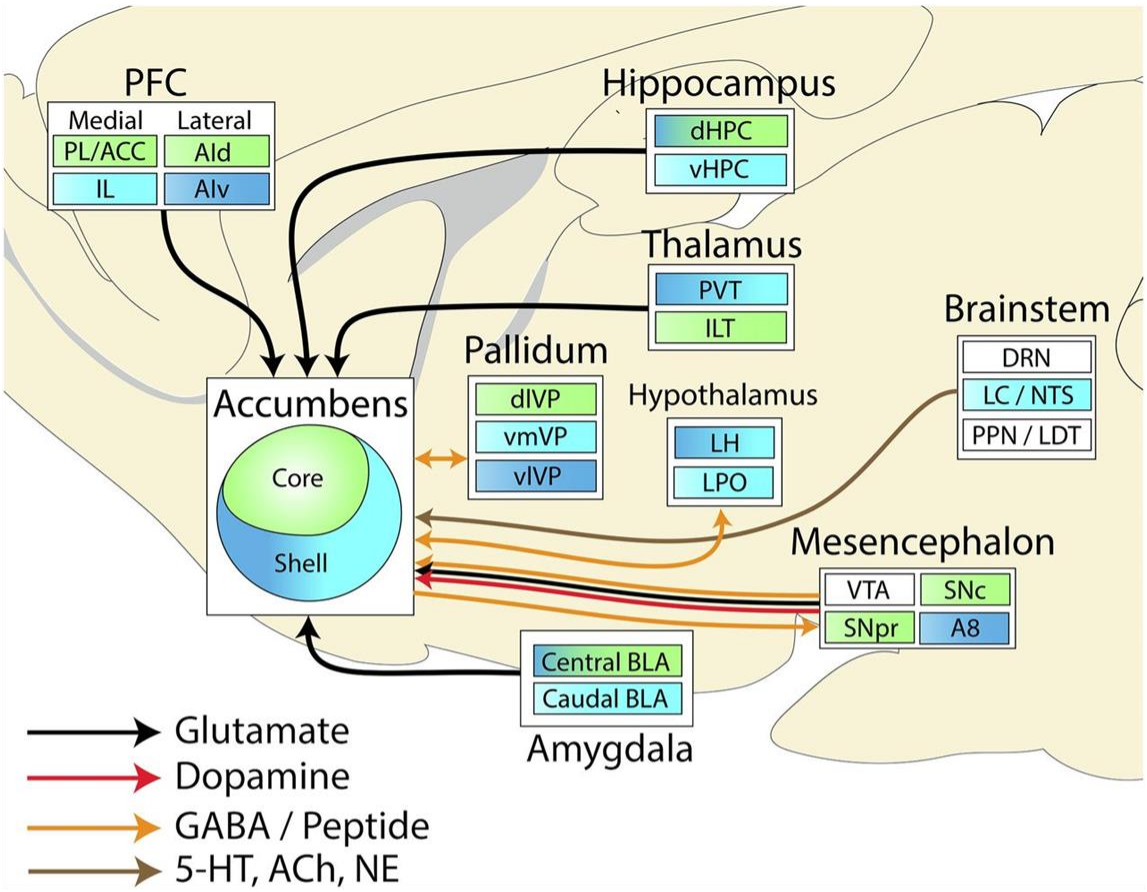

Figure 1.2: NAc connectivity. The NAc receives inputs from cortical, allocortical, thalamic, midbrain, and brainstem structures. In turn, it sends projections to other basal ganglia nuclei (VP and substantia nigra pars reticulata), nuclei in the mesencephalon, the hypothalamus, and the extended amygdala. Note that many structures project from different subareas to the NAcore or NAshell. For clarity, these projections have been color coded as projecting to the NAcore (green), medial NAshell (light blue), or lateral NAshell (dark blue); in reality, many regions project to both the NAcore and NAshell along topographical gradients (e.g., dorsoventral projections from the hippocampus terminating from lateral to medial parts of the accumbens; shown as color gradients in the figure). A number of regions project uniformly throughout the accumbens and are marked white. A8, retrorubral area; ACC, anterior cingulate cortex; AId, dorsal anterior insular; AIv, ventral anterior insular; dHPC, dorsal hippocampus; dlVP, dorsolateral ventral pallidum; DRN, dorsal raphe nucleus; IL, infralimbic cortex; ILT, interlaminar nuclei of the thalamus; LC, locus coeruleus; LH, lateral hypothalamus; LPO, lateral preoptic area; NTS, nucleus of the solitary tract; PL, prelimbic cortex; PPN, pedunculopontine nucleus; PVT, paraventricular nucleus of the thalamus; vlVP, ventrolateral ventral pallidum; vmVP, ventromedial ventral pallidum; SNc, substantia nigra pars compacta; SNpr, substantia nigra pars reticulata" **(Scofield et al., 2016)**

Epigenetics, Gene Expression, and Addiction

The field of epigenetics, first introduced conceptually by C.H. Waddington in 1939 as a way to explain stable patterns of cellular function associated with tissue differentiation during development, focuses on the stable covalent modifications to DNA structure that mediate mRNA transcription. Epigenetic modifications are crucial during embryonic development as they determine cell fate by producing long-term changes in gene expression i.e. turning on or off cell type-specific genes (Waddington, 1939; Jones et al., 1998). However, epigenetic modifications in the brain appear to play additional roles related to plasticity and, to date, there have been studied in relation to learning and memory (Day & Sweatt, 2010), regulation of motivated behaviors (Elvir, Duclot, Wang, & Kabbaj, 2017), and in addiction (Nestler, 2013; Renthal & Nestler, 2009).

DNA Methylation and Hydroxymethylation

DNA methylation is an epigenetic modification to base pairs within the DNA code that contributes to the regulation of gene expression. DNA methylation in eukaryotes is primarily produced by the covalent addition of a methyl group ($-CH_3$) to a cytosine nucleotide at position 5 of the pyrimidine ring via DNA methyltransferases (DNMTs) (Sweatt, Meaney, Nestler, & Akbarian, 2013; Wolffe & Matzke, 1999). The addition of methyl groups to cytosine serves a major function in transference of genetic material via haploid cells; extensive methylation of the genome produces missense and nonsense codons and halts gene expression for haploid cells (Wolffe & Matzke, 1999). After two haploid cells have met and fertilization has occurred, massive demethylation of the genome occurs in males (and moderate demethylation in females), which is in turn

followed by additional DNA methylation during gastrulation and embryonic development to produce stable, differentiated cell lines (Ehrlich et al., 1982; Kawai et al., 1994).

After embryonic-development, DNA methylation continues to occur throughout brain tissue in mammals and regulates gene expression in different neuronal and glial cells. For instance, DNA methylation that occurs in and around transcription factor binding sites serves to attenuate the binding of transcription factors to the DNA, which can in turn, repress or potentiate gene expression depending on whether the sequence serves to recruit a transcriptional repressor or activator (Sweatt et al., 2013). Alternatively, intra-genic DNA methylation can serve to repress spurious mRNA transcription (Neri et al., 2017), facilitate the expression of alternative splice variants (Malousi & Kouidou, 2012), or promote the occurrence of diseases states such as cancer (Jeziorska et al., 2017).

In mammals, DNA methylation is primarily produced by the addition of a methyl group (-CH_3) to cytosine (Sweatt et al., 2013). DNA methylation at transcription factor binding sites often interferes with the association of a transcription factor to the DNA, which can in turn repress or activate gene expression depending on whether the sequence is recognized by a transcriptional repressor or activator (Sweatt et al., 2013). DNA hydroxymethylation (5hmC) is another covalent modification that is highly prevalent in the mammalian brain—reaching only 10-20% of total 5mC levels in peripheral tissues, but up to 40% of 5mC levels in neurons—and has distinctive interactions with transcription factors (Globisch et al., 2010). The mechanism of converting 5mC and 5hmC has recently been elucidated and, briefly, ten-eleven translocation proteins 1 and 3 (Tet1 and Tet3 respectively) serve to oxidize methylated DNA into 5hmC (S. Ito et al.,

2010) and are expressed at higher levels in the PFC than in other tissues (Rudenko et al., 2013; Szwagierczak, Bultmann, Schmidt, Spada, & Leonhardt, 2010). Additionally, Tet proteins further convert 5hmC to 5-formylcytosine (5-fC), then 5-fC to 5-carboxylcytosine (5-caC), then 5-caC back into unmodified cytosine (Wu & Zhang, 2017) to complete the cycle.

Functionally, 5mc and 5hmc are distinct, with a transition to an open chromatin state associated with the conversion of 5mC to 5hmC (Wu & Zhang, 2011). Further studies have also indicated that DNA hydroxymethylation may facilitate gene activity, rather than repress it, as DNA methylation has been shown to do. For instance, a genome-wide map of human stem cells during embryonic development indicated a large amount of 5hmC within gene enhancer areas and gene bodies, as well as in DNA transcription binding sites for the proteins OCT4 and NANOG which are key proteins implicated in cell maturation (Stroud, Feng, Morey Kinney, Pradhan, & Jacobsen, 2011). Moreover, a screen for protein enrichment within DNA sites has indicated binding of various transcription factors to 5hmC and 5fC, further implicating 5hmC in transcriptional regulation (Iurlaro et al., 2013).

Recent studies have demonstrated global and site-specific alterations in DNA methylation in the PFC after experimenter-administered cocaine (Pol Bodetto et al., 2013; Tian et al., 2012) and methamphetamine (Numachi et al., 2004), as well as after cocaine self-administration (Baker-Andresen et al., 2015; Massart et al., 2015; Nielsen et al., 2012; Wright et al., 2015). Specifically, repeated cocaine injections increased DNA methylation of the protein phosphatase type-1 gene (PP1) in brain tissue, resulting in decreased mRNA and protein for PP1 in the PFC, CPu, NAC core, and NAC shell (Pol

Bodetto et al., 2013). Additionally, rats trained to self-administer cocaine for 6 h per day exhibited a wide range of changes in 5mC levels within the NAC after 1 and 30 days of forced abstinence, which were associated with decreases in mRNA expression (Massart et al., 2015). Furthermore, potentiation of DNA methylation via DNA methyl transferases (DNMTs) or methyl-donors (i.e. SAM or MET) affect both cocaine-related behaviors. Repeated SAM pretreatment before daily cocaine injection has been shown to increase global DNA methylation and potentiate cocaine-induced locomotor sensitization (Anier, Zharkovsky, & Kalda, 2013). However, MET supplementation before daily cocaine self-administration attenuates cocaine locomotor sensitization and reduces cocaine-induced reinstatement responding after extinction (Wright et al., 2015). The discrepancies between observed locomotor behavior in these two studies signifies a distinct role for contingent cocaine administration regarding the function of DNA methylation. Indeed, previous studies have also shown context specific changes in epigenetic markers based on the timing of drug injection in relation to the presentation of Pavlovian conditioning (Ploense et al., 2013). Lastly, inhibition of Tet proteins in the hippocampus has been demonstrated to inhibit fear-extinction learning (Li, Wei, et al., 2014), and to attenuate the retrieval of cocaine-associated memories (Liu et al., 2017) indicating that 5mC conversion to 5hmC is involved in learning and memory retrieval processes.

Histone Modifications and Addiction

Another prominent epigenetic process is the modification of specific residues on the histone tails of chromatin. Histone tails can undergo methylation or demethylation (addition or subtraction of a methyl group) or undergo acetylation or deacetylation (addition or subtraction of an acetyl group). Histone acetyltransferases (HATs) serve to

add acetyl groups to histones, whereas histone deacetylases (HDACs) remove the acetyl group from a histone (Renthal & Nestler, 2009). Similarly, histone methyltransferases (HMTs) add methyl groups to histones and histone demethylases (HDMs) remove methyl groups from histones (Renthal & Nestler, 2009). Generally speaking, acetylation and demethylation of histones is associated with an open-chromatin state that permits mRNA transcription, whereas methylation and deacetylation of histones is associated with a closed-chromatin state that reduces mRNA transcription (for reviews see: Berger, 2007; Borelli, Nestler, Allis, & Sassone-Corsi, 2008).

Histone modifications are typically more dynamic than DNA methylation and hydroxymethylation, and are rapidly induced during learning. As such, they have been implicated in many fields including reinstatement for food reinforcement (Ploense et al., 2013), acquisition, extinction, and reconsolidation of fear memory (Bredy & Barad, 2008; Bredy et al., 2007), and reinstatement of drug-seeking (Taniguchi et al., 2017).

A number of histone modifications have been implicated in addiction, however, alterations in levels of HDAC5 have been linked to behaviorally relevant differences that separate acute cocaine administration from chronic cocaine administration (Renthal et al., 2007).

Inhibition of HDACs in the NAC promotes cocaine-induced locomotion and enhances the reinforcing properties of cocaine (Kumar et al., 2005; Renthal et al., 2007), and also potentiates cocaine-taking behavior (Sun et al., 2008). However, overexpression of HDACs results in reduced sensitivity to cocaine (Levine et al., 2005) and an attenuation of cocaine-taking behavior (Romieu et al., 2008). Although histone modifications are important in the regulation of cocaine-seeking behaviors, they are rapidly induced in

response to a variety of learning mechanisms and drug-associated cues. Therefore, I focused on DNA methylation and hydroxymethylation for this dissertation in order to determine if epigenetic markers persisted throughout forced abstinence.

Specific Gene Functions and Roles in Addiction

As previously stated, glutamatergic activity in the dmPFC is critical for cocaine seeking behaviors (Shin et al., 2017; Smith et al., 2008; Szumlinski et al., 2016). During cocaine abstinence, glutamate levels are reduced in the NAC (Miguens et al., 2008), as well as in the mPFC (Ben-Shahar et al., 2012). Additionally, glutamate levels are elevated upon re-exposure to cocaine cues and are necessary for cocaine-seeking during reinstatement procedures (McFarland et al., 2003). Furthermore, prolonged-access to cocaine results in differences in glutamate receptor expression during abstinence (Ary & Szumlinski, 2007; Ben-Shahar et al., 2009), extinction and reinstatement procedures (Pomierny-Chamiolo, Miszkiel, Frankowska, Bystrowska, & Filip, 2017). Therefore, I decided to investigate several glutamate-relevant genes in detail for my dissertation.

***Homer2* Function and Relevance to Addiction**

The *Homer2* gene encodes for the Homer2a/b protein, a scaffolding protein within the post-synaptic density. Homer2 is unique, as it is specifically located in the soluble and synaptic vesicle subfractions of neurons. Due to its location Homer2a/b is especially implicated in the modulating receptor proteins and glutamate transmission via anchoring metabotropic glutamate receptors (mGluR1 and mGluR5) to the synapse and crosslinking mGluRs to each other (Xiao et al., 1998), as well as linking postsynaptic density-95 proteins (Kim & Sheng, 2004), NMDA, IP3, and Ryanodine receptors (Shiraishi, Mizutani, Mikoshiba, & Furuichi, 2003; Szumlinski, Ary, & Lominac, 2008). The

extensive roles that Homer2 has in regulating receptor proteins in the synaptic density has made it a prime candidate for investigation in drug addiction studies.

Evidence for the role of Homer proteins in addiction is extensive, but varies based on experimental conditions. Homer proteins were first implicated in addiction in a 2001 study by Swanson et al., whereby rats receiving daily experimenter-administered injections of cocaine for 1 week exhibited decreased levels of mGluR1 and mGluR5 proteins as well as diminished expression of Homer1b/c protein 3 weeks after injections were ceased (Swanson, Baker, Carson, Worley, & Kalivas, 2001). Repeated cocaine injections have additionally been associated with increased hippocampal and PFC *Homer2,* mGluR1, NR2A, and NR2B protein, as well as decreased *Homer2, Homer1, mGluR1a, NR2a,* and *NR2b* protein within the NAC shell of rats and mice (Ary & Szumlinski, 2007). Rats allowed prolonged-access to cocaine self-administration show similar decreases in *Homer2* protein within the NAC core after 14 days of withdrawal (Ben-Shahar et al., 2009) and increases of Homer2 protein in the vmPFC after 3 and 30 days of withdrawal, and acute cocaine injection (Gould et al., 2015). Lastly, short-hairpin RNA (shRNA) will reverse observed increases in *Homer2* protein expression present in rats after cocaine self-administration within the vmPFC, and also reduced drug-seeking behavior in the rats primed with a cocaine injection (Gould et al., 2015).

Further research into the functional role of *Homer2* conducted by Szumlinski et al. in 2003 demonstrated that mice with a selective deletion of the *Homer2* gene exhibited greater cocaine place conditioning, locomotion, and increased glutamate and dopamine transmission within the ventral striatum after cocaine injection (Szumlinski, Toda, Middaugh, Worley, & Kalivas, 2003). Additional studies employing *Homer2* KO mice

demonstrate that they are phenotypically similar to cocaine treated rats, as they exhibit accelerated acquisition of cocaine self-administration and also exhibit reduced basal glutamate levels and sensitized increases in glutamate transmission in the nucleus accumbens after cocaine injection (Kalivas, Szumlinski, & Worley, 2004; Szumlinski et al., 2004). To compliment *Homer2* KO studies, subsequent research was conducted to investigate virus-mediated overexpression of *Homer2* within the nucleus accumbens. Overexpression of *Homer2* reversed the effects of repeated cocaine administration; it reduced cocaine-induced locomotion, returned basal glutamate to control levels, and reversed cocaine-induced increases in extracellular glutamate in rats (Szumlinski et al., 2006). Additionally, overexpression of *Homer2* within the medial PFC elevated basal glutamate levels, enhanced conditioned place preference for cocaine, and reduced NAC *Homer2* expression and reduced NAC basal glutamate (Ary et al., 2013).

Dlg4 **Function and Relevance to Addiction**

The *Dlg4* gene encodes for the postsynaptic density 95 (PSD-95) protein, a crucial scaffolding protein in neuronal synapses that anchors N-methyl-D-Aspartate (NMDA) receptors to the cytoskeleton (Wang & Peng, 2016), inhibits dopamine D1 receptors (Zhang et al., 2007), co-localizes with Homer and Shank proteins (Szumlinski et al., 2008), links NMDA receptors to various messenger proteins such as Src, Pyk2, SynGAP, and nNOS, and is crucial for synapse maturation (El-Husseini, Schnell, Chetkovich, Nicoll, & Bredt, 2000). Overexpression of PSD-95 results in some interesting synaptic changes including increased dendritic spine number and size, increased mGluR protein in the postsynaptic synapse, increased metabotropic excitatory postsynaptic currents (mEPSCs), and increased axon terminals in presynaptic neurons

(El-Husseini et al., 2000). Additionally, up-regulation of PSD-95 is associated with additional NMDA scaffolding, induction of late-phase long-term potentiation (LTP), and prevents the internalization of NMDA receptors (Roche et al., 2001; Williams et al., 2003). In contrast, transgenic PSD-95 KO mice have impaired synaptic maturation and NMDAR signaling, which can be reversed via overexpression of PSD-95 (Elias, Elias, Apostolides, Kriegstein, & Nicoll, 2008). These studies indicate that PSD-95 plays a key role in the development of mature, functioning synapses. As such, disruptions in PSD-95 expression (and glutamate signaling) have been documented in a number of neuropsychiatric conditions such as schizophrenia (Catts, Derminio, Hahn, & Weickert, 2015), Huntington's disease (Smith et al., 2014), depression (Feyissa, Chandran, Stockmeier, & Karolewicz, 2009; Zhao et al., 2012), and drug addiction (Carpenter-Hyland & Chandler, 2006; L. A. Knackstedt et al., 2010; Szumlinski et al., 2008).

A key feature in addiction is the disruption of glutamatergic signaling within limbic circuits. Therefore, the disruption of PSD-95 expression within these circuits is evident in humans and animals after repeated alcohol, opiate, and psychostimulant use. For instance, one study indicated that DAT -/-, NET -/-, VMAT2 -/- mice, as well as mice administered repeated injections of cocaine, exhibited reduced levels of PSD-95 in the NAC, caudate-putamen, and whole striatum (Yao et al., 2004). In another prominent study by Knackstedt et al. (2010), rats allowed access to sucrose or cocaine self-administration for 2 h per day for 12 days were subjected to 3 weeks of forced abstinence. Groups were then divided into "abstinence only" and "extinction" conditions. Only rats exposed to cocaine self-administration with extinction training exhibited increases in PSD-95 expression within the NAC core, as well as blunted long-term

depression (LTD) of the synapse (Knackstedt et al., 2010). Finally, in 2011, Ghasemzadeh et al. investigated glutamatergic plasticity after prolonged access to cocaine self-administration and extinction training. They found increased levels of GluR1, PSD-95, and actin proteins and decreased levels of mGluR5 protein within the postsynaptic density of the dmPFC (Ghasemzadeh et al., 2011).

Grin1 function and relevance to addiction

The final gene that I investigated, *Grin1* (aka *NMDAR1*), encodes for the NMDA receptor 1 subunit (NR1) of the NMDA receptor. There are 3 families of genes that encode for NMDA receptor subunits. NR1 only has one gene, 8 splice variants, and is mandatory for NMDA receptor expression. NR2 has four separate genes and four subunits with many different splice variants, and are distributed differentially based on a variety of factors. Lastly, NR3 has two genes encoding for two separate subunits, and negatively regulate the NMDA receptor complex. The immense variety of possible NMDA receptor subunits, splice variants, and configurations allows for incredible diversity of NMDA function and distribution, but the NR1 subunit is always present (Bai & Hoffman, 2009).

NMDA receptors are crucial glutamate receptors and have been investigated extensively in many different fields including learning and memory, synaptic development, LTP, LTD, mood disorders, neurodegenerative disorders, and addiction (for a full review, refer to Sweatt, 2016). Chronic cocaine administration results in unique patterns of area-specific changes in NMDA receptor expression during administration, withdrawal, and reinstatement. For example, patients that have died from cocaine overdose exhibited increased levels of mRNA and protein for NR1, CREB, GluR2,

GluR5, and KA2 within the ventral tegmental area (VTA) (Tang, Fasulo, Mash, & Hemby, 2003), as well as increased NR1 and GluR2/3 protein within the NAC (Hemby et al., 2005). Additionally, cocaine self-administration in rhesus monkeys resulted in elevated protein levels for NR1, GluR1, GluR2/3, and GluR5 within the NAC (Hemby, Tang, et al., 2005). In 2007, Ary & Szumlinski injected rats with 30 mg/kg of cocaine for 7 days and then collected tissue at 3 weeks of forced abstinence. The observed increased levels of *NR2A, NR2B, mGluR1a,* and *Homer2a/b* protein in the PFC and hippocampus, but decreased levels of these proteins within the NAC (Ary & Szumlinski, 2007). Two subsequent studies in 2009 reported differential glutamatergic receptor subunit expression between synaptosomal and whole tissue protein levels within the PFC and NAC after 1 day or 21 days of forced abstinence following repeated cocaine injections. NR1 expression was decreased in the synaptosomal subfraction of the dmPFC and vmPFC after 1 day of abstinence, whereas NR1 synaptosomal protein expression was increased following 21 days of forced abstinence within the dmPFC, vmPFC, NAC core, and NAC shell (Ghasemzadeh, Mueller, & Vasudevan, 2009; Ghasemzadeh, Vasudevan, & Mueller, 2009). Lastly, Ghasemzadeh et al. profiled glutamate receptor expression in the PFC and VTA in rats following cocaine self-administration procedures and forced abstinence in either the home-cage, the operant chamber, or after cocaine-extinction procedures for 12-14 days. They reported no changes in NR1 expression in any condition within the dmPFC or vmPFC, but there was decreased tissue NR1 after extinction procedures in the VTA, and decreased synaptosomal NR1 expression following forced abstinence in the home cage and operant chamber (Ghasemzadeh et al., 2011).

Npas4 function and relevance to addiction

Npas4, also known as *NxF*, is a gene that encodes for neuronal PAS domain protein 4, an immediate early gene (IEG) expressed exclusively in neurons and selectively activated by the depolarization of neurons (Ooe, Saito, Mikami, Nakatuka, & Kaneko, 2004; Sun & Lin, 2016; Zhang et al., 2009). *Npas4* is essential for excitatory/ inhibitory synapse balance (Lin et al., 2008), protection against neuronal overexcitation (Piechota et al., 2010), and cell survival (Zhang et al., 2009). It regulates these processes through activating a large suite of genes including the GABAergic genes $GABA_A$-γ2, GAD65, and $GABA_A$-β2/3, growth factors such as BDNF, other IEGs like Arc, c-Fos, and Zif268, and by recruiting RNA polymerase II to enhancer and promoter sites in the DNA (Lin et al., 2008; Ramamoorthi et al., 2011; Spiegel et al., 2014). The various functions of *Npas4* are essential for LTP, LTD, and synaptic remodeling that are intrinsic to various learning and memory processes. Therefore, it is not surprising that *Npas4* is intimately involved in long-term memory storage and retrieval. For instance, *Npas4* activation within the hippocampus is essential for contextual fear conditioning, and acute expression of *Npas4* via injection reverses short-term and long-term memory deficits present in *Npas4* KO mice (Ramamoorthi et al., 2011). Additionally, *Npas4* is required for the formation of new auditory fear memories in the lateral amygdala (LA), as well as the reactivation of fear memories in the LA (Ploski, Monsey, Nguyen, DiLeone, & Schafe, 2011). Lastly, chronic stress or corticosterone treatment reduce *Npas4* expression in the hippocampus and PFC, impair hippocampus-dependent fear memories, and decreased PFC activity (Drouet et al., 2015).

Although *Npas4* has been extensively investigated for the role it plays in long-term memory storage and retrieval regarding cognition and fear memories, there is

limited investigation on *Npas4* function and addiction. The first study to implicate *Npas4* expression after drug treatment utilized whole-genome microarray profiling of the mouse striatum after acute systemic injection of nicotine, cocaine, ethanol, methamphetamine, or heroin (Piechota et al., 2010). Piechota et al. found drug-dependent expression of *Npas4* mRNA after treatment with cocaine, methamphetamine, and heroin (Piechota et al., 2010). In 2012, Martin et al. performed another microarray of the NAC 1 h, 8 h, 16 h, and 24 h after acute injection of methamphetamine. *Npas4* mRNA expression was increased 1 h after acute methamphetamine injection, but decreased 24 h after acute methamphetamine injection (Martin et al., 2012). Although these prior studies are an important contribution to the field and discovery of genes relevant to addiction, they assessed the role of *Npas4* after drug-induced neuronal excitation but did not address the impact of chronic exposure to drugs of abuse which is widely associated with the development of psychostimulant addiction (e.g. see Koob & Volkow, 2009). The first study to investigate the impact of repeated drug exposure on *Npas4* found while acute injections of amphetamine did not increase *Npas4* protein expression in either the NAC or in the caudate-putamen, repeated administration of amphetamine did increase *Npas4* protein expression in the NAC, but not in the caudate-putamen (Guo et al., 2012). More recently, a causal role for *Npas4* expression within the NAC in cocaine conditioned place preference (CPP) and cocaine self-administration behaviors has been established (Taniguchi et al., 2017). This study demonstrated that (1) *Npas4* protein expression was vastly increased in animals exposed to CPP and cocaine injection vs home cage controls, and animals injected with *Npas4* shRNA had lower preference to the cocaine chamber in CPP than control animals; and (2) *Npas4* conditional knockout (cKO) mice exhibited

blunted acquisition of cocaine self-administration, higher drug-seeking during extinction training, and blunted acquisition of extinction training (Taniguchi et al., 2017).

Summary

In summary, preclinical and clinical data gathered to date suggests a role for the dmPFC in the incubation of cocaine craving after extended periods of forced abstinence. Additionally, research has shown that prolonged-access to cocaine is associated with heightened levels of cocaine craving, cocaine seeking after forced abstinence, protein expression and glutamatergic neurotransmission within the dmPFC. Furthermore, DNA methylation is a persistent epigenetic modification that may underlie observed changes in neuronal structure and receptor expression that occur during abstinence from cocaine, or re-exposure to cocaine cues. Therefore, I propose the following Specific Aims to determine the role of DNA methylation and mRNA expression within the dmPFC after different periods of abstinence from cocaine, and after re-exposure to cocaine-associated cues.

Specific Aims

My dissertation seeks to address the following specific aims:

Specific Aim 1:

Determine the role of behavioral contingency in the effect of prolonged access on cocaine intake escalation and changes in glutamatergic gene expression within the dmPFC. Previous reports have indicated that increased access to cocaine administration results in an escalation of cocaine intake. However, it is yet unknown whether it is the amount of total cocaine intake, or the contingency of cocaine intake that induces an addiction-like phenotype in animals. Although, non-contingent injections of cocaine have

also been demonstrated to produce different behavioral effects than SA cocaine. Therefore, I hypothesize that rats allowed 6 h of contingent-access to cocaine SA daily will exhibit an escalation of cocaine intake which will be accompanied by changes in mRNA transcription for addiction-relevant genes.

Specific Aim 2:

Characterize differences in basal levels of DNA methylation and mRNA transcription within the dmPFC after extended access to cocaine, limited access to cocaine, limited + yoked access to cocaine, and access to saline during withdrawal. Based on preliminary data acquired prior to this thesis (Appendix Table 1), Aim 2 seeks to utilize updated extraction methods to measure DNA methylation and mRNA expression in genes relevant to addiction (*Homer2*, *Dlg4*, *Grin1*, and *Npas4*) at three different withdrawal points (1, 14, and 60 days). Based on previous findings regarding the function of DNA methylation, as well as preclinical findings associated with *Homer2, Dlg4*, *Grin1,* and *Npas4* mRNA and protein expression after cocaine self-administration, I hypothesize that prolonged-access to cocaine self-administration will produce a unique pattern of DNA methylation that will continue throughout abstinence and will be accompanied by associated patterns of mRNA expression.

Specific Aim 3:

Characterize DNA methylation and mRNA expression after re-exposure to drug-associated cues following protracted withdrawal. Aim 1 was proposed to assess the basal state of rats undergoing forced abstinence from cocaine. However, home cage abstinence does not measure cocaine-seeking behavior in animals. Therefore, for Aim 2, I

propose to submit rats to 60 days of forced abstinence after saline-access or prolonged-access to cocaine, then re-exposure them to cues previously associated with cocaine, and measure DNA methylation, mRNA expression, and cocaine seeking behavior. I hypothesize that levels of DNA methylation observed in prolonged-access rats after 60 days of forced abstinence will persist upon cue re-exposure, and cue re-exposure will initiate context-specific mRNA transcription that aligns with DNA methylation.

Specific Aim 4:

Examine DNA methylation, hydroxymethylation, and transcription factor binding within the *Homer2* promoter after 1 day of forced abstinence. Data acquired during completion of Aim 1 resulted in unexpected levels of DNA methylation in relation to mRNA expression. Both limited- and prolonged-access rats exhibited decreased DNA methylation, yet only prolonged-access rats exhibited increased mRNA expression. Therefore, I hypothesize that factors other than DNA methylation mediate mRNA transcription, such as DNA hydroxymethylation or transcription factor binding to the *Homer2* promoter.

General Methods:

Chapter 2: Contributions of prolonged contingent and non-contingent cocaine exposure to escalation of cocaine intake and glutamatergic gene expression.

Abstract

Similar to the pattern observed in people with substance abuse disorders, laboratory animals will exhibit escalation of cocaine intake when the drug is available over prolonged periods of time. Here, I investigated the contribution of behavioral contingency of cocaine administration on escalation of cocaine intake and gene expression in the dorsal medial prefrontal cortex (dmPFC) in adult male rats. Rats were allowed to self-administer intravenous cocaine (0.25 mg/infusion) under either limited cocaine- (1h/day), prolonged cocaine- (6h/day), or limited cocaine- (1h/day) plus yoked cocaine-access (5h/day); a control group received access to saline (1h/day). One day after the final self-administration session, the rats were euthanized and the dmPFC was removed for quantification of mRNA expression of critical glutamatergic signaling genes, *Homer2, Grin1, and Dlg4*, as these genes and brain region have been previously implicated in addiction, learning, and memory. All groups with cocaine-access showed escalated cocaine intake during the first 10 minutes of each daily session, and within the first 1h of cocaine administration. Additionally, the limited-access + yoked group exhibited more non-reinforced lever responses during self-administration sessions than the other groups tested. Lastly, *Homer2, Grin1,* and *Dlg4* mRNA were impacted by both duration and mode of cocaine exposure. Only prolonged-access rats exhibited increases in mRNA expression for *Homer2, Grin1,* and *Dlg4* mRNA. Taken together, these

findings indicate that both contingent and non-contingent "excessive" cocaine exposure supports escalation behavior, but the behavioral contingency of cocaine-access has distinct effects on the patterning of operant responsiveness and changes in mRNA expression.

Introduction

Cocaine addiction is a chronic disorder that persists in spite of negative interpersonal, professional, and physical consequences, with the development of tolerance and increased cocaine intake serving as central diagnostic criteria for stimulant use disorders (APA, 2013). It has been argued that one major reason for the escalation of cocaine intake relates to gradual changes in motivation and learning processes associated with responses to the positive and negative reinforcing properties of cocaine (Koob, 2004). In order to model the differences in cocaine use observed in humans, differential access to cocaine self-administration has been employed as an avenue to study the behavioral and neurobiological aspects of drug-taking. Ahmed & Koob (1998) demonstrated that "limited" daily-access (1 h/ day) and "prolonged" daily-access conditions (6 h/ day) are distinct in their ability to model aspects of drug abuse/addiction in two major ways: 1) rats with prolonged cocaine-access escalate their cocaine intake across daily sessions, whereas the limited-access rats exhibit stable intake for several weeks and 2) rats with daily "prolonged" cocaine-access dramatically increase intake of cocaine for the 1st 10 minutes of self-administration while "limited" access rats do not

escalate cocaine intake within the 1st 10 minutes of self-administration (Ahmed & Koob, 1998). This differential pattern of responding has since been replicated with a number of procedural variations to determine the underlying changes in drug-taking behavior (Ahmed & Koob, 1999), neurocircuitry (Ahmed et al., 2002; Ben-Shahar et al., 2012; Robinson & Kolb, 2004) and cellular/ molecular function in rats (Ben-Shahar et al., 2009; Ben-Shahar et al., 2013) , as well as in other species (Henry et al., 2009; Nakamura et al., 2011).

Contingency of cocaine intake and addiction-like behaviors

Despite substantial study, it remains to be determined whether escalated cocaine intake (and the neurobiological changes associated there with) is dependent upon the amount of cocaine exposure or the act of self-administering the cocaine. One way to dissociate the relative contribution of total drug exposure from the behavioral contingency of that exposure is to employ yoked-access procedures (i.e. the administration of cocaine under the control of a separate self-administering rat). Rats receiving yoked-cocaine exhibit several features that are distinct from rats self-administering cocaine. First, self-administering rats exhibit greater dopamine levels than their yoked- counterparts (Hemby et al., 1997). Additionally, yoked rats exhibit differential corticosterone levels in systemic plasma (Galici et al., 2000) and within the brain (Palamarchouk et al. 2009). They also have a higher morbidity rate (Dworkin et al., 1995) and exhibit higher indices of distress (measured by ultrasonic vocalization) (Mutschler & Miczek, 1998). These studies demonstrate that, despite equivalent cocaine dosing, rats receiving non-contingent (yoked) cocaine show a unique behavioral and molecular profile that is distinct from rats self-administering cocaine.

To expand upon the role of behavioral contingency in cocaine-mediated behaviors, Kippin et al. (2006) employed a novel mixed self-administration/yoked cocaine exposure procedure in which rats received 1-h access to cocaine self-administration before receiving non-contingent cocaine infusions via yoking procedures during the last 5 hours of cocaine self-administration. In this study, the prolonged contingent-access rats exhibited greater cocaine-primed reinstatement of responding than their yoked counterparts. Therefore, in the present study, I employ several procedures to assess cocaine-taking behavior in rats. I exposed rats to limited-access, prolonged-access, and limited + yoked-access procedures (in the same manner as Kippin et al., 2006) to determine the impact of contingent and non-contingent "excessive" cocaine exposure on the escalation of cocaine intake as well as operant responding for cocaine to determine how behavioral contingency of cocaine delivery influences these aspects of cocaine addiction-related behavior.

Dorsal PFC in addiction

The dorsomedial prefrontal cortex (dmPFC) is dysregulated in human cocaine addicts (Verdejo-Garcia et al., 2015) and this dysregulation is linked to aberrant learning and plasticity that is attributed to anomalies in glutamate transmission (Kalivas, Volkow, & Seamans, 2005; Pascoli et al., 2014; Ruan & Yao, 2017). To explore the potential neurobiology underpinning the effects of cocaine on dmPFC as a function of contingency, I measured the levels of mRNA for *Homer2*, *Grin1*, and *Dlg4* within the dorsomedial prefrontal cortex (dmPFC). Homer2 is a glutamate receptor scaffolding protein that is up-regulated within PFC by both non-contingent and contingent cocaine administration (Ary and Szumlinski, 2007; Ary et al., 2013; Ben-Shahar et al., 2009;

Gould et al., 2013) and while it remains to be determined whether or not cocaine-induced increases in PFC Homer2 protein expression reflects increased gene transcription, Homer2 expression bi-directionally regulates both basal and cocaine-induced changes in extracellular glutamate levels within PFC (Ary et al., 2013) to influence cocaine-conditioned approach behavior in place-conditioning models (Ary et al., 2013) and cocaine-primed reinstatement of lever-pressing behavior in operant-conditioning models (Gould et al., 2013). *Grin1* mRNA encodes the obligatory N-methyl-D-aspartate (NMDA) receptor sub-unit GluN1 within the mammalian brain (Bai & Hoffman, 2009); this receptor is widely implicated and critical for many forms of plasticity (Bear, 1996; Hopf, 2017; Sweatt, 2016; Thiels, Xie, Yeckel, Barrionuevo, & Berger, 1996), and is up-regulated in the PFC following cocaine exposure (Ary & Szumlinski, 2007; Blanco et al., 2014; Hemby, Horman, & Tang, 2005). *Dlg4* encodes the sequence for postsynaptic density-95 (PSD-95), a receptor scaffolding protein that regulates plasticity and learning through its interactions with the NMDA receptor (Wang & Peng, 2016). PSD-95 expression in the PFC is increased after prolonged withdrawal from cocaine (Ghasemzadeh, Vasudevan, & Mueller, 2009; McIntosh, Howell, & Hemby, 2013) and following extinction testing during prolonged exposure to cocaine self-administration (Ghasemzadeh et al., 2011).

Methods

Subjects:

Male Sprague-Dawley rats were pair-housed in a 12-h reverse light-dark cycle room and had *ad libitum* access to food and water (except as noted below). The housing

and care of the rats followed the guidelines set forth by the "Guide for the Care and Use of Laboratory Rats, 8th Edition" (IACUC, 2011).

Surgery:

Male Sprague-Dawley rats weighing 300-350g were deeply anesthetized using ketamine (60mg/kg) and xylazine (10mg/kg). Chronic indwelling catheters were constructed using a bent steel cannula with a screw-type connector (Plastics One, Roanoke, VA), SILASTIC tubing (11 cm, i.d. 0.64 mm, o.d. 1.19 mm, Dow Corning, Midland, MI), Prolite polypropylene monofilament mesh (Atrium Medical Corporation, Hudson, NH), a silicon ball 2.5 cm from the end, and methyl methacrylate dental cement. The catheters were implanted and maintained as I have reported previously (Ben-Shahar et al., 2013; Kerstetter, Aguilar, Parrish, & Kippin, 2008) . Naïve rats were left in the vivarium and handled daily, but had no access to behavioral training or surgery; they were euthanized at the same age as the rats undergoing behavioral training.

Behavioral Training:

Food-training and cocaine self-administration utilized standard operant chambers (Med Associates Inc., St. Albans, VT, USA) and were conducted during a fixed time in the dark phase of the rat's circadian cycle each day. Before surgical implantation of the jugular catheters, the rats were restricted to 20 g of food for 1 week, and trained on a fixed ratio 1 (FR1) schedule of food reinforcement for two 16 h training sessions where each right lever-press was associated with a 45 mg food pellet. After recovery from the surgery, the rats were placed on a fixed ratio 1 (FR1) schedule of reinforcement for intravenous (IV) cocaine (0.1mL at 0.25 mg/infusion in 0.9% saline) or saline for 1h/day

for 5 days. Each active lever-press was associated with a 4 sec infusion of cocaine or saline and a 20 sec timeout was signaled with a 20 sec light cue above the active lever. On the 6th day, the cocaine rats were divided into limited (1 h/day) cocaine-access, prolonged (6 h/day) cocaine-access, and limited-access + yoked-access (1 h access followed by 5 h of "yoked" exposure) treatment groups and continued the FR1 schedule of reinforcement for an additional 15 days. During yoked exposure, rats remained in their chambers for an additional 5 h with the levers retracted to eliminate the opportunity to perform the operant response. During this time, yoked rats received a 4 sec cocaine infusion every time a paired prolonged-access rat self-administered an infusion, but without cue light presentation.

Tissue Collection and mRNA Quantification:

Twenty-four hours after the last self-administration session, the animals were sacrificed via rapid decapitation, and their brains were frozen over ice and dissected into 0.5 mm sections with a metal brain mold (Braintree Scientific, Braintree, MA). The dmPFC was dissected out at 3.24 to 2.74 mm anterior to Bregma and stored at -80 Celsius (Figure 2.1). The frozen dmPFC was added to 600 µL of buffer RLT (Qiagen DNA/RNA/Protein extraction kit) and homogenized with the Qiagen TissueRuptor for 30 sec. mRNA was then extracted through use of the AllPrep DNA/RNA/protein extraction kit provided by Qiagen in accordance to the protocol provided by the manufacturer. RNA

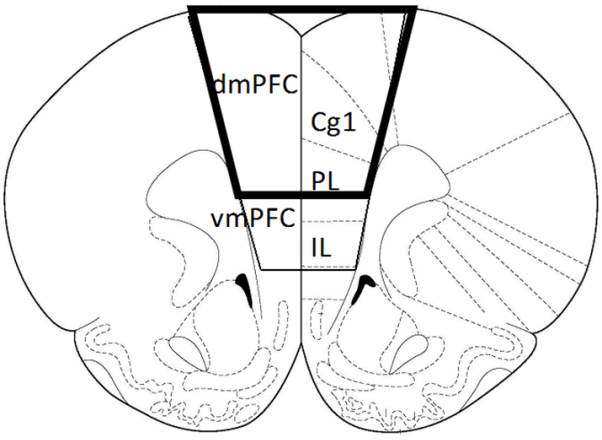

Figure 2.1: dmPFC dissection of the rat brain. The brain was dissected horizontally between bregma +3.24 and bregma +2.74 (Paxinos & Watson, 2006).

was eluted from the spin column with 50µL of nuclease-free water. RNA (500 ng/sample) was incubated with 2µL of gDNA Wipeout Buffer (Qiagen) at 42°C for 2 minutes then cooled over ice. 1µL of Reverse Transcription Master Mix (Qiagen), 1µL of RT primer mix (Qiagen), and 4µL of Quantiscript RT Buffer (Qiagen) were added to the reaction mixture and incubated in an Eppendorf MasterCycler at 42°C for 18 minutes to amplify the product, then incubated at 95°C for 3 minutes to inactivate the reverse transcriptase. A reverse transcriptase-negative reaction was carried out in parallel with the samples from 500ng of pooled sample RNA.

Levels of mRNA were assessed in triplicate using quantitative real time pcr (qRT-PCR) (Biorad) on the BioRad CFX96 Touch Real-Time system. Negative controls

Target	Accession Number	Forward (5'->3')	Reverse (5'->3')	Amplicon Length (bp)	Annealing Temp	Primer Concentration
Homer2 mRNA	NM_053309	GAGTGCTGCCAATGTGAAGA	TTGATCTCACCGCACTGTTC	195	61C	30nM
Grin1 mRNA	NM_001270610.1	CGGCTCTTGGAAGATACAGC	GTGGGAGTGAAGTGGTCGTT	156	60C	20nM
Dlg4 mRNA	NM_019621.1	CCGACAAGTTTGGATCCTGT	CCCATAGAGGTGGCTGTTGT	164	60C	20nM
Gapdh mRNA	NM_017008.4	AGAACATCATCCCTGCATCC	AGGAGACAACCTGGTCCTCA	240	61C	20nM
βActin mRNA	NM_031144	TGTCACCAACTGGGACGATA	GGGGTGTTGAAGGTCTCAAA	165	63C	20nM
Tubb5 mRNA	NM_139254	TGAGGCCTCCTCTCACAAGT	TGCAGGCAGTCACAATTCTC	237	62C	20nM

Table 2.1: Primer concentrations, size, and run conditions for qPCR experiments. Primers were created using Primer3 software and verified with PrimerBLAST and a 2% agarose gel.

consisted of a DNA-negative sample and a reverse-transcriptase free sample. Standard curves were run on each pcr plate with 3x serial dilutions ranging from 50.0ng/uL to 1.85ng/uL. The data were normalized using three control genes (Gapdh, βActin, and Tubb5) and dmPFC tissue from naïve age-matched rats according to the equations outlined by Hellemans et al. in 2007 (Table 2.1).

Statistical Analyses:

The self-administration data for individual sessions during differential access to cocaine were compared to baseline responding (average of days 6, 7, & 8 of differential access) at 10 min (i.e. "loading phase"; e.g. Ahmed & Koob, 1998) and 1 h intervals to compare across all four conditions, as well as across the entire 6 h sessions for the prolonged-access and limited-access + yoked groups. Separate two-way, between-within (group X day), repeated measures ANOVAs, were conducted for the numbers of cocaine infusions followed by Dunnett post-hoc comparisons to deconstruct significant interactions/main effects using the Prism 6 statistical software (Graphpad). Dunnett's post-hoc tests were used to compare cocaine intake during differential access to baseline responding. Non-reinforced responding (i.e. during the time-out period) was analyzed separately from total responding via a two-way, between-within (group X day), repeated measures ANOVAs followed by Tukey's post-hoc comparison for the first 10 min and 1 h of self-administration to assess the efficiency of behavioral responding at each day before (days 4 & 5) and after differential access (days 6 to 20). Inactive lever presses were also analyzed but no significant effects or interactions were detected and in all cases, mean inactive lever presses were < 5. Additionally, self-administration data was separated into separate 10 min blocks on day 6 and day 20 to assess loading during initial daily access. A two-way ANOVA (time block X condition) was conducted to assess the differences in cocaine intake during 10-minute time blocks for the first hour of self-administration in all four experimental groups during the 6^{th} day and 20^{th} day of self-administration; Tukey's post-hoc comparison was used to compare cocaine intake between each 10 minute time block and between access conditions. Lastly, analyses of normalized quantitative PCR data were performed by one-way MANOVA for *Homer2*,

Grin1, and *Dlg4* mRNA and decomposed via post-hoc LSD tests using SPSS Statistics 24 (IBM). All graphics were plotted by using the Prism 6 statistical software (Graphpad).

Results

Cocaine intake escalates in rats allowed to self-administer cocaine

A two-way repeated measures ANOVA of cocaine intake (mg/kg) during the loading phase of self-administration sessions (first 10 minutes) revealed significant within-group effects of time ($F_{15, 2055} = 8.049$, $p < 0.0001$ Figure 2.2A), between-group effects of treatment ($F_{3, 137} = 82.32$, $p < 0.0001$, Figure 2A), and an interaction between time and treatment ($F_{45, 2055} = 2.88$, $p < 0.0001$ Figure 2A). Within-group (aka time) differences were assessed via Dunnett's *post-hoc* test and revealed increases in cocaine intake for day 20 vs baseline in the limited-access group, days 12, 18, 19, and 20 vs baseline in prolonged-access group ($p<0.05$), and days 11 through 20 vs baseline in limited-access + yoked rats ($p<0.05$) (Figure 2.2A). These results indicate an escalation of cocaine intake in all cocaine-access groups for the first 10 minutes of self-administration. The limited + yoked-access rats exhibited escalation first (day 11), which was slightly faster than prolonged-access rats (day 12), and much faster than limited-access rats (day 20). However, limited + yoked-access rats had a slightly lower baseline than prolonged-access rats, so the difference in escalation between the two groups is likely negligible. Additionally, between-group (aka treatment) differences were assessed via Tukey's *post-hoc* comparison test, and revealed that all cocaine-access conditions had significantly higher intake than the saline-access condition (Figure 2.2A)

The first hour of drug intake (mg/kg) during self-administration was also assessed via a two-way repeated measures ANOVA and revealed significant within-group effects

of time ($F_{15, 1875} = 4.121$, $p < 0.0001$ Figure 2.2B), between-group effects of treatment ($F_{3, 125} = 88.64$, $p < 0.0001$ Figure 2B), and an interaction between time and treatment ($F_{45, 1875} = 1.385$, $p < 0.05$; Figure 2.2B). Dunnett's multiple comparisons *post-hoc* analysis showed increased cocaine intake in the limited-access rats for day 20 vs baseline ($p < 0.05$), prolonged-access rats for days 18 & 20 vs baseline ($p < 0.05$), and in limited-access + yoked rats for days 14 & 19 vs baseline ($p < 0.05$). These data indicate a distinct difference in the time of onset for escalated cocaine intake between prolonged-access and limited-access + yoked rats.

Additionally, I divided cocaine intake for the first 1 h of self-administration into 10-minute blocks and assessed intake on day 6 and day 20 of self-administration between different conditions (within-subjects effects), as well as cocaine intake between days and conditions (between-subjects effects) with a three-way repeated measures ANOVA. Within-subjects tests revealed significant effects of minutes (aka 10-min time blocks, $F_{5, 270} = 61.940$, $p < 0.0001$), minutes x condition ($F_{5, 272} = 8.771$, $p < 0.0001$), minutes x day ($F_{5, 270} = 3.975$, $p < 0.0001$), and minutes x day x condition ($F_{5, 272} = 3.439$, $p < 0.005$) (Figure 2.2C). Within-subject effects were assessed via Dunnett's *post hoc* test and revealed significant increases in cocaine intake during the first 10 minutes of self-administration for all cocaine groups compared to other time points ($p < 0.05$). Between-subjects tests revealed a significant effect of day ($F_{1, 274} = 4.171$, $p < 0.05$), where day 20 has higher intake than day 6, as well as significant effects of condition ($F_{3, 274} = 68.624$, $p < 0.0001$). Tukey's *post-hoc* comparison revealed significantly higher levels of intake for all cocaine-access conditions compared to the saline-access condition ($p < 0.0001$). These results indicate that cocaine-intake was highest during the first 10 minutes of self-

administration (aka binge behavior), and that intake increased between day 6 and day 20 for all cocaine-access conditions (aka escalation, Figure 2.2C).

Lastly, I looked at total cocaine intake between prolonged-access and limited + yoked-access rats. A two-way repeated measures ANOVA was run to assess any differences in total cocaine intake (mg/kg) over the full 6 h of the experiment (Figure 2.2D). The results revealed a significant effect of time ($F_{14, 392} = 6.874$, $p < 0.0001$), but no effect for treatment ($F_{1, 28} = 0.0318$, $p = 0.8597$), however there was a significant interaction between time and treatment ($F_{14, 392} = 1.779$, $p < 0.05$). Dunnett's *post-hoc* comparison revealed that both the prolonged-access and limited-access + yoked conditions exhibited escalated cocaine intake from day 12 to day 20. Additionally, there was no difference between prolonged-access and limited-access + yoked conditions. These data indicate that both groups had increased cocaine exposure from baseline and, expectedly, as the majority of daily intake in both groups was controlled by prolonged-access rats, there is no observable difference in cocaine exposure between these two conditions (Figure 2.2D).

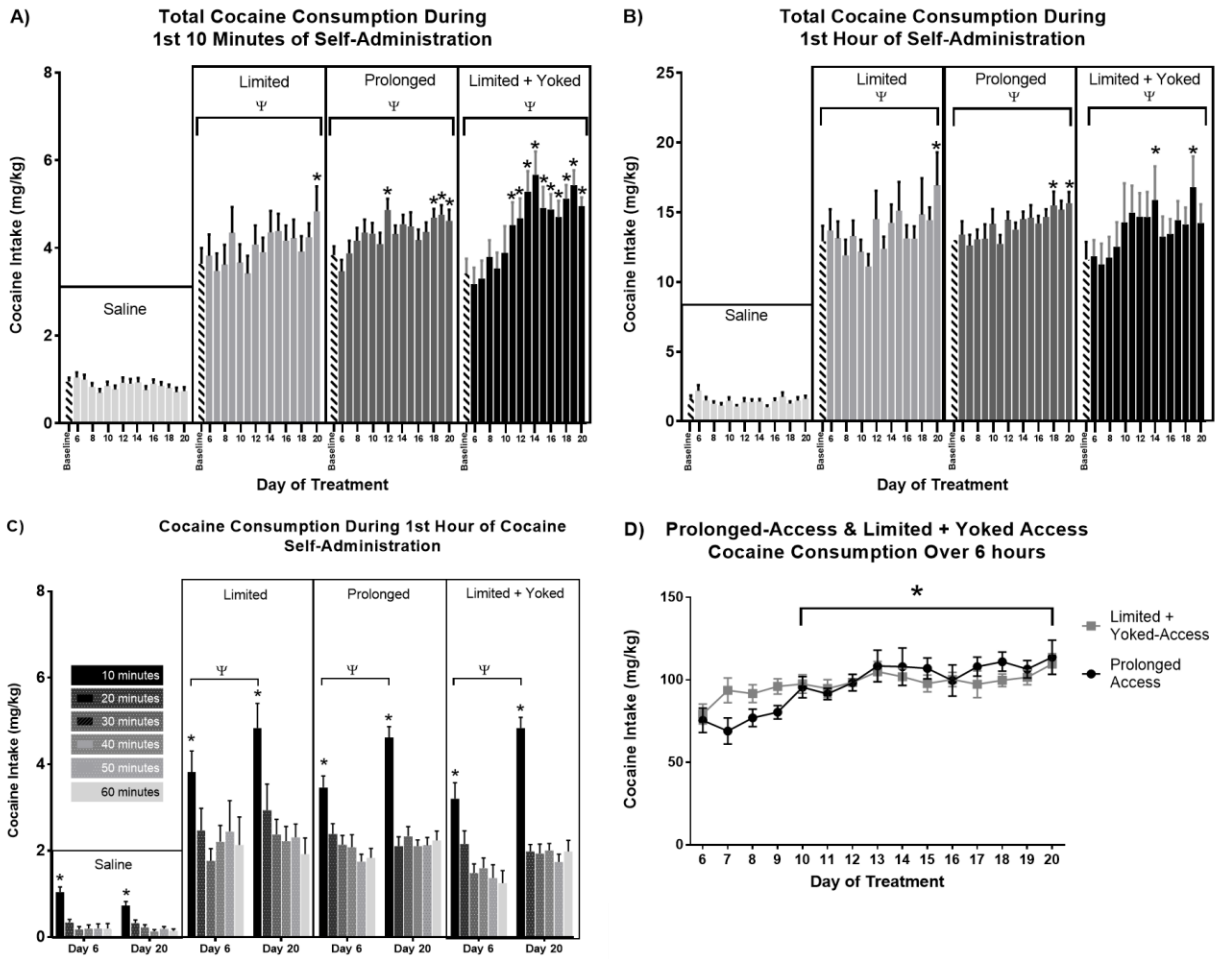

Figure 2.2: Cocaine intake during self-administration. * = within-group differences, ψ = between-group differences. **A) 1st 10 min of self-administration.** Limited-access rats escalated cocaine intake on day 20 of self-administration. Prolonged-access rats exhibited escalated cocaine intake on days 12, 18, 19, and 20 of self-administration. Limited + yoked rats escalated cocaine intake in between days 11 and 20 of self-administration (*, $p < 0.05$). All cocaine-access groups had higher intake than saline-access groups (ψ, $p < 0.0001$) **B) 1st 1 hour of self-administration.** Limited-access rats escalated cocaine intake on day 20. Prolonged-access rats escalated cocaine intake on days 18 & 20. Limited + yoked-access rats escalated cocaine intake on days 14 & 19 (*, $p < 0.05$). All cocaine-access groups had higher intake than saline-access groups (ψ, $p < 0.0001$) **C) Cocaine intake recorded every 10 minutes during 1st hour of self-administration.** Limited-access, prolonged-access, and limited + yoked-access rats all displayed increased cocaine intake during the first 10 minutes of self-administration sessions on both day 6 and day 20 of self-administration. Additionally, prolonged-access and limited + yoked-access rats exhibited increased cocaine intake within the 1st 10 minutes of self-administration between day 6 and day 20. (* $p < 0.05$, ψ $p < 0.05$) **D) Total daily cocaine intake for prolonged- and limited + yoked-access rats.** Cocaine intake escalated in both groups beginning on the 10th day of extended access to cocaine (* $p < 0.05$). Total cocaine intake did not differ between prolonged- and limited + yoked-access rats.

Non-reinforced Lever Responding

In addition to cocaine intake, I analyzed non-reinforced lever responding. A two-way repeated measures ANOVA of the numbers of non-reinforced active lever-responses (aka active lever responses – number of infusions) during the first 10 min of self-

administration revealed an effect of treatment ($F_{3, 134} = 5.236$, $p < 0.005$) and an interaction between time and treatment ($F_{51, 2278} = 2.183$, $p<0.0001$, Figure 2.3A). Dunnett's *post-hoc* comparison was used to assess within-group differences and indicated a significant increase in non-reinforced lever-responding for only the limited-access + yoked-access rats on days 12, 13, 14, 15, 18, and 19 versus baseline ($p<0.05$, Figure 2.3A). Furthermore, between-group differences were analyzed via Tukey's *post-hoc* test; limited + yoked-access rats exhibited significantly higher non-reinforced lever responding than other groups on days 10 to 19 ($p < 0.05$, Figure 2.3A). These data indicate that for the first 10 min of self-administration, the limited-access + yoked animals exhibited significantly more non-reinforced lever responding than limited-access and prolonged-access conditions well after differential-access to cocaine was initiated.

Non-reinforced lever responding was also assessed over the course of the first 1 h of self-administration. A two-way repeated measures ANOVA of the non-reinforced lever responding during the first 1 h of self-administration revealed a significant effect of time ($F_{17, 2210} = 4.31$, $p < 0.001$), and effect of treatment ($F_{3, 130} = 7.979$, $p < 0.0001$), and an interaction between time and treatment ($F_{51, 2210} = 1.379$, $p<0.05$; Figure 2.3B). Dunnett's multiple comparisons *post-hoc* analysis was used to break down within-group effects and revealed a significantly larger number of non-reinforced active lever responses for limited-, prolonged-, and limited + yoked-access conditions on the 4th day of self-administration ($p < 0.05$). In addition, Tukey's *post-hoc* comparisons revealed significant between-group effects; all cocaine rats engaged in more non-reinforced lever responses than saline-access rats on day 4 of self-administration, and the limited + yoked-access rats had more non-reinforced lever responses than all other groups on days 12, 14,

and 18 of self-administration (Figure 3B). These data indicated two main points, 1) before differential access, all cocaine-access rats exhibited non-reinforced lever responding at day 4 of self-administration and 2) only limited-access + yoked-access rats continued non-reinforced lever responding later in the experiment.

Lastly, inactive-lever responding was analyzed via a two-way repeated measures ANOVA. There was no effect of time, nor was there an interaction between time and treatment, but there was a main effect of treatment ($F_{3, 133} = 9.769$, $p < 0.0001$). Tukey's *post-hoc* test was used to assess between-group differences and revealed more inactive-lever responding in the saline-access rats compared to the prolonged-access rats on days 10, 11, 12, 14, and 19 of self-administration (Figure 2.3C). However, the mean differences (~ 3 lever responses) between these two conditions are not particularly meaningful in comparison to the total number of lever responses for cocaine-administration. Therefore, these data indicate a minor increase in inactive-lever responses within the saline-access rats.

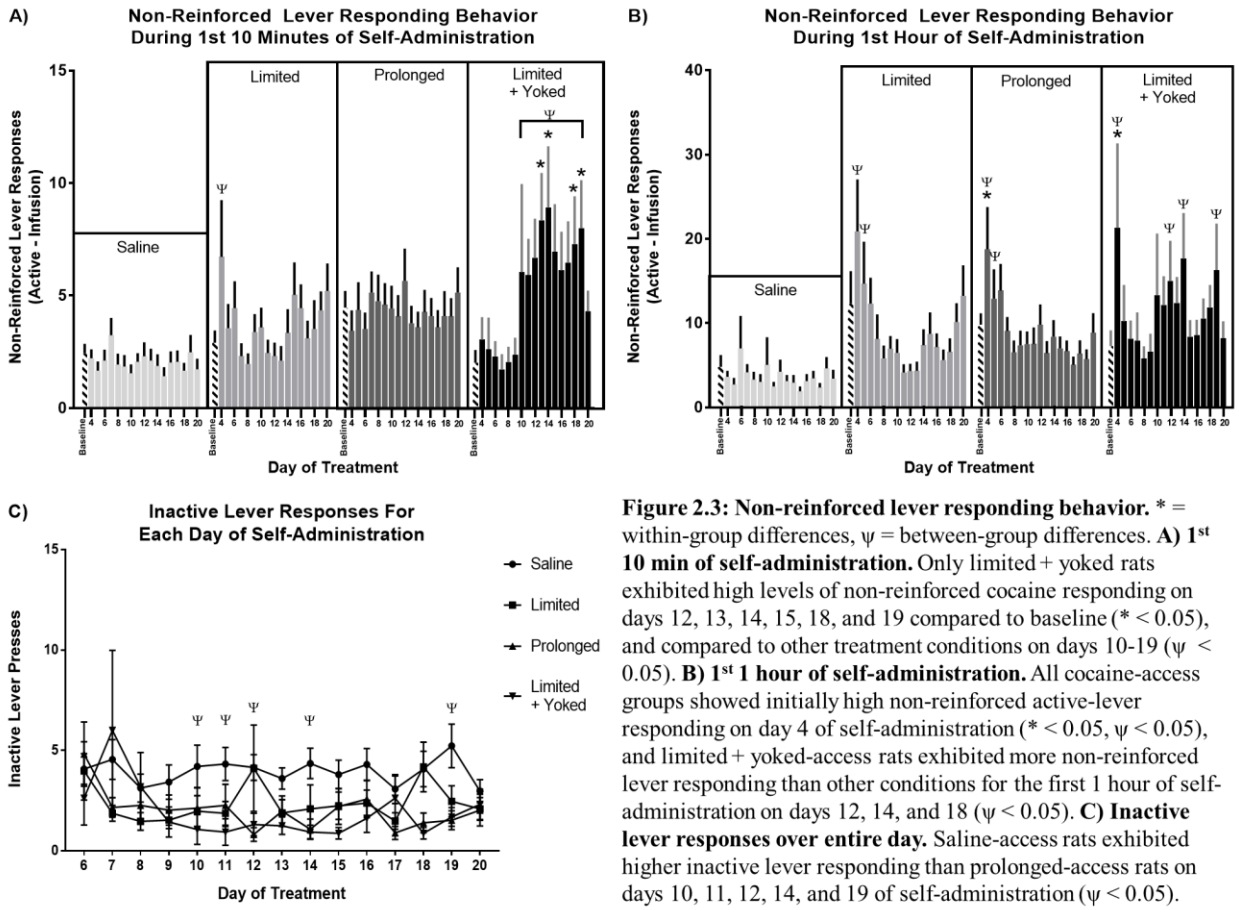

Figure 2.3: Non-reinforced lever responding behavior. * = within-group differences, ψ = between-group differences. **A) 1st 10 min of self-administration.** Only limited + yoked rats exhibited high levels of non-reinforced cocaine responding on days 12, 13, 14, 15, 18, and 19 compared to baseline (* < 0.05), and compared to other treatment conditions on days 10-19 (ψ < 0.05). **B) 1st 1 hour of self-administration.** All cocaine-access groups showed initially high non-reinforced active-lever responding on day 4 of self-administration (* < 0.05, ψ < 0.05), and limited + yoked-access rats exhibited more non-reinforced lever responding than other conditions for the first 1 hour of self-administration on days 12, 14, and 18 (ψ < 0.05). **C) Inactive lever responses over entire day.** Saline-access rats exhibited higher inactive lever responding than prolonged-access rats on days 10, 11, 12, 14, and 19 of self-administration (ψ < 0.05).

Homer2, Grin1, and Dlg4 mRNA expression is increased following prolonged-access to cocaine self-administration:

A one-way MANOVA of mRNA expression for *Homer2, Grin1,* and *Dlg4* resulted in a significant main effect of condition (Hotelling's trace = 0.652, $F_{12, 110}$) = 1.991, $p < 0.05$). LSD *post-hoc* pairwise comparisons revealed greater *Homer2* mRNA expression in the prolonged-access group relative to the naïve, saline, and limited-access + yoked groups (figure 2.4A) LSD *post-hoc* pair-wise comparisons also revealed increased *Grin1* mRNA expression in the prolonged-access group compared to naïve, saline, limited-access, and limited-access + yoked groups (figure 2.4B). Lastly, LSD *post-hoc* pair-wise comparisons revealed increased levels of *Dlg4* mRNA expression in

the prolonged-access group compared to the naïve and limited-access + yoked access groups (figure 2.4C). These data indicate that prolonged-access rats have a unique molecular phenotype, even though rats in the limited-access + yoked group received equivalent amounts of cocaine and escalated cocaine intake at about the same rate as the prolonged-access rats.

Additionally, Pearson correlation coefficients were calculated for total cocaine intake versus mRNA expression. *Homer2* expression was correlated positively with cocaine exposure for both prolonged-access groups ($R^2 = 0.1975$, $p < 0.05$), and limited-access ($R^2 = 0.4763$, $p < 0.05$) groups, whereas mRNA expression correlated negatively for the limited + yoked access group ($R^2 = 0.4046$, $p < 0.05$) (Figure 2.4D, E, F). Pearson correlation coefficients failed to reveal significant correlations between total cocaine intake versus *Grin1* and *Dlg4* mRNA (ps > 0.05).

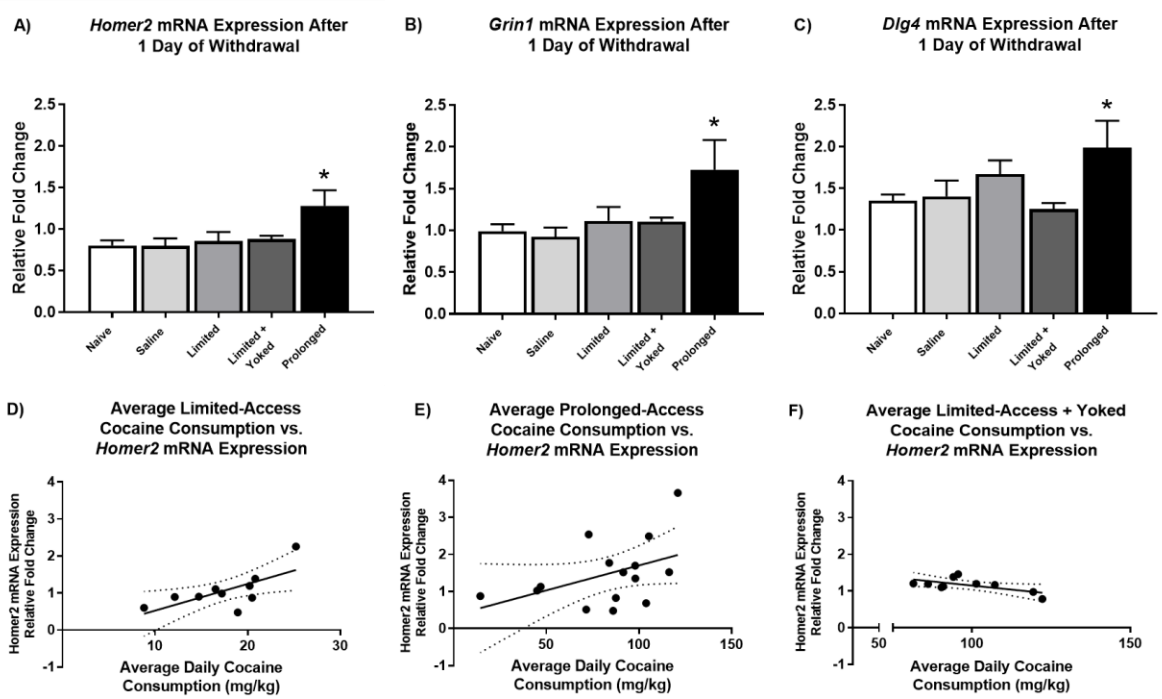

Figure 2.4: mRNA expression for glutamatergic genes in the dmPFC A) Prolonged-access to cocaine resulted in increased levels of *Homer2* mRNA within the dmPFC after 1 day of withdrawal (* $p < 0.05$). **B)** Prolonged-access to cocaine resulted in increased levels of *Grin1* mRNA within the dmPFC after 1 day of withdrawal (* $p < 0.05$). **C)** Prolonged-access to cocaine resulted in increased levels of *Dlg4* mRNA within the dmPFC after 1 day of withdrawal (* $p < 0.05$). **D)** *Homer2* mRNA was positively correlated with total cocaine infusions in the prolonged access group ($R^2 = 0.1975$, $p < 0.05$). **E)** *Homer2* mRNA was positively correlated with total cocaine infusions in the limited access group ($R^2 = 0.4763$, $p < 0.05$). **F)** *Homer2* mRNA was negatively correlated with total cocaine infusions in the limited + yoked access group ($R^2 = 0.4046$, $p < 0.05$).

Discussion

The major finding of the present study is that rats self-administering cocaine under all three cocaine-access conditions exhibited escalation of cocaine intake, but with distinct temporal and molecular profiles. Rats in all self-administration conditions escalated their cocaine intake during the "loading phase" of self-administration (i.e. the first 10 minutes, Figure 2.2A, C), during the first 1 h of self-administration, and over entire daily sessions (Figures 2.2B, D). Additionally, both prolonged-access and limited + yoked-access conditions escalated cocaine intake faster than limited-access animals. However, despite equivalent "excessive" cocaine exposure, the associated patterns of

active lever responding are distinct between prolonged-access and limited-access + yoked groups, with the limited-access + yoked condition exhibiting more non-reinforced lever responding on the active lever during the first 10 min of self-administration (i.e. during the time-out period which did not result in cocaine infusion; see Figure 3). This suggests that partially distinctive behavioral mechanisms may underlie the escalation of cocaine intake induced by contingent versus non-contingent cocaine exposure. Furthermore, I observed an overall increase in *Homer2, Grin1,* and *Dlg4* mRNA expression only in the prolonged-access rats, and total cocaine exposure and *Homer2* mRNA expression are positively correlated in both prolonged- and limited-access conditions but negatively correlated in the limited + yoked-access condition indicating distinct neurobiological consequences of both amount and mode of cocaine exposure. Thus, the present study demonstrates that escalation of cocaine-taking induced by differential cocaine-access, both with respect to session duration and behavioral contingency of cocaine delivery, can produce distinct behavioral and neurobiological consequences.

The finding that all cocaine-taking groups exhibited an escalation of drug intake, but at different rates and to different degrees, is generally consistent with prior findings. Although the capacity of prolonged drug-access to escalate drug-taking, over that observed under limited-access conditions, is a highly replicable finding (e.g. Ahmed and Koob, 1998; Ben-Shahar et al., 2004; Ben-Shahar et al., 2013), an escalation of cocaine intake is also reported in rats self-administering cocaine during slightly longer (2 h) daily sessions, albeit to a lesser extent than counterparts with daily 6-h-access (Mandt, Copenhagen, Zahniser, & Allen, 2015). Further, some rat strains exhibit escalation under daily 1 h sessions of cocaine-access (Perry et al., 2006), particularly when allowed to

self-administer cocaine over a protracted test period (e.g. 75 days) (Belin et al., 2009). The present report extends the literature on escalation by demonstrating that cocaine intake during the initial 10 minutes of the self-administration paradigm (i.e., the loading phase; see Ahmed & Koob, 1998) also escalates with drug experience in limited-, prolonged-, and limited + yoked-access rats, but at somewhat different rates (Figure 2.2). Thus, it appears that the loading phase (i.e. first 10 min) is more sensitive to escalation of intake than the overall duration of daily access.

The escalation of cocaine intake observed in the limited + yoked-access condition was additionally associated with a pronounced, but transient, increase in non-reinforced lever-responding during cocaine access (Figure 2.3). Marked differences in non-reinforced responding have been reported in the absence of differences in cocaine intake; e.g. in female relative to male rats (Fuchs et al., 2005; Kippin et al., 2006; Kosten & Zhang, 2008); thus, the two measures appear generally dissociable. In the case of the limited + yoked-access rats in the present study, the rats had acquired responding for cocaine, accompanied by low levels of non-reinforced responding which was markedly elevated, particularly during the loading phase (Figure 3A), following exposure to the yoking procedure. This disruption of "efficient" (i.e. lever presses are not tied to the drug reinforcer) operant behavior elicited by the addition of non-contingent cocaine exposure is likely due to discontinuous reinforcement schedules. Further, contextual cues can modulate escalation behavior; when rats are allowed alternating days of 1 h and 6 h access to cocaine with differential cues, they only escalate during the 6 h sessions (Beckmann et al., 2012). An equally-viable explanation for increased non-reinforced lever responding induced by non-contingent drug exposure may pertain to differences in

the aversive, stress-inducing, and glucocorticoid-releasing properties of cocaine delivered under yoked procedures (Twining, Bolan, & Grigson, 2009) which have been implicated in the processes underlying escalation (Mantsch et al., 2007; 2008). Thus, the combination of yoked-cocaine with subsequent re-introduction to the operant chamber with differential cues (i.e. lever extension and operant light) appears to serve as a potent elicitor of lever-responding. Furthermore, although it appears that limited + yoked-access rats escalate intake faster than prolonged-access rats, it is evident that escalation within the limited + yoked-access rats occurs alongside excessive non-reinforced lever responding (i.e. on days 12, 14, and 18, Figure 2.3 A, B).

Given the central role of drug-induced neuroadaptations, particularly alterations in glutamate function, in theories of addiction (see e.g. Kalivas & Volkow, 2005), it is critical to discern the role of behavioral contingency in neurobiological changes associated with escalating drug intake. Here, I identified differences between the prolonged-access and limited + yoked-access conditions; I examined the dmPFC which has been implicated in the escalation of cocaine intake (Smith, Ward, & Roberts, 2008) for changes in the expression levels of molecular markers implicated in addiction (i.e. *Homer2, Grin1,* and *Dlg4* mRNA). Consistent with prior findings (Ben-Shahar et al., 2009), I observed increased levels of *Homer2* mRNA only in the prolonged-access condition, (Figure 2.4A). Furthermore, the level of intake in the limited- and prolonged-access conditions correlated positively with *Homer2* mRNA expression, whereas cocaine exposure in the limited + yoked-access group correlated negatively with *Homer2* mRNA expression (Figure 4D-F). In addition, I also observed increases in *Grin1* and *Dlg4* mRNA within the dmPFC of the prolonged-access rats only (Figures 2.4B-C). Overall,

the differences between prolonged-access and limited + yoked-access conditions are consistent with other studies employing yoked procedures (Krawczyk et al., 2013; Ma et al., 2013; McFarland et al., 2003; Radley et al., 2015). However, the present finding furthers this literature by demonstrating that contingent and non-contingent cocaine exposure induces distinct neurobiological changes even when both are associated with escalation of cocaine intake, with only the prolonged-access condition producing elevation of several genes that are suggestive of enhanced glutamatergic signaling.

The increases in expression of glutamate-related genes observed, specifically following prolonged contingent access to cocaine, relates to the current literature concerning enhanced glutamate neurotransmission and cocaine-specific neuroplasticity in the prefrontal cortex. Repeated contingent-access to cocaine results in cocaine-specific synaptic plasticity including: increased dendritic spine density (Frankfurt, Salas-Ramirez, Friedman, & Luine, 2011), increased long-term potentiation (LTP) in the PFC (Huang, Lin, & Hsu, 2006), and lowered induction threshold for inducing cocaine-specific LTP (Ruan & Yao, 2017). Further, cocaine self-administration results in increased excitatory postsynaptic currents (EPSCs) between mPFC and NAC D1 medium spiny neuron synapses, and optogenetic inhibition of these synapses eliminates cocaine seeking behaviors (Pascoli et al., 2014). Lastly, I have previously demonstrated that prolonged-access to cocaine enhances cue-elicited glutamate release during protracted withdrawal (Shin et al., 2016), and increased expression of the NMDA GluN2b receptor subunits during early and later withdrawal (Szumlinski et al., 2016). Other groups have also shown increases in NMDA, as well as AMPA and Kainate, receptor subunits after withdrawal from contingent cocaine self-administration (Crespo, Oliva, Ghasemzadeh,

Kalivas, & Ambrosio, 2002; Ghasemzadeh, Vasudevan, Mueller, Seubert, & Mantsch, 2009; Tang, Wesley, Freeman, Liang, & Hemby, 2004). These previous studies, as well as the current report (Figure 2.3) indicate that cocaine-specific plasticity of glutamatergic receptors develops with repeated contingent-access to cocaine, and is necessary for cocaine-seeking behavior.

Briefly, *Homer2* is a gene encoding for a scaffolding protein that interacts with Group1 metabotropic glutamate receptors (mGluRs) and NMDA receptors and has been implicated in addictive behaviors (c.f., Szumlinski et al., 2008). Homer2a/b protein within the mPFC is increased following prolonged-access to cocaine (Ben-Shahar et al., 2009) and repeated cocaine injections (Ary & Szumlinski, 2007). Further, viral-mediated *Homer2b* overexpression in the mPFC increases basal glutamate levels and cocaine-conditioned reward in mice, whereas *Homer2b* knockdown reduces basal glutamate in this area (Ary et al., 2013). Additionally, *Grin1* encodes for the obligatory NR1 subunit of NMDA receptors, while *Dlg4* encodes for PSD-95, a scaffolding protein that regulates NMDA receptor function (Bai & Hoffman, 2009). Increases in NR1 protein within the PFC have previously been observed in response to repeated cocaine injections (Kovacs, Lajtha, & Sershen, 2010) and cocaine self-administration (Hemby, Horman, et al., 2005). Furthermore, NR1 is essential for cocaine-mediated learning; mice expressing a mutant version of the NR1 subunit (which reduces calcium flow through the NMDA receptor) fail to form conditioned place preference and locomotor sensitization in response to repeated cocaine exposure (Heusner & Palmiter, 2005). PSD-95 is critical for synaptic plasticity and regulation of NMDA receptor location and function (Wang & Peng, 2016). PSD-95 is also implicated in behavioral plasticity associated with chronic cocaine

administration (Yao et al., 2004), extinction of cocaine self-administration (Knackstedt et al., 2010), as well as prolonged withdrawal from cocaine (Ghasemzadeh, Vasudevan, & Mueller, 2009). Thus, our RNA data is generally consistent with findings examining protein levels of glutamatergic signaling molecules with the increases in RNA observed here coinciding or preceding latent increases in protein.

Escalation of drug intake is an important diagnostic criterion of addiction in humans and an integral component in various theories of addiction. Therefore, understanding the behavioral and neurobiological underpinnings of escalation is likely to facilitate addiction management programs in humans. In addition to facilitating cocaine intake, prolonged daily access to cocaine is associated with several behavioral changes, such as reduced brain reward function (Ahmed et al., 2002; Ahmed & Koob, 2005), increased breakpoints for cocaine reinforcement under progressive ratio schedules (Paterson & Markou, 2004; Wee, Orio, Ghirmai, Cashman, & Koob, 2009), diminished aversive properties of cocaine (Ben-Shahar, Posthumus, Waldroup, & Ettenberg, 2008), increased extinction responding during protracted withdrawal (Ferrario et al., 2005), as well as increased responding during cocaine-primed and cue-induced reinstatement of cocaine-seeking (Ahmed & Cador, 2006; Kippin et al., 2006; Knackstedt & Kalivas, 2007; Mantsch, Yuferov, Mathieu-Kia, Ho, & Kreek, 2004). Similarly, other approaches to modeling "excessive" intake also produce increases in measures of cocaine-taking and -seeking behaviors (Véronique Deroche-Gamonet, Belin, & Piazza, 2004; Roberts, Morgan, & Liu, 2007). Further study is required to determine the relations between nature of cocaine exposure and induction of addiction-like behavior, as well as between behavioral and molecular outcomes. To this end, the present study demonstrates

behavioral contingency plays an important role in the nature of the behavioral and molecular changes induced by cocaine exposure.

Chapter 3: Impact of Prolonged Contingent and Non-Contingent Cocaine Exposure on Gene Expression and DNA Methylation During Protracted Withdrawal from Cocaine.

Abstract

Cocaine addiction is a chronic disorder whereby cocaine craving in response to drug related cues and relapse to drug taking persist, and even intensify, through protracted drug-free abstinence periods. Studies employing animal models observe similar patterns in addicts with rat as well as other species exhibiting cocaine-seeking that not only persists through protracted periods of drug withdrawal as well as time-dependent intensification of these behaviors. Additionally, there are long term changes in neuron structure, receptor function, and neurotransmission associated with abstinence from cocaine in humans and animals. DNA methylation is an epigenetic modification to the DNA structure that mediates mRNA expression to confer different cell types, but has recently been implicated in learning and memory mechanisms. The long-term control that DNA methylation has over gene expression in animals makes it a prime candidate for

controlling gene expression over the course of abstinence in animals with previous drug experience. Thus, in the present study, I exposed rats to saline (1 h) or limited (1 h), prolonged (6 h), or limited + yoked (1 h contingent + 5 h non-contingent) access to cocaine for 15 days followed by protracted withdrawal due to forced abstinence for 1, 14, or 60 days. I then dissected the dmPFC and measured levels of methylated DNA or mRNA for *Homer2, Dlg4, Npas4,* and *Grin1* via digital PCR. Briefly, rats exposed to saline, limited-cocaine, or prolonged-cocaine, experienced time-dependent changes in DNA methylation in *Homer2*, *Dlg4*, *Npas4,* and *Grin1*. However, changes in mRNA expression did not exhibit strong correlation changed in DNA methylation, but rather by drug-access condition. In conclusion, DNA methylation is dynamic over abstinence, but mRNA expression appears to be stable.

Introduction

Although the gradual escalation of cocaine intake discussed in Chapter 2 is an important aspect of addiction, the chronic nature of addiction is observed during protracted cocaine-free periods with addicts reporting both drug craving and exhibiting relapse vulnerability for years after last drug use (APA, 2013). Similarly, animal models of addiction indicate that the impact of repeated exposure to cocaine induces changes that are very long-lasting. For instance, rats exhibit pronounced cocaine-seeking (as measured by responses on a operandum previously reinforced by cocaine) for periods of at least 6 months after the last self-administration session (Kerstetter et al., 2008) and similarly,

sensitization to the psychomotor effects of stimulants can last for up to 1 year in rats (Paulson, Camp, & Robinson, 1991). Additionally, time-dependent changes in neurotransmitter function and receptor expression are also observed following cocaine exposure and coincide with change in the reports of drug craving in addict. For instance, cravings in cocaine addicts have been reported to rise over the first few months of drug abstinence (Gawin et al., 1989; Parvaz, Moeller, & Goldstein, 2016). Similarly, after cocaine self-administration, rats re-exposed to the self-administration conditions (i.e. an extinction test) exhibited time-dependent increases in cocaine-seeking behavior for at least 90 days followed by recovery by 180 days of cocaine withdrawal (Tran-Nguyen et al., 1998; Grimm et al., 2001; Lu et al., 2005; Kerstetter et al al 2008). for 14 days, rats exposed to forced abstinence for 1 day, 1 week, or 1 month, experienced progressive and time-dependent increases in cocaine seeking behavior and extracellular dopamine levels within the amygdala during extinction, cue-reinstatement, and drug-reinstatement procedures (Tran-Nguyen et al., 1998). Additionally, Grimm et al. (2001) demonstrated that rats previously given prolonged-access to cocaine (6h/ day) will exhibit time-dependent increases in non-reinforced lever responding (on a lever previously associated with cocaine reinforcement) when the cues associated with cocaine administration (light & sound) are present (aka cued re-exposure) (Grimm et al., 2001). Furthermore, Grimm et al. (2003) showed progressive, time-dependent increases in BDNF expression in the VTA, NAC, and amygdala after 1, 30, and 90 days of forced abstinence. Lastly, in 2016, Shin et al. demonstrated cue-dependent increases in extracellular glutamate within the vmPFC after prolonged abstinence from cocaine (Shin et al., 2016). Together, these

studies demonstrate that forced abstinence from cocaine induces progressive, time-dependent changes in neuronal function.

Incubation of cocaine craving has mostly been investigated in animals allotted prolonged-access to cocaine, as they exhibit an "addictive phenotype" (Ahmed & Koob, 1998). For example, prolonged cocaine self-administration has been shown to have long-lasting effects on reward function as measured, for example, by shifts in intracranial self-stimulation (Markou & Koob, 1991, 1992) and increased reinstatement of cocaine seeking behavior (Grimm et al., 2001; Kippin et al., 2006; Tran-Nguyen et al., 1998). Chronic cocaine administration is also accompanied by changes in neuroplasticity after acute withdrawal such as increased dendritic spine density (Dobi, Seabold, Christensen, Bock, & Alvarez, 2011; Dumitriu et al., 2012), alterations in glutamate receptor proteins (Ben-Shahar et al., 2013; Ben-Shahar et al., 2009), intracellular signaling (McGinty, Zelek-Molik, & Sun, 2014), and neurotransmission (Addy, Daberkow, Ford, Garris, & Wightman, 2010; Ben-Shahar et al., 2012). These initial changes in neuroplasticity are continued for extended time periods and may precipitate altered reward-related learning and time-dependent increases in cocaine-seeking behavior (aka, the incubation of craving) (Grimm et al., 2001; Tran-Nguyen et al., 1998). However, it remains to be elucidated how such changes in neurocircuitry are produced and, how they are maintained for prolonged periods of time. Recent developments in the understanding of the regulation of gene expression suggests that epigenetic mechanisms, such as DNA methylation, are involved in the neural plasticity induced by cocaine administration and provide a likely candidate for the long-term maintenance of cocaine-induced alterations in gene expression (Freeman et al., 2008; Freeman et al., 2010).

DNA methylation is a long-lasting epigenetic marker that regulates addiction-like behaviors and gene transcription within the brain

Drug addiction, as previously mentioned, is a chronic, long-lasting disorder that is associated with the aberrant expression of receptor proteins, neurotransmitter release and reuptake, and synaptic structure. Each of these elements is ultimately controlled by mRNA expression that relies on a specific epigenetic state. In fact, recent studies have demonstrated global and site-specific reductions in DNA methylation after experimenter-administered cocaine (Pol Bodetto et al., 2013; Tian et al., 2016; Tian et al., 2012) and methamphetamine (Numachi et al., 2004) in both the PFC and NAC. Additionally, acute injections of cocaine upregulated DNMT3A and DNMT3B mRNA within the nucleus accumbens of mice, and were associated with increased locomotor sensitization, while repeated cocaine did not increase DNMT mRNA expression. Furthermore, inhibition of DNMT3A and DNMT3B via zebularine reduced DNA methylation of the PP1C (protein phosphatase 1c) gene, increased PP1C mRNA, and delayed the onset of cocaine-induced behavioral sensitization in animals with acute injections of cocaine (Anier, Malinovskaja, Aonurm-Helm, Zharkovsky, & Kalda, 2010).

DNA methylation has also been recently implicated in cocaine self-administration studies. Baker-Andresen et al. (2015) showed a unique pattern of global DNA methylation and mRNA expression in the dmPFC of mice after 1 or 21 days of abstinence from daily cocaine self-administration (2 h per day). Additionally, the same mice underwent 5mC-mediated alternative splicing of Cpeb4 mRNA after 21 days of abstinence and relapse-testing (Baker-Andresen et al., 2015). Conversely, supplementation of DNA methylation via methyl-donors serves to attenuate cocaine CPP

(Tian et al., 2012) and cocaine-seeking behaviors after self-administration and during cocaine-primed reinstatement (Wright et al., 2015). Although inhibition of methylation via zebularine or dAZA will potentiate cocaine intake during self-administration (Fonteneau et al., 2017).

Since DNA methylation is a persistent and long-lasting change associated with drugs of abuse, learning, and memory, and can regulate mRNA expression to control neuronal structure and function, it is a prime candidate for investigation to study the incubation of craving during forced abstinence from cocaine. Therefore, for the present study, I investigated changes in DNA methylation and mRNA expression across different cocaine-access conditions after 1, 14, and 60 days of forced abstinence from cocaine self-administration to determine the role of epigenetic modifications on glutamatergic gene expression.

Methods

Animals housing, care, and surgical procedures

Male Sprague-Dawley rats (250-300 g) were pair-housed in a reverse light:dark (12h:12h, lights off at 8 am) cycle room with ad-libitum access to food and water. The housing and care of the rats followed the guidelines set forth by the "Guide for the Care and Use of Laboratory Rats, 8th Edition" (IACUC, 2011).

Rats were deeply anesthetized using 2-3% isoflurane gas and implanted with chronic indwelling catheters constructed with a bent steel cannula with a screw-type connector (Plastics One, Roanoke, VA), SILASTIC tubing (11 cm, i.d. 0.64 mm, o.d. 1.19 mm, Dow Corning, Midland, MI), Prolite polypropylene monofilament mesh (Atrium Medical Corporation, Hudson, NH), methyl methacrylate cement, and a silicon

ball 2.5 cm from the end used to secure the catheter to the vein. Postoperative care lasted for 4 days; rats were visually inspected, weighed, and the catheters were maintained as we have reported previously, and tested once weekly with 2.0 mg of Methohexital Sodium (Brockton, MA) (Ben-Shahar et al., 2013; Kerstetter et al., 2008).

Cocaine self-administration, forced withdrawal, & dmPFC dissection

Food training and cocaine self-administration utilized standard operant chambers (Med Associates Inc., St. Albans, VT, USA) during the dark cycle at a controlled time each day. Before surgical implantation of the jugular catheters, the rats were restricted to 20 g of food daily and trained on a fixed ratio 1 (FR1) schedule of food reinforcement for two 16 h training sessions where each right lever press was associated with the delivery of a 45 mg food pellet (Ben-Shahar et al., 2012). After recovery from the surgery, the rats were placed on a fixed ratio 1 (FR1) with a 20 second time-out signaled by the activation of the cue light above the lever. Initial sessions lasted 1 h per day for the first 5 days with either cocaine or saline infusions available as reinforcers. Each "active" lever depression (right lever) was associated with a 4 s infusion of vehicle (0.1 mL saline) or cocaine (0.25 mg/ 0.1 mL); "inactive" lever depression (left lever) resulted in no infusion or stimulus (Ben-Shahar et al., 2012). On the 6^{th} day, the rats receiving cocaine were put on one of three programs, limited-access (1 h access per day), prolonged-access (6 h contingent access per day), and limited + yoked-access (1 h contingent + 5 hours yoked access per day) to cocaine. Additionally, separate age-matched rats were used as naïve controls; they arrived the same time as experimental rats, but were not subjected to behavioral conditioning and were sacrificed at the same time as the experimental rats. After self-administration training, all rats were put through forced abstinence for 1, 14, or 60 days

and were sacrificed via rapid decapitation. Then, their brains were removed, and the dorsal medial prefrontal cortex (dmPFC) was dissected out at 2.20 to 3.20 mm anterior to Bregma (Figure 2.1) via the Braintree brain mold (Braintree, MA), placed in a 0.5 mL microcentrifuge tube, frozen on dry ice, and stored at -80°C for further analysis.

DNA Methyl-binding-domain genome-wide assay

For genome-wide micro-array analyses, a Methyl-binding-domain (MBD) assay was carried out following the manufacturer's protocol (Active motif) to purify the methylated DNA from rats that had undergone 1 day of forced abstinence from cocaine and had their dmPFC dissected (Figure 2.1). Briefly, genomic DNA was digested with proteinase K and RNase A sequentially, and purified by using the Qiagen DNeasy extraction column according to the manufacturer's protocol (Qiagen). Purified genomic DNA was sonicated into average fragments of 500 base-pairs (bp) by using the Covaris S2 system (Covaris) and heat-denatured (95 ˚C, 10 min). An aliquot of sonicated genomic DNA was saved as input for later use. 1 µg of fragmented genomic DNA was used for the Active Motif immunoprecipitated (IP) MBD assay. We then treated the DNA beads with proteinase K for at least 3 h at 55 ˚C and purified the captured, methylated DNA by phenol-chloroform extraction followed by ethanol precipitation.

For the phenol chloroform extraction and ethanol precipitation: An equal volume (as the sample) of phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v) was added to the eluted DNA. The tube was vortexed for ~ 15 s and spun down in a centrifuge at 12,000 g for 5 min at room temperature. The top, aqueous layer was removed and added to a second microcentrifuge tube (MCT) and 4 uL glycogen (5 mg/ mL), 1 sample volume 5 M ammonium acetate, and 2.5 sample volumes of ice-cold 100% ethanol were added.

The tube was vortexed for ~ 15 s and then incubated at -80 °C for at least 2 h. The tube was then centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was pipetted off, and 500 mL of ice-cold 70% ethanol was added. The tube was once more centrifuged at 12,000 g for 10 min at 4 °C. The resulting supernatant was discarded, and the DNA pellets were air-dried for 5 minutes. The pellets were then resuspended in 50 uL of sterile DNase-free water for PCR quantification.

For quantitative real-time PCR analysis, 10 ng of input genomic DNA and 1/30 of the immunoprecipitated (IP) methylated DNA was used for each PCR reaction. MBD-qPCR was performed in the Rotor Gene thermocycler (Qiagen) using SYBR Green mix (Qiagen). Reactions were done in duplicates and standard curves were calculated on serial dilutions of input genomic DNA. For microarray analyses, we amplified input and MBD enriched DNA using a whole genome amplification kit version 2 (Sigma). The 4X72K array was used for microarray experiments (Roche). 1.5 µg of amplified DNA sample were then subjected for sample Cy3/5 labelling, hybridization, and washing according to the manufacturer's protocol (Roche). Two-color array scanning was performed by GenePix 4000B scanner (Roche) according to manufacturer's instructions. Data analysis was performed using NimbleScan v2.5 (Roche). For identification of probes that associated with significant changes in DNA methylation, ChIPMonk version 1.2.3 (https://www.bioinformatics.babraham.ac.uk/projects/chipmonk/) was used as previously reported (Farthing et al., 2008).

DNA and mRNA extraction and purification for digital PCR

For digital PCR analyses, frozen dmPFC tissue from all rats that were subjected to 60 days of forced abstinence was added to 600 µL of buffer RLT (Qiagen, #79216) and

homogenized with the Qiagen TissueRuptor for 30 s. DNA and mRNA were then extracted by the AllPrep DNA/RNA/protein extraction kit (Qiagen, #80004) in accordance to the manufacturer's protocol. RNA was eluted from the spin column with 50 µL of nuclease-free water. DNA was eluted from the spin column with 100 uL of 8 mM NaOH heated to 70 °C, and the pH was then neutralized with 12 µL of 0.1 M HEPES and 1.1 µL of 100 mM EDTA.

For the analysis of methylated DNA, extracted genomic DNA (1 µg) was fragmented to 400 bp fragments by the Covaris M220 system via the automated protocol. The size of the DNA fragments were confirmed via gel electrophoresis on a 2% gel, at 110V, for 75 minutes and visualized with the Lycor Gel Analyzer. To isolate the methylated DNA (5mC) the fragmented DNA then underwent methylated DNA immunoprecipitation (MeDIP) with a 5-methylcytosine antibody (Active Motif, 55009) and protein G magnetic beads according to the manufacturer's protocol. The DNA was then purified via phenol-chloroform extraction and ethanol precipitation as mentioned previously.

For the analysis of mRNA expression: 500 ng of mRNA from each extracted sample was reverse transcribed according to the manufacturer's protocol (Qiagen, 205311), except that samples were incubated with genomic DNA wipeout buffer in an Eppendorf MasterCycler at 42 °C for 18 minutes, then incubated at 95 °C for 3 min to inactivate the reverse transcriptase. A reverse transcriptase-negative reaction was carried out in parallel with the samples from 500 ng of pooled sample RNA.

Methylated DNA digital PCR design and analysis.

Primers for the methylated DNA samples (Table 1) were created using the UCSC genome browser (https://genome.ucsc.edu) to view the DNA code, primer3 (http://primer3.ut.ee) to find the best primers in the given region, and PrimerBlast (https://www.ncbi.nlm.nih.gov/tools/primer-blast) to validate the primer position and amplicon size in relation to the gene body. All primers were then tested with 50 ng of fragmented genomic DNA under multiple conditions (cycle duration, annealing temperature, primer concentration, etc) on a BioRad CFX96 Touch Real-Time system to determine primer efficiency and accuracy, and then visually assessed on the Lycor system after electrophoresis on a 2% agarose gel (110V, 75 minutes) to validate amplicon size. The final run conditions (Table 3.1) were used on experimental samples during quantification.

Digital real-time PCR (ddPCR) is a new method of DNA quantification to measure the absolute concentration of DNA in samples. In order to use this method, we first combined DNA samples with EvaGreen QX200 Supermix (BioRad), forward and reverse primer sets, and water (Table 3.1). Then, 20 μL of each sample were parsed into 10,000 to 20,000 droplets using ddPCR EvaGreen Droplet Generation Oil (BioRad) in the QX200 Droplet Generator, then transferred to a 96-well PCR plate. After the plate was loaded with droplets, the DNA was amplified on a BioRad CFX96 Touch Real-Time system according to previously tested conditions (Table 3.1). After amplification, the PCR plate was added to the QX200 digital PCR reader. The reader then measured the fluorescence of each individual droplet and used Poisson statistics to calculate the concentration (copies of DNA/ μL) of each sample. Post-run analyses were performed on

QuantaSoft software (BioRad), and the final concentration was determined by subtracting the baseline fluorescence samples from the amplified samples.

Gene Name	Forward Primer (5'-3')	Reverse Primer (5'-3')	Gene Location	Amplicon Location	Amplicon Distance to TSS	Amplicon Size	Primer Concentration	Annealing Temperature	Number of Cycles	µL QX200 EvaGreen	µL Primer	µL H2O Used	µL MeDIP DNA
Homer2	GGCTGCTCCT TTGTCTGACT	GGGCGGATTA GAAGTCCAGG	Chr 1: 143443300-143535579	Chr 1: 143531782-143531967	3344	185	2 nM	60 C	40	12	2	6	4
Dlg4	GGGAGAACTA GGTCCCCAAG	CACAAGCAGA GAGGCCCTAC	Chr 10: 56,625,845-56,655,543	Chr 10: 56626795-56626989	950	194	2 nM	60 C	40	12	2	6	4
Grin1	CCTGTTCCTCA TCTGGCAAT	TGTGGAGGAG ACCAGCTTCT	Chr 3: 2507745-2534664	Chr 3: 2535454-2535628	790	174	2 nM	60 C	40	12	2	6	4
Npas4	TCTACGGCGG ATGATGAAGC	TTAAATCGCCC AGCTACGCC	Chr 1: 220260138-220278177	Chr 1: 220278554-220278462	285	111	2 nM	60 C	40	12	2	6	4

Table 3.1: Primers for ddPCR of Methylated DNA in Gene Promoter Regions

mRNA digital PCR design and analysis

Primers for the mRNA samples (Table 3.2) were created using the Ensembl genome browser (https://www.ensembl.org/index.html) to view the transcript's cDNA code, primer3 (http://primer3.ut.ee) to find the best primers in the given region, and PrimerBlast (https://www.ncbi.nlm.nih.gov/tools/primer-blast) to validate the primer position and amplicon size in relation to the gene body. All primers were then tested with 50 ng of cDNA pooled from all experimental conditions under multiple PCR conditions (cycle duration, annealing temperature, primer concentration, etc) on a BioRad CFX96 Touch Real-Time system to determine primer efficiency and accuracy, and then visually assessed on the Lycor system after electrophoresis on a 2% agarose gel (110V, 75 minutes) to validate amplicon size. The final run conditions (Table 3.2) were used on experimental samples during quantification.

For mRNA quantification, we first combined cDNA samples (~12.5 ng/ µL) with EvaGreen QX200 Supermix (BioRad), forward and reverse primer sets, and water (Table

w). Then, 20 μL of each sample were parsed into 10,000 to 20,000 droplets using ddPCR EvaGreen Droplet Generation Oil (BioRad) in the QX200 Droplet Generator, and transferred to a 96-well PCR plate. After the plate was loaded with droplets, the DNA was amplified on a BioRad CFX96 Touch Real-Time system according to previously tested conditions (Table 3.2). After amplification, the PCR plate was added to the QX200 digital PCR reader. The reader then measured the fluorescence of each individual droplet and used Poisson statistics to calculate the concentration (copies of DNA/ μL) of each sample. Post-run analyses were performed on QuantaSoft software (BioRad), and the final cDNA concentration was determined by subtracting the baseline fluorescence samples from the amplified samples. ΔΔCT analysis was performed on the final concentrations of target DNA to normalize the expression of target genes against stable reference genes (*ActB* & *Gapdh*) and the experimental controls (naïve condition). The equations for this are as follows, where t = individual target sample concentration, r = individual reference gene sample concentration, R = mean of control genes for a specific sample, C = mean of control samples from experimental conditions normalized against the reference genes, and F = Final normalized concentration relative to control genes and experimental samples:

***Equation* 1**:

$$R = \frac{1}{n}\left(\sum_{i=1}^{n} r_i\ (of\ control\ genes)\right)$$

***Equation* 2**:

$$S = \frac{t}{R}$$

***Equation* 3**:

$$C = \frac{1}{n}\left(\sum_{i=1}^{n} S_i\ (of\ naive\ samples)\right)$$

***Equation* 4**:

$$F = \frac{S}{C}$$

Gene Name	RefSeq Number	Forward Primer (5'>3')	Reverse Primer (5'>3')	Amplicon Location	Amplicon Length (bp)	Primer Conc	Annealing Temp	Number of Cycles	EvaGreen (µL)	Primer (µL)	Water (µL)	cDNA (µL)
Gapdh mRNA	NC_005104.4	ATGATTCTACC CACGGCAAG	CTGGAAGATGG TGATGGGTT	Chr 5: 115649279-115649367	89	2 nM	60 C	40	12	2	6	4
βActin mRNA	NC_005111.4	GGGAAATCGT GCGTGACATT	GCGGCAGTG GCCATCTC	Chr 12: 13718030-13718105	79	2 nM	62 C	40	12	2	6	4
Grin1 mRNA	NC_005102.4	CGGCTCTTGGA AGATACAGC	GTGGGAGTGAA GTGGTCGTT	Chr 3: 2532666-2534181	156	2 nM	60 C	40	12	2	6	4
Dlg4 mRNA	NM_019621	BioRad Primer PCR*	BioRad Primer PCR*	Chr 10: 56385632-56392520	93	1 nM	58 C	40	12	1	7	4
Homer2 mRNA	NM_053309	BioRad Primer PCR*	BioRad Primer PCR*	Chr 1: 144387765-144388698	112	1 nM	58 C	40	12	1	7	4
Npas4 mRNA	NM_153626	BioRad Primer PCR*	BioRad Primer PCR*	Chr 1: 227193687-227193779	63	1 nM	58 C	40	12	1	7	4

Table 3.2: Primers for ddPCR of Methylated DNA in Gene Promoter Regions. *BioRad Primers are intellectual property of BioRad Inc. and can be purchased at www.BioRad.com

Statistics and graphing

All statistical analyses were conducted on IBM SPSS Statistics version 24. Bootstrapping was used to redistribute the mRNA and DNA methylation data over 1000 points in order to run parametric analyses on the data. DNA methylation and mRNA expression were assessed via separate 5 (access conditions) x 3 (forced abstinence periods) two-way ANOVAs for each target; One-way ANOVAs were utilized to breakdown factors between conditions and between forced abstinence time points when an interaction between condition x abstinence was observed. LSD post-hoc tests were used to break down interactions so that conditions and time points could be assessed in a pairwise manner. Pearson correlations were run pairwise between mRNA, DNA methylation, and total cocaine intake for each access condition in SPSS. Lastly, all graphs were created and edited using the Prism6 software. was used for one-way ANOVAs with bootstrapping on all ddPCR data, and then graphed via Prism6 software.

Results

Prolonged-access to cocaine results in a vast variety of differences in DNA methylation throughout the genome of the dmPFC.

ChIPMonk software analysis detected 64101 changes in DNA methylation along various genomic sites after 1 day of forced abstinence from cocaine or saline self-administration. We filtered through the 1000s of changes in DNA methylation between various genes for glutamatergic genes relevant to addiction as targets for further analysis. Among the various genes, *Homer2*, *Grin1*, *Dlg4*, and *Npas4* exhibited at least 50% increases or decreases in DNA methylation between saline, limited-access, or prolonged-access conditions (Table 3), thus, 5MC changes in these genes were selected for further analyses involving verification (via digital PCR), assessment of functional outcome (gene expression), and tracking through up to 60 days of forced drug abstinence. Additionally, changes global DNA methylation was assessed in for limited- and prolonged-access rats in comparison to saline-access rats (Figure 3.1).

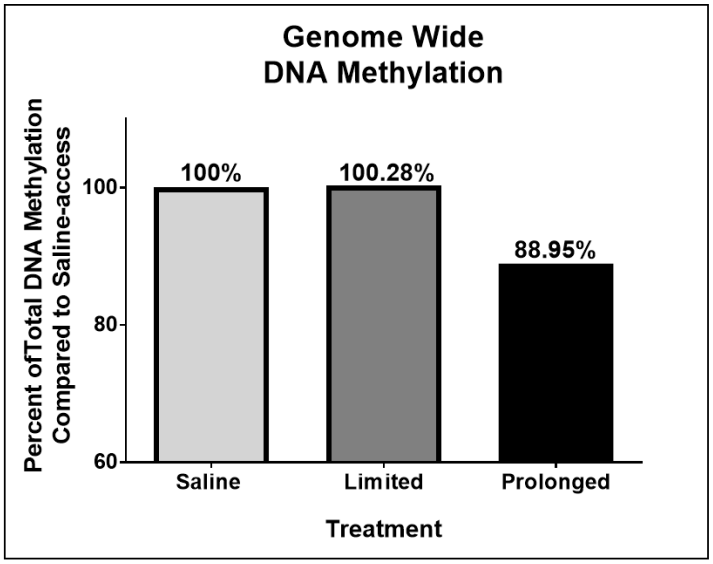

Figure 3.1: Global DNA Methylation. Saline-access served as a baseline. Limited-access rats exhibited a 0.28% increase in total levels of 5mC, whereas prolonged-access rats exhibited am 11.05% decrease in total 5mC across the genome.

Gene Symbol	Description	Distance from TSS	Prolonged-Access	Limited-Access	Saline-Access	Limited/ Saline	Prolonged/ Saline
Homer2	Homer protein homolog 2, Dendritic Scaffolding Protein	0-1000 bp	0.6784	1.1449	0.6820	↑ 1.6787	○ 0.9947
Homer2	Homer protein homolog 2, Dendritic Scaffolding Protein	0-538 bp	0.6164	0.3836	1.2762	↓ 0.3006	↓ 0.4830
Homer2	Homer protein homolog 2, Dendritic Scaffolding Protein	336-1336 bp	1.1700	0.6012	1.3324	↓ 0.4512	○ 0.8781
Homer2	Homer protein homolog 2, Dendritic Scaffolding Protein	1041-2041 bp	0.5555	0.8988	0.9665	○ 0.9300	↓ 0.5747
Homer2	Homer protein homolog 2, Dendritic Scaffolding Protein	1451-2451 bp	0.7942	0.9308	0.5239	↑ 1.7765	↑ 1.5157
Dlg4	Disks large homolog 4 (Postsynaptic densityprotein 95)(PSD-95)	0-1000 bp	1.4505	0.9037	2.8289	↓ 0.3194	↓ 0.5128
Dlg4	Disks large homolog 4 (Postsynaptic densityprotein 95)(PSD-95)	0-1000 bp	0.8476	1.2394	1.9450	↓ 0.6372	↓ 0.4358
Dlg4	Disks large homolog 4 (Postsynaptic densityprotein 95)(PSD-95)	0-1000 bp	0.6393	1.3204	1.3791	○ 0.9574	↓ 0.4635
Dlg4	Disks large homolog 4 (Postsynaptic densityprotein 95)(PSD-95)	0-1000 bp	1.0983	1.9532	3.3069	↓ 0.5907	↓ 0.3321
Npas4	Neuronal PAS domain-containing protein 4(Neuronal PAS4)(HLH-PAS transcription factor NXF)	0-1000 bp	0.6837	0.7471	1.7330	↓ 0.4311	↓ 0.3945
Npas4	Neuronal PAS domain-containing protein 4(Neuronal PAS4)(HLH-PAS transcription factor NXF)	0-1000 bp	0.6872	0.7187	1.7131	↓ 0.4195	↓ 0.4012
Npas4	Neuronal PAS domain-containing protein 4(Neuronal PAS4)(HLH-PAS transcription factor NXF)	455-1455 bp	0.9267	2.6720	1.0519	↑ 2.5401	○ 0.8809
Npas4	Neuronal PAS domain-containing protein 4(Neuronal PAS4)(HLH-PAS transcription factor NXF)	1055-2055 bp	1.4480	0.7260	0.6947	○ 1.0451	↑ 2.0845
Grin1	Glutamate [NMDA] receptor subunit zeta-1Precursor (N-methyl-D-aspartate receptor subunitNR1)	721-1721 bp	0.0268	0.0444	0.5056	↓ 0.0879	↓ 0.0530
Grin1	Glutamate [NMDA] receptor subunit zeta-1Precursor (N-methyl-D-aspartate receptor subunitNR1)	941-1941 bp	0.7263	1.0322	0.4499	↑ 2.2945	↑ 1.6146

Table 3.3: Selected genes and levels of DNA methylation for control, limited-access, and prolonged-access rats taken from a genome-wide methylated DNA array after 1 day of forced abstinence. There are both increases and decreases in prolonged cocaine-access *Homer2, Dlg4, Npas4, and Grin1* DNA methylation depending on the location of the probe.

Homer2 DNA methylation and mRNA expression

A two-way ANOVA between condition and abstinence for *Homer2* DNA methylation revealed significant a significant interaction between condition and abstinence ($F_{8, 114} = 2.00$, $p < 0.05$). To deconstruct the interaction, I performed one-way ANOVAs for between-condition effects for each day of abstinence, and within-condition effects during abstinence. There were no significant effects for naïve, limited-, or prolonged-access conditions, but effects were observed in limited + yoked- ($F_{2, 23} = 7.610$, $p < 0.005$) and saline-access ($F_{2, 21} = 8.153$, $p < 0.005$) conditions. LSD *post-hoc* tests revealed differences in *Homer2* DNA methylation for saline- and limited + yoked-access rats between days 1, 14, and 60 (Figure 3.2A). Additionally, there were main effects of condition ($F_{4, 114} = 3.604$, $p < 0.01$) with LSD posthoc tests revealed significant effects of condition for all self-administration conditions versus naïve conditions ($p < 0.05$), and of abstinence ($F_{2, 114} = 7.412$, $p < 0.001$) with LSD *post-hoc* tests indicating

significant effects of abstinence at 1 and 60 days of abstinence versus 14 days of abstinence ($p < 0.05$). Furthermore, *Homer2* DNA methylation was negatively correlated with total cocaine intake in limited + yoked-access rats [$r(23) = -0.425$, $p < 0.05$, Figure 3.2D]. These data indicate time-dependent increases in DNA methylation that are inconsistent across cocaine conditions.

A two-way ANOVA between condition and abstinence for *Homer2* mRNA expression revealed a significant main effect of condition ($F_{4, 198} = 4.173$, $p < 0.005$), but no effects of abstinence nor interactions between condition and abstinence were detected. LSD *post-hoc* tests revealed significant decreases in *Homer2* mRNA expression for limited-, prolonged-, and limited + yoked-access rats (Figure 3.2B). Lastly, there was a positive correlation between *Homer2* mRNA expression and total cocaine intake in prolonged-access rats [$r(16) = 0.533$, $p < 0.05$, Figure 3.2C]. Although there was no direct correlation observed between DNA methylation and mRNA expression for *Homer2*, the patterning of the data suggests DNA methylation may partially mediate mRNA expression. Samples with higher DNA methylation exhibited lower levels of mRNA expression, but this effect was not specific for mRNA expression, as mRNA was also reduced even when DNA methylation wasn't altered.

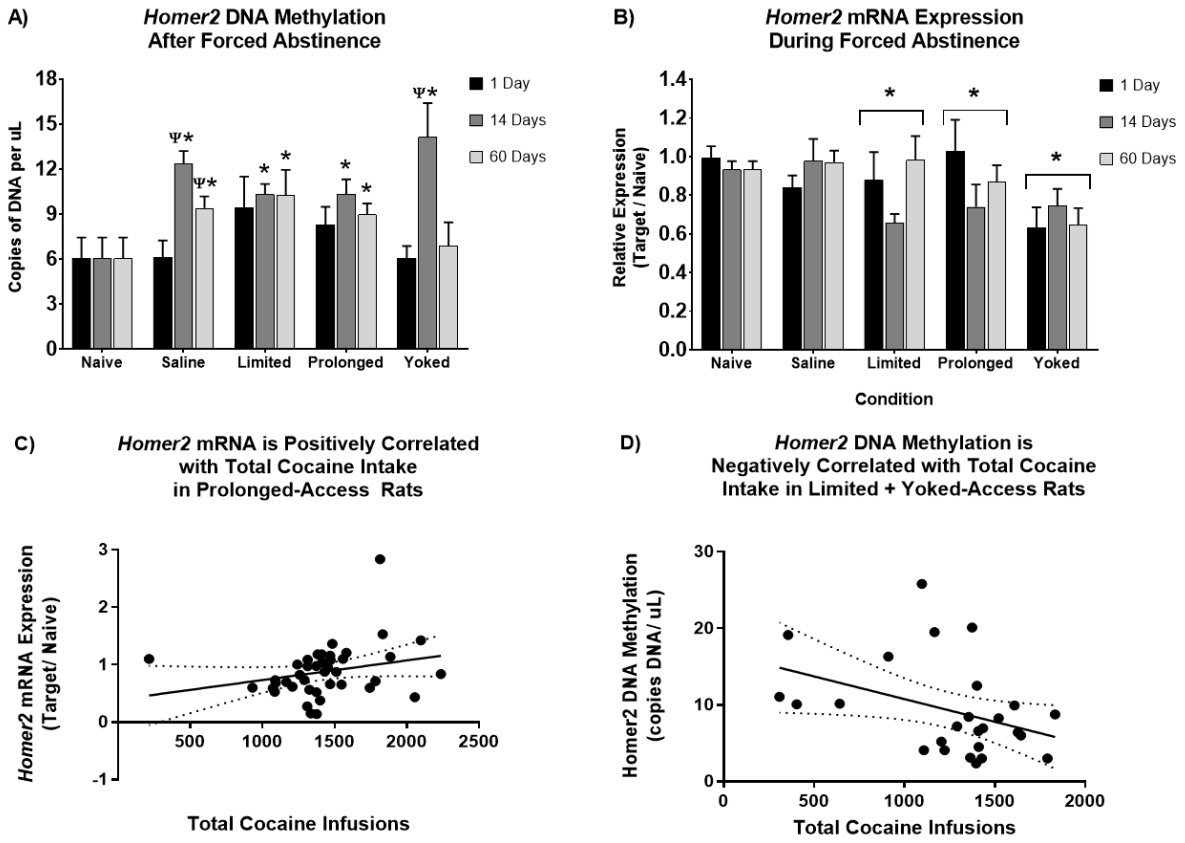

Figure 3.2: *Homer2* DNA methylation and mRNA expression after forced abstinence. A) *Homer2* DNA methylation was increased after 14 and 60 days of abstinence in saline-, limited-, and prolonged-access rats, but only increased after 14 days in limited + yoked-access rats (*, $p < 0.05$). Additionally, *Homer2* DNA methylation changed during abstinence in limited-access and limited + yoked-access rats (Ψ, $p < 0.05$). **B)** *Homer2* mRNA expression was decreased in limited + yoked-access rats (*, $p < 0.05$). **C)** *Homer2* mRNA expression was positively correlated with total cocaine intake in prolonged-access rats across all days of abstinence ($r = 0.533$, $p < 0.05$). **D)** *Homer2* DNA methylation was negatively correlated with total cocaine intake in limited + yoked-access rats for all days of abstinence ($r = -0.425$, $p < 0.05$).

Dlg4 DNA methylation and mRNA expression

A two-way ANOVA between condition and abstinence for *Dlg4* DNA methylation revealed significant main effects of abstinence ($F_{2, 110} = 6.350$, $p < 0.005$), however, there were not significant main effects of condition, or an interaction between condition and abstinence. LSD *post-hoc* tests revealed a significant increase in DNA methylation between 1 day versus 14 and 60 days of abstinence for self-administration conditions (Figure 3.3A).

A two-way ANOVA between condition and abstinence for *Dlg4* mRNA expression revealed a significant main effect of condition ($F_{4, 205} = 3.927$, $p < 0.005$), but no effects of abstinence or interactions between condition and abstinence were detected. LSD *post-hoc* tests revealed a significant increase in *Dlg4* mRNA expression for rats in the limited + yoked-access condition (Figure 3.3B). Lastly, Pearson correlations were run between DNA methylation, mRNA, and total cocaine intake, however, there were no significant correlations detected between any of these factors.

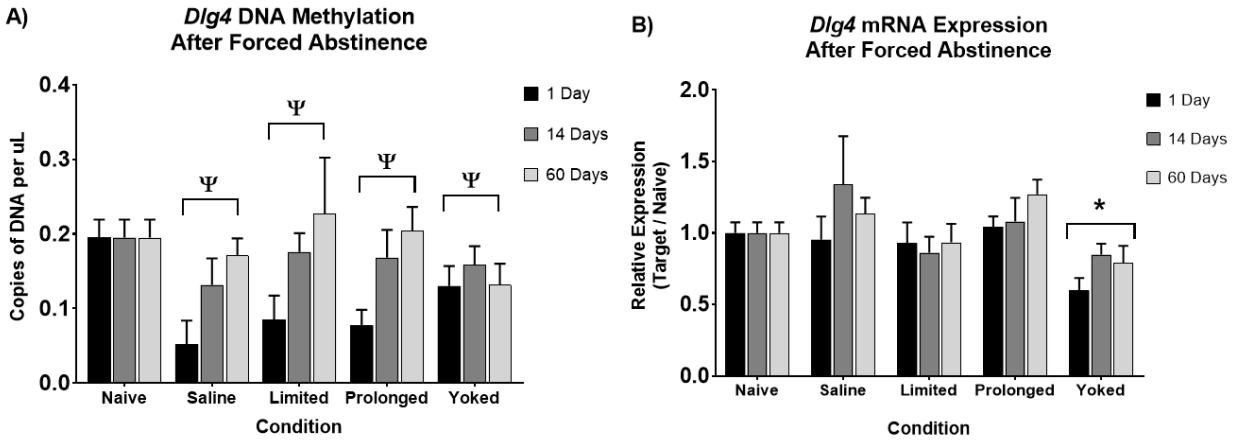

Figure 3.3: *Dlg4* DNA methylation and mRNA expression after forced abstinence. A) *Dlg4* DNA methylation increased over the course of withdrawal (Ψ, $p < 0.05$). **B)** *Dlg4* mRNA expression was lower in limited + yoked-access rats compared to other groups (*, $p < 0.05$).

Npas4 DNA methylation and mRNA expression

A two-way ANOVA for *Npas4* DNA methylation between condition and abstinence revealed a significant interaction between condition and abstinence ($F_{8, 110} = 2.776$, $p < 0.01$) but no significant main effects of either condition or abstinence (ps > 0.05). To deconstruct the interaction, I ran separate one-way ANOVAs for each abstinence period and between each access condition to break down the interaction; only the significant effects are reported below. First, abstinence periods were investigated by condition. One-way ANOVA of saline-access rats revealed a significant effect of

abstinence ($F_{2, 20} = 8.954$, $p < 0.001$), and LSD *post-hoc* analysis indicated significantly more DNA methylation after 1 day of abstinence than after 14 or 60 days of abstinence in saline-access rats (Figure 3.4A). Second, a One-way ANOVA of prolonged-access rats revealed a significant effect of abstinence ($F_{3, 24} = 3.017$, $p < 0.05$), and LSD *post-hoc* testing revealed a significant increase in DNA methylation for *Npas4* after 60 days of abstinence (Figure 3.4A). Third, a one-way ANOVA of limited + yoked-access rats during abstinence revealed a significant effect of abstinence ($F_{2, 22} = 3.888$, $p < 0.05$), and LSD *post-hoc* tests indicated a significant increase in DNA methylation of *Npas4* after 14 days of abstinence (Figure 3.4A). Interactions were further broken down to detect differences between access-conditions at different abstinence points. A one-way ANOVA of rats after 1 day of abstinence revealed a significant effect of condition ($F_{4, 37} = 3.621$, $p < 0.05$), and LSD *post-hoc* tests indicated significantly higher DNA methylation in the saline-access condition than in other conditions (Figure 3.4A). Lastly, a one-way ANOVA of rats after 14 days of abstinence revealed a significant effect of condition ($F_{4, 29} = 2.798$, $p < 0.05$), and LSD *post-hoc* testing revealed significantly higher *Npas4* DNA methylation in limited + yoked-access rats than in other conditions (Figure 3.4A).

A two-way ANOVA for *Npas4* mRNA between condition and abstinence revealed an interaction between condition and abstinence ($F_{8, 203} = 2.149$, $p < 0.05$) as well as a significant main effect of condition ($F_{4, 203} = 9.041$, $p < 0.0001$) but no main effect of abstinence. To deconstruct the interaction, I ran separate one-way ANOVAs for each abstinence period and between each access condition to break down the interaction; only the significant effects are reported below. First, a one-way ANOVA for saline-access rats indicated a significant effect of abstinence on *Npas4* mRNA expression ($F_{2, 41}$

= 8.404, $p < 0.001$), and LSD *post-hoc* tests indicated significantly higher mRNA expression after 60 days of abstinence than at 1 or 14 days of abstinence (Figure 3.4B). Secondly, a one-way ANOVA for prolonged-access rats indicated a marginally significant effect of abstinence on *Npas4* mRNA expression ($F_{2, 38} = 2.464$, $p = 0.099$), and LSD *post-hoc* tests indicated significantly higher mRNA expression after 60 days of abstinence than at 1 day of abstinence ($p < 0.05$, Figure 3.4B). Third, a one-way ANOVA for limited + yoked-access rats indicated a significant effect of abstinence on *Npas4* mRNA expression ($F_{2, 38} = 5.203$, $p < 0.05$), and LSD *post-hoc* tests indicated significantly higher mRNA expression after 14 days of abstinence than at 1 or 60 days of abstinence (Figure 3.4B). In addition to analyzing time-course effects between groups, we also performed one-way ANOVAs for each abstinence time point to determine effects between conditions. A one-way ANOVA for condition after 1 day of abstinence revealed a significant effect of condition on *Npas4* mRNA expression ($F_{4, 67} = 5.631$, $p < 0.001$), and LSD post-hoc tests indicated lower levels of *Npas4* mRNA in all conditions compared to naïve rats (Figure 3.4B). A one-way ANOVA for condition after 14 days of abstinence revealed a significant effect of condition on *Npas4* mRNA expression ($F_{4, 60} = 3.781$, $p < 0.01$), and LSD post-hoc tests indicated lower levels of *Npas4* mRNA in all access conditions compared to naive rats (Figure 3.4B). A one-way ANOVA for condition after 60 days of abstinence revealed a significant effect of condition on *Npas4* mRNA expression ($F_{4, 75} = 4.071$, $p < 0.005$), and LSD post-hoc tests indicated lower levels of *Npas4* mRNA in limited- and limited + yoked-access rats compared to naive rats (Figure 3.4B).

In addition to the ANOVAs, pairwise Pearson correlations between mRNA, DNA methylation, and total intake revealed significant negative correlations between levels of methylated *Npas4* DNA and *Npas4* mRNA. Specifically, these effects were observed in both naïve [$r(23) = -0.605$, $p < 0.005$, Figure 3.4C] and saline-access [$r(19) = -0.465$, $p < 0.05$, Figure 3.4D] conditions. However, correlations were not observed in any of the cocaine-access conditions, although there was a marginally significant positive correlation between methylated DNA and mRNA for the limited + yoked-access rats [$r(22) = 0.345$, $p = 0.098$]. The presence of correlations between methylated DNA and mRNA in the two control conditions (naïve and saline) indicate a disruption in *Npas4* expression in relation to DNA methylation after exposure to cocaine.

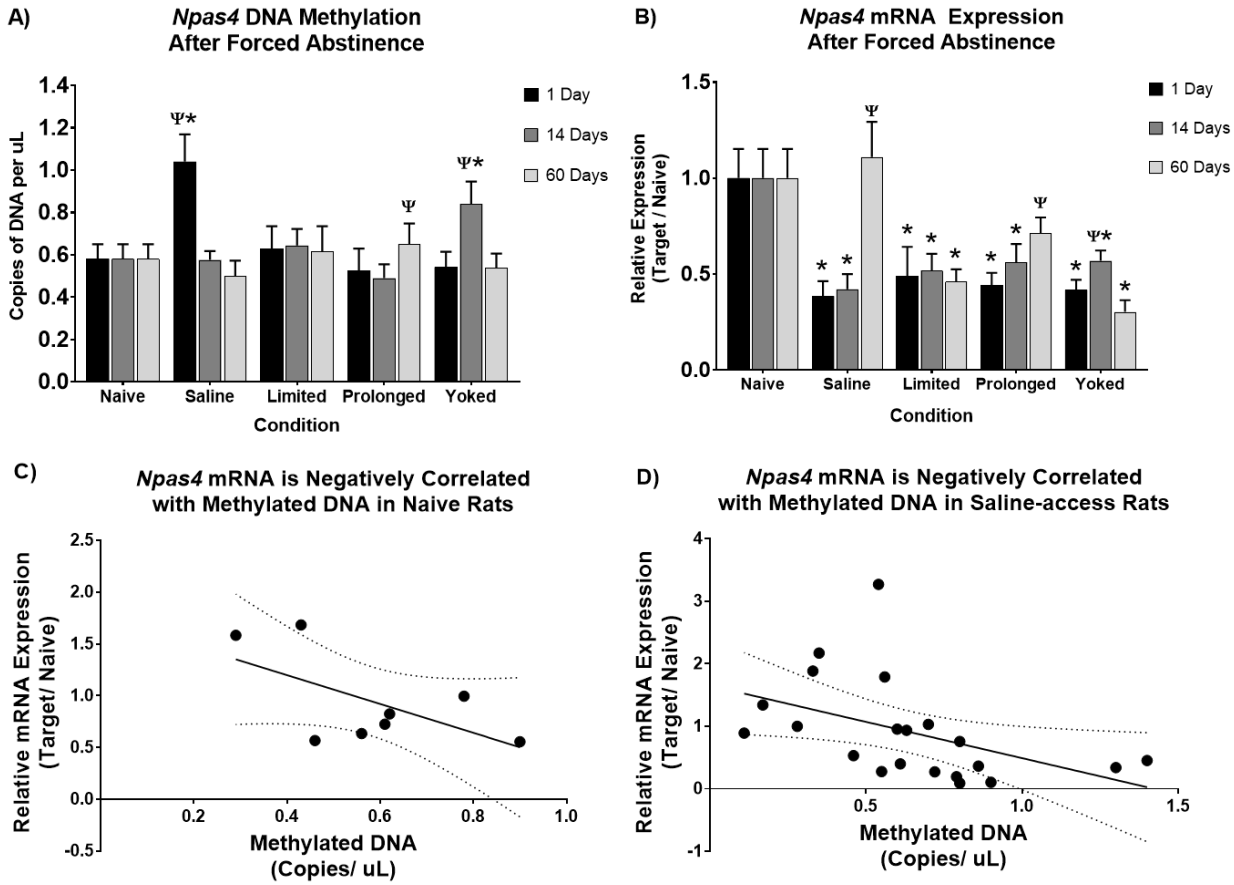

Figure 3.4: Npas4 DNA methylation and mRNA expression after forced abstinence. A) *Npas4* DNA methylation was higher in saline-access rats after 1 day of abstinence and in limited + yoked-access rats after 14 days of abstinence (*, $p < 0.05$). Additionally, DNA methylation changed over the course of abstinence (Ψ, $p < 0.05$). **B)** *Npas4* mRNA expression was lower in limited- and limited + yoked access rats after all abstinence periods, in saline- and prolonged-access rats after 1 and 14 days of abstinence (*, $p < 0.05$). Additionally, *Npas4* mRNA expression changed over the course of abstinence (Ψ, $p < 0.05$). **C, D)** *Npas4* mRNA is negatively correlated with *Npas4* DNA methylation in naïve ($r = -0.605$, $p < 0.005$) and saline-access rats ($r = -0.465$, $p < 0.05$).

Grin1 DNA methylation and mRNA expression

A two-way ANOVA for *Grin1* DNA methylation between condition and abstinence revealed an interaction between condition and abstinence ($F_{8, 100} = 2.198$, $p < 0.05$) and a significant main effect of abstinence ($F_{2, 100} = 5.497$, $p < 0.005$) but there was no main effect of condition ($p > 0.05$). To deconstruct the interaction, I ran separate one-way ANOVAs for each abstinence period and between each access condition; only the

significant effects are reported below. First, for saline-access rats, there was a significant effect of abstinence ($F_{2, 20} = 4.849$, $p < 0.05$), and LSD *post-hoc* tests indicated decreases in *Grin1* DNA methylation after 14 and 60 days of abstinence (Figure 3.5A). Next, a one-way ANOVA of limited-access rats revealed a marginally significant effect of abstinence on *Grin1* DNA methylation ($F_{2, 17} = 3.325$, $p = 0.060$) and LSD *post-hoc* tests indicated lower levels of *Grin1* DNA methylation after 14 days of abstinence ($p < 0.05$, Figure 3.5A). Between condition effects were assessed with one-way ANOVAs during each day of abstinence. A one-way ANOVA of *Grin1* DNA methylation after 14 days of abstinence revealed a significant effect of condition ($F_{4, 27} = 4.455$, $p < 0.01$), and LSD *post-hoc* tests indicated a decrease in *Grin1* DNA methylation for saline, limited- and prolonged- access conditions. Lastly, a one-way ANOVA of *Grin1* DNA methylation after 60 days of abstinence revealed a significant effect of condition ($F_{4, 39} = 2.683$, $p < 0.05$), and LSD *post-hoc* tests indicated decreased DNA methylation for *Grin1* in only the saline-access rats (Figure 3.5A). A two-way ANOVA was also conducted for *Grin1* mRNA expression and revealed a significant main effect of condition ($F_{4, 201} = 3.249$, $p < 0.05$), but no effects of abstinence nor an interaction between abstinence and condition. LSD *post-hoc* tests indicated decreased *Grin1* mRNA in all conditions compared to the naïve condition (Figure 3.5B). Lastly, there were no correlations observed between drug intake, *Grin1* mRNA, of *Grin1* DNA methylation.

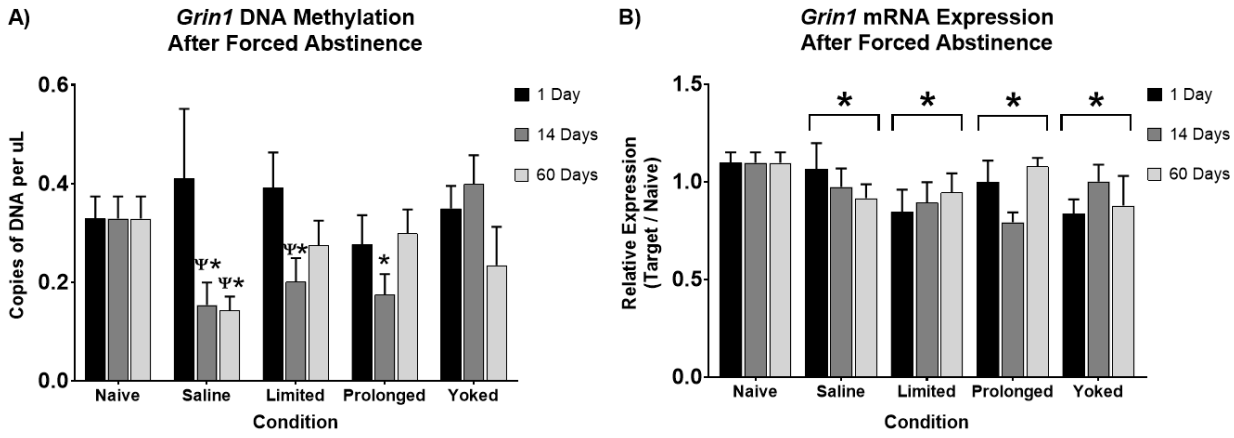

Figure 3.5: *Grin1* DNA methylation and mRNA expression after forced abstinence. A) *Grin1* DNA methylation was reduced in saline-access rats after 14 and 60 days of abstinence, and in limited- and prolonged-access rats after 14 days of abstinence (*, $p < 0.05$). Additionally, *Grin1* DNA methylation changed over the course of abstinence (Ψ, $p < 0.05$). **B)** *Grin1* mRNA expression was lower than naïve controls in all self-administration groups (*, $p < 0.05$).

Discussion

The current report provides an examination of DNA methylation and mRNA expression within the dmPFC of rats following multiple periods of withdrawal from cocaine. Overall, we found gene-specific changes in both DNA methylation and mRNA levels as a consequence of behavioral training (cocaine or saline self-administration) relative to naïve control tissue which were dynamic throughout the period of forced abstinence investigated. Notably, correlation between DNA methylation and mRNA for individual genes failed to reach significance indicating DNA methylation poorly predicts gene expression under the present conditions.

Arguably, the most novel findings of the present study concern dynamic changes in DNA methylation at different abstinence time points. We initially expected to see stable levels of DNA methylation in response to cocaine training, primarily because prior literature concerning epigenetic mechanisms describe DNA methylation as a persistent marker within genes, largely related to its critical role in determination of cell-type during

developmental differentiation (Hellman & Chess, 2007; Jaenisch & Bird, 2003). Persistent DNA methylation has additionally been observed in current and recovered nicotine addicts. They both exhibited lower levels of Monoamine Oxidase (MAO) DNA methylation, and higher levels of MAO protein decades after ceasing tobacco use compared to non-smokers (Launay et al., 2009). Additionally, animals exposed to cocaine self-administration have exhibited persistent changes in DNA methylation after 30 days of forced abstinence from cocaine in both the corpus callosum and the nucleus accumbens (Massart et al., 2015; Nielsen et al., 2012). However, unlike these studies, we did not observe persistent levels of DNA methylation in the dmPFC, but rather, time-dependent alterations in DNA methylation over the course of abstinence in rats self-administering cocaine and saline. (Figures 3.2 to 3.5, Table 3.4).

Although the observed time-dependent fluctuations in DNA methylation during abstinence were initially surprising to us, they are not entirely unprecedented within the field of epigenetics or addiction. Specifically, former tobacco smokers will experience changes in whole-blood genome-wide DNA methylation throughout withdrawal, indicating that their systems to recover to "non-smoker" levels (Gao, Jia, Zhang, Breitling, & Brenner, 2015; Zeilinger et al., 2013). Additionally, in 2014, Baker-Andresen et al. showed that self-administration of cocaine, but not passive cocaine-injection, resulted in both persistent methylation changes in 29 different genes, as well as time-dependent changes in DNA methylation in 28 different genes within the mPFC of mice across 21 days of forced abstinence (Baker-Andresen et al., 2015). The Baker-Andresen et al. results are particularly relevant to the present report because they indicate that abstinence from cocaine can attenuate or potentiate epigenetic modifications relevant

to addiction (as we also observed, Table 3.4), are independent of further cocaine administration, and likely rely upon memory consolidation and retrieval associated with prior cocaine use. To further this point, cocaine addiction is well known to involve alternating periods of binge/ intoxication of cocaine, withdrawal/ negative affect from cocaine, and preoccupation/ anticipation for cocaine during abstinence (Koob & Volkow, 2009), and as such, is a drug-induced disorder of learning and memory (Tronson & Taylor, 2013). Specifically, the "preoccupation/ anticipation" phase of addiction is associated with areas of the brain implicated in memory consolidation and retrieval (i.e. the hippocampus, BLA, insula, and mPFC) (Koob & Volkow, 2009). DNA methylation and other epigenetic modifications are not just implicated in, but are necessary for memory formation (Day & Sweatt, 2010), maintenance (Halder et al., 2016), and reconsolidation (Maddox & Schafe, 2011). As such, we can conclude that the dynamic changes we observed in DNA methylation for *Homer2, Dlg4, Npas4,* and *Grin1* within the dmPFC of limited- and prolonged-access rats are likely due to cocaine-associated memories during the abstinence period, particularly because these changes were not observed in limited + yoked-access rats. In addition to cocaine-dependent DNA methylation, we also observed changes in DNA methylation over the course of abstinence for saline-access rats. However, these rats were not "pure" controls, as they were trained for food reinforcers on an FR1 schedule before being trained for saline self-administration, so it is possible that the same learning and memory mechanisms mentioned above apply to food conditioning as well.

mRNA Expression Exhibits Dependence Upon Cocaine-Access Condition but Apparent Independence from DNA Methylation.

In addition to the dynamic changes in DNA methylation discussed above, DNA methylation did not appear to account for variance in mRNA expression for cocaine-access rats during abstinence. Although *Npas4* DNA methylation was negatively correlated with *Npas4* mRNA in naïve and saline-access rats but not in cocaine experienced rats. Although we expected to observe a wide range of changes in mRNA expression driven by DNA methylation (Jones, 2012; Jones et al., 1998), we instead observed consistent mRNA changes throughout abstinence that differed between the saline and cocaine-access conditions, indicating that experimental conditions had more effect on mRNA than levels of DNA methylation (Table 3.4). The saline-access rats were distinct from cocaine-access rats in terms of mRNA expression as they do not exhibit decreases in *Homer2* (Figure 3.2B) expression during abstinence, even if they do exhibit similar patterns of mRNA expression for *Dlg4* (Figure 3.3B), *Npas4* (Figure 3.4B), and *Grin1* (Figure 3.5B). These effects can primarily be explained by the function of the genes. *Dlg4* (Roche et al., 2001; Williams et al., 2003), *Npas4* (Ploski et al., 2011), and *Grin1* (Sweatt, 2016) are all genes that are implicated in various learning and memory studies, while *Homer2* has specifically been linked to cocaine self-administration numerous times (for review see (Szumlinski et al., 2008).

First, in addition to the dynamic changes in DNA methylation, we partially verified DNA methylation observed in the NimbleGen array (Table 3.3) with ddPCR for methylated DNA in *Dlg4* and *Npas4* gene promoters in limited- and prolonged-access rats, (Table 3.4). However, DNA methylation for *Grin1* and *Homer2* were not confirmed for the array for a couple of possible reasons. First, the array is a dataset that used the dmPFC from a single rat (n = 1), rather than multiple independent samples from separate

rats, which is not statistically valid for hypothesis testing (rather thresholds in the form of fold changes are set as presumed significance), and thus requires external validation due the propensity to produce misleading results. Secondly, the NimbleGen array data encompasses DNA methylation over a 1000 bp range of DNA, whereas digital PCR covers an area 150-250 bps in length, and averages the number of changes in DNA methylation over the 1000 bp expanse of DNA. However, even with these issues, the NimbleGen array data provides a non-biased analysis of epigenetic changes (i.e. forward epi-genetic). Based on those changes, we selectively validated specific targets in promoter regions which are of potential relevance to PFC hypofrontality, largely as mediators of excitatory neurotransmission.

In summary, we employed to approaches to assess 5mc changes (array and digital pcr validation) following multiple cocaine access conditions which revealed highly gene-specific changes which were dynamic across cocaine-free periods and, surprisingly, unrelated to mRNA changes associated with cocaine X withdrawal effects. One basic interpretation of these results is that 5mc fails does not predict the mRNA expression relevant to cocaine exposure and withdrawal. This is certainly not an entirely surprising result as gene expression is modulated by myriad transcription factors as well as other epigenetic factors, including histone modifications and other DNA modifications at cytosine nucleotides. Transcription factors are highly sensitive to environmental factors (Prast et al., 2014; Wang et al., 2016; Wei et al., 2012) including conditional stimuli paired previously with biological meaningful stimuli such as cocaine (Prast et al., 2014). As such, Chapter 4 investigates the ability of re-exposure to the cocaine associated cues in the absence of cocaine (i.e. extinction conditions) to induce changes in mRNA relative

to the patterns of cocaine-induced 5mc changes observed in the present chapter. Conversely, 5OHMC has been identified as a DNA modification that is functional and functionally distinct form 5MC as well as being form from 5mc. Given 5MC and 5OHMC are both sensitive to experience and have distinct contributions to gene expression, Chapter 5 investigates the inter-relation between gene expression and these multiple DNA modifications following cocaine exposure.

Gene	Distance from TSS	Condition	NimbleGen DNA Array	MeDIP ddPCR: Days of Abstinence			mRNA ddPCR: Days of Abstinence		
			1 Day	1 Day	14 Days	60 Days	1 Day	14 Days	60 Days
Homer2	*3159 to 3344 bp*	*Saline*	*n.s*	*n.s*	↑	↑	*n.s*	*n.s*	*n.s*
		Limited	*n.s*	*n.s*	↑	↑	↓	↓	↓
		Prolonged	*n.s*	*n.s*	↑	↑	↓	↓	↓
		Yoked		*n.s*	↑	*n.s*	↓	↓	↓
Dlg4	*756 to 950 bp*	*Saline*	↑	↓	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
		Limited	↓	↓	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
		Prolonged	↓	↓	*n.s*	*n.s*	*n.s*	*n.s*	*n.s*
		Yoked		*n.s*	*n.s*	*n.s*	↓	↓	↓
Npas4	*295 to 396 bp*	*Saline*	↑	↑	*n.s*	*n.s*	↓	↓	*n.s*
		Limited	↓	*n.s*	*n.s*	↑	↓	↓	↓
		Prolonged	↓	*n.s*	↑	*n.s*	↓	↓	*n.s*
		Yoked		*n.s*	*n.s*	*n.s*	↓	↓	↓
Grin1	*790 to 964 bp*	*Saline*	↓	*n.s*	↓	↓	↓	↓	↓
		Limited	↓	*n.s*	↓	*n.s*	↓	↓	↓
		Prolonged	↓	*n.s*	↓	*n.s*	↓	↓	↓
		Yoked		*n.s*	*n.s*	*n.s*	↓	↓	↓

Table 3.4: Summary of Molecular Changes. MeDIP and NimbleGen Array data for *Dlg4* and *Homer2* was partially replicated with MeDIP and digital pcr (ddPCR) techniques at 1 day post-abstinence. Patterns of mRNA expression are distinct depending upon access-condition and abstinence period, but do not appear to be influenced by DNA methylation.

Chapter 4:

The effect of cocaine cue re-exposure after prolonged cocaine self-administration and forced abstinence on DNA methylation and mRNA expression in the rat dmPFC

Abstract

In the previous chapter, I observed that DNA methylation incubates over the course of abstinence, but does not seem to predict basal mRNA transcription. One possible reason for this discrepancy is that mRNA expression relies on transcription factor binding and/ or immediate early gene activation that occurs with context-specific neuronal excitation. As such, I tested the hypothesis that cocaine exposure-induced changes in 5MC will more highly predict gene expression when there is a driver of gene expression, in this case, re-exposure to the self-administration condition in the absence of cocaine. To test this, I trained rats to self-administer either saline (1 h per day) or cocaine (6 h per day) for 15 days, then exposed them to 60 days of forced abstinence. After abstinence half of the rats were put back in self-administration chambers and re-exposed to cues for 2 h, while the other half were left in their home cages. After the cue manipulation, all rats were sacrificed, the dmPFC was dissected out, and DNA and mRNA were extracted. Methylated DNA was purified using methylated DNA immuno-precipitation, and mRNA was reverse-transcribed into cDNA; levels of each were then quantified via digital PCR procedures. Interestingly, I observed cue-induced changes in DNA methylation within *Homer2, Dlg4, Npas4,* and *Grin1* promoter regions. These context-specific changes indicate that DNA methylation is much more dynamic than

previously shown. Additionally, I saw changes in mRNA expression for *Homer2*, *Dlg4*, and *Npas4* that appear to be highly reflective of the changes in DNA methylation. These results indicate re-exposure to cocaine cues induces.gene-specific mRNA expression via rapid changes in DNA methylation.

Introduction

As discussed in previous chapters, the gradual escalation of cocaine intake and incubation of cocaine craving that occurs over extended periods of forced abstinence are integral aspects of an addictive phenotype. Specifically, Grimm et al. (2001) demonstrated that rats previously given prolonged-access to cocaine (6h/ day) will exhibit a time-dependent increase in non-reinforced lever responding (on a lever previously associated with cocaine reinforcement) when the cues associated with cocaine administration (light & sound) are present (aka cued-reinstatement). Furthermore, extended periods of forced abstinence have been associated with increased levels of GluN2B, mGluR1, mGluR5, and Homer2a/b protein within the dmPFC (Ben-Shahar et al., 2013; Gould et al., 2015; Szumlinski et al., 2016). Additionally, I demonstrated in chapter 3 that levels of DNA methylation also incubate between 1 and 60 days during forced abstinence, although they are not associated with changes in mRNA. Lastly, in 2013, Ben Shahar et al. demonstrated cue-elicited changes in glutamatergic protein expression after extended-abstinence.

The role of the prefrontal cortex in drug seeking under extinction and reinstatement conditions.

Human feelings of craving are hard to quantify behaviorally, but neuroimaging studies have identified a variety of neural substrates involved in the subjective experience of craving in humans. Studies of human cocaine addicts reveal a general reduction in baseline activity in the anterior cingulate cortex (ACC) and the orbital PFC (oPFC), as well as a reduction in cortical neuron populations (Goldstein & Volkow, 2002; Franklin et al., 2002). Interestingly, when human subjects are presented with drug-associated cues, there is an increase in activation of the ACC and oPFC (Goldstein & Volkow, 2002). The necessity of these areas in drug-seeking behavior in animals is reflected in rodent models whereby localized inactivation of the VTA to dPFC circuit, PFC to accumbens circuit, or accumbens to ventral pallidal circuit blocks drug-seeking behavior in animals trained to extinguish drug-related cues (McFarland & Kalivas, 2001). The dmPFC is also implicated in the reinstatement of cocaine-seeking behavior following forced abstinence (Berglind, Whitfield, LaLumiere, Kalivas, & McGinty, 2009; Szumlinski et al., 2016). Specifically, the dmPFC in necessary for explicit cue-induced and cocaine-primed reinstatement of cocaine administration (Fuchs et al., 2005). In addition to cocaine-paired cues, novel cues also induce reinstatement of cocaine-seeking, and are associated with cFos activation in the dmPFC (Bastle et al., 2012). Since changes in dmPFC functionality and composition underlie craving behavior in humans and drug-seeking in rodents, unravelling the molecular mechanisms underlying long term changes in the dmPFC are crucial to understanding cocaine addiction.

DNA methylation in addiction and after extinction and reinstatement procedures

Epigenetic mechanisms involve a number of substrates, including modifications directly on DNA such as addition of methyl or hydroxymethyl groups. In mammals, DNA methylation is primarily produced by the addition of a methyl group ($-CH_3$) to cytosine (Sweatt et al., 2013). DNA methylation at transcription factor binding sites often interferes with the association of a transcription factor to the DNA, which can in turn repress or activate gene expression depending on whether the sequence is recognized by a transcriptional repressor or activator (Sweatt et al., 2013). In the previous chapter, I indicated that differential access to cocaine induced a wide array of gene-specific DNA methylation changes within the dmPFC after 1 day of forced abstinence (Appendix Table 1), and in specific genes throughout abstinence. Additionally, previous reports have also indicated persistent changes in DNA methylation within the nucleus accumbens and corpus callosum after extended abstinence periods (Massart et al., 2015; Nielsen et al., 2012), and dynamic changes in DNA methylation that occur during the abstinence period (Baker-Andresen et al., 2015).

DNA methylation has also been implicated in extinction and reinstatement training procedures. For example, female mice have been shown to be resistant to fear extinction procedures, and also exhibit higher levels of 5mC in the BDNF promoter within the vmPFC (Baker-Andresen, Flavell, Li, & Bredy, 2013). Additionally, demethylation of gene promoters via Tet proteins is necessary for the expression of fear extinction (Li, Wei, et al., 2014; Rudenko et al., 2013). Similarly, levels of 5hmC are increased following cocaine-extinction procedures (Sadakierska-Chudy et al., 2017). Further evidence for DNA methylation in extinction learning is demonstrated in insects. In honeybees, DNA methylation promotes relearning (Biergans, Claudianos, Reinhard, &

Galizia, 2016), and pharmacological inhibition of DNMT alters extinction learning in a species-specific manner (Gong, Wang, Nieh, & Tan, 2016). However, previous reports have not investigated DNA methylation in relation to drug-seeking induced by re-exposure to drug cues after abstinence. Therefore, for the purposes of this study, I chose to perform a cued re-exposure procedure at 60 days post-abstinence for prolonged- and saline-access rats to determine the effect of cue re-exposure on DNA methylation and mRNA expression for *Homer2, Dlg4, Npas4,* and *Grin1* within the dmPFC.

Methods

Animals housing, care, and surgical procedures

Male Sprague-Dawley rats (250-300 g) were pair-housed in a reverse light:dark (12h:12h, lights off at 8 am) cycle room with ad-libitum access to food and water. The housing and care of the rats followed the guidelines set forth by the "Guide for the Care and Use of Laboratory Rats, 8th Edition" (IACUC, 2011).

Rats were deeply anesthetized using 2-3% isoflurane gas and implanted with chronic indwelling catheters constructed with a bent steel cannula with a screw-type connector (Plastics One, Roanoke, VA), SILASTIC tubing (11 cm, i.d. 0.64 mm, o.d. 1.19 mm, Dow Corning, Midland, MI), Prolite polypropylene monofilament mesh (Atrium Medical Corporation, Hudson, NH), methyl methacrylate cement, and a silicon ball 2.5 cm from the end used to secure the catheter to the vein. Postoperative care lasted for 4 days; rats were visually inspected, weighed, and the catheters were maintained as we have reported previously, and tested once weekly with 2.0 mg of Methohexital Sodium (Brockton, MA).

Cocaine self-administration, cued re-exposure & dmPFC dissection

Food training and cocaine self-administration utilized standard operant chambers (Med Associates Inc., St. Albans, VT, USA) during the dark cycle at a controlled time each day. Before surgical implantation of the jugular catheters, the rats were restricted to 20 g of food daily and trained on a fixed ratio 1 (FR1) schedule of food reinforcement for two 16 h training sessions where each right lever press was associated with the delivery of a 45 mg food pellet (Ben-Shahar et al., 2012). After recovery from the surgery, the rats were placed on a fixed ratio 1 (FR1) with a 20 second time-out signaled by the activation of the cue light above the lever. Initial sessions lasted 1 h per day for the first 5 days with either cocaine or saline infusions available as reinforcers. Each "active" lever depression (right lever) was associated with a 4 s infusion of vehicle (0.1 mL saline) or cocaine (0.25 mg/ 0.1 mL); "inactive" lever depression (left lever) resulted in no infusion or stimulus. On the 6^{th} day, the rats receiving cocaine began prolonged cocaine-access, where they were allowed access to cocaine for 6 h sessions daily for an additional 15 days; rats self-administering saline continued under 1 h access for 15 days (Figure 4.1). Additionally, separate rats were used as naïve controls; they arrived the same time as experimental rats, but were not subjected to behavioral conditioning and were sacrificed at the same time as the experimental rats.

After self-administration training, all rats were put through forced abstinence for 60 days (except naïve rats). The rats were further divided into two groups: rats with or without cue re-exposure (see below). The rats that did not undergo cue re-exposure were sacrificed via rapid decapitation, their brains were removed, and the dorsal medial prefrontal cortex (dmPFC) was dissected out at 2.20 to 3.20 mm anterior to Bregma

(Figure 2.1) via the Braintree brain mold (Braintree, MA), placed in a 0.5 mL microcentrifuge tube, frozen on dry ice, and stored at -80 °C for further analysis.

The cue re-exposure procedures were the same as the self-administration procedures except receiving response-contingent cocaine infusions, thus, these procedures re-exposed the rats to the contextual cues as well as response-contingent discrete cues. Specifically, rats that underwent cue re-exposure testing had their catheters flushed, as previously stated for maintenance, and were re-introduced into the same operant chamber as they had during self-administration training. Cue re-exposure lasted for 2 h, where each depression on the "active" lever was associated with activation of the infusion pump for 4 s (no fluid was attached) and activation of the light cue above the active lever for 20 s. Immediately after cued-reinstatement testing finished, the rats were sacrificed via rapid decapitation, their brains were removed, and the dorsal medial prefrontal cortex (dmPFC) was dissected out at 2.20 to 3.20 mm anterior to Bregma (Figure 2.1) via the Braintree brain mold (Braintree, MA), placed in a 0.5 mL microcentrifuge tube, frozen on dry ice, and stored at -80 °C for further analysis.

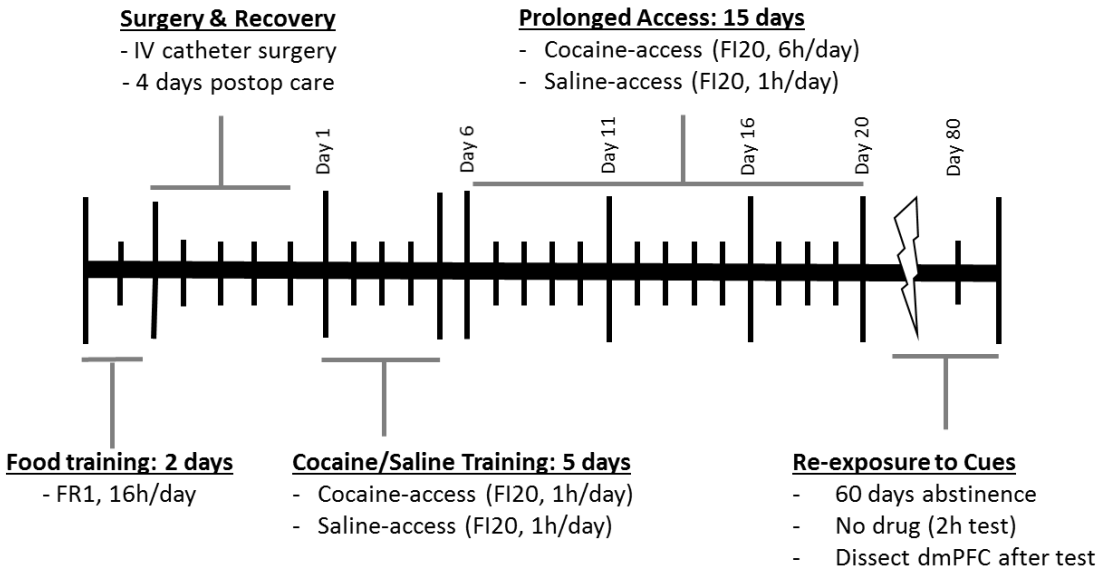

Figure 4.1: Experimental timeline. Rats were trained to lever press with food for 2 days. Surgery was then performed and followed by 4 days of postoperative care. Rats moved to cocaine and saline self-administration for 5 days following training. Cocaine rats were then introduced to 6h daily training sessions for 15 days. After cocaine training, rats underwent 60 days of forced abstinence. On the last day of abstinence, the rats were reintroduced to the operant chambers and allowed to press the lever previously associated with either saline or cocaine for 2 hours, then they were decapitated and the dmPFC was removed and stored at -80 C.

DNA and mRNA extraction and purification for digital PCR

The DNA and mRNA extraction procedures, as well as digital PCR design and analyses are identical to Chapter 3. However, to introduce them once more: for digital PCR analyses, frozen dmPFC tissue from all rats that were subjected to 60 days of forced abstinence was added to 600 µL of buffer RLT (Qiagen, #79216) and homogenized with the Qiagen TissueRuptor for 30 s. DNA and mRNA were then extracted by the AllPrep DNA/RNA/protein extraction kit (Qiagen, #80004) in accordance to the manufacturer's protocol. RNA was eluted from the spin column with 50 µL of nuclease-free water. DNA was eluted from the spin column with 100 uL of 8 mM NaOH heated to 70 °C, and the pH was then neutralized with 12 µL of 0.1 M HEPES and 1.1 µL of 100 mM EDTA.

For the analysis of methylated DNA, extracted genomic DNA (1 µg) was fragmented to 400 bp fragments by the Covaris M220 system via the automated protocol. The size of the DNA fragments were confirmed via gel electrophoresis on a 2% gel, at 110V, for 75 minutes and visualized with the Lycor Gel Analyzer. To isolate the methylated DNA (5mC) the fragmented DNA then underwent methylated DNA immunoprecipitation (MeDIP) with a 5-methylcytosine antibody (Active Motif, 55009) and protein G magnetic beads according to the manufacturer's protocol. The DNA was then purified via phenol-chloroform extraction and ethanol precipitation as mentioned previously.

For the analysis of mRNA expression: 500 ng of mRNA from each extracted sample was reverse transcribed according to the manufacturer's protocol (Qiagen, 205311), except that samples were incubated with genomic DNA wipeout buffer in an Eppendorf MasterCycler at 42 °C for 18 minutes, then incubated at 95 °C for 3 min to inactivate the reverse transcriptase. A reverse transcriptase-negative reaction was carried out in parallel with the samples from 500 ng of pooled sample RNA.

Methylated DNA digital PCR design and analysis

Primers for the methylated DNA samples (Table 3.1) were created using the UCSC genome browser (https://genome.ucsc.edu) to view the DNA code, primer3 (http://primer3.ut.ee) to find the best primers in the given region, and PrimerBlast (https://www.ncbi.nlm.nih.gov/tools/primer-blast) to validate the primer position and amplicon size in relation to the gene body. All primers were then tested with 50 ng of fragmented genomic DNA under multiple conditions (cycle duration, annealing temperature, primer concentration, etc) on a BioRad CFX96 Touch Real-Time system to

determine primer efficiency and accuracy, and then visually assessed on the Lycor system after electrophoresis on a 2% agarose gel (110V, 75 minutes) to validate amplicon size. The final run conditions (Table 3.1) were used on experimental samples during quantification.

Digital real-time PCR (ddPCR) is a new method of DNA quantification to measure the absolute concentration of DNA in samples. In order to use this method, we first combined DNA samples with EvaGreen QX200 Supermix (BioRad), forward and reverse primer sets, and water (Table 3.1). Then, 20 μL of each sample were parsed into 10,000 to 20,000 droplets using ddPCR EvaGreen Droplet Generation Oil (BioRad) in the QX200 Droplet Generator, then transferred to a 96-well PCR plate. After the plate was loaded with droplets, the DNA was amplified on a BioRad CFX96 Touch Real-Time system according to previously tested conditions (Table 3.1). After amplification, the PCR plate was added to the QX200 digital PCR reader. The reader then measured the fluorescence of each individual droplet and used Poisson statistics to calculate the concentration (copies of DNA/ μL) of each sample. Post-run analyses were performed on QuantaSoft software (BioRad), and the final concentration was determined by subtracting the baseline fluorescence samples from the amplified samples.

mRNA digital PCR design and analysis

Primers for the mRNA samples (Table 2) were created using the Ensembl genome browser (https://www.ensembl.org/index.html) to view the transcript's cDNA code, primer3 (http://primer3.ut.ee) to find the best primers in the given region, and PrimerBlast (https://www.ncbi.nlm.nih.gov/tools/primer-blast) to validate the primer position and amplicon size in relation to the gene body. All primers were then tested with

50 ng of cDNA pooled from all experimental conditions under multiple PCR conditions (cycle duration, annealing temperature, primer concentration, etc) on a BioRad CFX96 Touch Real-Time system to determine primer efficiency and accuracy, and then visually assessed on the Lycor system after electrophoresis on a 2% agarose gel (110V, 75 minutes) to validate amplicon size. The final run conditions (Table 3.2) were used on experimental samples during quantification.

For mRNA quantification, we first combined cDNA samples (~12.5 ng/ μL) with EvaGreen QX200 Supermix (BioRad), forward and reverse primer sets, and water (Table w). Then, 20 μL of each sample were parsed into 10,000 to 20,000 droplets using ddPCR EvaGreen Droplet Generation Oil (BioRad) in the QX200 Droplet Generator, and transferred to a 96-well PCR plate. After the plate was loaded with droplets, the DNA was amplified on a BioRad CFX96 Touch Real-Time system according to previously tested conditions (Table 3.2). After amplification, the PCR plate was added to the QX200 digital PCR reader. The reader then measured the fluorescence of each individual droplet and used Poisson statistics to calculate the concentration (copies of DNA/ μL) of each sample. Post-run analyses were performed on QuantaSoft software (BioRad), and the final cDNA concentration was determined by subtracting the baseline fluorescence samples from the amplified samples. ΔΔCT analysis was performed on the final concentrations of target DNA to normalize the expression of target genes against stable reference genes (*ActB* & *Gapdh*) and the experimental controls (naïve condition). The equations for this are as follows, where t = individual target sample concentration, r = individual reference gene sample concentration, R = mean of control genes for a specific sample, C = mean of control samples from experimental conditions normalized against

the reference genes, and F = Final normalized concentration relative to control genes and experimental samples:

***Equation* 1**: $$R = \frac{1}{n}\left(\sum_{i=1}^{n} r_i\ (of\ control\ genes)\right)$$

***Equation* 2**: $$S = \frac{t}{R}$$

***Equation* 3**: $$C = \frac{1}{n}\left(\sum_{i=1}^{n} S_i\ (of\ naive\ samples)\right)$$

***Equation* 4**: $$F = \frac{S}{C}$$

Statistics and graphing

All statistical analyses were conducted on IBM SPSS Statistics version 24. Bootstrapping was used to redistribute the mRNA and DNA methylation data over 1000 points in order to run parametric analyses on the data. DNA methylation and mRNA expression were assessed via one-way ANOVAs for condition and followed with LSD tests to compare each condition to another. Pearson correlations were run pairwise between mRNA, DNA methylation, total cocaine intake, and active lever responding during cue re-exposure for each access condition in SPSS. Self-administration and cue re-exposure behavior was assessed with Prism 6 software (Graphpad). Separate two-way repeated measures ANOVAs were used for drug intake for days 1-5 and days 6-20 (Day X Condition), and were followed by Dunnett's post hoc test to compare all behavior to either the first day of SA, or to baseline intake. Additionally, a two-way ANOVA was used to test lever responding after cue re-exposure (Lever X Condition) and broken down via Tukey's post-hoc test to compare lever pressing behavior within conditions. Lastly, all graphs were created and edited using Prism6 software.

Results

Prolonged-access to cocaine results in escalation of cocaine intake and cocaine-seeking during a cue re-exposure procedure.

Rats allowed to self-administer cocaine, but not saline, exhibited increased intake during the first 5 sessions (Figure 4.2A). A two-way repeated measures ANOVA was conducted between time and condition (saline versus cocaine) which revealed a significant interaction between time and drug ($F_{4, 180} = 3.944$, $p < 0.005$) as well as main effects of time ($F_{4, 180} = 7.193$, $p < 0.0001$) and condition ($F_{1, 45} = 18.35$, $p < 0.0001$). Dunnett's post-hoc test was used to compare each self-administration day to the first day of self-administration. While the cocaine-access rats exhibited stable responding for cocaine during the first 5 days of self-administration, saline-access rats decreased responding on days 2, 3, 4, and 5 of self-administration which is indicative of extinction training for previously administered food pellets (Figure 4.2A).

As reported in Chapter 2, when rats were given prolonged access (6 h per day) to cocaine, they steadily increased cocaine consumption in a time-dependent manner, whereas saline rats exhibit low levels of responding (Figure 4.2B). A two-way repeated measures ANOVA between time and drug revealed a significant interaction between time and drug ($F_{15,675} = 2.672$, $p < 0.0001$) as well as significant main effects of both time ($F_{15, 675} = 2.207$, $p < 0.005$) and drug ($F_{1, 45} = 368.2$, $p < 0.0001$). Dunnett's post-hoc test was used to compare each day of self-administration to a baseline (average of days 6, 7, & 8). The prolonged-access rats showed significant increases in cocaine intake from days 12 to 20 compared to baseline ($p < 0.05$, Figure 4.2B); there were no changes in saline intake across time.

When the saline and cocaine rats were re-exposed to the self-administration environment after 60 days of forced abstinence, cocaine rats, but not saline rats, exhibited pronounced drug seeking behavior (Figure 4.2C). A two-way ANOVA was performed for condition (saline vs cocaine) and lever type (inactive vs active) revealed an interaction between condition and lever type ($F_{1, 36} = 9.638$, $p < 0.005$) as well as significant main effects for both self-administration condition ($F_{1, 36} = 17.51$, $p < 0.0005$) and lever type ($F_{1, 36} = 17.69$, $p < 0.0005$). Tukey's post-hoc test was used to compare responding between both levers in saline and cocaine conditions. Responding on the active lever in the prolonged-access cocaine group was significantly higher than all of the other conditions ($p < 0.0001$, Figure 4.2C); there were no differences between any of the other conditions (Figure 4.2C). Additionally, this data indicates that although responding on the active lever is much lower than in the cocaine condition, saline rats do still respond on the active lever (29.85 ± 5.612 presses).

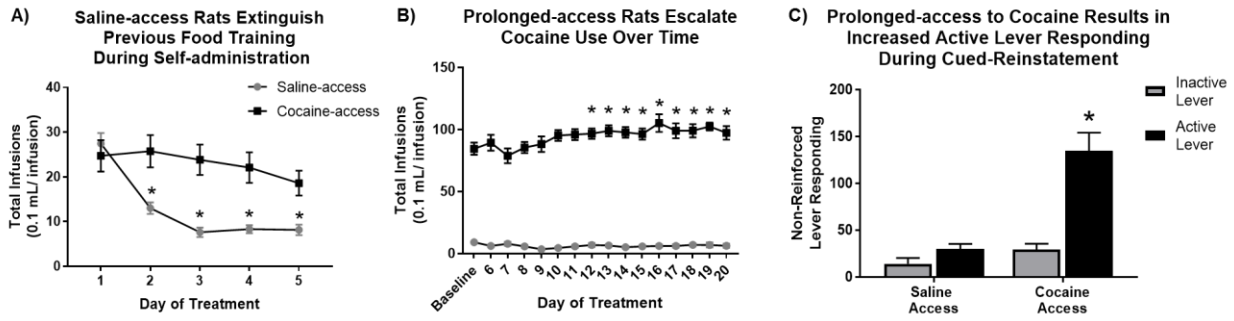

Figure 4.2: A) Saline-access rats extinguish previous food training during the first 5 days of self-administration ($p < 0.005$), whereas cocaine-access animals don't show a change in behavior during the switch from food to cocaine reinforcers **B)** Time-course of self-administration behavior during prolonged-access to cocaine. Prolonged-access cocaine rats escalated cocaine use between days 12 and 20 ($p < 0.05$). There were no observed changes in saline self-administration during the course of the experiment. **C)** Non-reinforced responding on the "inactive" and "active" levers during cued-reinstatement. Responding on the active lever during cued-reinstatement was significantly higher in the prolonged-access cocaine rats than responding on the inactive lever, or for responding in the saline-access rats on either lever ($p < 0.0001$).

Investigation of the effect of cue re-exposure at 60 days of forced abstinence on *Homer2* DNA methylation and *Homer2* mRNA expression in the dmPFC.

A One-way ANOVA was performed for condition and indicated a significant main effect for condition ($F_{4, 34} = 2.976$, $p < 0.05$). A post-hoc LSD test was performed between conditions and indicated a significant increase in levels of methylated DNA in the prolonged cocaine-access rats with cue re-exposure versus naïve control rats and the prolonged cocaine-access rats that did not receive cue-reinstatement (Figure 4.3A).

Additionally, analysis of *Homer2* mRNA expression was conducted via a one-way ANOVA with bootstrapping, where the samples were re-distributed over 1000 points to normalize the data. A significant main effect was observed ($F_{4, 65} = 2.714$, $p < 0.05$). A post-hoc LSD test was performed between conditions and a significant decrease in *Homer2* mRNA was detected in the prolonged cocaine-access rats with cue re-exposure compared to both naïve control rats and the prolonged cocaine-access rats that did not receive cue-reinstatement (Figure 4.3B).

Lastly, Pearson correlations were run between *Homer2* 5mC, *Homer2* mRNA, total cocaine intake, and active lever responding during cue re-exposure. Interestingly, *Homer2* DNA methylation was negatively correlated with total cocaine consumption in rats that were not subject to cue re-exposure [$r(7) = -0.6823$, $p < 0.05$] (Figure 4.3C). However, there were no observed correlations between methylated *Homer2* DNA, *Homer2* mRNA, and active lever responding during cued reinstatement.

These data indicate a unique mechanism in rats exposed to prolong access that have undergone 60 days of forced abstinence. First, the methylated *Homer2* DNA and *Homer2* mRNA data, indicate that as levels of *Homer2* DNA methylation went up,

Homer2 mRNA decreased, but only in animals exposed to cued reinstatement. Additionally, the correlation data suggests that before cue re-exposure, there is a negative correlation between total consumed cocaine and *Homer2* DNA methylation that disappears upon cued reinstatement procedures.

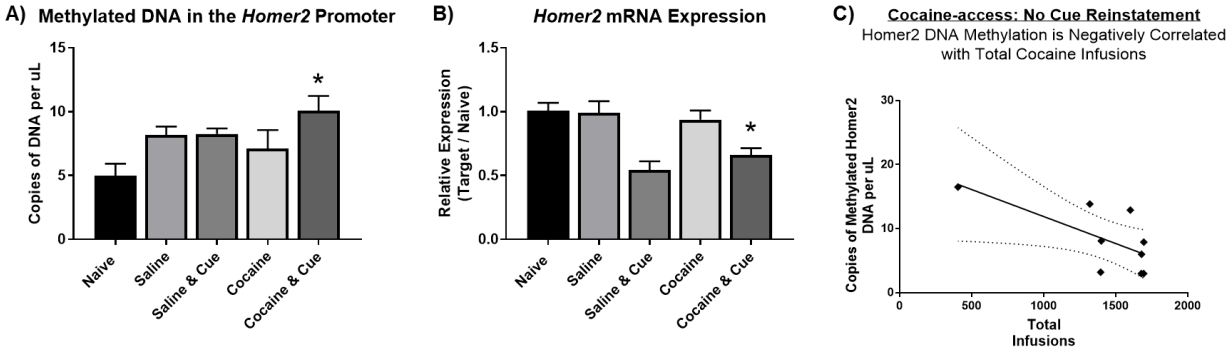

Figure 4.3: A) Methylated DNA of the *Homer2* Promoter in the dmPFC. *Homer2* DNA methylation is increased in rats given prolonged access to cocaine and cued reinstatement after 60 days of forced abstinence ($p < 0.05$) **B)** Levels of *Homer2* mRNA in the dmPFC. *Homer2* mRNA is decreased in rats given prolonged access to cocaine and cued reinstatement after 60 days of forced abstinence ($p < 0.05$) **C)** Cocaine consumption is negatively correlated with *Homer2* DNA methylation after 60 days of forced abstinence ($r = -0.6823$, $p < 0.05$).

Investigation of the effect of cue re-exposure at 60 days of forced abstinence on *Dlg4* DNA methylation and *Dlg4* mRNA expression in the dmPFC.

A One-way ANOVA was performed for *Dlg4* DNA methylation and indicated a significant main effect of condition ($F_{4, 31} = 3.536$, $p < 0.05$). A post-hoc LSD test was performed between conditions and indicated a significant increase in levels of methylated DNA in both the cocaine-access and saline-access rats that underwent cue re-exposure versus all other conditions (Figure 4.4A).

Additionally, analysis of *Dlg4* mRNA expression was conducted via a one-way ANOVA. A significant main effect of condition was observed ($F_{4, 65} = 10.527$, $p < 0.0001$). A post-hoc LSD test was performed between conditions and a significant

decrease in *Dlg4* mRNA was detected in the both cocaine-access and saline-access rats that underwent cued re-exposure compared to naïve and home-cage rats (Figure 4.4B).

Lastly, pairwise Pearson correlations were run for both *Dlg4* methylated DNA, *Dlg4* mRNA, total cocaine intake, and active lever responding during cue re-exposure. *Dlg4* mRNA was positively correlated with active lever presses in cocaine-access rats that underwent cued reinstatement procedures [$r(10) = 0.5946$, $p < 0.05$] (Figure 4.4C). However, no other correlations were detected.

These data reveal that *Dlg4* promoter methylation is increased by re-exposure to the conditioning environment in both the cocaine- and saline-access rats which correlates with reduced mRNA levels. Together this indicates the expression of this gene is modulated by cue re-exposure independent of the nature of the reinforcer as well as independent of prior extinction.

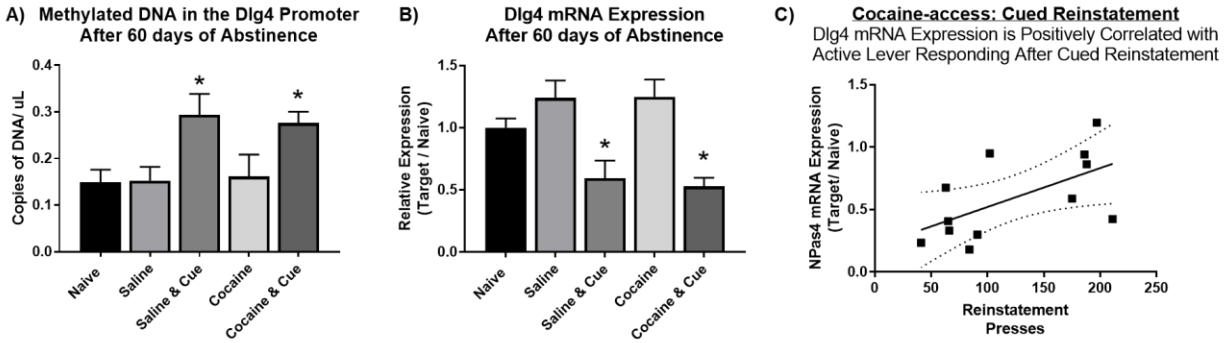

Figure 4.4: A) Methylated DNA of the *Dlg4* Promoter in the dmPFC. *Dlg4* DNA methylation is increased in both saline-access and cocaine-access rats that underwent cued-reinstatement after 60 days of forced abstinence ($p < 0.05$) **B)** Levels of *Dlg4* mRNA in the dmPFC. *Dlg4* mRNA is decreased in both saline-access and cocaine-access rats that underwent cued reinstatement after 60 days of forced abstinence ($p < 0.05$) **C)** Active lever responding during cued-reinstatement is correlated with *Dlg4* mRNA expression ONLY in cocaine-access rats that underwent cued-reinstatement after 60 days of forced abstinence ($r = 0.5946$, $p < 0.05$).

Investigation of the effect of cue re-exposure at 60 days of forced abstinence and on *Npas4* DNA methylation and *Npas4* mRNA expression in the dmPFC.

A One-way ANOVA of *Npas4* DNA methylation was performed and indicated a significant main effect for condition ($F_{4, 34} = 3.013$, $p < 0.05$). A post-hoc LSD test was performed between conditions and revealed a significant decrease in levels of methylated DNA in the saline-access rats with cue re-exposure versus all other conditions; no effect was observed in cocaine-access rats (Figure 4.5A).

Additionally, analysis of *Npas4* mRNA expression was conducted via a one-way ANOVA as well and a significant main effect of condition was observed ($F_{4, 60} = 3.998$, $p < 0.05$). A post-hoc LSD test was performed between conditions and a significant decrease in *Npas4* mRNA was detected in the saline-access rats that underwent cued re-exposure compared to home-cage rats; no effect was observed in cocaine-access rats (Figure 4.5B).

Lastly, pairwise Pearson correlations were run for both *Npas4* methylated DNA, *Npas4* mRNA, total drug intake, and active lever responding during cue re-exposure. *Npas4* mRNA was positively correlated with active lever presses in saline-access rats during cued re-exposure procedures [$r(3) = 0.9441$, $p < 0.05$] (Figure 4.5C).

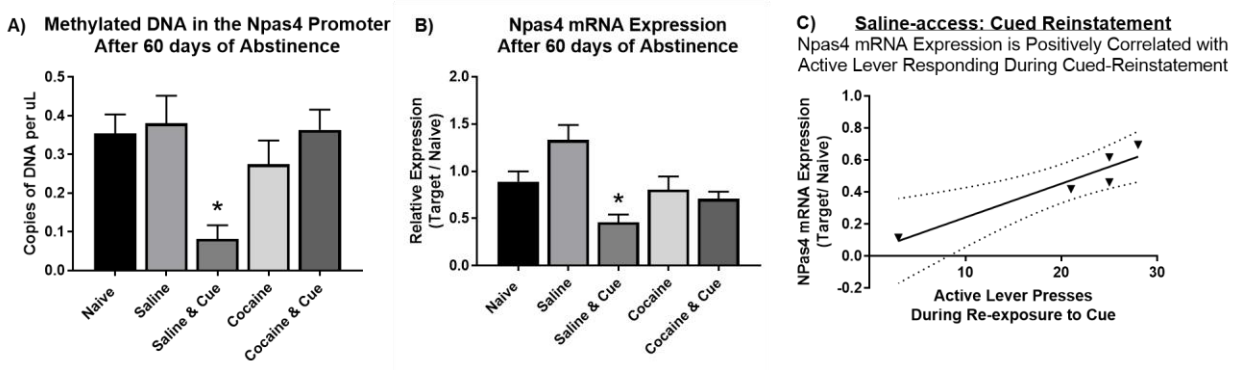

Figure 4.5: A) Methylated DNA of the *NPas4* Promoter in the dmPFC. *NPas4* DNA methylation is decreased in saline-access rats with cued-reinstatement after 60 days of forced abstinence ($p < 0.05$) **B)** Levels of *NPas4* mRNA in the dmPFC. *NPas4* mRNA is decreased in saline-access rats given that underwent cued reinstatement after 60 days of forced abstinence ($p < 0.05$) **C)** Active lever responding during cued-reinstatement is correlated with *NPas4* mRNA expression ONLY in saline-access rats that underwent cued-reinstatement after 60 days of forced abstinence ($r = 0.9441$, $p < 0.05$).

Investigation of the effect of cue re-exposure at 60 days of forced abstinence and on *Grin1* DNA methylation and *Grin1* mRNA expression in the dmPFC.

A One-way ANOVA of *Grin1* methylated DNA was performed for condition and indicated a significant main effect of condition ($F_{4, 33} = 5.914$, $p < 0.05$). A post-hoc LSD test was performed between conditions and revealed a significant decrease in levels of methylated DNA in both the cocaine-access and saline-access rats that underwent cue re-exposure as well as in home-cage saline-access rats (Figure 4.6A).

Additionally, analysis of *Grin1* mRNA expression was conducted via a one-way ANOVA, but no effect of condition was observed for *Grin1* mRNA ($F_{4, 62} = 0.776$, $p = 0.545$, Figure 4.6B). Additionally, pairwise Pearson correlations were run for *Grin1* methylated DNA, *Grin1* mRNA, total cocaine intake, and active lever responding during cue re-exposure, but no significant correlations were detected.

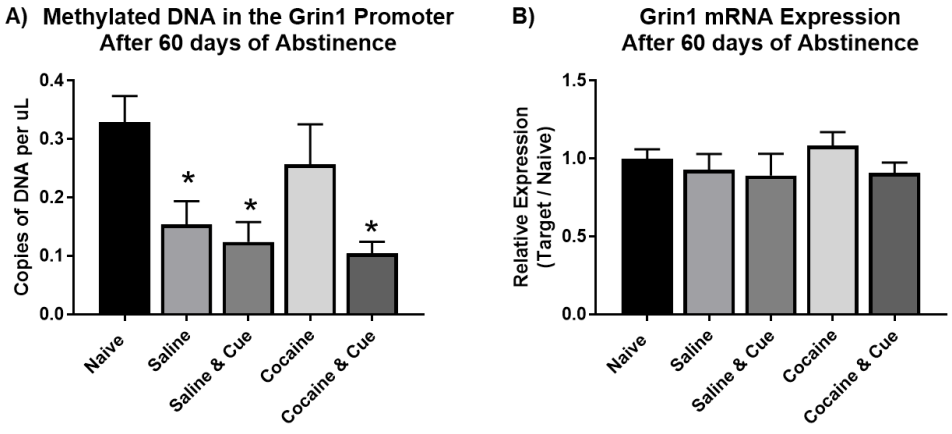

Figure 4.6: A) Methylated DNA of the *Grin1* Promoter in the dmPFC. *Grin1* DNA methylation is decreased in both saline-access and cocaine-access rats that underwent cued-reinstatement as well as saline-access rats that did not undergo cued-reinstatement testing after 60 days of forced abstinence ($p < 0.05$) **B)** Levels of *Grin1* mRNA in the dmPFC. *Grin1* mRNA is not different in any of the conditions ($p < 0.05$)

Discussion

DNA methylation is dynamic after cue re-exposure

DNA methylation has typically been associated with long-term, stable regulation of mRNA expression in differentiating cell types (Jaenisch & Bird, 2003) and in gene silencing of X-chromosomes in females (Hellman & Chess, 2007). As such, we initially hypothesized that DNA methylation would be altered throughout abstinence due to cocaine self-administration (Baker-Andresen et al., 2015; Nielsen et al., 2012), and that re-exposure to cocaine cues would elicit a transcriptional response from the DNA and affect downstream mRNA and subsequent protein expression, as has been shown previously (Ben-Shahar et al., 2013; Ben Shahar et al., 2009). However, here, we demonstrate rapid, environmentally-induced changes in DNA methylation as well as mRNA expression within the dmPFC of rats that occur after extended abstinence from cocaine self-administration and subsequent re-exposure to cocaine-associated cues. Specifically, we observed increased DNA methylation within both the Homer2 and Dlg4 promoter regions that correspond to decreased mRNA expression after rats have been re-exposed to cocaine-cues after extended abstinence (Table 4.1).

In addition to the cocaine-related changes in Homer2 and Dlg4 DNA methylation and mRNA, we observed rapid changes in both Npas4 and Grin1 DNA methylation in response to the re-exposure of cues in both saline-access and cocaine-access rats. There was evidence of decreased DNA methylation within the Npas4 gene promoter as well as decreased levels of Npas4 mRNA expression for rats re-exposed to saline-associated cues (Table 4.1). Also, we observed decreased DNA methylation within the Grin1 gene promoter for rats exposed to cocaine-cues, as well as in both groups of saline-access rats (cue and no-cue), however mRNA levels were not affected for this gene (Figure 4.6). These novel findings counter the popular idea of DNA methylation that once induced is highly stable as we are observing changes within a 2 h period following initial re-exposure to environmental cues. Further, these data qualify the notion that DNA methylation is consistently an inhibitory epigenetic mark that decreases mRNA expression, as I observed both decreased DNA methylation as well as decreased mRNA expression in rats re-exposed to saline-cues, while we observed no changes in mRNA for Grin1 even though DNA methylation was decreased in multiple conditions (Table 4.1).

Gene	Condition	MeDIP ddPCR	mRNA ddPCR
Homer2	Saline	n.s	n.s
	Saline + Cue	n.s	n.s
	Cocaine	n.s	n.s
	Cocaine + Cue	↑	↓
Dlg4	Saline	n.s	n.s
	Saline + Cue	↑	↓
	Cocaine	n.s	n.s
	Cocaine + Cue	↑	↓
Npas4	Saline	n.s	n.s
	Saline + Cue	↓	↓
	Cocaine	n.s	n.s
	Cocaine + Cue	n.s	n.s
Grin1	Saline	↓	n.s
	Saline + Cue	↓	n.s
	Cocaine	n.s	n.s
	Cocaine + Cue	↓	n.s

Table 4.1: Summary of Molecular Changes. DNA methylation corresponded to mRNA expression in rats re-exposed to cues.

Our current findings are unexpected and novel because 1) we demonstrate for the first time that DNA methylation is more transient than previously thought. Re-exposure to cocaine-relevant cues after extended abstinence results in altered levels of DNA

methylation and associated mRNA expression, and 2) we demonstrate that DNA methylation is not always inversely correlated with mRNA expression. Decreases in DNA methylation can be associated with decreases in mRNA expression (as with Npas4), as well as have no effect on mRNA (as with Grin1).

Homer2 DNA methylation is elevated and *Homer2* mRNA expression in attenuated after re-exposure to cocaine cues.

The *Homer2* gene encodes for the Homer2a/b protein, a scaffolding protein within the post-synaptic density. Due to its location Homer2 is especially implicated in the modulating receptor proteins and glutamate transmission via anchoring metabotropic glutamate receptors (mGluR1 and mGluR5) to the synapse and crosslinking mGluRs to each other (Xiao et al., 1998), as well as linking postsynaptic density-95 proteins (Kim & Sheng, 2004), NMDA, IP3, and Ryanodine receptors (Shiraishi et al., 2003; Szumlinski et al., 2008). The extensive roles that Homer2 has in regulating receptor proteins in the synaptic density has made it a prime candidate for investigation in drug addiction studies.

Here, we expand upon the *Homer2* literature and demonstrate the role of DNA methylation after prolonged abstinence from cocaine and re-exposure to cocaine cues. Re-exposure to cocaine cues (without cocaine) resulted in increased DNA methylation of the *Homer2* promoter, and decreased *Homer2* mRNA within the dmPFC (Figure 4.3A, B). These results are interesting because they imply that DNA methylation occurs dynamically during new learning paradigms. The rats were trained to administer cocaine and escalated their behavior over time, which is typically associated with increased levels of Homer2 protein in the PFC after withdrawal and cocaine injection (Gould et al., 2015).

However, in the present study, we did the opposite and trained rats to stop associating cocaine cues with cocaine (via cue re-exposure), and saw the opposite effect: cocaine mRNA was decreased after extinction training, and was likely induced by a dynamic increase in DNA methylation.

In conjunction with the *Homer2* mRNA and DNA methylation data, we observed a negative correlation between *Homer2* DNA methylation and total cocaine consumption in home cage withdrawal animals, but not within cue-exposed animals (Figure 4.3C, D). This is primarily interesting because it suggests that home cage withdrawal rats experience a reduction in DNA methylation as a function of increased cocaine intake that could facilitate *Homer2* mRNA expression upon re-exposure to cocaine or cocaine training. However, since cue re-exposed rats entered a new learning paradigm whereby cocaine cues were no longer associated with cocaine, the correlation between cocaine and DNA methylation disappeared.

Dlg4 **DNA methylation is elevated and** ***Dlg4*** **mRNA expression is attenuated after re-exposure to both cocaine and saline cues.**

The *Dlg4* gene encodes for the postsynaptic density 95 (PSD-95) protein, a crucial scaffolding protein in neuronal synapses that anchors N-methyl-D-Aspartate (NMDA) receptors to the cytoskeleton (Wang & Peng, 2016), inhibits dopamine D1 receptors (Zhang et al., 2007), co-localizes with Homer and Shank proteins (Szumlinski et al., 2008), links NMDA receptors to various messenger proteins such as Src, Pyk2, SynGAP, and nNOS, and is crucial for synapse maturation (El-Husseini et al., 2000).

In the present study, we demonstrate increased levels of *Dlg4* DNA methylation and decreased levels of *Dlg4* mRNA within the dmPFC after 60 days of forced abstinence from cocaine self-administration and re-exposure to cocaine cues (Figure 4.4A, B). These results initially appear to be opposite those of a previous report (Ghasemzadeh et al., 2011) since they demonstrated increased levels of PSD-95 in the PSD after 14 days of abstinence with extinction training (Ghasemzadeh et al., 2011). However, the experimental designs between the current study and their study are drastically different. The changes that they observed occurred after 14 full days of extinction training for 6 h per day, whereas I only provided rats with a single 2 h long session after 60 days of forced home-cage abstinence. Thus, their experimental paradigm investigated PSD-95 expression after rats have completely extinguished cocaine self-administration, whereas we show dynamic changes in *Dlg4* DNA methylation and mRNA expression that occur at the very start of extinction training and before rats are able to extinguish cocaine-seeking behavior (Figure 4.2B). Furthermore, we did observe results that support the findings in the Ghasemzadeh et al. study from 2011. We found a positive correlation between active lever presses during cue re-exposure and *Dlg4* mRNA expression (Figure 4C). These data imply higher transcription of *Dlg4* mRNA as extinction training progresses. As such, we can confirm the existence of increased *Dlg4* DNA methylation and associated reductions in *Dlg4* mRNA expression during the onset of extinction training in cocaine-access rats. Furthermore, increased extinction lever responding is associated with increased *Dlg4* mRNA expression, which is similar to the results presented by Ghasemzadeh et al. in 2011.

In addition to the changes in DNA methylation and mRNA expression for *Dlg4* in the cocaine self-administration rats, we observed a similar pattern of DNA methylation and mRNA expression within rats re-exposed to saline cues (Figures 4.4A, B). Saline is not considered to be reinforcing when administered intravenously, and as such, most animals will not readily self-administer saline solution. However, the rats in our experiment did not merely administer saline solution; they were initially food deprived and trained to respond for the active lever with food pellets on an FR1 schedule of reinforcement. Hence, each "saline-access training session" was an extinction trial for food reinforcers, and our behavioral data verifies this assertion (Figure 4.2A). Therefore, after extinction training, 60 days of forced abstinence, and cue re-exposure, the saline-access rats exhibited increased DNA methylation and decreased mRNA expression for *Dlg4*. The observed decreases in *Dlg4* mRNA are indicative of LTD within the dmPFC for cues associated with food reinforcement.

Npas4 DNA methylation and mRNA expression are reduced after re-exposure to saline cues.

Npas4, also known as *NxF*, is a gene that encodes for neuronal PAS domain protein 4, an immediate early gene (IEG) expressed exclusively in neurons and selectively activated by the depolarization of neurons (Ooe et al., 2004; Sun & Lin, 2016; Zhang et al., 2009). *Npas4* is essential for inhibitory synapse formation, balancing excitatory and inhibitory synapses (Lin et al., 2008), protection against neuronal overexcitation (Piechota et al., 2010), and cell survival (Zhang et al., 2009). It regulates these processes through activating a large suite of genes including the GABAergic genes

$GABA_A$-γ2, GAD65, and $GABA_A$-β2/3, growth factors such as BDNF, other IEGs like Arc, c-Fos, and Zif268, and by recruiting RNA polymerase II to enhancer and promoter sites in the DNA (Lin et al., 2008; Ramamoorthi et al., 2011; Spiegel et al., 2014).

In the present study I observed a significant decrease in DNA methylation and mRNA expression for saline-access rats exposed to cues, however, I did not observe any changes in *Npas4* DNA methylation or mRNA expression in either home-cage or cue re-exposed cocaine-access rats, nor in home-cage saline-access rats (Figure 4.5A, B). Additionally, there was a positive correlation between *Npas4* mRNA and active lever presses after re-exposure to cues in saline-access rats. These results are unexpected for 2 main reasons: 1) the saline-access animals were considered the "behavioral controls" allowed to self-administer inert vehicle, thus, re-exposure to saline cues was not expected to impact IEGs associated with memory consolidation and drugs of abuse, and 2) increased DNA methylation is typically associated with reduced mRNA expression (i.e. Figures 4.3 & 4.4), so mRNA would have been expected to be increased in association with decreased DNA methylation. Thus, I consider several possible explanations for these patterns of results.

First, as mentioned previously, the saline-access group is not a "pure control". Saline-access rats were exposed to food reinforcement of lever pressing prior to saline-access so each saline training trial was a food extinction trial. There is a well-established relation between extinction (i.e. re-exposure to cues and lever) of food reinforcement and increased blood corticosterone in rats (de Boer, de Beun, Slangen, & van der Gugten, 1990). Additionally, corticosterone injection or repeated stress procedures have been shown to reduce *Npas4* expression within the hippocampus and PFC (Coover, Goldman,

& Levine, 1971; Drouet et al., 2015). Therefore, it is possible that the decreased *Npas4* mRNA expression is linked to re-activation of prior established extinction memories and potentially to extinction related "stress"-like responses.

Secondly, although DNA methylation typically reduces mRNA expression, it does not "necessarily" reduce mRNA expression. There are many different types of epigenetic modifications that can influence mRNA expression, including histone modifications, inhibitory transcription factor binding the DNA promoter, and microRNA (miR). Histone acetylation tends to open up DNA for transcription, whereas histone deacetylation has the effect of coiling up DNA to prevent transcription. In 2017, Taniguchi et al. profiled the role of the enzyme histone deacetylase 5 (HDAC5) on *Npas4* expression in the nucleus accumbens. HDAC5 was shown to bind within the *Npas4* promoter region in the NAC and block gene transcription, whereas HDAC5 deletion elevated *Npas4* transcription. *Npas4* is only expressed within neuronal cells. Transcriptional inhibition of *Npas4* is mainly performed in cell populations via RE-1 silencing transcription factor (REST) (Bersten, Wright, McCarthy, & Whitelaw, 2014). The binding sequence for REST (aka RE-1) contains a CPG binding site for DNA methylation (Otto et al., 2007), and occurs in several places along the gene promoter (up to 2500 bp upstream of TSS) and within the gene body for *Npas4*, including the area that we assessed for DNA methylation with ddPCR (e.g. Table 1 & Bersten et al., 2014). Although I didn't assess REST binding in the current study, it is highly likely that the demethylation observed after re-exposure to cues associated with the saline-access condition (including prior food reinforcement) allowed for REST to bind to the *Npas4* promoter 285-396 bp upstream from TSS and attenuate *Npas4* mRNA transcription.

Lastly, microRNAs are short bits of non-coding RNA that bind the mRNA and inhibit them from reaching the rough ER/ ribosomes or tag it for degradation. MiR-224 and miR-203 have been previously been implicated in silencing *Npas4* transcription. Additionally, miR-224 is expressed concurrently with the $GABA_A$ receptor (they are both encoded on the GABRE gene), which is initiated with *Npas4* protein activation (Bersten et al., 2014). Therefore, it is possible that the stress associated with "saline" cue re-exposure and subsequent synaptic activity initiates a downstream genetic program that 1) activates HDAC5 to inhibit *Npas4* transcription, 2) demethylates the RE-1 site, allowing REST to bind and decrease *Npas4* mRNA transcription, or 3) initiates transcription of $GABA_A$ receptors and miR-224 via *Npas4* protein activation to negatively inhibit further *Npas4* mRNA expression and protein translation.

It is clear that more research needs to be done concerning *Npas4* in learning, memory, and addiction. Future experiments should simplify training parameters for control groups, and cocaine self-administration studies should investigate levels of *Npas4* after context-specific learning occurs such as after drug-primed reinstatement after abstinence. *Npas4* has relative unique IEG properties as it responds to depolarization but upregulates inhibitory elements of the synapse as well as is overexpressed in response to repeated, but not acute, psychostimulant administration. The upregulation of inhibitory synapses could potentially underlie observed changes in excitatory/inhibitory balance in cortical activity during drug abstinence.

***Grin1* DNA methylation is reduced in rats re-exposed to cocaine cues and in saline-access rats regardless of re-exposure to saline cues but there is no apparent relation to gene expression.**

The final gene that I investigated, *Grin1* (aka *NMDAR1*), encodes for the NMDA receptor 1 subunit (NR1) of the NMDA receptor. There are 3 families of genes that encode for NMDA receptor subunits. NR1 only has one gene, 8 splice variants, and is obligatory for NMDA receptor expression, while NR2 and NR3 are non-obligatory, have multiple genes encoding for various subunits and splice variants, and are distributed differentially based on a variety of factors. Lastly, NR3 has two genes encoding for two separate subunits, and negatively regulate the NMDA receptor complex (Bai & Hoffman, 2009). NMDA receptors are crucial glutamate receptors and have been investigated extensively in many different fields including learning and memory, synaptic development, LTP, LTD, mood disorders, neurodegenerative disorders, and addiction (for a full review, refer to Sweatt, 2016). For instance, patients that have died from cocaine overdose exhibited increased levels of mRNA and protein for NR1, CREB, GluR2, GluR5, and KA2 within the ventral tegmental area (VTA) (Tang et al., 2003), as well as increased NR1 and GluR2/3 protein within the NAC (Hemby, Tang, et al., 2005). Additionally, animal studies have reported a variety of changes in NMDA receptor levels after chronic cocaine injection (Ghasemzadeh, Mueller, et al., 2009; Ghasemzadeh, Vasudevan, & Mueller, 2009), self-administration (Hemby, Tang, et al., 2005), and extinction from cocaine cues (Ghasemzadeh et al., 2011).

In the present report, I observed no changes in *Grin1* mRNA within the dmPFC after 60 days of forced abstinence (Figure 4.6B). However, I did observe decreased levels of *Grin1* DNA methylation after 60 days of abstinence within saline home-cage, saline cue re-exposed, and cocaine re-exposed rats (Figure 4.6A). These results are striking because there was differential DNA methylation between the two cocaine-access groups,

but not between saline-access groups. As mentioned previously, both saline-access conditions were technically food-extinction conditions, and our results indicate zero difference between these two conditions. However, it is important to note that "food-extinction" resulted in decreased methylation within the *Grin1* promoter.

The demethylation of the *Grin1* promoter in cue re-exposed cocaine-access rats demonstrates another example of a context-specific DNA methylation. Since 5mC levels for home-cage cocaine-access rats did not differ from naïve controls, we can infer that exposure to cocaine cues without cocaine elicited the demethylation observed. This conclusion is supported by evidence from fear conditioning and fear extinction experiments. In 2014, Li et al. demonstrated that rats undergoing extinction training from conditioned foot shock exhibited increased decreased levels of DNA methylation (5mC), but increased levels of DNA hydroxymethylation (5hmC) in the *gephyrin* gene, as well as increased levels of Tet3 mRNA within the PFC. Furthermore, Tet3 shRNA disrupted fear extinction memory, lowered 5hmC, and reduced 5mC levels within the PFC (Li, Wei, et al., 2014). Although we didn't observe changes in mRNA for *Grin1*, it is possible that re-exposure to cocaine cues as well as food cues initiated a transcriptional program that opened up the gene for potential activation, however, without the associated food or cocaine stimuli, *Grin1* mRNA transcription did not occur as seen in animals presented with cocaine (Hemby, Tang, et al., 2005).

Conclusions

In conclusion, we demonstrate a novel role for DNA methylation in cocaine addiction. We identified rapid, context-specific DNA methylation patterns in addiction-relevant genes that occur in response to re-exposure to cocaine cues. Additionally, we

have provided evidence to show that changes in DNA methylation serve to upregulate, downregulate, or even have no effect on levels of mRNA, and the role of DNA methylation is location and context specific.

Chapter 5:

Prolonged-Access to Cocaine Induces Distinct *Homer2* DNA Methylation, Hydroxymethylation, and Transcriptional Profiles in the Dorsomedial Prefrontal Cortex of Sprague-Dawley Rats

Abstract

DNA methylation is a key determinant of gene expression and is implicated in neuroplasticity and memory; however, the extent to which this epigenetic modification is involved in neuroplasticity associated with drug addiction has not been fully elucidated. Here I examined the relationship between DNA methylation and gene expression within the dorsal medial prefrontal cortex (dmPFC) following limited cocaine self-administration (1h/ day), prolonged cocaine self-administration (6h/ day), and saline self-administration (1h/ day). Rats were fitted with intravenous catheters and allowed to lever press for saline or cocaine (0.25 mg/ kg/ infusion) in the different access conditions for 20 days. Prolonged-access rats exhibited escalation in cocaine intake over the course of training, while limited-access rats did not escalate cocaine intake. Additionally, limited-access and prolonged-

access rats exhibited unique *Homer2* epigenetic profiles and mRNA expression. In prolonged-access rats, *Homer2* mRNA levels in the dmPFC were increased, which was accompanied by decreased DNA methylation, increased DNA hydroxymethylation, and decreased p300 binding within the *Homer2* promoter; limited-access animals exhibited decreased DNA methylation, decreased DNA hydroxymethylation, and increased p300 binding within the *Homer2* promoter. These data indicate that distinct epigenetic profiles are induced by limited- versus prolonged-access self-administration conditions that contribute to transcriptional profiles and lend support to the notion that covalent modification of DNA is critically involved in addiction-like changes in cocaine-seeking behavior.

Introduction

Cocaine addiction is a chronic disorder involving periods of cocaine binging that escalate over time (Dackis & O'Brien, 2001). Similar to the observation that increased drug use leads to more severe consequences in human drug addicts, rats allowed prolonged-access to cocaine exhibit distinct behavioral profiles from rats allowed more limited-access. For instance, prolonged (6h/day), but not limited (1h/day), daily access to cocaine leads to an escalation (i.e. a progressive increase) of cocaine-taking across days (Ahmed & Koob, 1998). Additionally, rats subjected to prolonged-access to cocaine

exhibit greater increases the motivation for rats to seek cocaine during progressive ratio tests (Paterson & Markou, 2003) increased cue-induced and cocaine-primed reinstatement of cocaine-seeking following extinction (Kippin et al., 2006), and resistance to punishment-induced suppression of cocaine-taking (Ducret et al., 2016). Lastly, prolonged-access, but not limited-access, induces an enduring reduction in brain reward function as evidenced by a progressive and persistent increase in the brain self-stimulation reward thresholds (Ahmed, Kenny, Koob, & Markou, 2002). These various features indicate that prolonged daily access to cocaine induces a more "addiction-like" phenotype compared to more restricted access (Ahmed, 2012; Edwards & Koob, 2013).

The role of the prefrontal cortex in addiction.

The prefrontal cortex is widely implicated in several aspects of the addiction process and is specifically linked to neurobiological changes produced by prolonged-access to cocaine. For instance, extensive neuroimaging and neuropsychological evidence from clinical studies indicate that chronic drug abusers show deficits in PFC-dependent cognitive function (Bolla et al., 2004) with similar deficits observed in PFC function following prolonged access to cocaine in animal models (George et al., 2008). The mPFC has also been implicated in facilitating cocaine reinforcement the mPFC is widely implicated in reinstatement of cocaine-seeking behavior (reviewed in e.g. Kalivas et al., 2005) and mPFC lesions increase enhance acquisition of cocaine reinforcement as well as responding under a second-order schedule of cocaine reinforcement (Weissenborn et al., 1996). Such PFC alterations also contribute to impairment of executive control over behavior, including behavioral inhibition processes, which may contribute to addiction by the transition from voluntary to habitual responding (Ito et al., 2004).

Within the mPFC, the dorsal regions (comprised of the anterior cingulate cortex and prelimbic cortex; here after dmPFC) are implicated in aspects of the addiction-like alterations associated with prolonged access to cocaine with emerging data supporting a role for altered glutamate signaling being linked to escalated drug taking. The dmPFC is critical for the sensitization of cocaine reinforcement (as measured by increased breakpoints under a progressive ratio schedule of reinforcement) that occurs during prolonged access to cocaine in rats (Smith et al., 2008). Basal glutamate levels in the mPFC were diminished after 17 days of prolonged access to cocaine, but were not changed in limited access rats (Ben-Shahar et al., 2012). Prolonged-access, but not limited-access, increases expression of several glutamate-related signaling molecules within the dmPFC (Ploense et al, in press) and increases Homer 1b/c, GluNR2b and GluNR2a protein in a time-dependent fashion (Ben Shahar et al., 2009). Interestingly, the increase in GluNR2b persists for up to 30 days following prolonged-access to cocaine and inhibition of this receptor subunit decreases the elevated cocaine-seeking during extinction conditions produced by prolonged-access to cocaine (Szumlinski et al., 2016). Recent developments in the understanding of the regulation of gene expression suggest that epigenetic mechanisms are involved in the neural plasticity induced by exposure to cocaine (Freeman et al., 2010), and provide a likely candidate for the long-term maintenance of cocaine-induced alterations in gene expression within the prefrontal cortex.

DNA methylation and hydroxymethylation affect gene expression

Epigenetic mechanisms involve a number of substrates, including modifications directly on DNA such as addition of methyl or hydroxymethyl groups. In mammals,

DNA methylation is primarily produced by the addition of a methyl group (-CH_3) to cytosine (Sweatt et al., 2013). DNA methylation at transcription factor binding sites often interferes with the association of a transcription factor to the DNA, which can in turn repress or activate gene expression depending on whether the sequence is recognized by a transcriptional repressor or activator (Sweatt et al., 2013). DNA hydroxymethylation (5hmC) is another covalent modification that is highly prevalent in the mammalian brain—reaching 10-20% of total 5mC levels, and is especially prevalent in neurons (up to 40% of 5mC levels)—and has distinctive interactions with transcription factors (Globisch et al., 2010). The mechanistic relation between these two DNA modification has recently been elucidated and, briefly, ten-eleven translocation proteins 1 and 3 (Tet1 and Tet3 respectively) serve to oxidize methylated DNA into 5hmC (Ito et al., 2010), and are expressed at high levels in the PFC (Szwagierczak et al., 2010). Functionally, 5mc and 5hmc are distinct with the transition to an open chromatin state associated with the conversion of 5mC to 5hmC (Wu & Zhang, 2011). Recent studies have demonstrated global and site-specific alterations in DNA methylation in the PFC after experimenter-administered cocaine (Pol Bodetto et al., 2013; Tian et al., 2012) and methamphetamine (Numachi et al., 2004) as well as following cocaine self-administration (Baker-Andresen et al., 2015; Massart et al., 2015; Nielsen et al., 2012; Wright et al., 2015). However, no studies have examined the impact of cocaine on 5hmC or the specific impact of prolonged access to cocaine self-administration on DNA methylation—either 5mC or 5hmC—in the PFC.

Accordingly, the present study sought to determine changes in DNA methylation and the subsequent mRNA expression of the glutamate receptor scaffolding gene Homer2

within the dmPFC following the escalation of cocaine intake in a prolonged access model of addiction. Specifically, I examined 5mC, 5hmC, and binding of the transcriptional corepressor/modulator, p300, in the dmPFC following saline, limited-access cocaine, and prolonged-access cocaine self-administration in order to assess if each condition could induce a unique epigenetic state associated with differential gene expression.

Methods

Subjects

Male Sprague-Dawley rats (250-300 g) were pair-housed in a reverse light:dark (12h:12h, lights off at 8 am) cycle room with ad-libitum access to food and water. The housing and care of the rats followed the guidelines set forth by the "Guide for the Care and Use of Laboratory Rats, 8th Edition" (IACUC, 2011).

Surgery

Rats were deeply anesthetized using 60 mg/kg ketamine and 10 mg/kg of xylazine intramuscularly and implanted with chronic indwelling catheters constructed with a bent steel cannula with a screw-type connector (Plastics One, Roanoke, VA), SILASTIC tubing (11 cm, i.d. 0.64 mm, o.d. 1.19 mm, Dow Corning, Midland, MI), Prolite polypropylene monofilament mesh (Atrium Medical Corporation, Hudson, NH), methyl methacrylate cement, and a silicon ball 2.5 cm from the end used to secure the catheter to the vein. The catheters were then maintained as I have reported previously and tested once weekly with 2.0 mg of Methohexital Sodium (Brockton, MA).

Behavioral training

Food training and cocaine self-administration utilized standard operant chambers (Med Associates Inc., St. Albans, VT, USA) during the dark cycle at a controlled time each day. Before surgical implantation of the jugular catheters, the rats were restricted to 20 g of food daily and trained on a fixed ratio 1 (FR1) schedule of food reinforcement for two 16 h training sessions where each right lever press was associated with the delivery of a 45 mg food pellet. After recovery from the surgery, the rats were placed on a fixed ratio 1 (FR1) with a 20 second time-out signaled by the activation of the cue light above the lever. Initial sessions lasted 1 h per day for the first 5 days with either cocaine or saline infusions available as reinforcers. Each "active" lever depression (right lever) was associated with a 4 s infusion of vehicle (0.1 mL saline) or cocaine (0.25 mg/ 0.1 mL); "inactive" lever depression (left lever) resulted in no infusion or stimulus. On the 6^{th} day, the rats receiving cocaine were divided into groups that continued to self-administer cocaine under the same schedule of reinforcement during either limited (1 h) or prolonged (6 h) access sessions for an additional 15 days, and rats self-administering saline continued under 1 h access for the same amount of days.

Tissue collection and preparation

Twenty-four hours after the last self-administration session, rats were sacrificed via rapid decapitation and their brains were dissected over ice into 1 mm sections with a metal brain mold (Braintree Scientific, Braintree, MA). The dorsal medial prefrontal cortex (dmPFC) was dissected out at 3.20 to 2.20 mm anterior to Bregma (Figure 2.1), placed in a 0.5 mL microcentrifuge tube, frozen on dry ice, and stored at -80 °C. For molecular analyses, the frozen dmPFC was added to 600 µL of buffer RLT (Qiagen, 79216) and homogenized with the Qiagen TissueRuptor for 30 s. DNA and mRNA were

then extracted by the AllPrep DNA/RNA/protein extraction kit (Qiagen, 80004#) in accordance to the manufacturer's protocol. RNA was eluted from the spin column with 50 µL of nuclease-free water. DNA was eluted from the spin column with 100 uL of 8 mM NaOH heated to 70 °C, and the pH was then neutralized with 12 µL of 0.1 M HEPES and 1.1 µL of 100 mM EDTA.

Gene expression analyses

RNA (500 ng) from each sample was reverse transcribed according to the manufacturer's protocol (Qiagen, 205311), except that samples were incubated with genomic DNA wipeout buffer in an Eppendorf MasterCycler at 42 °C for 18 minutes, then incubated at 95 °C for 3 min to inactivate the reverse transcriptase. A reverse transcriptase-negative reaction was carried out in parallel with the samples from 500 ng of pooled sample RNA. In preliminary analyses, 6 reference genes were run against *Homer2* to assess stability. The 3 most stable reference genes, *Gapdh*, *Actβ*, and *Tubb3* were used as controls for normalization (Table 5.1). Levels of cDNA for *Homer 2* (Table 5.1) were assessed in triplicate using quantitative real time pcr (qRT-PCR) on the BioRad CFX96 Touch Real-Time system (Biorad, Richmond, CA). The reaction mixtures were optimized for each gene and are located in Table 1. Negative controls consisted of a Sybrgreen and primer-only sample and a reverse-transcriptase free sample. Standard curves were run on each pcr plate with 3x serial dilutions ranging from 50.0 ng/uL to 1.85 ng/uL. The data were normalized using the equations outlined by Hellemans et al. in 2007. (Hellemans et al., 2007).

Target	Accession Number	Forward (5'->3')	Reverse (5'->3')	Amplicon Length (bp)	Annealing Temp	Primer Concentration
HOMER2 mRNA	NM_053309	GAGTGCTGCCAATGTGAAGA	TTGATCTCACCGCACTGTTC	195	61C	30nM
GAPDH mRNA	NM_017008.4	AGAACATCATCCCTGCATCC	AGGAGACAACCTGGTCCTCA	240	61C	20nM
βACTIN mRNA	NM_031144	TGTCACCAACTGGGACGATA	GGGGTGTTGAAGGTCTCAAA	165	63C	20nM
TUBB5 mRNA	NM_139254	TGAGGCCTCCTCTCACAAGT	TGCAGGCAGTCACAATTCTC	237	62C	20nM
HOMER2 Promoter DNA	NC_005100.3	GGCTGCTCCTTTGTCTGACT	GGGCGGATTAGAAGTCCAGG	166	60C	5nM

Table 5.1: Primer concentrations, size, and run conditions for qPCR experiments. Primers were created using Primer3 software and verified with PrimerBLAST and a 2% agarose gel.

MeDip and hMeDIP-qPCR

Extracted DNA (1 µg) was fragmented to 200-600 bp fragments by Covaris S2 system under the following conditions: duty cycle = 5%, intensity = 3, cycles/burst = 200, time = 45 s, cycles = 6, temperature = 7 ºC. The size of the DNA fragments were confirmed through the BioRad Experion 1K DNA chip. To isolate 5mC or 5hmC, the fragmented DNA then underwent methylated DNA immunoprecipitation (MeDIP) with a 5-methylcytosine antibody or a 5-hydroxymethylcytosine antibody and protein G magnetic beads according to the manufacturer's protocol (Active Motif, 55009 & 55010) and then subjected to qPCR with the BioRad CFX96 thermocycler (Table 5.1). The normalized values were calculated by ΔΔCT with input controls as a reference and the average value of saline controls for normalization.

Chromatin immunoprecipitation (ChIP)

Frozen dmPFC punches were added to 550 uL of ice-cold phosphate buffer solution in water (PBS) in a glass douncer, and "dounced" until the tissue was fully homogenized, then moved to a 2.0 mL MCT over ice. Separate samples of yeast were simultaneously resuspended in 500 µL of ice-cold PBS. Then, 31.25 µL of 16%

formaldehyde in methanol (Thermoscientific, NC9658705) was added to each experimental and yeast sample and incubated at RT with end to end rotation for 5 min. 25 µL of 2.5 M glycine was added to each tube and incubated at RT with end to end rotation for 10 min to stop the reaction. Samples were centrifuged at maximum speed for 1 min at 4 °C. Each sample was subsequently washed twice with 500 µL of ice-cold PBS and centrifuged at maximum speed for 1 min at 4 °C. After washing, 500 µL of SDS lysis buffer (5 mL SDS, 1 mL 0.5 M EDTA, 2.5 mL 1 M Tris HCl pH 8.0, 41.5 mL water) with protease inhibitor cocktail was added to experimental samples and yeast samples, and both were incubated over ice for 10 min. Each yeast sample (500 µL) was added to the experimental samples followed by 10 times of pipetting. Each sample was fragmented down to 500 bp by Covaris M220 system (75 W peak intensity power; 200 cycle per burst; 25 min; in a 6 ºC water bath). The ChIP experiment was performed with an antibody against p300 and used magnetic protein dynabeads following the manufactor's protocol (Life technology, 10004D).

Statistics and Graphing

All statistics were completed using Prism 6 statistical software. Cocaine intake, active presses, and inactive presses were assessed via separate two-way repeated measures ANOVAs followed by Dunnett's post-hoc tests to compare responding to baseline behavior. qPCR results were analyzed with one-way ANOVAs followed by Dunnett's post-hoc tests to compare limited and prolonged-access conditions to saline controls.

Results

Cocaine self-administration behavior escalates in prolonged, but not limited, access rats

Rats in the prolonged-access condition exhibited an increase in cocaine consumption over time, whereas limited-access rats maintained steady cocaine consumption (Figure 5.1A), replicating previous reports (Ben-Shahar et al., 2004; Ben-Shahar et al., 2012). A two-way ANOVA on total cocaine infusions during differential access by day yielded significant main effects of time ($F_{14, 644} = 7.058$, $p<0.0001$), treatment ($F_{2, 46} = 359$, $P<0.0001$), and a time x treatment interaction ($F_{28, 644} = 4.996$, $p<0.0001$). Dunnett's post-hoc analysis of time revealed an increase in cocaine consumption from day 10 until day 20 in the prolonged-access condition, but no increases were observed in the limited-access or saline-access conditions (Figure 5.1A).

Rats allowed prolonged access to cocaine exhibited an increase in active lever pressing over time (Figure 5.1B), but not inactive lever pressing (Figure 5.1C). A two-way ANOVA on total active lever depressions by day during differential access yielded significant main effects of time ($F_{14, 630} = 1.861$, $p<0.05$), treatment ($F_{2, 45} = 161.7$, $p<0.0001$), and a time x treatment interaction ($F_{28,630} = 2.171$, $p<0.001$). Dunnett's post-hoc analysis of time revealed an increase in active lever depressions in on days 13, 15, and 18 in the prolonged access condition, but there were no observed increases in the limited access or saline access conditions (Figure 5.1B). For inactive lever pressing, all comparisons failed to reach statistical significance (all $F < 1.0$, all $p > 0.10$; Figure 5.1C).

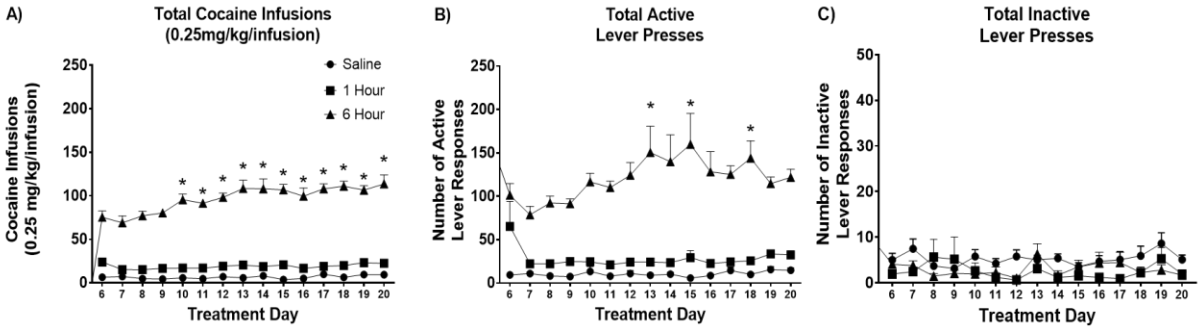

Figure 5.1: Cocaine seeking and taking escalate during prolonged access. **A)** Prolonged-access (6h) rats exhibited an escalation in cocaine consumption from day 10 to day 20 of differential cocaine access (* $p < 0.05$). **B)** Prolonged-access rats exhibited an escalation in active lever responding during differential cocaine access on day 15 of the experiment (* $p < 0.05$) **C)** Neither saline, limited-access, or prolonged-access rats exhibited increases in inactive lever responding at any point during the experiment.

Prolonged-access cocaine self-administration elevates the expression and induces a unique epigenetic state on the promoter of *Homer2*.

Given prior studies implicating Homer2 proteins in addiction neurobiology, expression levels of this gene were determined at 1 day following the last self-administration session, which revealed elevated mRNA following prolonged-access to cocaine relative to all other groups (Figure 5.2A). A one-way ANOVA of *Homer2* mRNA expression resulted in a significant main effect of treatment ($F_{2,39}=4.066$, $p<0.05$). A post-hoc Tukey's analysis revealed greater mRNA in the prolonged-access rats relative to the control (saline) rats ($p < 0.05$), however, mRNA did not differ between limited-access and controls ($p > 0.05$), thus, *homer2* expression is only elevated by prolonged access to cocaine.

Next, I sought to determine the underlying epigenetic basis of the impact of prolonged-access to cocaine on *Homer2* expression. First, I examined 5mC and 5hMC surrounding the *Homer2* promoter using MeDIP preparations followed by qPCR. For 5mC, there was a significant main effect of treatment ($F_{2, 14}=9.102$, $p < 0.005$) and

Tukey's posthoc analysis revealed lower DNA methylation in both the limited and prolonged access conditions relative to saline controls (ps < 0.05, Figure 5.2B). Conversely, for 5hmC, there was a significant main effect of treatment ($F_{2, 11}$=4.834, p < 0.05), however, Tukey's posthoc analysis revealed decreased 5hmC the limited-access compared to the prolonged-access conditions (p < 0.05, Figure 5.2C).

Lastly, given the role of the histone acetyltransferase p300 as a transcriptional corepressor, I examined its binding to the *Homer2* promoter following self-administration employing ChIP preparations followed by qPCR. One-way ANOVA revealed a significant main effect of treatment on p300 occupancy at the *Homer2* promoter ($F_{2,15}$=4.110, p < 0.05) and Tukey's posthoc analysis revealed increased p300 occupancy in limited access rats relative to both saline control and prolonged access rats (p < 0.05, Figure 5.2D). These data demonstrate a unique epigenetic profile for each condition, which sets a framework for understanding the differential effect of limited and prolonged access to cocaine on gene expression in the dmPFC.

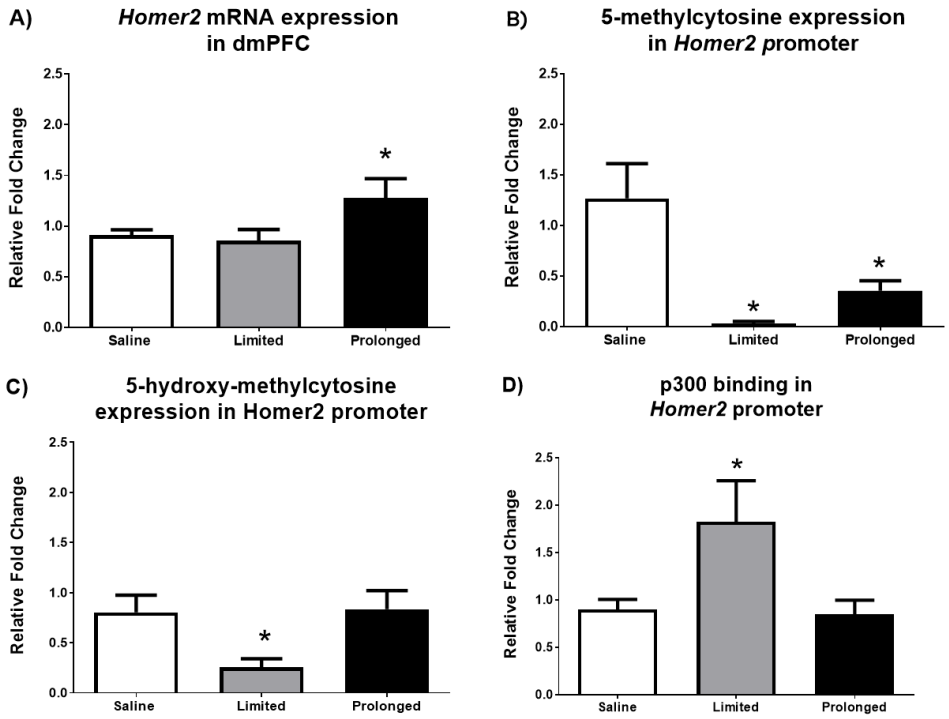

Figure 5.2: Impact of cocaine intake on *Homer2* transcriptional and epigenetic states. A) *homer2* relative expression in the dmPFC. *homer2* mRNA levels were elevated in the 6h access condition relative to controls (* $p<0.05$). B) DNA methylation within the *Homer2* promoter was reduced in both the 1h and 6h conditions relative to saline controls (* $p<0.05$). C) DNA hydroxymethylation within the *Homer2* promoter was reduced in 1h relative to 6h rats (* $p<0.05$). D) ChIP for the p300 transcription factor revealed increased p300 transcription factor binding in 1h relative to 6h and saline rats (* $p<0.05$).

Discussion

The PFC is a structure of major interest in addiction because addicts exhibit both functional abnormalities in this area in imaging studies, as well as, deficits in PFC-dependent tasks which are reproduced in animal models involving high cocaine intake (George & Koob, 2010). Further, preclinical data indicate that the dmPFC plays a critical role in both the escalation of cocaine intake (Smith et al., 2008) and elevated cocaine-seeking during extinction (Szumlinski et al., 2016). The long-term nature of these behavioral changes led us to hypothesize that changes in dmPFC function are mediated

by alterations in the epigenetic machinery which regulates gene expression. Here, I report that prolonged-access to cocaine induces a distinct epigenetic state at the *Homer2* promoter, which is associated with elevation of *Homer2* mRNA whereas limited-access induces a different epigenetic state which is not associated with altered mRNA expression.

Homer2, a scaffolding protein linking metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (aka AMPA and NMDA) within the PFC has been implicated in addictive behaviors (Ben-Shahar et al., 2009; Kalivas et al., 2003). Increased expression of Homer2a/b protein within the mPFC has been observed during abstinence in rats allowed daily prolonged (6h)-access to cocaine (Ben-Shahar et al., 2009). Moreover, *Homer2b* mRNA overexpression in the mPFC has been shown to increase basal glutamate levels and conditioned place preference behavior in mice, while *Homer2b* mRNA knockdown resulted in reduced basal glutamate within the dmPFC (Ary et al., 2013). Thus, in the present report, I performed a detailed investigation of the impact of cocaine self-administration on epigenetic changes within the *Homer2* gene promoter and assessed the levels of mRNA expression associated with the associated gene's state. Here, it is demonstrated that a unique epigenetic and transcriptional pattern in the dmPFC of rats exhibiting escalation of cocaine intake following prolonged-access to cocaine (Figure 5.1). Specifically, the dmPFC from the prolonged-access condition expressed increased *Homer2* mRNA (Figure 5.2A) and reduced 5mC, whereas the limited-access condition expressed basal mRNA (as defined by the saline control), reduced 5mC, and reduced 5hmC, but increased p300 binding in the *Homer2* promoter (Figure 5.2). Functionally, there is extensive evidence supporting the notion that DNA

methylation hinders binding of transcription factor binding to the DNA promoter (Sweatt et al., 2013), thus, the low levels of 5mC in the prolonged-access relative to saline conditions (Figure 5.2B) is a likely mediator of increase in *Homer2* mRNA in the prolonged access conditions (Figure 5.2A).

The observation that the relations between 5hmC but not 5mC is distinctive between limited- and prolonged-access conditions has multiple potential underpinnings. This pattern suggests that cocaine intake produces an initial de-methylation of the *Homer2* promoter (likely mediated by Tet enzymes), as it was observed in both prolonged- and limited-access conditions (Figure 5.2B). However, cocaine intake in the limited-access condition is further associated with lower levels of DNA methylation than in the prolonged-access, as well as a decrease of 5hmC within the *Homer2* promoter, which is indicative of greater Tet1 or Tet3 function in the limited-access rats than in the prolonged-access rats (Figure 5.2C). Only one study has investigated the role of Tet enzymes in cocaine addiction, but it showed repeated cocaine treatment reduced Tet1 activity in the NAC, and Tet1 knockdown within the NAC increased cocaine conditioned place preference (CPP) (Feng et al., 2015). From this, we can speculate that prolonged-access rats (having had more cocaine access) may have reduced Tet1 function in the PFC as well, so limited-access rats appear to have "enhanced" Tet-activity only in comparison to prolonged-access rats, but further investigation needs to be performed to elucidate the true nature of Tet enzymes after cocaine self-administration.

In addition to differences in 5mC and 5hmC distribution, limited-access rats are distinct from prolonged-access rats with regards to p300 binding of the *Homer2* promoter; p300 binding to the *Homer2* promoter is elevated in the limited-access

condition relative to saline and prolonged-access conditions (Figure 5.2D). This can be partially explained by differences in 5hmC levels within the *Homer2* promoter, as 5hmC can affect gene transcription through binding of CFP1 and $H3K4^{me3}$ (Wu & Zhang, 2011). Further, although p300/CBP associated factor (PCAF) can be a transcriptional coactivator (Wei et al., 2012), it has recently been shown to function as a transcriptional corepressor as well (Yoshimochi, Daitoku, & Fukamizu, 2010). p300 and creb binding protein (CBP) form a complex, PCAF, to recruit the transcriptional corepressor ATF4 to the promoter of the immediate early gene zif268 (Wei et al., 2012). This mechanism provides a second explanation for the difference in transcriptional states of limited- versus prolonged-access conditions.

In contrast to changes observed in the prolonged-access condition, the limited-access to cocaine condition had the same level of mRNA as saline controls, but exhibited reduced 5mC, and reduced 5hmC, and increased p300 binding (Figure 5.2) in the *Homer2* promoter. In the limited-access condition the lack of increased *Homer2* mRNA expression, even though 5mC was reduced, appears to be the consequence of concurrent decreased 5hmC, which may in turn have reduced the recruitment of ZFPs containing CFP1 within the promoter, and in turn increased binding of the repressive transcription factor, p300 (see Figure 5.3).

Given that Homer2 protein serves to scaffold mGluRs (as well as other proteins), the effects on DNA methylation observed here may form the basis for alterations in many proteins associated with excitatory receptor complexes over the course of abstinence. Homer2a/b protein levels in the mPFC are reported to increase following prolonged-access but are only become evident at 14 days of abstinence (Ben-Shahar et al., 2009)

indicating that the change in epigenetic markings on the promoter and increased mRNA of *Homer2* mRNA precede a progressive accumulation of Homer2 protein that emerges during protracted abstinence. Additionally, mGluR1 and mGluR5 proteins were elevated in the dmPFC during cocaine-reinforced responding at 3 days of abstinence (Ben-Shahar et al., 2013) as well as altered ionotropic receptor currents induced by the presentation of cocaine-relevant cues (Gipson et al., 2013). The present findings of distinct epigenetic profiles and increased *Homer2* mRNA produced by prolonged-access to cocaine may underlie previously reported deficits in the neurocircuitry of the PFC (Kalivas, 2008).

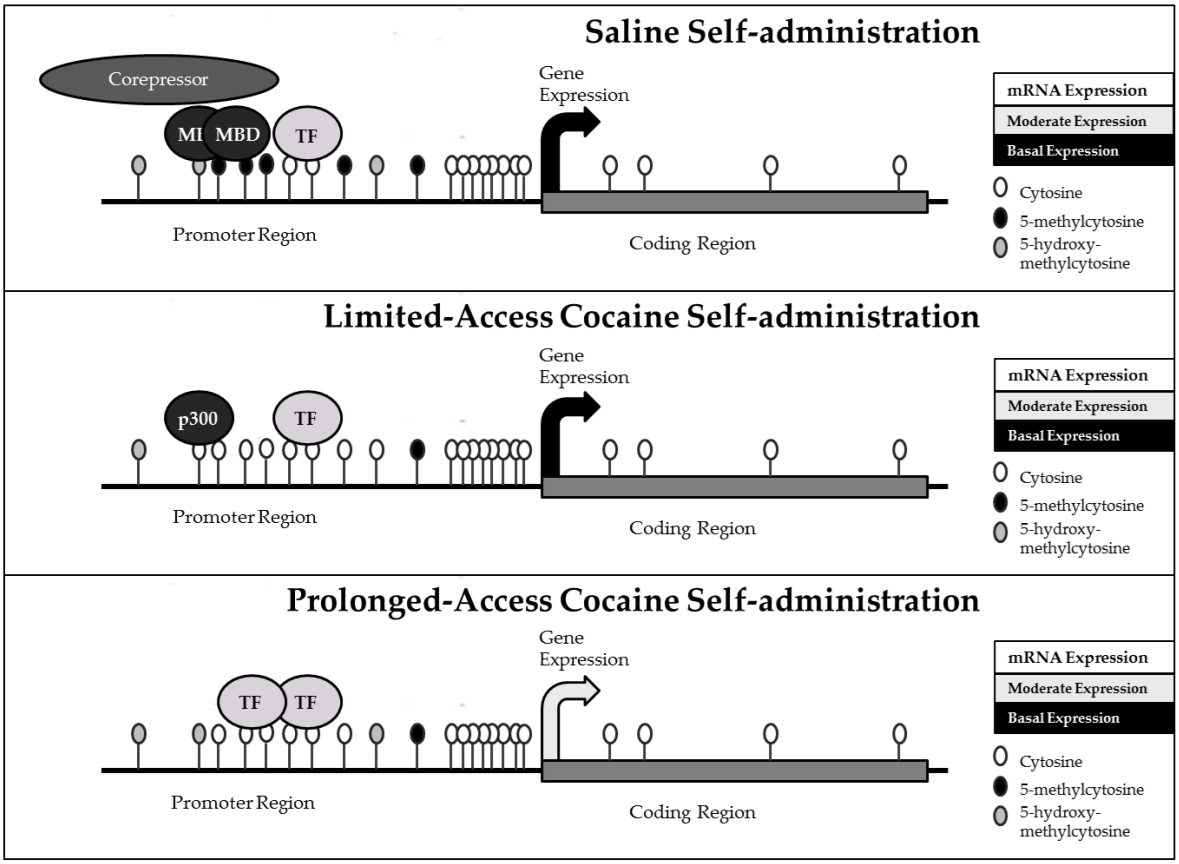

Figure 5.3: Theoretical schematic of *Homer2* data. Excessive 5mC accumulation in the saline rats lowers mRNA transcription; lower 5hmC and increased p300 binding in the limited access rats lowers mRNA transcription; low 5mC and increased 5hmC in prolonged access rats creates an open transcriptional state that potentiates *Homer2* mRNA transcription.

In conclusion, our data indicates that gene-specific changes in DNA methylation, DNA hydroxymethylation, and HAT activity within the dmPFC is associated with prolonged cocaine self-administration. Importantly, extended access to cocaine, which is accompanied by an "addicted-like behavioral profile" (Ahmed & Koob, 1998; Grimm et al., 2001), induces unique epigenetic modifications within the promoter of the *Homer2* gene, which are distinct from that which occurs under limited access conditions. Also of critical relevance to understanding the neurobiological bases of addiction, addicts report drug cravings that are not only highly enduring but also appear to intensify over the course of drug withdrawal in a time-dependent fashion (Gawin & Kleber, 1986). Accordingly, determination of the molecular status of long and short-term regulators of gene expression across not only different cocaine histories but also withdrawal periods will be necessary to develop a fuller understanding of the epigenetic basis of addiction.

Chapter 6:

General discussion and conclusions

Summary of findings

The data presented in this dissertation are the first to examine DNA methylation changes and mRNA expression in the dmPFC between differential cocaine-access conditions at different withdrawal times. Initial data for this project (Appendix Table 1) indicated genome-wide changes in DNA methylation after 1 day of abstinence between saline-, limited-, and prolonged-access conditions. In prioritizing the gene targets for validation, I noticed that there were approximately 18,000 changes in DNA methylation within promoter regions with a 2-fold difference between conditions. However, the majority of the genes were non-neuronal and only a few of the genes were implicated in excitatory or inhibitory signaling, which lead me to select four targets (based on fold changes and gene function), *Homer2*, *Dlg4*, *Grin1* and *Npas4*, for follow up validation in my thesis.

Since DNA methylation can be induced by repeated, experimenter-administrated cocaine injections (Cassel et al., 2006), it was unclear whether a decrease in global DNA methylation in prolonged-access rats was associated with total cocaine intake, or with changes in neurons that are associated with transition into a drug-addicted state. Therefore, in Chapter 2 (Aim 1 of the thesis), I added another cocaine-access condition, limited + yoked-access (rats were allowed 1 h of contingent cocaine SA followed by 6 h of yoked cocaine-access), to dissect the difference between limited- vs prolonged-access into the “excessive” cocaine exposure versus the “excessive cocaine-taking behavior (in

addition to exposure) as well as mRNA expression for glutamatergic genes in comparison to saline-, limited-, prolonged-access, and training-naïve rats.

The major findings of Chapter 2 were 1) all cocaine-access rats gradually escalate cocaine intake during the 1st 10 min and 1 h of SA, but at different rates, and 2) only prolonged-access rats exhibit increases in *Homer2*, *Dlg4*, and *Grin1* mRNA expression after 1 day of forced abstinence. Additionally, increased cocaine responding during the 1st 10 min and 1 h of SA in limited + yoked-access rats was associated with excessive non-reinforced responding due in part to mixed contingent and non-contingent cocaine training. The results of this first study indicated a unique behavioral and molecular phenotype for prolonged-access rats that differentiated them from limited-access rats in terms of total cocaine consumption, and from limited + yoked-access rats in relation to cues associated with cocaine intake.

To follow up on the Chapter 2 results (and complete Aim 2 of my dissertation), I once again allowed rats to self-administer cocaine under naïve, saline-, limited-, prolonged-, or limited + yoked-access to cocaine conditions, then measured DNA methylation and mRNA expression for the genes *Homer2*, *Dlg4*, *Npas4*, and *Grin1* after 1, 14, and 60 days of forced abstinence. The primary take-away from Study 2 (Chapter 3) is that DNA methylation incubated over the course of forced abstinence from cocaine. This is only the second instance of dynamic changes in 5mC that occur over the course of cocaine-abstinence (Baker-Andresen et al., 2015), but the first such instance of 5mC levels changing in glutamate-associated genes during abstinence as well as the first investigate the impact of different amount of cocaine exposure. Additionally, I observed consistent patterns of mRNA expression that were dependent upon access-condition,

rather than levels of 5mC. *Homer2* mRNA expression was decreased in all cocaine-access rats, *Dlg4* mRNA expression was decreased only in limited + yoked-access rats, while *Npas4* and *Grin1* mRNA were decreased across all access-conditions compared to naïve rats. Thus, Chapter 3 indicated condition and abstinence-dependent changes in basal levels of 5mC, as well as condition-dependent changes in basal mRNA expression.

Although the methylated DNA from Chapter 3 was a novel finding, the mRNA data in study 2 does not exactly align with study 1: *Homer2, Dlg4,* and *Grin1* were elevated after 1 day of abstinence in Study 1, whereas *Homer2* and *Grin1* are decreased throughout abstinence (Table 3.4). One possible explanation for this discrepancy between studies involves a difference in statistical analyses. Chapter 2 utilized a one-way ANOVA of condition, and it was simple to assess differences between conditions with an LSD *post-hoc* test. However, Chapter 3 utilized a two-way ANOVA of condition x abstinence, so when no interaction was detected between condition and abstinence, only the main effects were reported. In this manner, decreases in mRNA expression at 14 or 60 days of abstinence for *Homer2*, *Dlg4*, and *Grin1* could have been more influential to the overall the calculated average (mean) and standard deviation of the prolonged-access condition than mRNA expression after 1 day of abstinence. In addition, different techniques were utilized to quantify mRNA expression between Chapters 2 and 3 (differences are discussed below).

Chapter 3 also revealed a somewhat surprisingly limited predictive relation between DNA methylation and mRNA expression which led me to further probe into the relationship between DNA methylation and mRNA expression. The observation of an incubation of DNA methylation throughout the course of forced-abstinence might not

mediate basal mRNA during abstinence, but could possibly have another form functional relevance. Specifically, I hypothesized that levels of DNA methylation observed in prolonged-access rats after 60 days of forced abstinence will persist upon re-exposure to cocaine cues, and that re-exposure to cocaine cues will initiate context-specific mRNA transcription that aligns with DNA methylation. These hypotheses were tested in Chapter 4 which led to a novel and interesting discovery: re-exposure to cocaine-related (contextual and discrete stimuli as well as the opportunity to engage in lever responding) cues after protracted abstinence results in rapid changes in DNA methylation and associated mRNA expression. After cue re-exposure, *Homer2* 5mC increased while *Homer2* mRNA decreased in prolonged-access rats, *Dlg4* 5mC increased while *Dlg4* mRNA decreased in cocaine- and saline-access rats, and lastly, both *Npas4* 5mC and mRNA decreased in saline-access animals. These results are similar to a previously published report by Li et al. (2014) regarding fear extinction procedures; mice that were subjected to fear conditioning as well as a 2 hour extinction session for fear conditioning exhibited an increase in 5hmC within the hippocampus (Li et al., 2014). Additionally, mice exposed to contextual fear conditioning exhibit both increases in DNMT mRNA and methylated DNA for a memory suppression gene (PP1) as well as decreases in 5mC for a memory promoting gene (Reelin) within the hippocampus (Miller & Sweatt, 2007). Moreover, the increase in DNA methylation for PP1 was associated with a decrease in PP1 mRNA, while the decrease in Reelin 5mC was associated with an increase in Reelin mRNA, indicating that DNA methylation changes rapidly in the hippocampus during memory consolidation in a context specific manner (Miller & Sweatt, 2007).

Although I expected to observe widespread and gene-specific changes in epigenetic and gene expression following cocaine, I did not initially anticipate DNA methylation or mRNA changes to occur in saline-access rats, yet they were observed for both *Dlg4* and *Npas4* 5mC and mRNA. One explanation for these specific changes in saline-access rats were trained to lever press for food and the saline-access and re-exposure test would represent extinction condition for the food-seeking behavior. Both *Dlg4* and *Npas4* expression have implicated in consolidation and retrieval of previous memories. For instance, PSD-95 (the protein *Dlg4* encodes for) is increased in the mPFC after prolonged-access to cocaine SA and extinction procedures (Ghasemzadeh et al., 2011). Although Ghasemzadeh et al. observed an increase in PSD-95 protein after extinction training, I demonstrated a decrease in *Dlg4* mRNA in Chapter 4. However, the study by Ghasemzadeh et al. used rats that had been through 10 days of extinction training post-SA, with no abstinence period, whereas I exposed rats to 60 days of forced abstinence and then tested them for 2 h under extinction conditions. Another possible explanation could be that presentation of the light cue with saline injection serves as a primary reinforcer. Mice and rats have been previously shown to engage in "operant sensation seeking" behaviors and self-administer lights or sounds in the absence of a typical reinforcer such as food or cocaine (Olsen & Winder, 2010). Sensation-seeking behaviors have also been linked to addiction; rodents that engage in high sensation-seeking behavior engage in more drug-seeking behavior when subsequently trained to administer drugs (Gancarz, Robble, Kausch, Lloyd, & Richards, 2012, 2013). Lastly, operant sensation-seeking and drug-seeking behaviors both activate the same neural substrates (Olsen & Winder, 2009), and require mGluR5 receptors (Olsen, Childs,

Stanwood, & Winder, 2010). Therefore, the observed changes in DNA methylation and mRNA expression for saline-access rats could be due to either prior training with a food reinforcer, or a product of operant-sensation seeking since the dmPFC is activated in both behaviors.

Npas4 has also been implicated in learning and memory in relation to fear memories. It is essential for contextual fear conditioning as well as retrieval of auditory fear memories (Ploski, Monsey, Nguyen, DiLeone, & Schafe, 2011; Ramamoorthi et al., 2011). Additionally, *Npas4* expression is attenuated after corticosterone injection and after repeated stress (Drouet et al., 2015). Interestingly, extinction of operant behavior (including food) in rats has been observed to increase blood corticosterone (Coover et al., 1971; de Boer et al., 1990). Therefore, it is possible that continued food extinction training in rats during cue re-exposure after abstinence increased plasma corticosterone and decreased *Npas4* expression. It is also interesting that rats re-exposed to cocaine cues did not exhibit similar changes in *Npas4* DNA methylation or mRNA expression. However, in 2001, Peltier et al. demonstrated that extinction from cocaine SA did produce increased levels of corticosterone in rats, but only in rats administering 0.5 mg/kg of cocaine per infusion (Peltier, Guerin, Dorairaj, & Goeders, 2001); the rats in Chapter 4 only administered 0.25 mg/kg of cocaine per infusion during cocaine SA. Therefore, potential stress-associated effects of extinction were not observed in cocaine-access rats after re-exposure to cues.

While pursuing Chapter 5, I initially observed decreased *Homer2* DNA methylation within dmPFC in both limited- and prolonged-access rats, but only increased *Homer2* mRNA in prolonged-access rats. As such, only assessing DNA methylation

proved to be insufficient to explain differences in mRNA expression. Therefore, for Aim 3, I hypothesized that DNA hydroxymethylation or transcription factor binding, in addition to DNA methylation within the *Homer2* promoter, serve to mediate mRNA transcription in the dmPFC of prolonged-access rats after 1 day of forced abstinence. In testing this hypothesis, I observed decreased *Homer2* DNA methylation within dmPFC in both limited- and prolonged-access rats, decreased *Homer2* hydroxymethylation in limited-access rats, increased p300 transcription factor binding in limited-access rats, and increased *Homer2* mRNA expression in only the prolonged-access rats. Briefly, I have previously discussed that high levels of DNA methylation repress mRNA transcription (Jones et al., 1998), hydroxymethylation is associated with enhanced gene expression (Stroud et al., 2011), and p300 binding can inhibit mRNA transcription (Wei et al., 2012; Yoshimochi et al., 2010). Additionally, enhanced *Homer2* expression in the PFC is associated with enhanced cocaine seeking behaviors (Ary et al., 2013), which are observed in rats after protracted abstinence and re-exposure to cocaine cues (Grimm et al., 2001; Tran-Nguyen et al., 1998). To summarize the above, I observed various epigenetic modifications associated with the limited-access condition, but did not observe increases in *Homer2* mRNA expression as with the prolonged-access condition. Additionally, prolonged-access rats exhibit an addiction-like phenotype (Ben-Shahar, Moscarello, Jacob, Roarty, & Ettenberg, 2005) escalate cocaine intake faster than limited-access rats (Chapter 2). As such, I propose that limited-access rats exhibit some behavioral and molecular similarities with prolonged-access rats (such as escalated intake and decreased *Homer2* DNA methylation), but also maintain compensatory mechanisms to prevent an addictive phenotype, such as a reduction in 5hmC and increased p300

binding of the *Homer2* promoter in the dmPFC. In this manner, it is possible that limited-access rats maintain a drug-induced homeostasis via compensatory epigenetic mechanisms to maintain baseline *Homer2* mRNA expression, whereas prolonged-access rats undergo an allostatic shift and new baseline for *Homer2* expression.

After completion of Aims 1-4, I observed a few discrepancies between qPCR and ddPCR studies. Initially, I utilized quantitative real-time PCR (qPCR) to complete Aims 1 & 4, as this was the technology available. However, for Aims 2 & 3, I performed digital droplet PCR (ddPCR), because it was purportedly more accurate and more sensitive than standard qPCR. Therefore, there was some variation in experimental design and implementation between the studies which are explained below. First, in order to perform qPCR, I created and tested my own primer sets for mRNA levels, whereas in digital PCR, I purchased primer sets from the manufacturer. Thus, I may have detected different splice variants of *Homer2*, *Dlg4,* and *Grin1* mRNA between studies 1 & 4 versus 2 & 3. Additionally, qPCR and ddPCR work on very different principles and measure different things. The main difference between qPCR and ddPCR is that qPCR measures the level of fluorescence from fluorescently bound DNA in a single well during PCR amplification, whereas in ddPCR, a reaction mixture containing DNA and fluorescent marker are packaged in 10,000 to 20,000 droplets of DNA, amplified in a thermocycler, then each single fluorescent droplet is physically counted to produce an absolute concentration of DNA. Thus, the greatest limitation of qPCR is that it cannot reliably quantify low concentrations of DNA, because they may not produce enough of a fluorescent signal. However, ddPCR overcomes the limitation of low sample concentration because it can parse samples into tens of thousands of droplets, and

measures fluorescence within each droplet to determine an absolute concentration of DNA per µL of sample (examples of qPCR vs ddPCR experiments are attached in Appendix Figure 1).

In addition to the discrepancies between studies, I observed some unexpected changes to DNA methylation and mRNA expression in the saline-access condition. Saline-access rats were intended to serve as a behavioral control, however, they are not completely naïve to behavioral training, as they were initially trained under an FR1 schedule for food pellets before surgery. Food reinforcement prior to saline administration resulted in both food extinction learning during the first 5 days of self-administration (Figure 4.1A), as well as slight reinstatement of food responding during cue re-exposure (Figure 4.1B). The saline-access rats also exhibited changes to 5mC and mRNA expression that were similar to cocaine-access conditions for glutamatergic genes (i.e. in chapters 3 & 4). Re-exposure to food cues has previously been shown to elicit neuronal responses and activation of genetic machinery (i.e. mRNA transcription) in cortical-striatal networks (Schiltz, Bremer, Landry, & Kelley, 2007). Additionally, food extinction results in increased levels of blood corticosterone and alters mRNA transcription for genes associated with learning and memory (Coover et al., 1971, de Boer et al., 1990; Drouet et al., 2015). Therefore, the changes that I observed as well as previously published studies indicate that the saline-access condition was in reality a food-extinction condition, and indicate that changes in 5mC and mRNA for glutamatergic genes are involved in multiple forms of learning.

Interpretations and general implications of findings.

The experimental chapters comprising my dissertation introduce several novel discoveries regarding the recruitment of epigenetic mechanisms in the course of cocaine exposure of relevance to understanding addiction. I have demonstrated changes in mRNA levels for glutamatergic genes within the dmPFC of rats allowed contingent prolonged-access to cocaine, incubation of DNA methylation from acute to protracted abstinence, cue-elicited changes in DNA methylation and corresponding mRNA expression, and lastly, unique molecular phenotypes for limited- and prolonged-access rats that correspond to differential levels of *Homer2* mRNA. Together, these results broaden the field of epigenetics and addiction research, and suggest a unique and dynamic system of gene regulation within the dmPFC that is responsive to amount of cocaine access, time of abstinence, and re-exposure to cues.

First, my observation on dynamic changes in DNA methylation within the dmPFC during abstinence and in response to cocaine cues is intriguing. The above results in Chapters 3 & 4 indicate an adaptation in gene regulation that is unique in neural tissue (and stem cells), as neurons become quickly methylated or demethylated in response to learning, that has not been shown in other tissue types. Prior evidence also supports my findings, whereby extinction learning is aided through Tet-mediated demethylation of DNA promoter regions (Li, Wei, et al., 2014; Sadakierska-Chudy et al., 2017). Additionally, the abundance of Tet proteins and 5hmC within cortical and hippocampal tissues also support the notion of fast cycling of methylated DNA in neurons (Kriaucionis & Heintz, 2009). Fast and dynamic regulation of DNA methylation/ hydroxymethylation within neurons might also be intrinsically tied to the adaptive nature of learning and underlie the plasticity of mammalian brains to respond to change, as in the case of fear

conditioning or reinforcement. However, dynamically changing DNA methylation could also underscore addiction in humans and addiction-like behaviors in animals, as the neural substrates affected by drugs of abuse (i.e. cocaine) are the same substrates activated in seeking natural reinforcement (such as food or water). Suppose, for instance, that DNA methylation in the dmPFC was altered in response to receiving a salient food reinforcer, dmPFC DNA methylation could then be further altered after subsequent pairings of food to cues (such as location or smell), then the presentation of the food and/ or food cues could subsequently produce a desire to obtain the food. After many successive pairings, this process could hypothetically occur in the absence of food or food cues, and occur during memories of food. In this same manner, cocaine could hypothetically hijack learning and reinforcement, and direct the PFC to recall memories of cocaine and potentiate the process of "craving".

Second, I have consistently asserted that changes in levels 5mC and mRNA indicate a unique molecular phenotype. Although this is true, I would be remiss if I did not discuss the implications and inherent flaws of only measuring mRNA as an experimental outcome. To put it simply, mRNA is merely a message sent from a DNA blueprint, and is intended to be translated into a protein, but can be disrupted along the way. Eukaryotic cells highly complicated self-regulated systems designed to maintain optimal function and internal homeostasis. As such, processes such as mRNA transcription and protein translation can be halted as needed. The primary regulation of mRNA transcripts exists in the form of microRNAs (MiRs), short (~20 bp) fragments of RNA that bind to mRNA to inhibit it and tag it for degradation (for review see Kosik & Krichevsky, 2005). MicroRNAs respond in an activity-dependent manner and are

implicated in learning processes (Bredy, Lin, Wei, Baker-Andresen, & Mattick, 2011) and in addiction (Most, Workman, & Harris, 2014). Specifically, the microRNAs MiR-124 and Let-7d are suppressed after chronic cocaine treatment (Chandrasekar & Dreyer, 2009), and viral overexpression of MiR-124 and Let-7d inhibit cocaine-seeking behavior, whereas lentiviral inhibition of MiR-124 and Let-7d potentiate cocaine-seeking behavior (Chandrasekar & Dreyer, 2011). Furthermore, MiR-124 and Let-7d overexpression increased cocaine extinction learning (i.e. less time to reach extinction), whereas MiR-124 and Let-7d suppression decreased cocaine extinction learning (i.e. more time to reach extinction) (Chandrasekar & Dreyer, 2011). Lastly, MiR-124 and Let-7d regulate plasticity-related proteins that are implicated in addiction (Chandrasekar & Dreyer, 2011). These results indicate a fast, modulatory role of MiRs in response to cocaine or cocaine cues, and could potentially explain the decreases in mRNA across abstinence that I observed in Study 3 (Table 3.4). As such, potential discrepancies between mRNA and protein expression do not necessarily mean that mRNA is meaningless. In fact, these discrepancies could be due to allostatic mechanisms that control gene expression during forced abstinence.

Third, I must reiterate that mRNA is only a messenger, it is a signal from the DNA to become protein and lasts for only a short period within a cell, whereas receptor proteins (i.e. Homer2, PSD-95, and Grin1) are more stable and can be transported to synapses to influence dendrite size and shape in response to neural signals such as a cocaine challenge (for example, see Shen et al., 2009). As such, mRNA and proteins exhibit unique temporal properties from each other and can represent different aspects of behavioral studies. I have previously demonstrated that both levels of *Homer2* 5mC and

mRNA are affected by a 2 h re-exposure to cocaine cues (increased 5mC, decreased mRNA). However, the observed decreases in *Homer2* mRNA after cue re-exposure presented above (Figure 4.3B) initially appear to be contrary to behavioral studies indicating that Homer2 protein expression in the dmPFC is essential for cocaine-seeking behavior (Ary et al., 2013), although Homer2 protein in the dmPFC has not yet been quantified after 2 h of cue re-exposure (Gould et al., 2015). However, since mRNA and proteins exist within cells for different periods of time, it is possible that over the course of the 2 h cue re-exposure, *Homer2* mRNA was initially increased, allowing for Homer2 protein to be transiently expressed and aid cocaine-seeking behavior, but decrease in response to the absence of cocaine over the 2 h cue re-exposure session.

Future directions

Although I have established a new role for DNA methylation of the dmPFC after protracted abstinence and cue re-exposure, there is still a lot more research that needs to be done. First, for the majority of this dissertation, I have focused on 5mC levels in the dmPFC. However, as exampled in Study 4, 5hmC and protein binding to DNA fragments are necessary to fully describe how epigenetic modifications influence mRNA expression. Therefore, follow-up studies for the current report should at the very minimum incorporate levels of 5hmC, but should also investigate binding of common IEGs and transcription factors such as CREB, ARC, and Zif268.

Additionally, qPCR and ddPCR have allowed me to view DNA methylation much more specifically than via a methylated DNA array (i.e. NimbleGen array), but I still only observed changes in 5mC or 5hmC levels at a resolution of 150 to 250 bp. Recent advances in gene sequencing technology in the Bredy laboratory have allowed for cell-

type-specific ultrasequencing of methylated CpG regions that will allow for specific probing of cell populations that project to distinct anatomical regions (i.e. layer V pyramidal neurons in the PFC that project to the NAC or the VTA) with single base-pair resolution (Li, Baker-Andresen, Zhao, Marshall, & Bredy, 2014). This technique would also be capable of detecting changes in 5hmC and extend the role that hydroxymethylation has during cocaine abstinence and during cocaine-seeking.

Lastly, although the above studies elucidated a novel role for DNA methylation after cocaine self-administration followed by abstinence and re-exposure to conditioned cues, I did not perform causal manipulations on DNA methylation or hydroxymethylation. Therefore, I believe that future studies should target the dmPFC and increase 5mC within the dmPFC via protein overexpression of DNMT or inhibition of Tet1/ 3 via gene knockdown or siRNA, and decrease 5mC within the dmPFC via protein overexpression of Tet1/ 3, or inhibition of DNMT via gene knockdown or shRNA at multiple time points after cue re-exposure (i.e. 5 min, 30 min, and 1 day). These techniques have been performed previously to elucidate the role of DNA methylation in fear learning (Li, Wei, et al., 2014), but have not yet been applied to the study of drug-seeking behavior after cocaine self-administration. After determining the role of 5mC and 5hmC in cue-elicited drug seeking, modification of global, system-wide DNA methylation before or after re-exposure to drug cues with SAM or MET can potentially be used in clinical settings, as these compounds are in vitamin supplements that are widely available and in use by humans.

References

Addy, Daberkow, Ford, Garris, & Wightman. (2010). Sensitization of rapid dopamine signaling in the nucleus accumbens core and shell after repeated cocaine in rats. J Neurophysiol, 104(2), 922-931. doi:10.1152/jn.00413.2010

Ahmed. (2012). The science of making drug-addicted animals. Neuroscience, 211, 107-125. doi:10.1016/j.neuroscience.2011.08.014

Ahmed, S., & Cador, M. (2006). Dissociation of psychomotor sensitization from compulsive cocaine consumption. Neuropsychopharmacology, 31(3), 9.

Ahmed, S., Kenny, P., Koob, G., & Markou, A. (2002a). Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. Nat Neurosci, 5(7), 2.

Ahmed, S., & Koob, G. (1999). Long-lasting increase in the set point for cocaine self-administration after escalation in rats. Psychopharmacology (Berl), 146(3), 303-312.

Ahmed, S. H., & Koob, G. F. (1998). Transition from moderate to excessive drug intake: change in hedonic set point. Science, 282(5387), 298-300.

Ahmed, S. H., & Koob, G. F. (2005). Transition to drug addiction: a negative reinforcement model based on an allostatic decrease in reward function. Psychopharmacology (Berl), 180(3), 473-490. doi:10.1007/s00213-005-2180-z

Anier, K., Malinovskaja, K., Aonurm-Helm, A., Zharkovsky, A., & Kalda, A. (2010). DNA methylation regulates cocaine-induced behavioral sensitization in mice. Neuropsychopharmacology, 35(12), 2450-2461. doi:10.1038/npp.2010.128

Anier, K., Zharkovsky, A., & Kalda, A. (2013). S-adenosylmethionine modifies cocaine-induced DNA methylation and increases locomotor sensitization in mice. Int J Neuropsychopharmacol, 16(9), 2053-2066. doi:10.1017/s1461145713000394

APA. (2013). Diagnostic and statistical manual of mental disorders (DSM-5®): American Psychiatric Pub.

Ary, A. W., Lominac, K. D., Wroten, M. G., Williams, A. R., Campbell, R. R., Ben-Shahar, O., . . . Szumlinski, K. K. (2013). Imbalances in prefrontal cortex CC-Homer1 versus CC-Homer2 expression promote cocaine preference. J Neurosci, 33(19), 8101-8113. doi:10.1523/jneurosci.1727-12.2013

Ary, A. W., & Szumlinski, K. K. (2007). Regional differences in the effects of withdrawal from repeated cocaine upon Homer and glutamate receptor expression: a two-species comparison. Brain Res, 1184, 295-305. doi:10.1016/j.brainres.2007.09.035

Bai, G., & Hoffman, P. W. (2009). Frontiers in Neuroscience

Transcriptional Regulation of NMDA Receptor Expression. In A. M. Van Dongen (Ed.), Biology of the NMDA Receptor. Boca Raton (FL): CRC Press/Taylor & Francis

Taylor & Francis Group, LLC.

Baker-Andresen, D., Flavell, C. R., Li, X., & Bredy, T. W. (2013). Activation of BDNF signaling prevents the return of fear in female mice. Learn Mem, 20(5), 237-240. doi:10.1101/lm.029520.112

Baker-Andresen, D., Zhao, Q., Li, X., Jupp, B., Chesworth, R., Lawrence, A. J., & Bredy, T. (2015). Persistent variations in neuronal DNA methylation following cocaine self-administration and protracted abstinence in mice. Neuroepigenetics, 4, 1-11. doi:10.1016/j.nepig.2015.10.001

Bastle, R. M., Kufahl, P. R., Turk, M. N., Weber, S. M., Pentkowski, N. S., Thiel, K. J., & Neisewander, J. L. (2012). Novel cues reinstate cocaine-seeking behavior and induce Fos

protein expression as effectively as conditioned cues. Neuropsychopharmacology, 37(9), 2109-2120. doi:10.1038/npp.2012.60

Bear, M. F. (1996). A synaptic basis for memory storage in the cerebral cortex. Proc Natl Acad Sci U S A, 93(24), 13453-13459.

Belin, D., Balado, E., Piazza, P. V., & Deroche-Gamonet, V. (2009). Pattern of intake and drug craving predict the development of cocaine addiction-like behavior in rats. Biol Psychiatry, 65(10), 863-868. doi:10.1016/j.biopsych.2008.05.031

Ben-Shahar, O., Ahmed, S. H., Koob, G. F., & Ettenberg, A. (2004). The transition from controlled to compulsive drug use is associated with a loss of sensitization. Brain Res, 995(1), 46-54.

Ben-Shahar, O., Moscarello, J., Jacob, B., Roarty, M., & Ettenberg, A. (2005). Prolonged daily exposure to IV cocaine results in tolerance to its stimulant effects. Pharmacology Biochemistry and Behavior, 82(2), 411-416. doi:10.1016/j.pbb.2005.09.013

Ben-Shahar, O., Posthumus, E., Waldroup, S., & Ettenberg, A. (2008). Heightened drug-seeking motivation following extended daily access to self-administered cocaine. Prog Neuropsychopharmacol Biol Psychiatry., 32(3), 7.

Ben-Shahar, O., Sacramento, A., Miller, B., Webb, S., Wroten, M., Silva, H., . . . Szumlinski, K. (2013). Deficits in ventromedial prefrontal cortex group 1 metabotropic glutamate receptor function mediate resistance to extinction during protracted withdrawal from an extensive history of cocaine self-administration. J Neurosci, 33(2), 495-506a. doi:10.1523/jneurosci.3710-12.2013

Ben-Shahar, O., Szumlinski, K., Lominac, K., Cohen, A., Gordon, E., Ploense, K., . . . Woodward, N. (2012). Extended access to cocaine self-administration results in reduced

glutamate function within the medial prefrontal cortex. Addict Biol, 17(4), 746-757. doi:10.1111/j.1369-1600.2011.00428.x

Ben Shahar, O., Obara, I., Ary, A., Ma, N., Mangiardi, M., Medina, R., & Szumlinski, K. (2009). Extended Daily Access to Cocaine Results in Distinct Alterations in Homer 1b/c and NMDA Receptor Subunit Expression Within the Medial Prefrontal Cortex. Synapse, 63, 10. doi:10.1002/syn.20640

Berger, S. L. (2007). The complex language of chromatin regulation during transcription. Nature, 447(7143), 407-412. doi:10.1038/nature05915

Berglind, W. J., Whitfield, T. W., Jr., LaLumiere, R. T., Kalivas, P. W., & McGinty, J. F. (2009). A single intra-PFC infusion of BDNF prevents cocaine-induced alterations in extracellular glutamate within the nucleus accumbens. J Neurosci, 29(12), 3715-3719. doi:10.1523/jneurosci.5457-08.2009

Bersten, D. C., Wright, J. A., McCarthy, P. J., & Whitelaw, M. L. (2014). Regulation of the neuronal transcription factor NPAS4 by REST and microRNAs. Biochim Biophys Acta, 1839(1), 13-24. doi:10.1016/j.bbagrm.2013.11.004

Biergans, S. D., Claudianos, C., Reinhard, J., & Galizia, C. G. (2016). DNA Methylation Adjusts the Specificity of Memories Depending on the Learning Context and Promotes Relearning in Honeybees. Front Mol Neurosci, 9, 82. doi:10.3389/fnmol.2016.00082

Blanco, E., Pavon, F. J., Palomino, A., Luque-Rojas, M. J., Serrano, A., Rivera, P., . . . Rodriguez de Fonseca, F. (2014). Cocaine-induced behavioral sensitization is associated with changes in the expression of endocannabinoid and glutamatergic signaling systems in the mouse prefrontal cortex. Int J Neuropsychopharmacol, 18(1). doi:10.1093/ijnp/pyu024

Bolla, K., Ernst, M., Kiehl, K., Mouratidis, M., Eldreth, D., Contoreggi, C., . . . London, E. (2004). Prefrontal cortical dysfunction in abstinent cocaine abusers. J Neuropsychiatry Clin Neurosci, 16(4), 456-464. doi:10.1176/appi.neuropsych.16.4.456

Borrelli, E., Nestler, E. J., Allis, C. D., & Sassone-Corsi, P. (2008). Decoding the epigenetic language of neuronal plasticity. Neuron, 60(6), 961-974. doi:10.1016/j.neuron.2008.10.012

Bredy, T. W., & Barad, M. (2008). The histone deacetylase inhibitor valproic acid enhances acquisition, extinction, and reconsolidation of conditioned fear. Learn Mem, 15(1), 39-45. doi:10.1101/lm.801108

Bredy, T. W., Lin, Q., Wei, W., Baker-Andresen, D., & Mattick, J. S. (2011). MicroRNA regulation of neural plasticity and memory. Neurobiol Learn Mem, 96(1), 89-94. doi:10.1016/j.nlm.2011.04.004

Bredy, T. W., Wu, H., Crego, C., Zellhoefer, J., Sun, Y. E., & Barad, M. (2007). Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learn Mem, 14(4), 268-276. doi:10.1101/lm.500907

Carpenter-Hyland, E. P., & Chandler, L. J. (2006). Homeostatic plasticity during alcohol exposure promotes enlargement of dendritic spines. Eur J Neurosci, 24(12), 3496-3506. doi:10.1111/j.1460-9568.2006.05247.x

Cassel, S., Carouge, D., Gensburger, C., Anglard, P., Burgun, C., Dietrich, J. B., . . . Zwiller, J. (2006). Fluoxetine and cocaine induce the epigenetic factors MeCP2 and MBD1 in adult rat brain. Mol Pharmacol, 70(2), 487-492. doi:10.1124/mol.106.022301

Catts, V. S., Derminio, D. S., Hahn, C. G., & Weickert, C. S. (2015). Postsynaptic density levels of the NMDA receptor NR1 subunit and PSD-95 protein in prefrontal cortex from people with schizophrenia. NPJ Schizophr, 1, 15037. doi:10.1038/npjschz.2015.37

Cervo, L., & Samanin, R. (1995). Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. Brain Res, 673(2), 242-250.

Chandrasekar, V., & Dreyer, J. L. (2009). microRNAs miR-124, let-7d and miR-181a regulate cocaine-induced plasticity. Mol Cell Neurosci, 42(4), 350-362. doi:10.1016/j.mcn.2009.08.009

Chandrasekar, V., & Dreyer, J. L. (2011). Regulation of MiR-124, Let-7d, and MiR-181a in the accumbens affects the expression, extinction, and reinstatement of cocaine-induced conditioned place preference. Neuropsychopharmacology, 36(6), 1149-1164. doi:10.1038/npp.2010.250

Coover, G. D., Goldman, L., & Levine, S. (1971). Plasma corticosterone increases produced by extinction of operant behavior in rats. Physiol Behav, 6(3), 261-263.

Crespo, J. A., Oliva, J. M., Ghasemzadeh, M. B., Kalivas, P. W., & Ambrosio, E. (2002). Neuroadaptive changes in NMDAR1 gene expression after extinction of cocaine self-administration. Ann N Y Acad Sci, 965, 78-91.

Dackis, C. A., & O'Brien, C. P. (2001). Cocaine dependence: a disease of the brain's reward centers. J Subst Abuse Treat, 21(3), 111-117.

Day, J. J., & Sweatt, J. D. (2010). DNA methylation and memory formation. Nat Neurosci, 13(11), 1319-1323. doi:10.1038/nn.2666

de Boer, S. F., de Beun, R., Slangen, J. L., & van der Gugten, J. (1990). Dynamics of plasma catecholamine and corticosterone concentrations during reinforced and extinguished operant behavior in rats. Physiol Behav, 47(4), 691-698.

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for Addiction-like Behavior in the Rat. Science, 305(5686), 1014-1017. doi:10.1126/science.1099020

Deroche-Gamonet, V., Piat, F., Le Moal, M., & Piazza, P. V. (2002). Influence of cue-conditioning on acquisition, maintenance and relapse of cocaine intravenous self-administration. Eur J Neurosci, 15(8), 1363-1370.

Dobi, A., Seabold, G. K., Christensen, C. H., Bock, R., & Alvarez, V. A. (2011). Cocaine-induced plasticity in the nucleus accumbens is cell specific and develops without prolonged withdrawal. J Neurosci, 31(5), 1895-1904. doi:10.1523/jneurosci.5375-10.2011

Drouet, J. B., Fauvelle, F., Maunoir-Regimbal, S., Fidier, N., Maury, R., Peinnequin, A., . . . Canini, F. (2015). Differences in prefrontal cortex GABA/glutamate ratio after acute restraint stress in rats are associated with specific behavioral and neurobiological patterns. Neuroscience, 285, 155-165. doi:10.1016/j.neuroscience.2014.10.058

Ducret, E., Puaud, M., Lacoste, J., Belin-Rauscent, A., Fouyssac, M., Dugast, E., . . . Belin, D. (2016). N-acetylcysteine Facilitates Self-Imposed Abstinence After Escalation of Cocaine Intake. Biol Psychiatry, 80(3), 226-234. doi:10.1016/j.biopsych.2015.09.019

Dumitriu, D., Laplant, Q., Grossman, Y. S., Dias, C., Janssen, W. G., Russo, S. J., . . . Nestler, E. J. (2012). Subregional, dendritic compartment, and spine subtype specificity in cocaine regulation of dendritic spines in the nucleus accumbens. J Neurosci, 32(20), 6957-6966. doi:10.1523/jneurosci.5718-11.2012

Dworkin, S. I., Mirkis, S., & Smith, J. E. (1995). Response-dependent versus response-independent presentation of cocaine: differences in the lethal effects of the drug. Psychopharmacology (Berl), 117(3), 262-266.

Edwards, G. (1986). The Alcohol Dependence Syndrome: a concept as stimulus to enquiry★. British Journal of Addiction, 81(2), 171-183.

Edwards, S., & Koob, G. F. (2013). Escalation of drug self-administration as a hallmark of persistent addiction liability. Behav Pharmacol, 24(5-6), 356-362. doi:10.1097/FBP.0b013e3283644d15

Ehrlich, M., Gama-Sosa, M. A., Huang, L. H., Midgett, R. M., Kuo, K. C., McCune, R. A., & Gehrke, C. (1982). Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. Nucleic Acids Res, 10(8), 2709-2721.

El-Husseini, A. E., Schnell, E., Chetkovich, D. M., Nicoll, R. A., & Bredt, D. S. (2000). PSD-95 involvement in maturation of excitatory synapses. Science, 290(5495), 1364-1368.

Elias, G. M., Elias, L. A., Apostolides, P. F., Kriegstein, A. R., & Nicoll, R. A. (2008). Differential trafficking of AMPA and NMDA receptors by SAP102 and PSD-95 underlies synapse development. Proc Natl Acad Sci U S A, 105(52), 20953-20958. doi:10.1073/pnas.0811025106

Elvir, L., Duclot, F., Wang, Z., & Kabbaj, M. (2017). Epigenetic regulation of motivated behaviors by histone deacetylase inhibitors. Neurosci Biobehav Rev. doi:10.1016/j.neubiorev.2017.09.030

Ettenberg, A., Raven, M. A., Danluck, D. A., & Necessary, B. D. (1999). Evidence for opponent-process actions of intravenous cocaine. Pharmacol Biochem Behav, 64(3), 507-512.

Everitt, B. J. (2017). Drug Cues, Conditioned Reinforcement, and Drug Seeking: The Sequelae of a Collaborative Venture With Athina Markou. Biol Psychiatry. doi:10.1016/j.biopsych.2017.09.013

Everitt, B. J., Morris, K. A., O'Brien, A., & Robbins, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience, 42(1), 1-18.

Farthing, C. R., Ficz, G., Ng, R. K., Chan, C. F., Andrews, S., Dean, W., . . . Reik, W. (2008). Global mapping of DNA methylation in mouse promoters reveals epigenetic reprogramming of pluripotency genes. PLoS Genet, 4(6), e1000116. doi:10.1371/journal.pgen.1000116

Feltenstein, M. W., & See, R. E. (2008). The neurocircuitry of addiction: an overview. Br J Pharmacol, 154(2), 261-274. doi:10.1038/bjp.2008.51

Feng, J., Shao, N., Szulwach, K. E., Vialou, V., Huynh, J., Zhong, C., . . . Nestler, E. J. (2015). Role of Tet1 and 5-hydroxymethylcytosine in cocaine action. Nat Neurosci, 18(4), 536-544. doi:10.1038/nn.3976

Ferrario, C. R., Gorny, G., Crombag, H. S., Li, Y., Kolb, B., & Robinson, T. E. (2005). Neural and Behavioral Plasticity Associated with the Transition from Controlled to Escalated Cocaine Use. Biological psychiatry, 58(9), 751-759. doi:10.1016/j.biopsych.2005.04.046

Feyissa, A. M., Chandran, A., Stockmeier, C. A., & Karolewicz, B. (2009). Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in

major depression. Prog Neuropsychopharmacol Biol Psychiatry, 33(1), 70-75. doi:10.1016/j.pnpbp.2008.10.005

Fonteneau, M., Filliol, D., Anglard, P., Befort, K., Romieu, P., & Zwiller, J. (2017). Inhibition of DNA methyltransferases regulates cocaine self-administration by rats: a genome-wide DNA methylation study. Genes Brain Behav, 16(3), 313-327. doi:10.1111/gbb.12354

Frankfurt, M., Salas-Ramirez, K., Friedman, E., & Luine, V. (2011). Cocaine alters dendritic spine density in cortical and subcortical brain regions of the postpartum and virgin female rat. Synapse, 65(9), 955-961. doi:10.1002/syn.20918

Freeman, W., Patel, K., Brucklacher, R., Lull, M., Erwin, M., Morgan, D., . . . Vrana, K. (2008). Persistent alterations in mesolimbic gene expression with abstinence from cocaine self-administration. Neuropsychopharmacology, 33(8), 11.

Freeman, W. M., Lull, M. E., Patel, K. M., Brucklacher, R. M., Morgan, D., Roberts, D. C., & Vrana, K. E. (2010). Gene expression changes in the medial prefrontal cortex and nucleus accumbens following abstinence from cocaine self-administration. BMC Neurosci, 11, 29. doi:10.1186/1471-2202-11-29

Fuchs, R. A., Evans, K. A., Ledford, C. C., Parker, M. P., Case, J. M., Mehta, R. H., & See, R. E. (2005). The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology, 30(2), 296-309. doi:10.1038/sj.npp.1300579

Galici, R., Pechnick, R. N., Poland, R. E., & France, C. P. (2000). Comparison of noncontingent versus contingent cocaine administration on plasma corticosterone levels in rats. Eur J Pharmacol, 387(1), 59-62.

Gancarz, A. M., Robble, M. A., Kausch, M. A., Lloyd, D. R., & Richards, J. B. (2012). Association between locomotor response to novelty and light reinforcement: sensory reinforcement as a rodent model of sensation seeking. Behav Brain Res, 230(2), 380-388.

Gancarz, A. M., Robble, M. A., Kausch, M. A., Lloyd, D. R., & Richards, J. B. (2013). Sensory reinforcement as a predictor of cocaine and water self-administration in rats. Psychopharmacology (Berl), 226(2), 335-346. doi:10.1007/s00213-012-2907-6

Gao, X., Jia, M., Zhang, Y., Breitling, L. P., & Brenner, H. (2015). DNA methylation changes of whole blood cells in response to active smoking exposure in adults: a systematic review of DNA methylation studies. Clin Epigenetics, 7. doi:10.1186/s13148-015-0148-3

Gawin, F., & Kleber, H. (1986). Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations. Arch Gen Psychiatry, 43(2), 7.

Gawin, F. H., Kleber, H. D., Byck, R., Rounsaville, B. J., Kosten, T. R., Jatlow, P. I., & Morgan, C. (1989). Desipramine facilitation of initial cocaine abstinence. Arch Gen Psychiatry, 46(2), 117-121.

George, O., & Koob, G. F. (2010). Individual differences in prefrontal cortex function and the transition from drug use to drug dependence. Neurosci Biobehav Rev, 35(2), 232-247. doi:10.1016/j.neubiorev.2010.05.002

George, O., Mandyam, C. D., Wee, S., & Koob, G. F. (2008). Extended access to cocaine self-administration produces long-lasting prefrontal cortex-dependent working memory impairments. Neuropsychopharmacology, 33(10), 2474-2482. doi:10.1038/sj.npp.1301626

Gerber, G. J., & Wise, R. A. (1989). Pharmacological regulation of intravenous cocaine and heroin self-administration in rats: a variable dose paradigm. Pharmacol Biochem Behav, 32(2), 527-531.

Ghasemzadeh, M. B., Mueller, C., & Vasudevan, P. (2009). Behavioral sensitization to cocaine is associated with increased glutamate receptor trafficking to the postsynaptic density after extended withdrawal period. Neuroscience, 159(1), 414-426. doi:10.1016/j.neuroscience.2008.10.027

Ghasemzadeh, M. B., Vasudevan, P., Giles, C., Purgianto, A., Seubert, C., & Mantsch, J. R. (2011). Glutamatergic plasticity in medial prefrontal cortex and ventral tegmental area following extended-access cocaine self-administration. Brain Res, 1413, 60-71. doi:10.1016/j.brainres.2011.06.041

Ghasemzadeh, M. B., Vasudevan, P., & Mueller, C. (2009). Locomotor sensitization to cocaine is associated with distinct pattern of glutamate receptor trafficking to the postsynaptic density in prefrontal cortex: early versus late withdrawal effects. Pharmacol Biochem Behav, 92(3), 383-392. doi:10.1016/j.pbb.2008.12.004

Ghasemzadeh, M. B., Vasudevan, P., Mueller, C., Seubert, C., & Mantsch, J. R. (2009). Region specific alterations in glutamate receptor expression and subcellular distribution following extinction of cocaine self-administration. Brain Res. doi:10.1016/j.brainres.2009.01.047

Gipson, C. D., Kupchik, Y. M., Shen, H., Reissner, K. J., Thomas, C. A., & Kalivas, P. W. (2013). Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation. Neuron, 77(5), 867-872. doi:10.1016/j.neuron.2013.01.005

Globisch, D., Münzel, M., Müller, M., Michalakis, S., Wagner, M., Koch, S., . . . Carell, T. (2010). Tissue distribution of 5-hydroxymethylcytosine and search for active demethylation intermediates. PLoS ONE, 5(12), e15367.

Goeders, J. E., Murnane, K. S., Banks, M. L., & Fantegrossi, W. E. (2009). Escalation of food-maintained responding and sensitivity to the locomotor stimulant effects of cocaine in mice. Pharmacol Biochem Behav, 93(1), 67-74. doi:10.1016/j.pbb.2009.04.008

Gong, W., Neill, D., & Justice, J. B., Jr. (1997). 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. Brain Res, 754(1-2), 103-112.

Gong, Z., Wang, C., Nieh, J. C., & Tan, K. (2016). Inhibiting DNA methylation alters olfactory extinction but not acquisition learning in Apis cerana and Apis mellifera. J Insect Physiol, 90, 43-48. doi:10.1016/j.jinsphys.2016.05.007

Gould, A. T., Sacramento, A. D., Wroten, M. G., Miller, B. W., von Jonquieres, G., Klugmann, M., . . . Szumlinski, K. K. (2015). Cocaine-elicited imbalances in ventromedial prefrontal cortex Homer1 versus Homer2 expression: implications for relapse. Addict Biol, 20(1), 148-157. doi:10.1111/adb.12088

Grimm, J. W., Hope, B. T., Wise, R. A., & Shaham, Y. (2001). Neuroadaptation. Incubation of cocaine craving after withdrawal. Nature, 412(6843), 141-142. doi:10.1038/35084134

Grimm, J. W., Lu, L., Hayashi, T., Hope, B. T., Su, T. P., & Shaham, Y. (2003). Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci, 23(3), 742-747.

Guo, M. L., Xue, B., Jin, D. Z., Liu, Z. G., Fibuch, E. E., Mao, L. M., & Wang, J. Q. (2012). Upregulation of Npas4 protein expression by chronic administration of amphetamine in rat nucleus accumbens in vivo. Neurosci Lett, 528(2), 210-214. doi:10.1016/j.neulet.2012.07.048

Halder, R., Hennion, M., Vidal, R. O., Shomroni, O., Rahman, R. U., Rajput, A., . . . Bonn, S. (2016). DNA methylation changes in plasticity genes accompany the formation and maintenance of memory. Nat Neurosci, 19(1), 102-110. doi:10.1038/nn.4194

Hao, Y., Martin-Fardon, R., & Weiss, F. (2010). Behavioral and functional evidence of metabotropic glutamate receptor 2/3 and metabotropic glutamate receptor 5 dysregulation in cocaine-escalated rats: factor in the transition to dependence. Biological psychiatry, 68(0350767c-96a0-5f8b-34fe-f9051c7b4ceb), 240-248. doi:10.1016/j.biopsych.2010.02.011

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., & Vandesompele, J. (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol, 8(2), R19. doi:10.1186/gb-2007-8-2-r19

Hellman, A., & Chess, A. (2007). Gene body-specific methylation on the active X chromosome. Science, 315(5815), 1141-1143. doi:10.1126/science.1136352

Hemby, S. E., Co, C., Koves, T. R., Smith, J. E., & Dworkin, S. I. (1997). Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. Psychopharmacology (Berl), 133(1), 7-16.

Hemby, S. E., Horman, B., & Tang, W. (2005). Differential regulation of ionotropic glutamate receptor subunits following cocaine self-administration. Brain Res, 1064(1-2), 75-82. doi:10.1016/j.brainres.2005.09.051

Hemby, S. E., Tang, W., Muly, E. C., Kuhar, M. J., Howell, L., & Mash, D. C. (2005). Cocaine-induced alterations in nucleus accumbens ionotropic glutamate receptor subunits in human and non-human primates. J Neurochem, 95(6), 1785-1793. doi:10.1111/j.1471-4159.2005.03517.x

Heusner, C. L., & Palmiter, R. D. (2005). Expression of mutant NMDA receptors in dopamine D1 receptor-containing cells prevents cocaine sensitization and decreases cocaine preference. J Neurosci, 25(28), 6651-6657. doi:10.1523/jneurosci.1474-05.2005

Hopf, F. W. (2017). Do specific NMDA receptor subunits act as gateways for addictive behaviors? Genes Brain Behav, 16(1), 118-138. doi:10.1111/gbb.12348

Huang, C.-C., Lin, H.-J., & Hsu, K.-S. (2006). Repeated cocaine administration promotes long-term potentiation induction in rat medial prefrontal cortex. Cerebral cortex, 17(8), 1877-1888.

IACUC. (2011). Guide for the Care and Use of Laboratory Animals: Eighth Edition: The National Academies Press.

Isaac, W. L., Nonneman, A. J., Neisewander, J., Landers, T., & Bardo, M. T. (1989). Prefrontal cortex lesions differentially disrupt cocaine-reinforced conditioned place preference but not conditioned taste aversion. Behav Neurosci, 103(2), 345-355.

Ito, R., Robbins, T. W., & Everitt, B. J. (2004). Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. Nat Neurosci, 7(4), 389-397. doi:10.1038/nn1217

Ito, S., D'Alessio, A. C., Taranova, O. V., Hong, K., Sowers, L. C., & Zhang, Y. (2010). Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature, 466(7310), 1129-1133. doi:10.1038/nature09303

Iurlaro, M., Ficz, G., Oxley, D., Raiber, E. A., Bachman, M., Booth, M. J., . . . Reik, W. (2013). A screen for hydroxymethylcytosine and formylcytosine binding proteins suggests functions in transcription and chromatin regulation. Genome Biol, 14(10), R119. doi:10.1186/gb-2013-14-10-r119

Jaenisch, R., & Bird, A. (2003). Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet, 33 Suppl, 245-254. doi:10.1038/ng1089

Jeziorska, D. M., Murray, R. J. S., De Gobbi, M., Gaentzsch, R., Garrick, D., Ayyub, H., . . . Tufarelli, C. (2017). DNA methylation of intragenic CpG islands depends on their transcriptional activity during differentiation and disease. Proc Natl Acad Sci U S A, 114(36), E7526-e7535. doi:10.1073/pnas.1703087114

Jones, P. A. (2012). Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet, 13(7), 484-492. doi:10.1038/nrg3230

Jones, P. L., Veenstra, G. J., Wade, P. A., Vermaak, D., Kass, S. U., Landsberger, N., . . . Wolffe, A. P. (1998). Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet, 19(2), 187-191. doi:10.1038/561

Kalivas, P. (2007). Cocaine and amphetamine-like psychostimulants: neurocircuitry and glutamate neuroplasticity. Dialogues in clinical neuroscience, 9(e540c0c0-fb52-97cf-1a4d-fc8ec1f4bbbc), 389-486.

Kalivas, P. W. (2008). Addiction as a pathology in prefrontal cortical regulation of corticostriatal habit circuitry. Neurotox Res, 14(2-3), 185-189. doi:10.1007/bf03033809

Kalivas, P. W., McFarland, K., Bowers, S., Szumlinski, K., Xi, Z. X., & Baker, D. (2003). Glutamate transmission and addiction to cocaine. Ann N Y Acad Sci, 1003, 169-175.

Kalivas, P. W., Szumlinski, K. K., & Worley, P. (2004). Homer2 gene deletion in mice produces a phenotype similar to chronic cocaine treated rats. Neurotox Res, 6(5), 385-387.

Kalivas, P. W., Volkow, N., & Seamans, J. (2005). Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron, 45(5), 647-650. doi:10.1016/j.neuron.2005.02.005

Kawai, J., Hirose, K., Fushiki, S., Hirotsune, S., Ozawa, N., Hara, A., . . . Watanabe, S. (1994). Comparison of DNA methylation patterns among mouse cell lines by restriction landmark genomic scanning. Mol Cell Biol, 14(11), 7421-7427.

Kerstetter, K. A., Aguilar, V. R., Parrish, A. B., & Kippin, T. E. (2008). Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. Psychopharmacology (Berl), 198(1), 63-75. doi:10.1007/s00213-008-1089-8

Kim, E., & Sheng, M. (2004). PDZ domain proteins of synapses. Nat Rev Neurosci, 5(10), 771-781. doi:10.1038/nrn1517

Kippin, T. E., Fuchs, R. A., & See, R. E. (2006). Contributions of prolonged contingent and noncontingent cocaine exposure to enhanced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl), 187(1), 60-67. doi:10.1007/s00213-006-0386-3

Kirkland Henry, P., Davis, M., & Howell, L. L. (2009). Effects of cocaine self-administration history under limited and extended access conditions on in vivo striatal

dopamine neurochemistry and acoustic startle in rhesus monkeys. Psychopharmacology (Berl), 205(2), 237-247. doi:10.1007/s00213-009-1534-3

Knackstedt, L., & Kalivas, P. (2007). Extended access to cocaine self-administration enhances drug-primed reinstatement but not behavioral sensitization. J Pharmacol Exp Ther., 322(3), 7.

Knackstedt, L. A., Moussawi, K., Lalumiere, R., Schwendt, M., Klugmann, M., & Kalivas, P. W. (2010). Extinction training after cocaine self-administration induces glutamatergic plasticity to inhibit cocaine seeking. J Neurosci, 30(23), 7984-7992. doi:10.1523/jneurosci.1244-10.2010

Koob, G., & Volkow, N. (2009). Neurocircuitry of Addiction. Neuropsychopharmacology, 35(1), 217-238. doi:10.1038/npp.2009.110

Koob, G. F. (2004). Allostatic view of motivation: implications for psychopathology. Nebr Symp Motiv, 50, 1-18.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. Neuropsychopharmacology, 35(1), 217-238. doi:10.1038/npp.2009.110

Kosik, K. S., & Krichevsky, A. M. (2005). The Elegance of the MicroRNAs: A Neuronal Perspective. Neuron, 47(6), 779-782. doi:10.1016/j.neuron.2005.08.019

Kovacs, K., Lajtha, A., & Sershen, H. (2010). Effect of nicotine and cocaine on neurofilaments and receptors in whole brain tissue and synaptoneurosome preparations. Brain Res Bull, 82(1-2), 109-117. doi:10.1016/j.brainresbull.2010.02.008

Krawczyk, M., Mason, X., DeBacker, J., Sharma, R., Normandeau, C. P., Hawken, E. R., . . . Dumont, E. C. (2013). D1 dopamine receptor-mediated LTP at GABA synapses

encodes motivation to self-administer cocaine in rats. J Neurosci, 33(29), 11960-11971. doi:10.1523/jneurosci.1784-13.2013

Kriaucionis, S., & Heintz, N. (2009). The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science, 324(5929), 929-930. doi:10.1126/science.1169786

Kumar, A., Choi, K. H., Renthal, W., Tsankova, N. M., Theobald, D. E., Truong, H. T., . . . Nestler, E. J. (2005). Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron, 48(2), 303-314. doi:10.1016/j.neuron.2005.09.023

Launay, J. M., Del Pino, M., Chironi, G., Callebert, J., Peoc'h, K., Megnien, J. L., . . . Rendu, F. (2009). Smoking induces long-lasting effects through a monoamine-oxidase epigenetic regulation. PLoS ONE, 4(11), e7959. doi:10.1371/journal.pone.0007959

Laviolette, S., & van der Kooy, D. (2004). The neurobiology of nicotine addiction: bridging the gap from molecules to behaviour. Nature reviews. Neuroscience, 5(d5f9f78f-ab8a-dbb5-da17-1ba091f0d853), 55-120. doi:10.1038/nrn1298

Lecca, D., Cacciapaglia, F., Valentini, V., Acquas, E., & Di Chiara, G. (2007). Differential neurochemical and behavioral adaptation to cocaine after response contingent and noncontingent exposure in the rat. Psychopharmacology (Berl), 191(3), 653-667. doi:10.1007/s00213-006-0496-y

Levine, A. A., Guan, Z., Barco, A., Xu, S., Kandel, E. R., & Schwartz, J. H. (2005). CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. Proc Natl Acad Sci U S A, 102(52), 19186-19191. doi:10.1073/pnas.0509735102

Li, X., Baker-Andresen, D., Zhao, Q., Marshall, V., & Bredy, T. W. (2014). Methyl CpG binding domain ultra-sequencing: a novel method for identifying inter-individual and cell-type-specific variation in DNA methylation. Genes Brain Behav, 13(7), 721-731. doi:10.1111/gbb.12150

Li, X., Wei, W., Zhao, Q. Y., Widagdo, J., Baker-Andresen, D., Flavell, C. R., . . . Bredy, T. W. (2014). Neocortical Tet3-mediated accumulation of 5-hydroxymethylcytosine promotes rapid behavioral adaptation. Proc Natl Acad Sci U S A, 111(19), 7120-7125. doi:10.1073/pnas.1318906111

Lin, Y., Bloodgood, B. L., Hauser, J. L., Lapan, A. D., Koon, A. C., Kim, T. K., . . . Greenberg, M. E. (2008). Activity-dependent regulation of inhibitory synapse development by Npas4. Nature, 455(7217), 1198-1204. doi:10.1038/nature07319

Liu, C., Sun, X., Wang, Z., Le, Q., Liu, P., Jiang, C., . . . Ma, L. (2017). Retrieval-induced Upregulation of Tet3 in Pyramidal Neurons of the Dorsal Hippocampus Mediates Cocaine-Associated Memory Reconsolidation. Int J Neuropsychopharmacol. doi:10.1093/ijnp/pyx099

Luis, C., Cannella, N., Spanagel, R., & Kohr, G. (2017). Persistent strengthening of the prefrontal cortex - nucleus accumbens pathway during incubation of cocaine-seeking behavior. Neurobiol Learn Mem, 138, 281-290. doi:10.1016/j.nlm.2016.10.003

Ma, Y. Y., Henley, S. M., Toll, J., Jentsch, J. D., Evans, C. J., Levine, M. S., & Cepeda, C. (2013). Drug-primed reinstatement of cocaine seeking in mice: increased excitability of medium-sized spiny neurons in the nucleus accumbens. ASN Neuro, 5(4), 257-271. doi:10.1042/an20130015

Maddox, S. A., & Schafe, G. E. (2011). Epigenetic alterations in the lateral amygdala are required for reconsolidation of a Pavlovian fear memory. Learn Mem, 18(9), 579-593. doi:10.1101/lm.2243411

Malousi, A., & Kouidou, S. (2012). DNA hypermethylation of alternatively spliced and repeat sequences in humans. Mol Genet Genomics, 287(8), 631-642. doi:10.1007/s00438-012-0703-y

Mandt, B. H., Copenhagen, L. I., Zahniser, N. R., & Allen, R. M. (2015). Escalation of cocaine consumption in short and long access self-administration procedures. Drug Alcohol Depend, 149, 166-172. doi:10.1016/j.drugalcdep.2015.01.039

Mantsch, J., Yuferov, V., Mathieu-Kia, A., Ho, A., & Kreek, M. (2004). Effects of extended access to high versus low cocaine doses on self-administration, cocaine-induced reinstatement and brain mRNA levels in rats. Psychopharmacology (Berl), 175(1), 11.

Markou, A., & Koob, G. F. (1991). Postcocaine anhedonia. An animal model of cocaine withdrawal. Neuropsychopharmacology, 4(1), 17-26.

Markou, A., & Koob, G. F. (1992). Bromocriptine reverses the elevation in intracranial self-stimulation thresholds observed in a rat model of cocaine withdrawal. Neuropsychopharmacology, 7(3), 213-224.

Martin, T. A., Jayanthi, S., McCoy, M. T., Brannock, C., Ladenheim, B., Garrett, T., . . . Cadet, J. L. (2012). Methamphetamine causes differential alterations in gene expression and patterns of histone acetylation/hypoacetylation in the rat nucleus accumbens. PLoS ONE, 7(3), e34236. doi:10.1371/journal.pone.0034236

Massart, R., Barnea, R., Dikshtein, Y., Suderman, M., Meir, O., Hallett, M., . . . Yadid, G. (2015). Role of DNA methylation in the nucleus accumbens in incubation of cocaine craving. J Neurosci, 35(21), 8042-8058. doi:10.1523/jneurosci.3053-14.2015

McFarland, K., Lapish, C. C., & Kalivas, P. W. (2003). Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci, 23(8), 3531-3537.

McGinty, J. F., Zelek-Molik, A., & Sun, W. L. (2014). Cocaine self-administration causes signaling deficits in corticostriatal circuitry that are reversed by BDNF in early withdrawal. Brain Res. doi:10.1016/j.brainres.2014.09.050

McGlinchey, E. M., James, M. H., Mahler, S. V., Pantazis, C., & Aston-Jones, G. (2016). Prelimbic to Accumbens Core Pathway Is Recruited in a Dopamine-Dependent Manner to Drive Cued Reinstatement of Cocaine Seeking. J Neurosci, 36(33), 8700-8711. doi:10.1523/jneurosci.1291-15.2016

McIntosh, S., Howell, L., & Hemby, S. E. (2013). Dopaminergic dysregulation in prefrontal cortex of rhesus monkeys following cocaine self-administration. Front Psychiatry, 4, 88. doi:10.3389/fpsyt.2013.00088

Meil, W. M., & See, R. E. (1996). Conditioned cued recovery of responding following prolonged withdrawal from self-administered cocaine in rats: an animal model of relapse. Behav Pharmacol, 7(8), 754-763.

Miguens, M., Del Olmo, N., Higuera-Matas, A., Torres, I., Garcia-Lecumberri, C., & Ambrosio, E. (2008). Glutamate and aspartate levels in the nucleus accumbens during cocaine self-administration and extinction: a time course microdialysis study. Psychopharmacology (Berl), 196(2), 303-313. doi:10.1007/s00213-007-0958-x

Miller, C. A., & Sweatt, J. D. (2007). Covalent modification of DNA regulates memory formation. Neuron, 53(6), 857-869. doi:10.1016/j.neuron.2007.02.022

Most, D., Workman, E., & Harris, R. A. (2014). Synaptic adaptations by alcohol and drugs of abuse: changes in microRNA expression and mRNA regulation. Front Mol Neurosci, 7, 85. doi:10.3389/fnmol.2014.00085

Mutschler, N. H., & Miczek, K. A. (1998). Withdrawal from a self-administered or non-contingent cocaine binge: differences in ultrasonic distress vocalizations in rats. Psychopharmacology (Berl), 136(4), 402-408.

Nader, M. A. (2016). Animal models for addiction medicine: From vulnerable phenotypes to addicted individuals. Prog Brain Res, 224, 3-24. doi:10.1016/bs.pbr.2015.07.012

Nakamura, M., Gao, S., Okamura, H., & Nakahara, D. (2011). Intrathecal cocaine delivery enables long-access self-administration with binge-like behavior in mice. Psychopharmacology (Berl), 213(1), 119-129. doi:10.1007/s00213-010-2021-6

Neri, F., Rapelli, S., Krepelova, A., Incarnato, D., Parlato, C., Basile, G., . . . Oliviero, S. (2017). Intragenic DNA methylation prevents spurious transcription initiation. Nature, 543(7643), 72-77. doi:10.1038/nature21373

Nestler E.J., H. S. E., & Malenka R.C. (2009). Molecular Neuropharmacology: A Foundation for Clinical Neuroscience (2 ed.): McGraw Hill.

Nestler, E. J. (2013). Cellular basis of memory for addiction. Dialogues Clin Neurosci, 15(4), 431-443.

NIDA. (2016). Cocaine. Retrieved from https://www.drugabuse.gov/publications/research-reports/cocaine

Nielsen, D. A., Huang, W., Hamon, S. C., Maili, L., Witkin, B. M., Fox, R. G., . . . Moeller, F. G. (2012). Forced Abstinence from Cocaine Self-Administration is Associated with DNA Methylation Changes in Myelin Genes in the Corpus Callosum: a Preliminary Study. Front Psychiatry, 3, 60. doi:10.3389/fpsyt.2012.00060

Numachi, Y., Yoshida, S., Yamashita, M., Fujiyama, K., Naka, M., Matsuoka, H., . . . Sora, I. (2004). Psychostimulant alters expression of DNA methyltransferase mRNA in the rat brain. Ann N Y Acad Sci, 1025, 102-109. doi:10.1196/annals.1316.013

Olsen, C. M., Childs, D. S., Stanwood, G. D., & Winder, D. G. (2010). Operant sensation seeking requires metabotropic glutamate receptor 5 (mGluR5). PLoS ONE, 5(11), e15085. doi:10.1371/journal.pone.0015085

Olsen, C. M., & Winder, D. G. (2009). Operant sensation seeking engages similar neural substrates to operant drug seeking in C57 mice. Neuropsychopharmacology, 34(7), 1685-1694. doi:10.1038/npp.2008.226

Olsen, C. M., & Winder, D. G. (2010). Operant sensation seeking in the mouse. J Vis Exp(45). doi:10.3791/2292

Ooe, N., Saito, K., Mikami, N., Nakatuka, I., & Kaneko, H. (2004). Identification of a novel basic helix-loop-helix-PAS factor, NXF, reveals a Sim2 competitive, positive regulatory role in dendritic-cytoskeleton modulator drebrin gene expression. Mol Cell Biol, 24(2), 608-616.

Otto, S. J., McCorkle, S. R., Hover, J., Conaco, C., Han, J. J., Impey, S., . . . Mandel, G. (2007). A new binding motif for the transcriptional repressor REST uncovers large gene networks devoted to neuronal functions. J Neurosci, 27(25), 6729-6739. doi:10.1523/jneurosci.0091-07.2007

Ouachikh, O., Dieb, W., Durif, F., & Hafidi, A. (2014). Anterior ventral tegmental area dopaminergic neurons are not involved in the motivational effects of bromocriptine, pramipexole and cocaine in drug-free rats. Behav Brain Res, 262, 1-7. doi:10.1016/j.bbr.2013.12.021

Palamarchouk, V., Smagin, G., & Goeders, N. E. (2009). Self-administered and passive cocaine infusions produce different effects on corticosterone concentrations in the medial prefrontal cortex (MPC) of rats. Pharmacol Biochem Behav, 94(1), 163-168. doi:10.1016/j.pbb.2009.08.003

Parvaz, M. A., Moeller, S. J., & Goldstein, R. Z. (2016). Incubation of Cue-Induced Craving in Adults Addicted to Cocaine Measured by Electroencephalography. JAMA Psychiatry, 73(11), 1127-1134. doi:10.1001/jamapsychiatry.2016.2181

Pascoli, V., Terrier, J., Espallergues, J., Valjent, E., O'Connor, E. C., & Lüscher, C. (2014). Contrasting forms of cocaine-evoked plasticity control components of relapse. Nature, 509(7501), 459-464.

Paterson, N. E., & Markou, A. (2003). Increased motivation for self-administered cocaine after escalated cocaine intake. Neuroreport, 14(17), 2229-2232. doi:10.1097/01.wnr.0000091685.94870.ba

Paterson, N. E., & Markou, A. (2004). Prolonged nicotine dependence associated with extended access to nicotine self-administration in rats. Psychopharmacology, 173(1), 64-72. doi:10.1007/s00213-003-1692-7

Paulson, P. E., Camp, D. M., & Robinson, T. E. (1991). Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine

concentrations during amphetamine withdrawal in rats. Psychopharmacology (Berl), 103(4), 480-492.

Peltier, R. L., Guerin, G. F., Dorairaj, N., & Goeders, N. E. (2001). Effects of saline substitution on responding and plasma corticosterone in rats trained to self-administer different doses of cocaine. J Pharmacol Exp Ther, 299(1), 114-120.

Peoples, L. L., Kravitz, A. V., & Karine, G. (2010). Frontiers in Neuroscience

Application of Chronic Extracellular Recording Method to Studies of Cocaine Self-Administration: Method and Progress. In C. M. Kuhn & G. F. Koob (Eds.), Advances in the Neuroscience of Addiction. Boca Raton (FL): CRC Press/Taylor & Francis

Llc.

Perry, J. L., Morgan, A. D., Anker, J. J., Dess, N. K., & Carroll, M. E. (2006). Escalation of i.v. cocaine self-administration and reinstatement of cocaine-seeking behavior in rats bred for high and low saccharin intake. Psychopharmacology (Berl), 186(2), 235-245. doi:10.1007/s00213-006-0371-x

Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem, 16(5), 279-288. doi:10.1101/lm.1041309

Piechota, M., Korostynski, M., Solecki, W., Gieryk, A., Slezak, M., Bilecki, W., . . . Przewlocki, R. (2010). The dissection of transcriptional modules regulated by various drugs of abuse in the mouse striatum. Genome Biol, 11(5), R48. doi:10.1186/gb-2010-11-5-r48

Ploense, K. L., Kerstetter, K. A., Wade, M. A., Woodward, N. C., Maliniak, D., Reyes, M., … Kippin, T. E. (2013). Exposure to histone deacetylase inhibitors during Pavlovian

conditioning enhances subsequent cue-induced reinstatement of operant behavior. Behavioural Pharmacology, 24(3), 164-171.

Ploense, K. L., Vieira, P. A., Bubalo, L., Olivaria, G., Carr, A. E., Szumlinski, K. K., & Kippin, T. E. (In Press). Contributions of prolonged contingent and non-contingent cocaine exposure to escalation of cocaine intake and glutamatergic gene expression. Psychopharmacology.

Ploski, J., Monsey, M., Nguyen, T., DiLeone, R., & Schafe, G. (2011). The neuronal PAS domain protein 4 (Npas4) is required for new and reactivated fear memories. PLoS ONE, 6(8).

Ploski, J. E., Monsey, M. S., Nguyen, T., DiLeone, R. J., & Schafe, G. E. (2011). The neuronal PAS domain protein 4 (Npas4) is required for new and reactivated fear memories. PLoS ONE, 6(8), e23760. doi:10.1371/journal.pone.0023760

Pol Bodetto, S., Carouge, D., Fonteneau, M., Dietrich, J. B., Zwiller, J., & Anglard, P. (2013). Cocaine represses protein phosphatase-1Cbeta through DNA methylation and Methyl-CpG Binding Protein-2 recruitment in adult rat brain. Neuropharmacology, 73, 31-40. doi:10.1016/j.neuropharm.2013.05.005

Pomierny-Chamiolo, L., Miszkiel, J., Frankowska, M., Bystrowska, B., & Filip, M. (2017). Cocaine self-administration, extinction training and drug-induced relapse change metabotropic glutamate mGlu5 receptors expression: Evidence from radioligand binding and immunohistochemistry assays. Brain Res, 1655, 66-76. doi:10.1016/j.brainres.2016.11.014

Prast, J. M., Schardl, A., Schwarzer, C., Dechant, G., Saria, A., & Zernig, G. (2014). Reacquisition of cocaine conditioned place preference and its inhibition by previous social interaction preferentially affect D1-medium spiny neurons in the accumbens corridor. Front Behav Neurosci, 8, 317. doi:10.3389/fnbeh.2014.00317

Radley, J. J., Anderson, R. M., Cosme, C. V., Glanz, R. M., Miller, M. C., Romig-Martin, S. A., & LaLumiere, R. T. (2015). The Contingency of Cocaine Administration Accounts for Structural and Functional Medial Prefrontal Deficits and Increased Adrenocortical Activation. J Neurosci, 35(34), 11897-11910. doi:10.1523/jneurosci.4961-14.2015

Ramamoorthi, K., Fropf, R., Belfort, G. M., Fitzmaurice, H. L., McKinney, R. M., Neve, R. L., . . . Lin, Y. (2011). Npas4 regulates a transcriptional program in CA3 required for contextual memory formation. Science, 334(6063), 1669-1675. doi:10.1126/science.1208049

Renthal, W., Maze, I., Krishnan, V., Covington, H. E., 3rd, Xiao, G., Kumar, A., . . . Nestler, E. J. (2007). Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. Neuron, 56(3), 517-529. doi:10.1016/j.neuron.2007.09.032

Renthal, W., & Nestler, E. J. (2009). Chromatin regulation in drug addiction and depression. Dialogues Clin Neurosci, 11(3), 257-268.

Roberts, D. C. S., Morgan, D., & Liu, Y. (2007). How to make a rat addicted to cocaine. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 31(8), 1614-1624. doi:http://dx.doi.org/10.1016/j.pnpbp.2007.08.028

Robinson, T. E., & Kolb, B. (2004). Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology, 47, 33-46. doi:10.1016/j.neuropharm.2004.06.025

Roche, K. W., Standley, S., McCallum, J., Dune Ly, C., Ehlers, M. D., & Wenthold, R. J. (2001). Molecular determinants of NMDA receptor internalization. Nat Neurosci, 4(8), 794-802. doi:10.1038/90498

Romieu, P., Host, L., Gobaille, S., Sandner, G., Aunis, D., & Zwiller, J. (2008). Histone deacetylase inhibitors decrease cocaine but not sucrose self-administration in rats. J Neurosci, 28(38), 9342-9348. doi:10.1523/jneurosci.0379-08.2008

Ruan, H., & Yao, W.-D. (2017). Cocaine promotes coincidence detection and lowers induction threshold during Hebbian associative synaptic potentiation in prefrontal cortex. Journal of Neuroscience, 37(4), 986-997.

Rudenko, A., Dawlaty, M. M., Seo, J., Cheng, A. W., Meng, J., Le, T., . . . Tsai, L. H. (2013). Tet1 is critical for neuronal activity-regulated gene expression and memory extinction. Neuron, 79(6), 1109-1122. doi:10.1016/j.neuron.2013.08.003

Sadakierska-Chudy, A., Frankowska, M., Wydra, K., Jastrzebska, J., Miszkiel, J., & Filip, M. (2017). Increased 5-hydroxymethylation levels in the hippocampus of rat extinguished from cocaine self-administration. Hippocampus, 27(7), 811-821. doi:10.1002/hipo.22733

Schiltz, C. A., Bremer, Q. Z., Landry, C. F., & Kelley, A. E. (2007). Food-associated cues alter forebrain functional connectivity as assessed with immediate early gene and proenkephalin expression. BMC Biol, 5, 16. doi:10.1186/1741-7007-5-16

Shen, H. W., Toda, S., Moussawi, K., Bouknight, A., Zahm, D. S., & Kalivas, P. W. (2009). Altered dendritic spine plasticity in cocaine-withdrawn rats. J Neurosci, 29(9), 2876-2884. doi:10.1523/jneurosci.5638-08.2009

Shin, C. B., Serchia, M. M., Shahin, J. R., Ruppert-Majer, M. A., Kippin, T. E., & Szumlinski, K. K. (2016). Incubation of cocaine-craving relates to glutamate over-flow

within ventromedial prefrontal cortex. Neuropharmacology, 102, 103-110. doi:10.1016/j.neuropharm.2015.10.038

Shin, C. B., Templeton, T. J., Chiu, A. S., Kim, J., Gable, E. S., Vieira, P. A., . . . Szumlinski, K. K. (2017). Endogenous glutamate within the prelimbic and infralimbic cortices regulates the incubation of cocaine-seeking in rats. Neuropharmacology, 128, 293-300. doi:10.1016/j.neuropharm.2017.10.024

Shiraishi, Y., Mizutani, A., Mikoshiba, K., & Furuichi, T. (2003). Coincidence in dendritic clustering and synaptic targeting of homer proteins and NMDA receptor complex proteins NR2B and PSD95 during development of cultured hippocampal neurons. Mol Cell Neurosci, 22(2), 188-201.

Siegel, R. K. (1984). Changing patterns of cocaine use: longitudinal observations, consequences, and treatment. NIDA Res Monogr, 50, 92-110.

Smith, G. A., Rocha, E. M., McLean, J. R., Hayes, M. A., Izen, S. C., Isacson, O., & Hallett, P. J. (2014). Progressive axonal transport and synaptic protein changes correlate with behavioral and neuropathological abnormalities in the heterozygous Q175 KI mouse model of Huntington's disease. Hum Mol Genet, 23(17), 4510-4527. doi:10.1093/hmg/ddu166

Smith, M. A., Ward, S. J., & Roberts, D. C. (2008). Lesions of the dorsomedial frontal cortex block sensitization to the positive-reinforcing effects of cocaine. Pharmacol Biochem Behav, 88(3), 238-246. doi:10.1016/j.pbb.2007.08.007

Spanagel, R., & Weiss, F. (1999). The dopamine hypothesis of reward: past and current status. Trends Neurosci, 1999(22), 7.

Spiegel, I., Mardinly, A. R., Gabel, H. W., Bazinet, J. E., Couch, C. H., Tzeng, C. P., . . . Greenberg, M. E. (2014). Npas4 regulates excitatory-inhibitory balance within neural

circuits through cell-type-specific gene programs. Cell, 157(5), 1216-1229. doi:10.1016/j.cell.2014.03.058

Spyraki, C., Nomikos, G. G., Galanopoulou, P., & Daifotis, Z. (1988). Drug-induced place preference in rats with 5,7-dihydroxytryptamine lesions of the nucleus accumbens. Behav Brain Res, 29(1-2), 127-134.

Stewart, J. (1983). Conditioned and unconditioned drug effects in relapse to opiate and stimulant drug self-adminstration. Prog Neuropsychopharmacol Biol Psychiatry, 7(4-6), 591-597.

Stroud, H., Feng, S., Morey Kinney, S., Pradhan, S., & Jacobsen, S. E. (2011). 5-Hydroxymethylcytosine is associated with enhancers and gene bodies in human embryonic stem cells. Genome Biol, 12(6), R54. doi:10.1186/gb-2011-12-6-r54

Su, Z. I., Wenzel, J., Ettenberg, A., & Ben-Shahar, O. (2014). Prior extended daily access to cocaine elevates the reward threshold in a conditioned place preference test. Addict Biol, 19(5), 826-837. doi:10.1111/adb.12053

Sun, J., Wang, L., Jiang, B., Hui, B., Lv, Z., & Ma, L. (2008). The effects of sodium butyrate, an inhibitor of histone deacetylase, on the cocaine- and sucrose-maintained self-administration in rats. Neurosci Lett, 441(1), 72-76. doi:10.1016/j.neulet.2008.05.010

Sun, X., & Lin, Y. (2016). Npas4: Linking Neuronal Activity to Memory. Trends Neurosci, 39(4), 264-275. doi:10.1016/j.tins.2016.02.003

Swanson, C. J., Baker, D. A., Carson, D., Worley, P. F., & Kalivas, P. W. (2001). Repeated cocaine administration attenuates group I metabotropic glutamate receptor-mediated glutamate release and behavioral activation: a potential role for Homer. J Neurosci, 21(22), 9043-9052.

Sweatt, J. D. (2016). Neural plasticity and behavior - sixty years of conceptual advances. J Neurochem, 139 Suppl 2, 179-199. doi:10.1111/jnc.13580

Sweatt, J. D., Meaney, M. J., Nestler, E. J., & Akbarian, S. (2013). Epigenetic Regulation in the Nervous System (1 ed.). Oxford, UK: Elsevier Inc. .

Swinford-Jackson, S. E., Anastasio, N. C., Fox, R. G., Stutz, S. J., & Cunningham, K. A. (2016). Incubation of cocaine cue reactivity associates with neuroadaptations in the cortical serotonin (5-HT) 5-HT2C receptor (5-HT2CR) system. Neuroscience, 324, 50-61. doi:10.1016/j.neuroscience.2016.02.052

Szumlinski, K. K., Abernathy, K. E., Oleson, E. B., Klugmann, M., Lominac, K. D., He, D. Y., . . . Kalivas, P. W. (2006). Homer isoforms differentially regulate cocaine-induced neuroplasticity. Neuropsychopharmacology, 31(4), 768-777. doi:10.1038/sj.npp.1300890

Szumlinski, K. K., Ary, A. W., & Lominac, K. D. (2008). Homers regulate drug-induced neuroplasticity: Implications for addiction. Biochem Pharmacol, 75(1), 112-133. doi:10.1016/j.bcp.2007.07.031

Szumlinski, K. K., Dehoff, M. H., Kang, S. H., Frys, K. A., Lominac, K. D., Klugmann, M., . . . Kalivas, P. W. (2004). Homer proteins regulate sensitivity to cocaine. Neuron, 43(3), 401-413. doi:10.1016/j.neuron.2004.07.019

Szumlinski, K. K., Toda, S., Middaugh, L. D., Worley, P. F., & Kalivas, P. W. (2003). Evidence for a relationship between Group 1 mGluR hypofunction and increased cocaine and ethanol sensitivity in Homer2 null mutant mice. Ann N Y Acad Sci, 1003, 468-471.

Szumlinski, K. K., Wroten, M. G., Miller, B. W., Sacramento, A. D., Cohen, M., Ben-Shahar, O., & Kippin, T. E. (2016). Cocaine Self-Administration Elevates GluN2B within dmPFC Mediating Heightened Cue-Elicited Operant Responding. J Drug Abuse, 2(2).

Szwagierczak, A., Bultmann, S., Schmidt, C. S., Spada, F., & Leonhardt, H. (2010). Sensitive enzymatic quantification of 5-hydroxymethylcytosine in genomic DNA. Nucleic Acids Res, 38(19), e181. doi:10.1093/nar/gkq684

Tang, W., Wesley, M., Freeman, W. M., Liang, B., & Hemby, S. E. (2004). Alterations in ionotropic glutamate receptor subunits during binge cocaine self-administration and withdrawal in rats. J Neurochem, 89(4), 1021-1033. doi:10.1111/j.1471-4159.2004.02392.x

Tang, W. X., Fasulo, W. H., Mash, D. C., & Hemby, S. E. (2003). Molecular profiling of midbrain dopamine regions in cocaine overdose victims. J Neurochem, 85(4), 911-924.

Taniguchi, M., Carreira, M. B., Cooper, Y. A., Bobadilla, A. C., Heinsbroek, J. A., Koike, N., . . . Cowan, C. W. (2017). HDAC5 and Its Target Gene, Npas4, Function in the Nucleus Accumbens to Regulate Cocaine-Conditioned Behaviors. Neuron, 96(1), 130-144.e136. doi:10.1016/j.neuron.2017.09.015

Thiels, E., Xie, X., Yeckel, M. F., Barrionuevo, G., & Berger, T. W. (1996). NMDA receptor-dependent LTD in different subfields of hippocampus in vivo and in vitro. Hippocampus, 6(1), 43-51. doi:10.1002/(SICI)1098-1063(1996)6:1<43::AID-HIPO8>3.0.CO;2-8

Tian, W., Wang, J., Zhang, K., Teng, H., Li, C., Szyf, M., . . . Zhao, M. (2016). Demethylation of c-MYB binding site mediates upregulation of Bdnf IV in cocaine-conditioned place preference. Sci Rep, 6, 22087. doi:10.1038/srep22087

Tian, W., Zhao, M., Li, M., Song, T., Zhang, M., Quan, L., . . . Sun, Z. S. (2012). Reversal of cocaine-conditioned place preference through methyl supplementation in mice: altering

global DNA methylation in the prefrontal cortex. PLoS ONE, 7(3), e33435. doi:10.1371/journal.pone.0033435

Tran-Nguyen, L. T., Fuchs, R. A., Coffey, G. P., Baker, D. A., O'Dell, L. E., & Neisewander, J. L. (1998). Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacology, 19(1), 48-59. doi:10.1016/s0893-133x(97)00205-4

Tronson, N. C., & Taylor, J. R. (2013). Addiction: a drug-induced disorder of memory reconsolidation. Curr Opin Neurobiol, 23(4), 573-580. doi:10.1016/j.conb.2013.01.022

Twining, R. C., Bolan, M., & Grigson, P. S. (2009). Yoked delivery of cocaine is aversive and protects against the motivation for drug in rats. Behav Neurosci, 123(4), 913-925. doi:10.1037/a0016498

Tzschentke, T. M. (2000). The medial prefrontal cortex as a part of the brain reward system. Amino Acids, 19(1), 211-219.

Verdejo-Garcia, A., Clark, L., Verdejo-Roman, J., Albein-Urios, N., Martinez-Gonzalez, J. M., Gutierrez, B., & Soriano-Mas, C. (2015). Neural substrates of cognitive flexibility in cocaine and gambling addictions. Br J Psychiatry, 207(2), 158-164. doi:10.1192/bjp.bp.114.152223

Voorn, P., Vanderschuren, L. J., Groenewegen, H. J., Robbins, T. W., & Pennartz, C. M. (2004). Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci, 27(8), 468-474. doi:10.1016/j.tins.2004.06.006

Waddington, C. (1939). Development as an epigenetic process. An introduction to modern genetics.

Wang, H., & Peng, R. Y. (2016). Basic roles of key molecules connected with NMDAR signaling pathway on regulating learning and memory and synaptic plasticity. Mil Med Res, 3(1). doi:10.1186/s40779-016-0095-0

Wang, Z. J., Martin, J. A., Mueller, L. E., Caccamise, A., Werner, C. T., Neve, R. L., . . . Dietz, D. M. (2016). BRG1 in the Nucleus Accumbens Regulates Cocaine-Seeking Behavior. Biol Psychiatry, 80(9), 652-660. doi:10.1016/j.biopsych.2016.04.020

Wee, S., Orio, L., Ghirmai, S., Cashman, J. R., & Koob, G. F. (2009). Inhibition of kappa opioid receptors attenuated increased cocaine intake in rats with extended access to cocaine. Psychopharmacology, 205(4), 565-575. doi:10.1007/s00213-009-1563-y

Wee, S., Specio, S. E., & Koob, G. F. (2007). Effects of dose and session duration on cocaine self-administration in rats. J Pharmacol Exp Ther, 320(3), 1134-1143. doi:10.1124/jpet.106.113340

Wei, W., Coelho, C. M., Li, X., Marek, R., Yan, S., Anderson, S., . . . Bredy, T. W. (2012). p300/CBP-associated factor selectively regulates the extinction of conditioned fear. J Neurosci, 32(35), 11930-11941. doi:10.1523/jneurosci.0178-12.2012

Weissenborn, R., Deroche, V., Koob, G. F., & Weiss, F. (1996). Effects of dopamine agonists and antagonists on cocaine-induced operant responding for a cocaine-associated stimulus. Psychopharmacology (Berl), 126(4), 311-322.

Will, M. J., Der-Avakian, A., Bland, S. T., Grahn, R. E., Hammack, S. E., Sparks, P. D., . . . Maier, S. F. (2004). Electrolytic lesions and pharmacological inhibition of the dorsal raphe nucleus prevent stressor potentiation of morphine conditioned place preference in rats. Psychopharmacology (Berl), 171(2), 191-198. doi:10.1007/s00213-003-1572-1

Williams, J. M., Guevremont, D., Kennard, J. T., Mason-Parker, S. E., Tate, W. P., & Abraham, W. C. (2003). Long-term regulation of N-methyl-D-aspartate receptor subunits and associated synaptic proteins following hippocampal synaptic plasticity. Neuroscience, 118(4), 1003-1013.

Willuhn, I., Burgeno, L. M., Everitt, B. J., & Phillips, P. E. (2012). Hierarchical recruitment of phasic dopamine signaling in the striatum during the progression of cocaine use. Proc Natl Acad Sci U S A, 109(50), 20703-20708. doi:10.1073/pnas.1213460109

Wise, R. (2004). Dopamine, learning and motivation. Nature reviews. Neuroscience, 5(ec5affba-3d9e-4cba-ed20-d274f2382e41), 483-577. doi:10.1038/nrn1406

Witten, I. B., Lin, S. C., Brodsky, M., Prakash, R., Diester, I., Anikeeva, P., . . . Deisseroth, K. (2010). Cholinergic interneurons control local circuit activity and cocaine conditioning. Science, 330(6011), 1677-1681. doi:10.1126/science.1193771

Wolffe, A. P., & Matzke, M. A. (1999). Epigenetics: regulation through repression. Science, 286(5439), 481-486.

Woolverton, W. L. (1992). Determinants of cocaine self-administration by laboratory animals. Ciba Found Symp, 166, 149-161; discussion 161-144.

Wright, K. N., Hollis, F., Duclot, F., Dossat, A. M., Strong, C. E., Francis, T. C., . . . Kabbaj, M. (2015). Methyl supplementation attenuates cocaine-seeking behaviors and cocaine-induced c-Fos activation in a DNA methylation-dependent manner. J Neurosci, 35(23), 8948-8958. doi:10.1523/jneurosci.5227-14.2015

Wu, H., & Zhang, Y. (2011). Mechanisms and functions of Tet protein-mediated 5-methylcytosine oxidation. Genes & development, 25(23), 2436-2452.

Wu, X., & Zhang, Y. (2017). TET-mediated active DNA demethylation: mechanism, function and beyond. Nat Rev Genet, 18(9), 517-534. doi:10.1038/nrg.2017.33

Xiao, B., Tu, J. C., Petralia, R. S., Yuan, J. P., Doan, A., Breder, C. D., . . . Worley, P. F. (1998). Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins. Neuron, 21(4), 707-716.

Yao, W. D., Gainetdinov, R. R., Arbuckle, M. I., Sotnikova, T. D., Cyr, M., Beaulieu, J. M., . . . Caron, M. G. (2004). Identification of PSD-95 as a regulator of dopamine-mediated synaptic and behavioral plasticity. Neuron, 41(4), 625-638.

Yoshimochi, K., Daitoku, H., & Fukamizu, A. (2010). PCAF represses transactivation function of FOXO1 in an acetyltransferase-independent manner. J Recept Signal Transduct Res, 30(1), 43-49. doi:10.3109/10799890903517947

Yu, F., Zhong, P., Liu, X., Sun, D., Gao, H. Q., & Liu, Q. S. (2013). Metabotropic glutamate receptor I (mGluR1) antagonism impairs cocaine-induced conditioned place preference via inhibition of protein synthesis. Neuropsychopharmacology, 38(7), 1308-1321. doi:10.1038/npp.2013.29

Zavala, A. R., Weber, S. M., Rice, H. J., Alleweireldt, A. T., & Neisewander, J. L. (2003). Role of the prelimbic subregion of the medial prefrontal cortex in acquisition, extinction, and reinstatement of cocaine-conditioned place preference. Brain Res, 990(1-2), 157-164.

Zeilinger, S., Kuhnel, B., Klopp, N., Baurecht, H., Kleinschmidt, A., Gieger, C., . . . Illig, T. (2013). Tobacco smoking leads to extensive genome-wide changes in DNA methylation. PLoS ONE, 8(5), e63812. doi:10.1371/journal.pone.0063812

Zhang, J., Vinuela, A., Neely, M. H., Hallett, P. J., Grant, S. G., Miller, G. M., . . . Yao, W. D. (2007). Inhibition of the dopamine D1 receptor signaling by PSD-95. J Biol Chem, 282(21), 15778-15789. doi:10.1074/jbc.M611485200

Zhang, S. J., Zou, M., Lu, L., Lau, D., Ditzel, D. A., Delucinge-Vivier, C., . . . Bading, H. (2009). Nuclear calcium signaling controls expression of a large gene pool: identification of a gene program for acquired neuroprotection induced by synaptic activity. PLoS Genet, 5(8), e1000604. doi:10.1371/journal.pgen.1000604

Zhao, J., Bao, A. M., Qi, X. R., Kamphuis, W., Luchetti, S., Lou, J. S., & Swaab, D. F. (2012). Gene expression of GABA and glutamate pathway markers in the prefrontal cortex of non-suicidal elderly depressed patients. J Affect Disord, 138(3), 494-502. doi:10.1016/j.jad.2012.01.013