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# 药物成瘾诱导相关大脑核团功能和行为改变的 DNA 甲基化机制*

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**摘 要** 药物成瘾会导致相关神经环路的结构和功能长期改变。大量新的研究证据表明, 在 DNA 序列不变的情况下, 药物成瘾可通过影响不同亚型 DNA 甲基转移酶(DNMTs)的表达, 使脑内多个相关核团发生 DNA 甲基化以及基因表达的改变, 进而导致神经元功能的可塑性变化。因此, DNA 甲基化被视作导致成瘾行为长期存在的可能机制之一。结合近几年来的重要发现, 本文将重点讨论相关脑区的 DNA 甲基化在成瘾行为发生发展过程中的作用, 以及成瘾药物影响 DNA 甲基化水平的可能机制, 并试图提出可深入的研究展望。

**关键词** 药物成瘾; DNA 甲基化; DNA 甲基转移酶(DNMTs)

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药物成瘾是一种反复发作的慢性脑疾病(Bloom, 1997)。大量证据提示, 成瘾药物可使脑内相关神经环路的结构和功能发生长时程改变, 这是成瘾行为长期存在的主要原因。神经环路的长时程改变需要染色体结构和基因表达的稳定变化, 表观遗传学改变可能是其内在机制。表观遗传学主要包括 DNA 甲基化、组蛋白修饰(包括乙酰化、甲基化、磷酸化、泛素化和苏素化)、染色体重塑和非编码 RNA (如 RNAi)等多种机制(Delcuve, Rastegar, & Davie, 2009), 其中 DNA 甲基化(DNA methylation)是相对稳定的一种, 常发生于 CpG 二核苷酸部位胞嘧啶的 C5 位置, 可以通过调控相关基因的永久沉默(Cavalli, 2006; Feng et al., 2006), 进而使得相关表型得到长期保持(Hyman, Malenka, & Nestler, 2006)。所以与其他表观遗传学修饰相比, DNA 甲基化可能在生命活动的长时程改变中发挥更重要的作用。之前对 DNA 甲基化的研究主要关注影响发育和癌症发生的机制, 而近年来发现, DNA 的甲基化还参与学习和长时程记忆的形成(Miller & Sweatt, 2007), 并可能在抑郁和药物成瘾等精神疾病中发挥重要作用(Tsankova, Renthal, Kumar, & Nestler, 2007)。

药物成瘾机制涉及奖赏、动机、情绪、学习记忆和执行功能等多种神经生物学过程。奖赏效应是人和动物反复摄取成瘾药物的主要原因之一(Volkow, Wang, Fowler, & Tomasi, 2012)。在药物奖赏过程中, 快感情绪的体验可与伴随的相关条件刺激匹配, 这种匹配被反复强化后存储在记忆系统中, 形成成瘾相关的长期记忆。奖赏伴随的快感情绪体验被认为是诱导成瘾形成或进而诱发复吸的最关键因素, 而撤药后引起的负性情绪体验也是驱动复吸的另一个重要原因(Koob, Caine, Parsons, Markou, & Weiss, 1997; Koob, Stinus, Le Moal, & Bloom, 1989)。本文将概述 DNA 甲基化在调节药物奖赏、成瘾情绪和记忆等相关核团功能活动的研究进展, 为药物成瘾机理的研究或研发新的干预方法拓展视角。

## 1 伏隔核的奖赏效应与 DNMTs 调控

大多数成瘾性药物主要是通过激活中脑边缘

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系统(mesolimbic system, MLS)的腹侧被盖区(ventral tegmental area, VTA)内多巴胺神经元群, 经其主要传出投射引起伏隔核(nucleus accumbens, NAc)内多巴胺的释放增加, 从而产生愉悦感, 以实现奖赏效应。除了 VTA 至 NAc 的投射通路以外, 脑内奖赏系统(reward system)还包括了参与该通路调节的杏仁核、海马以及内侧前额叶皮层(medial prefrontal cortex, mPFC)等多个核团或脑区。其中, VTA 和 NAc 是参与奖赏的关键核团。成瘾药物可以导致 NAc 发生突触可塑性的长期改变(Alcantara et al., 2011; Kasanetz et al., 2010), 染色质重塑(Chromatin remodeling)可能是其内在机制(Kumar et al., 2005)。

成瘾药物可以影响 NAc 中 DNA 的甲基化过程。催化 DNA 发生甲基化的 DNA 甲基转移酶(DNMTs)有 DNMT1、DNMT3a 和 DNMT3b 这 3 种亚型(Foulks et al., 2012)。有研究表明, 急性可卡因处理可导致 NAc 内神经元 DNA 甲基化(Anier, Malinovskaja, Aonurm-Helm, Zharkovsky, & Kalda, 2010), DNMT1 的表达下调(LaPlant et al., 2010), 但 DNMT3a 与 DNMT3b 表达水平的变化尚无定论(Anier et al., 2010; LaPlant et al., 2010); 而可卡因慢性处理及自身给药都可以降低 NAc 中 DNMT3a 的表达, 但 DNMT3b 不受影响(LaPlant et al., 2010)。

DNA 甲基化还可能介导慢性可卡因处理导致的 NAc 神经元树突棘数量的显著增加(LaPlant et al., 2010)。也有实验证实, 抑制 NAc 的 DNMTs 可影响可卡因引起的行为敏化, 并且表现出更强的条件位置偏爱(CPP)效应(Anier et al., 2010; LaPlant et al., 2010)。

目前, 阿片类药物成瘾与 DNA 甲基化相关性的研究还较少。不过最近 Tian 等人的工作表明, 甲基供体甲硫氨酸的处理可抑制可卡因 CPP 的获得但不能影响吗啡 CPP 的获得(Tian et al., 2012), 提示阿片类药物成瘾可能和精神兴奋剂类药物成瘾有不同的 DNA 甲基化调节机制。

## 2 正负情绪调控 DNA 甲基化

药物成瘾者在停止用药后会产生负性情绪状态(Koob & Nestler, 1997; Miller & Sweatt, 2007), 包括烦躁不安、抑郁、易激怒和焦虑等。据 Wager 等人的研究, 觅药行为带来的欣快感由 NAc 调节通路介导, 而消极情绪反应则由杏仁核来调节(Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008)。

急性或慢性可卡因处理都可以诱导 NAc 中的 DNMT3a 的表达降低(LaPlant et al., 2010), 可卡因戒断后, NAc 中 DNMT3a 表达增加(LaPlant et al., 2010), 提示成瘾药物导致的正负性情绪可能双向调节 DNA 甲基化水平。到目前为止, 阐释上述观点的直接证据仍然很少。然而, 对应激、抑郁样行为、恐惧和创伤后应激障碍(post-traumatic stress disorder, PTSD)等负性情绪导致的 DNA 甲基化的影响的研究可以提供一些间接的证据。

有大量的证据表明, 应激可以通过负性情绪机制增加实验动物对可卡因奖赏效应的敏感性(Covington et al., 2005; Prasad, Ulibarri, & Sorg, 1998; Schindler, Li, & Chavkin, 2010; Sorg & Kalivas, 1991; Tidey & Miczek, 1997), 并且应激可以引发负性情绪并诱导复吸(Shaham, Erb, & Stewart, 2000)。LaPlant 等人用社交挫败模型发现, 长期应激导致的抑郁样行为也可诱导 NAc 内 DNMT3a 的过表达, 表明 NAc 的甲基化对应激刺激导致的细胞和行为可塑性具有重要的作用(LaPlant et al., 2010), 抑郁症患者中也检测到了 DNMT1 表达的降低和 DNMT3b 表达的升高(Higuchi et al., 2011); DNMT 的抑制剂也可以导致抗抑郁行为(Sales et al., 2011)。还有研究表明, 酒精暴露可以使杏仁核一些基因的表达发生选择性改变(D'Addario et al., 2012), 提示成瘾与杏仁核 DNA 甲基化的相关性不容忽视。Megumi 等人的研究也表明, 甲基化 DNA 的结合蛋白 MeCP2 能增强基底外侧杏仁核(basolateral amygdala, BLA)调控的焦虑情绪(Adachi, Autry, Covington, & Monteggia, 2009)。情景恐惧条件化(contextual fear conditioning)会伴随大鼠海马中 DNMT 表达的上调(Miller & Sweatt, 2007), BDNF 转录及其 DNA 甲基化的变化可持续 24 小时(Mizuno, Dempster, Mill, & Giese, 2012), PTSD 样的大鼠中也发现海马部位甲基化的改变(Chertkow-Deutsher, Cohen, Klein, & Ben-Shachar, 2010)。在听觉恐惧条件反射(auditory Pavlovian fear conditioning)中也发现外侧杏仁核(lateral amygdala, LA)中 DNMT3A 表达会增加(Monsey, Ota, Akingbade, Hong, & Schafe, 2011)。综上表明, 药物成瘾可能通过引起

正负性情绪进而影响 DNA 的甲基化。

## 3　海马的甲基化强化成瘾记忆的获得和保持

成瘾相关的记忆的异常保持是导致复吸的重要原因之一。已有证据表明, 可卡因可以通过调节海马部位的甲基转移酶活动进而影响其突触可塑性(Krasnova et al., 2008; Levenson et al., 2006; Marie-Claire et al., 2003); 而成瘾药物使海马的突触效能降低可能导致了成瘾记忆被长久保持(Han et al., 2010; Miguens et al., 2011; Yang et al., 2004)。DNA 甲基化介导了 LTP (长时程增强)和 LTD (长时程抑制)的改变(Bailey, Kandel, & Si, 2004; Levenson et al., 2006; Sweatt, 2010), 这是成瘾异常记忆持续存在的重要机制(Han et al., 2010)。

近年来, 有一系列的证据表明, DNA 甲基化在海马依赖的记忆形成过程中具有重要作用(Feng et al., 2010; Miller & Sweatt, 2007), 抑制 DNMTs 可以显著抑制海马的突触传递功能(Nielsen et al., 2009)和 LTP(Feng et al., 2010), DNMT1 和 DNMT3a 介导了该过程(Feng et al., 2010); 海马的 DNA 甲基化还参与了记忆的获得和长时记忆的加工过程(Miller & Sweatt, 2007), CA1 还参与记忆的巩固过程(Daumas, Halley, Francés, & Lassalle, 2005)。本实验室先前的工作也表明, 海马的DNA甲基化还调控药物成瘾记忆的获取和保持, 而成瘾记忆的提取则依赖于 mPFC 而不是海马中 DNMTs 的活动(Han et al., 2010)。

还有研究表明, 在恐惧记忆的巩固中, 增加海马的 DNA 甲基化起到非常关键的作用(Miller & Sweatt, 2007)。我们的研究也发现, 海马 CA1 区 DNA 的甲基化对于成瘾记忆的巩固和维持也是必须的(数据待发表)。这些证据提示, 海马的 DNA 甲基化介导了成瘾记忆的形成、保持和提取过程的突触可塑性改变。

## 4　成瘾药物改变 DNA 甲基化的潜在分子机制

已有大量研究证明 DNA 甲基化在成瘾药物诱导核团功能发生长时程改变的过程中发挥重要作用。DNMT1 作为 cAMP 反应元件结合蛋白(cAMP-responsive element binding protein, CREB)的靶点, 其转录可能受到 CREB 的直接调控(Impey et al., 2004), 而 CREB 也可以通过另一个作用靶点 Sp1 (Impey et al., 2004), 来间接调节 DNMT 的表达(Kinney & Pradhan, 2011)。转录因子 CREB 可能是重要的调控因子。可卡因、安非他明和吗啡都可以增加 NAc 内 cAMP (环磷酸腺苷)水平, 并激活蛋白激酶 A (protein kinase A, PKA), 使 CREB 的磷酸化增加; 对于兴奋剂类药物如可卡因, 还可以通过 MAPK (促分裂原活化蛋白激酶)或是 CaMK (钙调蛋白激酶)途径激活 CREB (Nestler, 2004; Renthal & Nestler, 2008)。

成瘾性药物还可能通过影响组蛋白的乙酰化来调控 DNA 甲基化。可卡因或安非他明通过 CaMK 途径不仅可以磷酸化 CREB, 还会磷酸化组蛋白去乙酰酶 5 (HDAC5), 慢性酒精处理后的戒断则可以抑制 HDAC 的活性(Renthal & Nestler, 2008), 而 HDAC 的抑制剂可以下调 DNMT1 的水平(You et al., 2008), 降低 DNMT3B 的 mRNA 的稳定性(Xiong et al., 2005), 并诱导 DNA 的去甲基化(Hu et al., 2000; Selker, 1998)。此外, 由于 DNMT 的转录依赖于 Rb/E2F 通路(Kinney & Pradhan, 2011), 而 E2F 与 HDAC 存在相互作用(Singh, Johnson, & Chellappan, 2010), 所以 HDAC 也可能通过 E2F 来调控 DNMT 的表达。

DNMT 的表达也会受到小的非编码 RNA——MicroRNAs (miRNAs)的调节, miR-148, miR-152 和 miR-29 家族都可以直接调控 DNMT 的表达(Braconi, Huang, & Patel, 2010; Fabbri et al., 2007)。最近有观点认为成瘾性药物通过 miRNA 来改变基因表达(Duncan, 2012)。虽然有证据表明可卡因对 miR-8、miR-124, miR-132 和 miR-212 的影响会作用于 CREB (Chandrasekar & Dreyer, 2009; Eipper-Mains et al., 2011; Hollander et al., 2010; Vo et al., 2005), 但是慢性酒精和尼古丁处理可以改变 miR-152 及 miR-29 的表达水平这一结果则提示成瘾性药物也可能通过 miRNAs 来直接调节 DNA 的甲基化(Guo, Chen, Carreon, & Qiang, 2012; Li & van der Vaart, 2011)。

DNMTs 的表达发生改变后, 可以调控多种药物成瘾相关基因的甲基化。有研究表明可卡因处理可以导致蛋白质磷酸酶 1 的催化亚基(protein phosphatase-1 catalytic subunit, PP1c)启动子区域

DNA 甲基化增加, 相反 fosB 启动子处的甲基化降低, fosB 的转录上调(Anier et al., 2010), 多巴胺转运体基因 DAT (Nieratschker et al., 2012)、神经激肽 3 受体(neurokinin3-receptor, NK3-R)基因 TACR3 也可能受到影响(Barros et al., 2011), 最终影响到成瘾行为。

## 5 研究展望

DNA 甲基化是成瘾机制研究近年来关注的热点。深入研究 DNA 甲基化机制有助于理解药物成瘾行为长期性改变的病理基础。

以下几点展望值得期待：1)目前的研究仍然主要集中在 NAc 和海马等核团, 中脑边缘系统中其他重要核团有待深入, 比如与成瘾行为密切相关的 VTA、杏仁核等重要结构; 2)不同药物导致的甲基化机制仍需进一步了解, 例如, 阿片类药物成瘾的 DNA 甲基化机制还不清楚; 3)某些成瘾相关重要基因的甲基化如何影响药物成瘾行为, 例如, 多巴胺转运体 DAT 基因、fosB 基因, 也需要更多的探索; 4)DNA 的甲基化与组蛋白的修饰并不是孤立存在的, 二者之间存在复杂的相互作用关系(Ooi et al., 2007; Robertson & Wolffe, 2000), 同时, 对组蛋白修饰的研究也将有助于全面理解 DNA 甲基化在药物成瘾中的作用; 5)DNA 的主动去甲基化在哺乳动物中似乎也是存在的(Kouzmenko, Ohtake, Fujiki, & Kato, 2010), 去甲基化机制研究应该被重视; 6)成瘾药物盗用了自然奖赏通路, 产生更强的奖赏效应。那么, 自然奖赏物如美食、性等刺激, 如何影响基因和染色体修饰还需要更多的研究。

## 参考文献


Adachi, M., Autry, A. E., Covington, H. E., III., & Monteggia, L. M. (2009). MeCP2-mediated transcription repression in the basolateral amygdala may underlie heightened anxiety in a mouse model of rett syndrome. *Journal of Neuroscience, 29*(13), 4218–4227.

Alcantara, A. A., Lim, H. Y., Floyd, C. E., Garces, J., Mendenhall, J. M., Lyons, C. L., & Berlanga, M. L. (2011). Cocaine- and morphine-induced synaptic plasticity in the nucleus accumbens. *Synapse, 65*(4), 309–320.

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.

Bailey, C. H., Kandel, E. R., & Si, K. S. (2004). The persistence of long-term memory: A molecular approach to self-sustaining changes in learning-induced synaptic growth. *Neuron, 44*(1), 49–57.

Barros, M., Dempster, E. L., Illott, N., Chabrawi, S., Maior, R. S., Tomaz, C.,... Müller, C. P. (2011). Decreased methylation of the NK3 receptor coding gene (TACR3) after cocaine-induced place preference in marmoset monkeys. *Addiction Biology, 18*, 452–454.

Bloom, F. E. (1997). The science of substance abuse. *Science, 278*(5335), 15.

Braconi, C., Huang, N., & Patel, T. (2010). MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology, 51*(3), 881–890.

Cavalli, G. (2006). Chromatin and epigenetics in development: Blending cellular memory with cell fate plasticity. *Development, 133*(11), 2089–2094.

Chandrasekar, V., & Dreyer, J. L. (2009). MicroRNAs miR-124, let-7d and miR-181a regulate cocaine-induced plasticity. *Molecula and Cellular Neuroscience, 42*(4), 350–362.

Chertkow-Deutsher, Y., Cohen, H., Klein, E., & Ben-Shachar, D. (2010). DNA methylation in vulnerability to post-traumatic stress in rats: Evidence for the role of the post-synaptic density protein Dlgap2. *International Journal of Neuropsychopharmacology, 13*(3), 347–359.

Covington, H. E., Kikusui, T., Goodhue, J., Nikulina, E. M., Hammer, R. P., & Miczek, K. A. (2005). Brief social defeat stress: Long lasting effects on cocaine taking during a binge and *Zif268* mRNA expression in the amygdala and prefrontal cortex. *Neuropsychopharmacology, 30*(2), 310–321.

D'Addario, C., Caputi, F. F., Ekstrom, T. J., Di Benedetto, M., Maccarrone, M., Romualdi, P., & Candeletti, S. (2013). Ethanol induces epigenetic modulation of prodynorphin and pronociceptin gene expression in the rat amygdala complex. *Journal of Molecular Neuroscience, 49*, 312–319.

Daumas, S., Halley, H., Francés, B., & Lassalle, J. M. (2005). Encoding, consolidation, and retrieval of contextual memory: Differential involvement of dorsal CA3 and CA1 hippocampal subregions. *Learning & Memory, 12*(4), 375–382.

Delcuve, G. P., Rastegar, M., & Davie, J. R. (2009). Epigenetic control. *Journal of Cellular Physiology, 219*(2), 243–250.

Duncan, J. R. (2012). Current perspectives on the neurobiology of drug addiction: A focus on genetics and factors regulating gene expression. *ISRN Neurology, 2012*,

972607.

Eipper-Mains, J. E., Kiraly, D. D., Palakodeti, D., Mains, R. E., Eipper, B. A., & Graveley, B. R. (2011). MicroRNA-Seq reveals cocaine-regulated expression of striatal microRNAs. *RNA, 17*(8), 1529–1543.

Fabbri, M., Garzon, R., Cimmino, A., Liu, Z. F., Zanesi, N., Callegari, E.,... Croce, C. M. (2007). MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proceedings of the National Academy of Sciences of the United States of America, 104*(40), 15805–15810.

Feng, J., Zhou, Y., Campbell, S. L., Le, T., Li, E., Sweatt, J. D.,... Fan, G. P. (2010). Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nature Neuroscience, 13*(4), 423–430.

Feng, Y. Q., Desprat, R., Fu, H., Olivier, E., Lin, C. M., Lobell, A.,... Bouhassira, E. E. (2006). DNA methylation supports intrinsic epigenetic memory in mammalian cells. *PLoS Genetics, 2*(4), e65.

Foulks, J. M., Parnell, K. M., Nix, R. N., Chau, S., Swierczek, K., Saunders, M.,... Kanner, S. B. (2012). Epigenetic drug discovery: Targeting DNA methyltransferases. *Journal of Biomolecular Screening, 17*(1), 2–17.

Guo, Y. Q., Chen, Y. X., Carreon, S., & Qiang, M. (2012). Chronic intermittent ethanol exposure and its removal induce a different miRNA expression pattern in primary cortical neuronal cultures. *Alcoholism: Clinical and Experimental Research, 36*(6), 1058–1066.

Han, J., Li, Y. H., Wang, D. M., Wei, C. G., Yang, X. Y., & Sui, N. (2010). Effect of 5-aza-2-deoxycytidine microinjecting into hippocampus and prelimbic cortex on acquisition and retrieval of cocaine-induced place preference in C57BL/6 mice. *European Journal of Pharmacology, 642*(1-3), 93–98.

Higuchi, F., Uchida, S., Yamagata, H., Otsuki, K., Hobara, T., Abe, N.,Watanabe, Y. (2011). State-dependent changes in the expression of DNA methyltransferases in mood disorder patients. *Journal of Psychiatric Research, 45*(10), 1295–1300.

Hollander, J. A., Im, H. I., Amelio, A. L., Kocerha, J., Bali, P., Lu, Q., Kenny, P. J. (2010). Striatal microRNA controls cocaine intake through CREB signalling. *Nature, 466*(7303), 197–202.

Hu, J. F., Pham, J., Dey, I., Li, T., Vu, T. H., & Hoffman, A. R. (2000). Allele-specific histone acetylation accompanies genomic imprinting of the insulin-like growth factor II receptor gene. *Endocrinology, 141*(12), 4428–4435.

Hyman, S. E., Malenka, R. C., & Nestler, E. J. (2006). Neural mechanisms of addiction: The role of reward-related learning and memory. *Annual Review of Neuroscience, 29*, 565–598.

Impey, S., McCorkle, S. R., Cha-Molstad, H., Dwyer, J. M., Yochum, G. S., Boss, J. M., Goodman, R. H. (2004). Defining the CREB regulon: A genome-wide analysis of transcription factor regulatory regions. *Cell, 119*(7), 1041–1054.

Kasanetz, F., Deroche-Gamonet, V., Berson, N., Balado, E., Lafourcade, M., Manzoni, O., & Piazza, P. V. (2010). Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science, 328*(5986), 1709–1712.

Kinney, S. R. M., & Pradhan, S. (2011). Regulation of expression and activity of DNA (cytosine-5) methyltransferases in mammalian cells. *Progress in Molecular Biology and Translational Science, 101*, 311–333.

Koob, G. F., Caine, S. B., Parsons, L., Markou, A., & Weiss, F. (1997). Opponent process model and psychostimulant addiction. *Pharmacology Biochemistry and Behavior, 57*(3), 513–521.

Koob, G. F., & Nestler, E. J. (1997). The neurobiology of drug addiction. *Journal of Neuropsychiatry and Clinical Neurosciences, 9*(3), 482–497.

Koob, G. F., Stinus, L., Le Moal, M., & Bloom, F. E. (1989). Opponent process theory of motivation: Neurobiological evidence from studies of opiate dependence. *Neuroscience & Biobehavioral Reviews, 13*(2-3), 135–140.

Kouzmenko, A., Ohtake, F., Fujiki, R., & Kato, S. (2010). Hormonal gene regulation through DNA methylation and demethylation. *Epigenomics, 2*(6), 765–774.

Krasnova, I. N., Li, S. M., Wood, W. H., McCoy, M. T., Prabhu, V. V., Becker, K. G., Cadet, J. L. (2008). Transcriptional responses to reinforcing effects of cocaine in the rat hippocampus and cortex. *Genes, Brain and Behavior, 7*(2), 193–202.

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

LaPlant, Q., Vialou, V., Covington, H. E., Dumitriu, D., Feng, J. A., Warren, B. L., Nestler, E. J. (2010). Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nature Neuroscience, 13*(9), 1137–1143.

Levenson, J. M., Roth, T. L., Lubin, F. D., Miller, C. A., Huang, I. C., Desai, P., Sweatt, J. D. (2006). Evidence that DNA (cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. *Journal of Biological Chemistry, 281*(23), 15763–15773.

Li, M. D., & van der Vaart, A. D. (2011). MicroRNAs in

addiction: Adaptation's middlemen? *Molecular Psychiatry, 16*(12), 1159–1168.

Marie-Claire, C., Laurendeau, I., Canestrelli, C., Courtin, C., Vidaud, M., Roques, B., & Noble, F. (2003). *Fos* but not *Cart* (cocaine and amphetamine regulated transcript) is overexpressed by several drugs of abuse: A comparative study using real-time quantitative polymerase chain reaction in rat brain. *Neuroscience Letters, 345*(2), 77–80.

Miguens, M., Coria, S. M., Higuera-Matas, A., Fole, A., Ambrosio, E., & Del Olmo, N. (2011). Depotentiation of hippocampal long-term potentiation depends on genetic background and is modulated by cocaine self-administration. *Neuroscience, 187*, 36–42.

Miller, C. A., & Sweatt, J. D. (2007). Covalent modification of DNA regulates memory formation. *Neuron, 53*(6), 857–869.

Mizuno, K., Dempster, E., Mill, J., & Giese, K. P. (2012). Long-lasting regulation of hippocampal *Bdnf* gene transcription after contextual fear conditioning. *Genes, Brain and Behavior, 11*(6), 651–659.

Monsey, M. S., Ota, K. T., Akingbade, I. F., Hong, E. S., & Schafe, G. E. (2011). Epigenetic alterations are critical for fear memory consolidation and synaptic plasticity in the lateral amygdala. *PLoS One, 6*(5), e19958.

Nestler, E. J. (2004). Historical review: Molecular and cellular mechanisms of opiate and cocaine addiction. *Trends in Pharmacological Sciences, 25*(4), 210–218.

Nielsen, D. A., Yuferov, V., Hamon, S., Jackson, C., Ho, A., Ott, J., & Kreek, M. J. (2009). Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. *Neuropsychopharmacology, 34*(4), 867–873.

Nieratschker, V., Grosshans, M., Frank, J., Strohmaier, J., von der Goltz, C., El-Maarri, O., Rietschel, M. (2012). Epigenetic alteration of the dopamine transporter gene in alcohol-dependent patients is associated with age. *Addiction Biology*, doi: 10.1111/j.1369-1600.2012.00459.x

Ooi, S. K., Qiu, C., Bernstein, E., Li, K. Q., Jia, D., Yang, Z., Bestor, T. H. (2007). DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature, 448*(7154), 714–717.

Prasad, B. M., Ulibarri, C., & Sorg, B. A. (1998). Stress-induced cross-sensitization to cocaine: Effect of adrenalectomy and corticosterone after short- and long-term withdrawal. *Psychopharmacology, 136*(1), 24–33.

Renthal, W., & Nestler, E. J. (2008). Epigenetic mechanisms in drug addiction. *Trends in Molecular Medicine, 14*(8), 341–350.

Robertson, K. D., & Wolffe, A. P. (2000). DNA methylation in health and disease. *Nature Reviews Genetics, 1*(1), 11–19.

Sales, A. J., Biojone, C., Terceti, M. S., Guimaraes, F. S., Gomes, M. V., & Joca, S. R. (2011). Antidepressant-like effect induced by systemic and intra-hippocampal administration of DNA methylation inhibitors. *British Journal of Pharmacology, 164*(6), 1711–1721.

Schindler, A. G., Li, S., & Chavkin, C. (2010). Behavioral stress may increase the rewarding valence of cocaine-associated cues through a dynorphin/κ-opioid receptor-mediated mechanism without affecting associative learning or memory retrieval mechanisms. *Neuropsychopharmacology, 35*(9), 1932–1942.

Selker, E. U. (1998). Trichostatin A causes selective loss of DNA methylation in *Neurospora*. *Proceedings of the National Academy of Sciences of the United States of America, 95*(16), 9430–9435.

Shaham, Y., Erb, S., & Stewart, J. (2000). Stress-induced relapse to heroin and cocaine seeking in rats: A review. *Brain Research Reviews, 33*(1), 13–33.

Singh, S., Johnson, J., & Chellappan, S. (2010). Small molecule regulators of Rb-E2F pathway as modulators of transcription. *Biochimia et Biophysica Acta, 1799*(10-12), 788–794.

Sorg, B. A., & Kalivas, P. W. (1991). Effects of cocaine and footshock stress on extracellular dopamine levels in the ventral striatum. *Brain Research, 559*(1), 29–36.

Sweatt, J. D. (2010). Epigenetic mechanisms in memory formation. *International Journal of Developmental Neuroscience, 28*(8), 639.

Tian, W. P., Zhao, M., Li, M., Song, T. B., 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.

Tidey, J. W., & Miczek, K. A. (1997). Acquisition of cocaine self-administration after social stress: Role of accumbens dopamine. *Psychopharmacology, 130*(3), 203–212.

Tsankova, N., Renthal, W., Kumar, A., & Nestler, E. J. (2007). Epigenetic regulation in psychiatric disorders. *Nature Reviews Neuroscience, 8*(5), 355–367.

Vo, N., Klein, M. E., Varlamova, O., Keller, D. M., Yamamoto, T., Goodman, R. H., & Impey, S. (2005). A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. *Proceedings of the National Academy of Sciences of the United States of America, 102*(45), 16426–16431.

Volkow, N. D., Wang, G. J., Fowler, J. S., & Tomasi, D. (2012). Addiction circuitry in the human brain. *Annual Review of Pharmacology and Toxicology, 52*, 321–336.

Wager, T. D., Davidson, M. L., Hughes, B. L., Lindquist, M.

A., & Ochsner, K. N. (2008). Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron, 59*(6), 1037–1050.

Xiong, Y. N., Dowdy, S. C., Podratz, K. C., Jin, F., Attewell, J. R., Eberhardt, N. L., & Jiang, S. W. (2005). Histone deacetylase inhibitors decrease DNA methyltransferase-3B messenger RNA stability and down-regulate *de novo* DNA methyltransferase activity in human endometrial cells. *Cancer Research, 65*(7), 2684–2689.

Yang, Y., Zheng, X. G., Wang, Y. F., Cao, J., Dong, Z. F., Cai, J. X., Xu, L. (2004). Stress enables synaptic depression in CA1 synapses by acute and chronic morphine: Possible mechanisms for corticosterone on opiate addiction. *Journal of Neuroscience, 24*(10), 2412–2420.

You, J. S., Kang, J. K., Lee, E. K., Lee, J. C., Lee, S. H., Jeon, Y. J., Han, J. W. (2008). Histone deacetylase inhibitor apicidin downregulates DNA methyltransferase 1 expression and induces repressive histone modifications via recruitment of corepressor complex to promoter region in human cervix cancer cells. *Oncogene, 27*(10), 1376–1386.

# DNA Methylation Mechanisms Underlying the Changes of Drug Addiction-related Nuclei Function and Addictive Behavior

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**Abstract:** Drug addiction induces long-term changes in the structure and function of the addiction-related circuitries. Recent studies suggested that drug abuse induced the changes of DNA methylation and gene expression in addiction-related nuclei by modulating the expression of three types of DNA methyltransferases (DNMTs) without changing DNA sequences, which in turn changed the functional neural plasticity. Therefore, DNA methylation is regarded as one of the possible mechanisms underlying persistent addictive behavior. In this review we discuss recent advances in our understanding of how DNA methylation contribute to the addictive behavior and how abusive drugs regulate the DNA methylation, and further proposed a few important directions for future research in the field.

**Key words:** drug addiction; DNA methylation; DNA methyltransferases (DNMTs)