Published in final edited form as:

Neurobiol Learn Mem. 2016 November ; 135: 139–145. doi:10.1016/j.nlm.2016.08.012.

Impact of DNMT1 and DNMT3a forebrain knockout on depressive- and anxiety like behavior in mice

Michael J. Morris^{1,3}, Elisa S. Na^{2,3}, Anita E. Autry³, and Lisa M. Monteggia³

¹Department of Natural Sciences, University of Michigan – Dearborn, Dearborn, Michigan, 48198

²Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, Michigan, 48105

³Department of Neuroscience, The University of Texas Southwestern Medical Center, Dallas, Texas, 75390-9111

Abstract

DNA methylation has been shown to impact certain forms of synaptic and behavioral plasticity that have been implicated in the development in psychiatric disorders. DNA methylation is catalyzed by DNA methyltransferase (DNMT) enzymes that continue to be expressed in postmitotic neurons in the forebrain. Using a conditional forebrain knockout of DNMT1 or DNMT3a we assessed the role of these DNMTs in anxiety and depressive-like behavior in mice using an array of behavioral testing paradigms. Forebrain deletion of DNMT1 had anxiolytic and antidepressant-like properties as assessed by elevated plus maze, novelty suppressed feeding, forced swim, and social interaction tests. DNMT3a knockout mice, by contrast, did not exhibit significant behavioral alterations in these tests. Given the putative role of altered DNA methylation patterns in the development of schizophrenia, we also assessed DNMT1 and DNMT3a knockout mice in a prepulse inhibition task and found an enhanced prepulse inhibition of startle in DNMT1 knockouts relative to wild type mice, with no change evident in DNMT3a knockout mice. Our data suggest that DNMT1 and DNMT3a are distinctly involved in affective behavior and that DNMT1 may ultimately represent a potential target for treatment of certain affective behavioral disorders.

Keywords

DNA methylation; depression; anxiety; prepulse inhibition; behavior

Corresponding author: Lisa M. Monteggia, Ph.D., Department of Neuroscience, The University of Texas Southwestern Medical Center, 5233 Harry Hines Blvd, Dallas, TX 75390-9111, Tel 214-648-5548, Fax 214-648-4947, Lisa.monteggia@utsouthwestern.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

DNA methylation plays an essential role in several developmental processes (e.g., X-chromosome inactivation, genomic imprinting), in the maintenance of genome stability, and in perpetuating tissue-specific patterns of gene expression in mitotic cells (Li, 2002; Santos *et al.*, 2005; Lister *et al.*, 2013). Long thought to be a paragon of transcriptional stability and silencing, DNA methylation in the brain is now recognized as dynamic and is altered as a result of environmental input (Baker-Andresen *et al.*, 2013; Morris & Monteggia, 2014). Indeed, the methylation and demethylation of cytosine nucleotides responds rapidly to a myriad of environmental stimuli [(e.g., stressors, associative learning experiences, neurological insults, intake of drugs of abuse; for review see (Morris & Monteggia, 2014)]. The enzymes responsible for catalyzing the transfer of a methyl group to a cytosine nucleotide are the DNA methyltransferases (DNMTs) and expression of the DNMTs similarly responds in dynamic fashion to environmental stimuli (Feng *et al.*, 2005; Miller & Sweatt, 2007; Lubin *et al.*, 2008; Morris *et al.*, 2014). DNMTs are broadly subdivided into two categories – maintenance DNMT, DNMT1, recreates already established methylation patterns on hemimethylated, replicating DNA and the *de novo* DNMTs, DNMT3a and DNMT3b, establish initial methylation patterns on unmethylated DNA

Accumulating evidence implicates dynamic DNA methylation and demethylation as essential cellular mechanisms for certain forms of synaptic and behavioral plasticity. However, in spite of evidence that active DNA methylation/demethylation and DNMT expression are involved in adult behavior and synapse function, the role of specific isoforms of the DNMTs is still an active area of investigation. Conditional neuron-specific DNMT knockouts have yielded some insights regarding the role of the DNMTs in the adult central nervous system and in complex behavior. Using mice with conditional forebrain-specific double knockout of DNMT1 and DNMT3a in neurons, Feng and coworkers reported learning and hippocampal long-term potentiation (LTP) deficits that were not apparent with a single knockout of either DNMT1 or DNMT3a (Feng *et al.*, 2010). By contrast, using different behavioral and electrophysiological protocols, our laboratory recently reported that forebrain-specific knockout of DNMT3a led to learning and memory deficits and impairments in synaptic plasticity, however DNMT1 KO mice were indistinguishable from control mice (Morris *et al.*, 2014). Recent work in humans and in animal models suggests that aberrant DNA methylation and expression of DNMTs may be causal or contributing factors in a variety of psychiatric disorders including major depressive disorder, anxiety disorders, and schizophrenia (Grayson *et al.*, 2005; Murgatroyd & Spengler, 2012; Chouliaras *et al.*, 2013; Murphy *et al.*, 2013; Hing *et al.*, 2014; Murphy *et al.*, 2015). Indeed, data in humans implicate different DNMT isoforms in the etiology of distinct neuropsychiatric syndromes including suicide (DNMT3b) and schizophrenia (DNMT1) (Grayson *et al.*, 2005; Murphy *et al.*, 2013). Furthermore, a recent study reported that antidepressant medications specifically inhibit the activity of DNMT1 via inhibition of the histone methyltransferase G9a (Zimmermann *et al.*, 2012).

The goal of the present study was to characterize the role of DNMT1 and DNMT3a in affective behavior. To that end we tested mice with conditional forebrain deletion of each of these DNMTs in a series of behavioral tests to assess anxiety- and depressive-like behavior.

Our results demonstrate that DNMT1, but not DNMT3a, plays a significant role in mediating anxiety and depressive-like behavior. DNMT1 knockout mice had anxiolytic and antidepressant – like effects in several behavioral testing paradigms, while DNMT3a knockouts were phenotypically normal. These data further support the hypothesis that DNMT1 and DNMT3a are involved in distinct neurobehavioral processes in the adult and may have implications for future treatment strategies for mood disorders.

2. Materials and Methods

2.1 DNMT1 and DNMT3a conditional knockout mice

Adult male mice (10 – 18 weeks of age) were used for all experiments and were maintained on a 12 hr light/dark cycle with *ad libitum* access to food and water. Floxed DNMT1 and DNMT3a lines and the CaMKII-Cre93 line were on a mixed 129/BALBC background that was backcrossed to a C57BL/6 line for at least 10 generations. Male CaMKII-Cre93 mice were crossed with female floxed homozygous DNMT1 or DNMT3a mice, and the resulting male Cre-floxed heterozygous DNMT1 or DNMT3a were then crossed with female floxed homozygous DNMT1 or DNMT3a mice to generate conditional knockouts (KOs). Littermates derived from this mating paradigm that were specific for either DNMT1 KOs or DNMT3a KOs served as the comparison control groups for all experiments. Male DNMT1 KO mice and their CTL littermates or male DNMT3a KO mice and their control littermates were run in separate cohorts for the various behavioral tests with $n = 8-13$ per group for all experiments. The same cohorts of DNMT1 KO mice vs. littermate CTL or DNMT3a vs. littermate CTL were tested for, in chronological order, the open field test, elevated plus maze, social interaction test, and prepulse inhibition testing. Distinct cohorts were run in the forced swim test and other distinct cohorts in the novelty suppressed feeding test. All experiments were scored by an observer that was blind to mouse genotype. Genomic DNA was isolated from tails for genotyping by PCR analysis. The primer sequences used were as follows: Cre, Forward (5'-CCCAGAGAACCTGAA GATGTTC-3'), Reverse (5'-CGGCTATAC GTAACAGGGTG-3'); DNMT1, Forward (5'-GGGCCAGTTGTGTGACTTGG-3'), Reverse (5'-CTTGGGCCTG GATCTTGGGGATC-3'); DNMT3a, Forward (5'-CCTCTGGGGATTAACTCTTGGCCAG CCC-3'), Reverse (5'-CCTGTGTGCAGCAGACACTTCTTTGGCGTC-3'). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center at Dallas.

2.2 Quantitative assay of DNMT1 and DNMT3a expression by real time PCR

To assess expression of DNMT1 and DNMT3a in adult brain tissue, bilateral hippocampi, amygdala, frontal cortex, and cerebellum were dissected on an ice-cold glass dish and immediately placed on dry ice until storage at -80°C . Total RNA was extracted from 4–5 animals per group using Trizol reagent and precipitated with isopropanol. Total RNA was treated with DNase I to remove residual contaminating DNA. cDNA templates were synthesized using at least 250 ng RNA with Superscript III RNase reverse transcriptase for 60 minutes at 50°C in the presence of random hexamers. PCR was performed in triplicate using optimized primers and the following protocol: 95°C for 10 minutes followed by 40 cycles of 95°C for 0.15 seconds and 60°C for 1 minute. Primer sequences used were as

follows: DNMT1, Forward (5'-GGCCAGTTGTGTGACTTGG-3'), Reverse (5'-CTTG GGCCTGGATCTTG GGGATC-3'); DNMT3a, Forward (5'-CCTCTGGGGATTA AACTCTTGGCCAGCCC-3'), Reverse (5'-CCTGTGTGCAGCAGACAC TTCTTTGGCGTC-3'); GAPDH was chosen as a housekeeping gene due to similar mRNA expression across all sample templates. Relative gene expression of DNMT1 was calculated using the $2^{-\Delta\Delta C_t}$ method (Schmittgen & Livak, 2008) and it was expressed as fold-induction with respect to control.

2.3 Open field activity

DNMT1 and DNMT3a KO mice and CTL mice were placed in an open field chamber (W X L 39 × 39 cm) and their activity was monitored for 5 minutes using EthoVision software (Noldus Technologies). Amount of time spent in the center of the open field (14 × 14 cm), and the peripheral zones was scored. Zones of the open field were defined using the video tracking software and separated into a center region, peripheral region (5 cm around the periphery of the arena), and non-peripheral region (the space between the center and periphery). Experiments were carried out in dim lighting.

2.4 Elevated plus maze

Mice were placed in the center of a cross-shaped maze (each arm 33 cm × 5 cm) that was elevated 2 feet above the floor. The maze was composed of two open arms and two closed arms (closed arm height, 25 cm). Each mouse was tested individually and behavior was monitored for 5 minutes with a video tracking system. The time spent in the closed and open arms and the center of the maze was determined using EthoVision tracking software.

2.5 Novelty suppressed feeding test (NSF)

The NSF was conducted in an open-field apparatus (W X L 39 × 39 cm) in bright light. Prior to testing, animals were food-deprived for approximately 1 day. Testing was conducted at approximately 1500 h in a quiet room equipped with a video camera suspended over the top of the open-field box. A Petri dish containing 5–6 food pellets was pre-weighed and placed in the center square (14 × 14 cm) of the open-field chamber. Each animal was placed in a peripheral square of the open-field chamber (the same square was used as a starting position for each mouse) and allowed 10 minutes in the chamber. Latency to feed (sec) was recorded for each animal. The food deprivation procedure was subsequently repeated 5–7 days later and mice were tested to determine latency to feed in the home cage.

2.6 Forced swim test

Mice were placed in a 4000-mL Pyrex glass beaker containing 3000 mL of water at 24°C for 6 minutes. Time spent immobile and latency to the first appearance of immobility was quantified during the last 4 minutes of the trial and t-tests were conducted to assess between-group differences. Immobility was defined as when the mouse made only movements necessary to keep the nose above the water. Test sessions were recorded by a video camera positioned on the side of the cylinders, and later analyzed by an observer blind to the genotype.

2.7 Social Interaction Test

In this paradigm, we measured social interest, which was defined as the approach and time that an experimental mouse spent with a novel male C57BL/6 mouse (“target mouse”). An experimental mouse was placed in an open field chamber (39 cm × 39 cm) containing a wire mesh cage centered against one of the walls. Movements of the mouse were recorded with a video tracking system for 5 minutes. Following this initial exploration period, the mouse was removed from the arena and the target mouse was placed in the wire mesh cage. The experimental mouse was then re-introduced in the arena with movements recorded for another 5 minutes. The wire mesh cage allowed visual and olfactory interactions between mice, but prevented direct physical contact. The duration of time spent in the interaction zone in the absence and presence of the target mouse (T−, T+, respectively) were quantified, expressed as a ratio, and between groups differences were analyzed by t-test.

2.8 Prepulse inhibition and acoustic startle

Startle was measured using a SR-Lab Startle Response System (San Diego Instruments). Mice were placed into the Plexiglas holders and allowed to acclimate to the chamber and background white noise (70 dB) for 5 minutes. After the acclimation period, six startle stimuli (120 dB, 40 ms, white noise) were presented with an average interstimulus interval of 15 seconds (range 7–23 seconds), followed by 40-startle stimuli preceded by a prepulse stimulus (20 ms prepulse preceding the 120 dB stimulus by 100 ms). The vibrations caused by body movements of the mouse following a startle stimulus are piezoelectrically transduced into an analog signal. Prepulse intensities were 0, 4, 8 or 16-dB above the background noise and were presented in a pseudorandom order. The Plexiglas holders were cleaned with 70% ETOH and allowed to dry between mice.

2.9 Statistical Analysis

Data were analyzed by Student’s *t*-tests with a *p*-value of 0.05 considered statistically significant in all experiments.

3. Results

3.1 Assessment of forebrain specific deletion of DNMT1 or DNMT3a

In this study we utilized only male mice as our previous work had identified differences between male conditional DNMT1 or DNMT3a forebrain specific knockout mice in learning and memory and we wanted to test if these differences extended to affective behaviors (Morris *et al.*, 2014) and avoid the confounds of sex which we had previously observed in affective related behaviors in mice (Autry *et al.*, 2009). Previous work has shown that CamKII-Cre93-mediated knockout occurs at approximately 10–14 days postnatal and is specific for forebrain neurons (Morris *et al.*, 2013; Morris *et al.*, 2014). The forebrain deletion of DNMT1 or DNMT3a does not adversely affect survival, or gross brain size (Morris *et al.*, 2014). Consistent with a forebrain specific deletion, we found a reduction of DNMT1 and DNMT3a mRNA (~70–85 %) in forebrain structures including the hippocampus, amygdala, and frontal cortex, with no change in the cerebellum (Figure 1). We have previously shown that there does not appear to be compensation in the level of

DNMT3a or DNMT3b expression when DNMT1 is knocked down, and vice versa (Morris *et al.*, 2014). Forebrain deletion of DNMT1 or DNMT3a had no effect on body weight ($M \pm SD$, in grams: DNMT1 KO 24.77 ± 2.03 , CTL 24.07 ± 1.71 ; DNMT3a KO 25.42 ± 1.08 , CTL 26.42 ± 1.32 ; not shown)

3.2 Anxiety- like and depressive-like behavior is reduced in DNMT1 KO mice relative to wild type mice

Mice were tested for their behavior in an open field test, commonly used to assess general activity as well as anxiety-like behavior (Campos *et al.*, 2013). There was no effect of either DNMT1 or DNMT3a KO on time spent in the different zones of the open field apparatus, nor any difference in distance travelled during the test, however a trend toward time spent in the center zone ($p = 0.11$) was apparent in DNMT1 KO mice (Figure 2A–D). Therefore, we further tested for anxiety-like behavior using an elevated plus maze and found that DNMT1 KO mice spent significantly more time in the open arms and less time in the closed arms than littermate CTLs (Figure 2E), suggesting the KO has anxiolytic effects. No significant differences were observed in DNMT3a KO mice relative to CTL (Figure 2F).

The forced swim test is an animal model of behavioral despair sensitive to treatments with clinically effective antidepressant medications, which reduce the amount of time an animal spends immobile during the test (Porsolt *et al.*, 1977). DNMT1 KO mice exhibited reduced total immobility time as well as an increase in the latency to immobility (Figure 2G, I) suggesting that the KO had antidepressant- like effects. No statistically significant differences were observed in terms of latency to first occurrence of immobility or total immobility time in DNMT3a KO as compared with CTL mice (Figure 2J).

We further tested the DNMT1 KO mice to confirm the anxiolytic and antidepressant-like effects observed in the elevated plus maze and forced swim tests, respectively. The NSF has been pharmacologically validated as a model for anxiety that is selectively responsive to anxiolytics (Britton & Britton, 1981). The test pits the motivation of hunger following food deprivation against the tendency for normal mice to avoid the center of an open field chamber in brightly lit conditions. Mice with forebrain KO of DNMT1 showed a reduced latency to approach and begin eating a food pellet in an open field arena compared to CTL mice (Figure 3A). This effect could not be attributable to a non-specific decrease in the latency to consume food following deprivation, as a test conducted in the home cage revealed no differences in latency to eat in DNMT1 KO versus control (Figure 3A). In the social interaction test, a frequently used paradigm to assess depressive-like behavior (Toth & Neumann, 2013), DNMT1 KO mice displayed an increased ratio of time spent in the interaction zone when it contained a target mouse compared to when it did not, and this ratio was significantly higher than that observed in CTL mice (Figure 3B).

3.3 DNMT1 KO mice exhibit enhanced prepulse inhibition of startle responses

The prepulse inhibition test is a putative measure of sensory gating (Koch, 2013). Deficits in the ability to inhibit a startle response to an auditory stimulus when that stimulus is preceded by a smaller magnitude auditory stimulus are observed in human schizophrenic patients as well as in animal models of schizophrenia (Koch, 2013). Recent evidence suggests aberrant

DNA methylation as well as expression of DNMTs in schizophrenic patients (Guidotti *et al.*, 2000; Grayson *et al.*, 2005; Huang & Akbarian, 2007; Mill *et al.*, 2008; Matrisciano *et al.*, 2013). Therefore we tested DNMT1 and DNMT3a KO mice for their performance in a prepulse inhibition task. DNMT1 KO mice showed an enhanced inhibition of their startle response at all prepulse stimulus magnitudes tested (Figure 4A). There was a trend for diminished startle responses with high amplitude acoustic stimuli in the DNMT1 KO mice that did not reach statistical significance (110 dB, $p = 0.197$; 120 dB, $p = 0.315$; Figure 4C). By contrast DNMT3a KO mice were indistinguishable from CTL mice in the PPI test and in their responses to an auditory startle stimulus (Figure 4B, D).

4. Discussion

In the current study, we report an important role for DNMT1, an enzyme responsible for transferring methyl groups to DNA, in affective behavior. Our studies demonstrate the novel finding that DNMT1 deletion in the forebrain has anxiolytic and antidepressant-like effects in specific behavioral tasks commonly utilized to assess affective behavior. By contrast, we observed no statistically significant differences between DNMT3a KO mice and their littermate CTLs in any of these behavioral tests. Our data therefore demonstrate dissociable roles for distinct DNMT isoforms in adult brain function and complex behavior in several commonly used tests of anxiety and depressive-like behavior.

Anxiolytic effects were observed in the elevated plus maze and novelty suppressed feeding tests in DNMT1 KO mice. In addition, DNMT1 KO led to increased social interest in a social interaction test. Interestingly, DNMT1 KOs spent less time immobile and had an increased latency to first immobility in the forced swim test relative to CTL mice suggesting an antidepressant-like effect. Previous work has shown that overexpression of DNMT3a in the nucleus accumbens induced depressive-like behavior in mice, whereas inhibition of DNMTs using nonspecific DNMT inhibitors infused into the hippocampus had antidepressant-like effects in the forced swim test, while another study reported enhanced anxiety-related behaviors and memory deficits (LaPlant *et al.*, 2010; Sales *et al.*, 2011; Autry *et al.*, 2015). Similarly, histone deacetylase inhibitor infusion, which also de-represses gene expression, into the basolateral amygdala leads to anxiogenic behavior (Adachi *et al.*, 2009). Moreover, overexpression of DNMT3a in the medial prefrontal cortex is anxiolytic, whereas a knockdown is anxiogenic (Elliott *et al.*, 2016). Thus it appears that forebrain-wide vs. nucleus accumbens or medial prefrontal cortex-specific knockdown of DNMT3a may have contrasting effects on anxiety and depressive-like behavior and manipulations of DNMT1 vs. DNMT3a may, in some behavioral paradigms, have opposing effects. In future studies it will be important to clarify whether anxiety and depressive behavioral paradigms differentially impact DNMT1 versus DNMT3a expression as well as DNA methylation in a brain-region specific fashion. Indeed, recent studies highlight that post-mitotic neurons show a much more dynamic landscape of DNA modifications including methylation at both CG and non-CG sites, as well as hydroxymethylation, than previously appreciated (Lister *et al.*, 2013). These marks are presumably deposited preferentially by specific enzymes and these distinct marks likely recruit different transcription factors or repressor complexes as evidenced by preferential binding of MeCP2 to particular methyl sites in DNA (Kinde *et al.*, 2015).

Our data are consistent with past work showing that DNMT1 and DNMT3a exhibit distinct changes in expression in brain as a result of prior experience. For example, Sweatt and colleagues as well as our laboratory have reported robust changes in DNMT3a expression in the hippocampus following experience in an associative fear learning task, while DNMT1 expression did not change (Miller & Sweatt, 2007; Morris *et al.*, 2014). Moreover, chronic treatment with drugs of abuse has been shown to alter the expression of DNMT3a, and not DNMT1, in the forebrain, an effect consistent with a distinct role for these enzymes in adult behavior (LaPlant *et al.*, 2010).

Stress paradigms in rodents have repeatedly been shown to impact DNA methylation patterns in neurons. For instance differential methylation of the corticotropin-releasing factor gene results from varying stressors including maternal deprivation stress and exposure to chronic mild stress (Mueller & Bale, 2008; Sterrenburg *et al.*, 2012; Chen *et al.*, 2013) and exposing mice to varying diets either during gestation or later in development can produce lasting changes in the methylation state of DNA (Cooney *et al.*, 2002; Waterland *et al.*, 2006). Dietary L-methionine supplementation reverses the effects of poor maternal care behavior on DNA methylation as well as hypothalamic-pituitary-adrenal axis and behavioral responses to stress, providing further evidence that DNA methylation in neurons is modifiable (Weaver *et al.*, 2005; Weaver *et al.*, 2006). Cat exposure increases methylation of the BDNF gene in the dorsal hippocampus in rats although this stressor decreases methylation of the BDNF gene in the ventral hippocampus, with no change in the basolateral amygdala or the prefrontal cortex (Roth *et al.*, 2011). The functional relevance of bidirectional methylation of the BDNF gene in different brain regions, or how this is mediated is not yet clear.

Psychiatric disorders including schizophrenia, bipolar disorder, and major depressive disorder have been linked to aberrant DNA methylation. Mill and coworkers (2008) examined DNA from 125 postmortem brains of patients with schizophrenia, bipolar disorder, and patients with no known psychiatric disorder and concluded that DNA methylation is significantly altered in these psychiatric disorders (Mill *et al.*, 2008). Schizophrenia is associated with increased DNMT1 expression and hypermethylation of the *reelin* and GAD promoters, therefore it is intriguing that in our study forebrain KO of DNMT1 produced a phenotype in the prepulse inhibition task opposite from the deficits in inhibition of baseline startle observed in schizophrenic patients and animal models of schizophrenia (Guidotti *et al.*, 2000; Grayson *et al.*, 2005; Huang & Akbarian, 2007; Gavin & Sharma, 2010; Koch, 2013).

We have previously reported that forebrain KO of DNMT3a led to deficits in hippocampal LTP along with profound learning and memory deficits as assessed by several learning tasks that assay distinct forms of learning and memory (e.g, associative fear learning, conditioned taste aversion learning, object recognition memory). Conversely, in all cases of learning and memory task performance we found DNMT1 KO mice to be normal. Furthermore, hippocampal LTP was normal in DNMT1 KOs, in stark contrast to DNMT3a KOs which were impaired using a theta burst stimulus or more robust high frequency stimulation to induce LTP. Taken together, the results of previous work along with the current study demonstrate that DNMT1 and DNMT3a are involved in distinct processes in the adult brain

and that manipulations of either enzyme promote distinct behavioral and synaptic outcomes. Further studies are needed to clarify the roles of different DNMT isoforms and variable alterations in DNA methylation throughout the brain on complex behavior. Another important undertaking for future research is to determine the downstream gene targets that mediate the behavioral and electrophysiological phenotypes that result from specific manipulations of DNMT1 or DNMT3a in the adult brain.

Acknowledgments

This work was supported by National Institute of Health grant MH081060 (LMM) and a NARSAD Independent Investigator Award (MJM). We thank Dr. Rudolf Jaenisch for generously providing the floxed DNMT1, DNMT3a, and CaMKII-Cre93 mice. The authors would also like to thank Elizabeth Gordon, Melissa Maghoub, and Aroon Karra for assistance with breeding and genotyping of the mice. The authors would like to acknowledge members of the Monteggia laboratory for discussions and comments on the manuscript.

References

- Adachi M, Autry AE, Covington HE 3rd, Monteggia LM. MeCP2-mediated transcription repression in the basolateral amygdala may underlie heightened anxiety in a mouse model of Rett syndrome. *J Neurosci.* 2009; 29:4218–4227. [PubMed: 19339616]
- Autry AE, Adachi M, Cheng P, Monteggia LM. Gender-specific impact of brain-derived neurotrophic factor signaling on stress-induced depression-like behavior. *Biol Psychiatry.* 2009; 66:84–90. [PubMed: 19358977]
- Autry AE, Adachi M, Monteggia LM. Dynamic methylation driven by neuronal activity in hippocampal neurons impacts complex behavior. *Frontiers in Biology.* 2015; 10:439–447.
- Baker-Andresen D, Ratnu VS, Bredy TW. Dynamic DNA methylation: a prime candidate for genomic metaplasticity and behavioral adaptation. *Trends Neurosci.* 2013; 36:3–13. [PubMed: 23041052]
- Britton DR, Britton KT. A sensitive open field measure of anxiolytic drug activity. *Pharmacol Biochem Behav.* 1981; 15:577–582. [PubMed: 6117083]
- Campos AC, Fogaca MV, Aguiar DC, Guimaraes FS. Animal models of anxiety disorders and stress. *Rev Bras Psiquiatr.* 2013; 35(Suppl 2):S101–111. [PubMed: 24271222]
- Chen J, Torcia S, Xie F, Lin CJ, Cakmak H, Franciosi F, Horner K, Onodera C, Song JS, Cedars MI, Ramalho-Santos M, Conti M. Somatic cells regulate maternal mRNA translation and developmental competence of mouse oocytes. *Nat Cell Biol.* 2013; 15:1415–1423. [PubMed: 24270888]
- Chouliaras L, Mastroeni D, Delvaux E, Grover A, Kenis G, Hof PR, Steinbusch HW, Coleman PD, Rutten BP, van den Hove DL. Consistent decrease in global DNA methylation and hydroxymethylation in the hippocampus of Alzheimer's disease patients. *Neurobiol Aging.* 2013; 34:2091–2099. [PubMed: 23582657]
- Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. *J Nutr.* 2002; 132:2393S–2400S. [PubMed: 12163699]
- Elliott E, Manashirov S, Zwang R, Gil S, Tsoory M, Shemesh Y, Chen A. Dnmt3a in the Medial Prefrontal Cortex Regulates Anxiety-Like Behavior in Adult Mice. *J Neurosci.* 2016; 36:730–740. [PubMed: 26791204]
- Feng J, Chang H, Li E, Fan G. Dynamic expression of de novo DNA methyltransferases Dnmt3a and Dnmt3b in the central nervous system. *J Neurosci Res.* 2005; 79:734–746. [PubMed: 15672446]
- Feng J, Zhou Y, Campbell SL, Le T, Li E, Sweatt JD, Silva AJ, Fan G. Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nat Neurosci.* 2010; 13:423–430. [PubMed: 20228804]
- Gavin DP, Sharma RP. Histone modifications, DNA methylation, and schizophrenia. *Neurosci Biobehav Rev.* 2010; 34:882–888. [PubMed: 19879893]
- Grayson DR, Jia X, Chen Y, Sharma RP, Mitchell CP, Guidotti A, Costa E. Reelin promoter hypermethylation in schizophrenia. *Proc Natl Acad Sci U S A.* 2005; 102:9341–9346. [PubMed: 15961543]

- Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, Impagnatiello F, Pandey G, Pesold C, Sharma R, Uzunov D, Costa E. Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch Gen Psychiatry*. 2000; 57:1061–1069. [PubMed: 11074872]
- Hing B, Gardner C, Potash JB. Effects of negative stressors on DNA methylation in the brain: implications for mood and anxiety disorders. *Am J Med Genet B Neuropsychiatr Genet*. 2014; 165B:541–554. [PubMed: 25139739]
- Huang HS, Akbarian S. GAD1 mRNA expression and DNA methylation in prefrontal cortex of subjects with schizophrenia. *PLoS One*. 2007; 2:e809. [PubMed: 17726539]
- Kinde B, Gabel HW, Gilbert CS, Griffith EC, Greenberg ME. Reading the unique DNA methylation landscape of the brain: Non-CpG methylation, hydroxymethylation, and MeCP2. *Proc Natl Acad Sci U S A*. 2015; 112:6800–6806. [PubMed: 25739960]
- Koch M. Clinical relevance of animal models of schizophrenia. *Suppl Clin Neurophysiol*. 2013; 62:113–120. [PubMed: 24053035]
- LaPlant Q, Vialou V, Covington HE 3rd, Dumitriu D, Feng J, Warren BL, Maze I, Dietz DM, Watts EL, Iniguez SD, Koo JW, Mouzon E, Renthall W, Hollis F, Wang H, Noonan MA, Ren Y, Eisch AJ, Bolanos CA, Kabbaj M, Xiao G, Neve RL, Hurd YL, Oosting RS, Fan G, Morrison JH, Nestler EJ. Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nat Neurosci*. 2010; 13:1137–1143. [PubMed: 20729844]
- Li E. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet*. 2002; 3:662–673. [PubMed: 12209141]
- Lister R, Mukamel EA, Nery JR, Urich M, Puddifoot CA, Johnson ND, Lucero J, Huang Y, Dwork AJ, Schultz MD, Yu M, Tonti-Filippini J, Heyn H, Hu S, Wu JC, Rao A, Esteller M, He C, Haghghi FG, Sejnowski TJ, Behrens MM, Ecker JR. Global epigenomic reconfiguration during mammalian brain development. *Science*. 2013; 341:1237905. [PubMed: 23828890]
- Lubin FD, Roth TL, Sweatt JD. Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. *J Neurosci*. 2008; 28:10576–10586. [PubMed: 18923034]
- Matrisciano F, Tueting P, Dalal I, Kadriu B, Grayson DR, Davis JM, Nicoletti F, Guidotti A. Epigenetic modifications of GABAergic interneurons are associated with the schizophrenia-like phenotype induced by prenatal stress in mice. *Neuropharmacology*. 2013; 68:184–194. [PubMed: 22564440]
- Mill J, Tang T, Kaminsky Z, Khare T, Yazdanpanah S, Bouchard L, Jia P, Assadzadeh A, Flanagan J, Schumacher A, Wang SC, Petronis A. Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. *Am J Hum Genet*. 2008; 82:696–711. [PubMed: 18319075]
- Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. *Neuron*. 2007; 53:857–869. [PubMed: 17359920]
- Mo A, Mukamel EA, Davis FP, Luo C, Henry GL, Picard S, Urich MA, Nery JR, Sejnowski TJ, Lister R, Eddy SR, Ecker JR, Nathans J. Epigenomic Signatures of Neuronal Diversity in the Mammalian Brain. *Neuron*. 2015; 86:1369–1384. [PubMed: 26087164]
- Morris MJ, Adachi M, Na ES, Monteggia LM. Selective role for DNMT3a in learning and memory. *Neurobiol Learn Mem*. 2014; 115:30–37. [PubMed: 24937014]
- Morris MJ, Mahgoub M, Na ES, Pranav H, Monteggia LM. Loss of histone deacetylase 2 improves working memory and accelerates extinction learning. *J Neurosci*. 2013; 33:6401–6411. [PubMed: 23575838]
- Morris MJ, Monteggia LM. Role of DNA methylation and the DNA methyltransferases in learning and memory. *Dialogues Clin Neurosci*. 2014; 16:359–371. [PubMed: 25364286]
- Mueller BR, Bale TL. Sex-specific programming of offspring emotionality after stress early in pregnancy. *J Neurosci*. 2008; 28:9055–9065. [PubMed: 18768700]
- Murgatroyd C, Spengler D. Genetic variation in the epigenetic machinery and mental health. *Curr Psychiatry Rep*. 2012; 14:138–149. [PubMed: 22307409]
- Murphy TM, Mullins N, Ryan M, Foster T, Kelly C, McClelland R, O'Grady J, Corcoran E, Brady J, Reilly M, Jeffers A, Brown K, Maher A, Bannan N, Casement A, Lynch D, Bolger S, Buckley A, Quinlivan L, Daly L, Kelleher C, Malone KM. Genetic variation in DNMT3B and increased global

- DNA methylation is associated with suicide attempts in psychiatric patients. *Genes Brain Behav.* 2013; 12:125–132. [PubMed: 23025623]
- Murphy TM, O'Donovan A, Mullins N, O'Farrelly C, McCann A, Malone K. Anxiety is associated with higher levels of global DNA methylation and altered expression of epigenetic and interleukin-6 genes. *Psychiatr Genet.* 2015; 25:71–78. [PubMed: 25350786]
- Nugent BM, Wright CL, Shetty AC, Hodes GE, Lenz KM, Mahurkar A, Russo SJ, Devine SE, McCarthy MM. Brain feminization requires active repression of masculinization via DNA methylation. *Nat Neurosci.* 2015; 18:690–697. [PubMed: 25821913]
- Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature.* 1977; 266:730–732. [PubMed: 559941]
- Roth TL, Zoladz PR, Sweatt JD, Diamond DM. Epigenetic modification of hippocampal Bdnf DNA in adult rats in an animal model of post-traumatic stress disorder. *J Psychiatr Res.* 2011; 45:919–926. [PubMed: 21306736]
- Sales AJ, Biojone C, Terceti MS, Guimaraes FS, Gomes MV, Joca SR. Antidepressant-like effect induced by systemic and intra-hippocampal administration of DNA methylation inhibitors. *Br J Pharmacol.* 2011; 164:1711–1721. [PubMed: 21585346]
- Santos KF, Mazzola TN, Carvalho HF. The prima donna of epigenetics: the regulation of gene expression by DNA methylation. *Braz J Med Biol Res.* 2005; 38:1531–1541. [PubMed: 16172746]
- Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc.* 2008; 3:1101–1108. [PubMed: 18546601]
- Sterrenburg L, Gaszner B, Boerrigter J, Santbergen L, Bramini M, Roubos EW, Peeters BW, Kozicz T. Sex-dependent and differential responses to acute restraint stress of corticotropin-releasing factor-producing neurons in the rat paraventricular nucleus, central amygdala, and bed nucleus of the stria terminalis. *J Neurosci Res.* 2012; 90:179–192. [PubMed: 21922520]
- Toth I, Neumann ID. Animal models of social avoidance and social fear. *Cell Tissue Res.* 2013; 354:107–118. [PubMed: 23760888]
- Waterland RA, Lin JR, Smith CA, Jirtle RL. Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (Igf2) locus. *Hum Mol Genet.* 2006; 15:705–716. [PubMed: 16421170]
- Weaver IC, Champagne FA, Brown SE, Dymov S, Sharma S, Meaney MJ, Szyf M. Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *J Neurosci.* 2005; 25:11045–11054. [PubMed: 16306417]
- Weaver IC, Meaney MJ, Szyf M. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc Natl Acad Sci U S A.* 2006; 103:3480–3485. [PubMed: 16484373]
- Zimmermann N, Zschocke J, Perisic T, Yu S, Holsboer F, Rein T. Antidepressants inhibit DNA methyltransferase 1 through reducing G9a levels. *Biochem J.* 2012; 448:93–102. [PubMed: 22880885]

Deletion of DNMT1 in postnatal forebrain neurons results in anxiolytic and antidepressant-like responses.

Deletion of DNMT3a in postnatal forebrain neurons does not alter affective behavior

DNMT1 and DNMT3a are involved in distinct processes in adult brain and promote distinct behavioral outcomes

Figure 1.

Forebrain-specific knockout of DNMT1 and DNMT3a. DNMT1 and DNMT3a mRNA were reduced (~70–85%) as compared to CTL mice, with no change in the cerebellum, consistent with a forebrain-specific knockout.

Figure 2.

Depressive- and anxiety-like behavior in DNMT 1 and DNMT3a KO mice. (A–D) In the open field test neither DNMT1 ($n = 10$) nor DNMT3a knockout (KO) mice ($n = 10$) exhibited significant differences in time spent in the zones of the open field (A, B) or in total distance moved within the open field (C, D) relative to control littermates (CTL; $n = 10, 10$); “per” = peripheral region, “non-per” = non-peripheral region. (E, F) In the elevated plus maze DNMT1 KOs ($n = 13$) spent significantly greater time in the open arms of the maze relative to CTL [$n = 12$; $t(23) = 2.098$, $p = 0.048$] but significantly less in the closed arms [$t(23) = 2.346$, $p = 0.028$]; DNMT3a KO mice ($n = 10$) were indistinguishable from CTL mice ($n = 10$). (G–J) In the forced swim test, DNMT1 KO mice ($n = 12$) exhibited a reduction in total immobility time compared with CTLs [$n = 12$; $t(22) = 2.189$, $p = 0.04$] and a significantly increased latency to first immobility posture [$t(22) = 3.52$, $p = 0.002$]. Although a trend was apparent, DNMT3a KO mice ($n = 10$) did not differ from CTL mice ($n = 10$) in terms of total time spent immobile [$t(18) = 1.967$, $p = 0.07$] and no differences in latency to immobility were observed.

Figure 3.

Anxiolytic and antidepressant-like effects of DNMT1 KO. (A) In the novelty suppressed feeding test DNMT1 KO mice ($n = 12$) showed a significantly reduced latency to feed relative to CTL mice [$n = 12$; $t(22) = 2.144$, $p = 0.043$] but no change in latency to feed in the home cage following food deprivation. (B) Relative to CTL mice ($n = 8$) in the social interaction paradigm DNMT1 KO mice ($n = 8$) had an increased ratio of time spent in the interaction zone with target mouse present as compared to time spent with no target mouse present [$t(14) = 2.198$, $p = 0.045$]

Figure 4.

Prepulse inhibition and acoustic startle in DNMT1 and DNMT3a KO mice. (A, B) Relative to CTL mice ($n = 8$) DNMT1 KO mice ($n = 8$) exhibited enhanced prepulse inhibition of startle at each stimulus magnitude tested [PP4: $t(14) = 2.209$, $p = 0.044$; PP8: $t(14) = 2.694$, $p = 0.017$; PP16: $t(14) = 2.193$, $p = 0.046$] whereas DNMT3a KO mice ($n = 10$) were not different from CTL mice ($n = 10$). (C, D) Assessment of startle amplitudes revealed a trend for decreased startle response at 110 dB ($p = 0.197$). DNMT3a KO mice ($n = 10$) showed no change in startle response at any stimulus magnitude tested as compared to CTL ($n = 10$).