

Study on the Role of *Dnmt3a* Expression in the Dentate Gyrus of the Hippocampus in Reward Memory

ABSTRACT

Objective: Emotional memory has been associated with many psychiatric diseases. Understanding emotional memory could be beneficial in comprehending and discovering new therapies for diseases related to emotional memory, such as depression and post-traumatic stress disorder (PTSD). Our previous study revealed that *Dnmt3a* expression in the dentate gyrus (DG) contributes to fear memory. However, is there a correlation between *Dnmt3a* expression in the DG and reward memory? This study aims to explore the relationship between *Dnmt3a* expression and reward memory.

Methods: We induced fear memory (Fear group) or reward memory (Reward group) using fear conditioning and social interaction in females, respectively. We then measured the expression levels of *Dnmt3a* and *c-fos* after the retrieval of different types of memory. Additionally, we used a recombinant Adeno-Associated Virus (rAAV) to overexpress *Dnmt3a* in the DG and conducted conditioned place preference (CPP) tests to assess changes in reward memory.

Results: We observed a significant increase in *Dnmt3a* and *c-fos* expression in the Fear group compared with the Reward group. Overexpression of *Dnmt3a* in the DG led to an increase in time spent in the white box during CPP tests.

Conclusion: *Dnmt3a* expression levels varied after the retrieval of fear or reward memory, and overexpression of *Dnmt3a* in the DG enhanced reward memory. These findings suggest that *Dnmt3a* expression in the DG plays a role in reward memory.

Keywords: Behavior, emotional expression, psychiatry

Introduction

Emotional memories have been studied for decades, as they are linked to numerous psychological disorders, such as depression and post-traumatic stress disorder (PTSD). Depression is characterized by a lack of feelings of reward and memory, while PTSD is characterized by fear memories. Therefore, understanding the mechanisms of fear and reward memory is crucial for developing new therapies for these diseases.

Many brain regions are implicated in emotion-related psychological disorders. For instance, the volume of the hippocampus is associated with depression¹ and anxiety,² while the functions of the prefrontal cortex and amygdala are linked to depression and PTSD³ in clinical research. Studies in mice have also shown that the prefrontal cortex, amygdala, and hippocampus are related to emotional memory.⁴

Furthermore, specific molecular mechanisms play a role in emotional memory. For example, the expression of PKMzeta in the basal amygdala is linked to fear memory⁵ and anxiety.⁶ Additionally, N-methyl-D-aspartate (NMDA) receptors in the amygdala and medial prefrontal cortex are associated with fear memory,^{7,8} whereas in the ventral tegmental area (VTA) they are related to reward memory.⁹ Expression of epigenetic-related genes, such as *Dnmt3a1*, in the hippocampus is involved in fear memory¹⁰ and global methylation in the prefrontal cortex is involved in reward memory.¹¹ Moreover, HDAC3 expression in the amygdala is



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implicated in fear¹² while its expression in the hippocampus is linked to reward memory,¹³ These findings suggest that gene expression in brain regions may regulate emotional memories.

The dentate gyrus (DG) is mainly associated with spatial memory, but increasing evidence suggests that it plays an important role in the regulation of emotional memory.¹⁴ The DG can activate downstream neural circuits through spatial memory to induce pleasant or sad emotions, which has led to further research on the DG in depression and PTSD.^{15,16} Clinical studies have also found correlations between changes in neurons in the DG and depression and PTSD.¹⁷ However, it is unclear whether neurons in the DG regulate reward memory and fear memory differently.

DNA methylation (DNAm) is a key epigenetic mechanism relevant to PTSD.¹⁸ Key mechanisms of neurological development and function are epigenetically regulated through DNA methylation and histone modifications, and DNA methyltransferases play an important role in neuronal plasticity and learning memory.¹⁹ In neuronal cells, *Dnmt3a* expression is critical for neuronal maturation. Defects in *Dnmt3a* in forebrain neurons lead to deficits in synaptic plasticity and learning and memory.²⁰ DNA methylation affects animal behavior, and it has been found that a methyl donor-deficient diet during early childhood affects anxiety-like behaviors and fear memories, accompanied by alterations in the expression of a number of genes in the hippocampus and their methylation, and that parental fear memories affect the fear response of the offspring.²¹ The nucleus ambiguus (NAc) was found to be essential for the formation of cued fear memories in mice and to increase the expression of *Dnmt3a*; injection of a DNA methyltransferase inhibitor into the NAc after conditioning induced a nonadaptive fear response to neutral cues.²² Moreover, Elliott et al²³ found that *Dnmt3a* in the prefrontal cortex of mice modulated anxiety-depression-like behaviors.²³ These studies suggest that *Dnmt3a* plays a key role in the regulation of emotional memories such as fear and anxiety.

Our previous research has shown a correlation between *Dnmt3a* expression and neuronal activity in the DG and the extinction and renewal of fear memory.^{24,25} However, it is unclear whether *Dnmt3a* in the DG also interacts with reward memory. To compare changes in *Dnmt3a* expression in the DG caused by fear and reward memories in the same context, we established fear and reward memories in the same context and then retrieved them. Subsequently, we conducted staining for *Dnmt3a* and *c-fos*. Our results showed that compared with reward memory, fear memory induced more expression of *Dnmt3a* and *c-fos*. Furthermore, we found that the overexpression of *Dnmt3a* in the DG of mice could enhance reward memory. Our study indicates that *Dnmt3a* expression in the DG of mice plays an important role not only in fear memory but also in reward memory.

MAIN POINTS

- Different types of emotional memories cause different gene expression changes in the DG region.
- *Dnmt3a* expression in the DG region is associated with fear memories and reward memories, and it regulates neural activity and memory formation in the DG.
- *Dnmt3a* was only associated with the intensity of the memory but not the type of memory.

Methods

Animals

Two- to three-month-old adult C57BL/6J mice were utilized in the study. All experiments involved male subjects, except for the reward memory and CPP experiments, where female mice were used for social interaction. All mice were sourced from SPF-grade breeding stock at SPF (Beijing) Biotechnology Co. Ltd. The mice were reared in standard laboratory conditions, under temperature control (22 ± 1°C) and a reversed 12 hour light-dark cycle (8 AM-8 PM), in Dali University Laboratory Animal Center. Mice in each experiment were derived from the same batch of male mice of the same age. All animal experiments were performed according to the ARRIVE guidelines on the Care and Use of Experimental Animals and approved by the Dali University Animal Care and Use Committee (Approval No: 2022-P2-119, Date: 07/01/2022).

Fear and Reward Test

Lighting was obtained from SuperFcs (Shanghai XinRuan Information Technology Co. Ltd, Shanghai, China), and 75% alcohol was used as a cleaning agent. The experimental setup was performed using SuperFcs software, and training was performed on the first day. The experimental setup consisted of a stimulus period of 600 seconds with 1 cycle, which did not require adaptation in the fear box. Experimental mice were randomly assigned to a group set to receive 2 seconds of plantar shock with a current of 30 mA at the beginning and 2 seconds of plantar shock at 120 seconds intervals thereafter. In the other group, a female mouse was placed in the hollowed-out box, allowing the experimental mice to freely explore the environment. Mice were removed 1 minute after the end of the experiment to reduce stress behavior. After training, a test was performed the following day, where both groups of mice were placed in the background environment and the experimental mice were allowed to explore freely for 600 seconds. Freezing levels and head exploration time were analyzed using the SuperFcs or VisualTrack analysis systems (Shanghai XinRuan Information Technology Co. Ltd, Shanghai, China) on a computer, and the freezing threshold was set to at least more than 1 second without any movement.

Conditioned Place Preference

The conditioned place preference (CPP) chambers comprised of 2 compartments of equal size (30 cm × 30 cm × 30 cm), each with distinct tactile and visual cues (one compartment featured a black floor and walls, while the other compartment had a white floor and walls), separated by a guillotine door.

On the first day of testing, the procedure consisted of 2 stages. In the first stage, the mice were allowed to freely explore the box for 3 minutes before being removed. In the second stage, a female mouse was placed on one side of the white box to ensure contact with the test mouse while restricting movement. The test mouse was then placed in the box and allowed to freely explore for 10 minutes.

On the second day, the test mouse was placed in the box and allowed to freely explore for 3 minutes. The activity of the mice was recorded using AnyMaze software (Stoelting Co., Wood Dale, IL, USA).

Immunofluorescence Staining

Mice were anesthetized with pentobarbital sodium and perfused with 4% paraformaldehyde (PFA) and Phosphate-Buffered Saline

(PBS) through the heart. Two hours after the end of the final behavioral test, rats were anesthetized with sodium pentobarbital (450 mg/kg, i.p.) and then perfused transcardially with 40 mL PBS followed by 20 mL cold fresh 4% PFA. Brains were removed and fixed overnight in 4% PFA, and transferred to 30% sucrose for 48 hours for cryoprotection. Frozen sections were cut coronally (30 μ m) at different levels of the brain. Brain sections were treated with 0.5% Triton X-100, 10% goat serum, and 0.2% skim milk powder in PBS for 1 hour at room temperature. The sections were incubated with primary antibodies (anti-*Dnmt3a* [Rabbit], 1:500~1:1000, CST, #3598; anti-*c-fos* [Rabbit], 1:1000, Abcam, ab190289) overnight at room temperature, washed with PBS 3 times (each for 10 min), incubated with secondary antibodies (goat anti-Rabbit 488, 1:400, A11008; goat anti-Rabbit 555, 1:400, 4413S) for 1 hour in the dark at room temperature, and washed 3 times in PBS (each for 10 minutes). Finally, sections were incubated with Hoechst for 5 minutes and mounted on glass slides. Images were taken using an inverted microscope at 10X magnification (ix73; Olympus, Tokyo, Japan). Co-localization of *Dnmt3a* and *c-fos* positive cells were counted using ImageJ software; NIH, Bethesda, MD, USA.

Statistical Analysis

Behavioral data were recorded and analyzed using software (SuperFcs or Visualtrack systems, Shanghai, China; AnyMaze software (Stoelting Co., Wood Dale, IL, USA)). All statistical analyses were performed with the statistical analysis software package GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). Statistical charts were produced by GraphPad Prism 8.0 (GraphPad Software Inc., USA). Descriptive statistics of the data are shown as n (%), normalized variables (for parametric tests) are shown as "mean \pm standard error of the mean (SEM)," and non-normalized variables (for non-parametric tests) are shown as "median (min-max)." The normality of the variables was assessed using the Shapiro-Wilk test. Statistical differences between the 2 groups were assessed using the unpaired *t*-test (for parametric tests) and the Mann-Whitney U-test (for non-parametric tests). A 2-way Two-way Analysis of Variance (2-way ANOVA) was used for freezing percentage change every 180 seconds and in CPP experiments, and comparisons between groups were made using Bonferroni's multiple comparisons test. Mice tended to maintain an immobile defensive posture in a fearful state, and freezing percentage is the time that mice spend immobile over the total time. In CPP, due to the natural preference of mice for the black box, the time spent in the white box where the female mouse had been placed was used as a measure of whether CPP was achieved. Statistical significance was set at $P < .05$.

Results

The Mice Formed Fear/reward Memories by Context Fear Conditioning/with the Female

To investigate the changes in the DG region of the hippocampus following the formation of fear and reward memories in the same spatial context, we divided 2-month-old mice into 2 groups. One group of test mice underwent context fear conditioning on the first day, while the other group had a female mouse placed in the same context (ensuring contact between the female and test mice, but restricting the female's movement). On the second day, both groups of mice were allowed to freely explore the context for 10 minutes (Figure 1A. Experimental procedures. Fear, $n=8$; Reward, $n=8$). The mice were sacrificed 2 hours later. Behavioral results showed that

mice in the Fear group exhibited reduced activity (Figure 1B). The total distance traveled by the Fear group was significantly lower than that by the Reward group on the first day of training and the second day of testing (Table 1, unpaired *t*-test, $P < .001$)

The Reward group showed a significant increase in exploration of the area where the female mouse was placed (Figure 1C, Table 1, Day 1, $P=.016$; Day 2, $P=.049$). The number of head probes of mice in the 2 groups was recorded. On the first day, the number of head probes in the Reward group was significantly lower than that in the Fear group on Day 1 (Table 1, unpaired *t*-test, $P=.016$) and Day 2 (Table 1, unpaired *t*-test, $P=.049$) (Figure 1C). The freezing time percentage of mice in the 2 groups was recorded (Figure 1D). The freezing level in the Fear group was significantly higher than in the Reward group on Day 1 (Table 1, unpaired *t*-test, $P < .001$) and Day 2 (Table 1, $P < .001$) (Figure 1D). The total recorded time of freezing was divided into segments of 180 seconds each (Figure 1E, Tables 2 and 3). These results indicate that the mice formed fear/reward memories.

The Expression Levels of *Dnmt3a* and *c-fos* Differ After the Retrieval of Fear Memory and Reward Memory are Retrieved in the Same Context

After the behavioral tests, the mice were sacrificed. Staining results showed that, compared with the Reward group, the Fear group had significantly increased expression levels of *Dnmt3a* (Figure 2, Table 4; unpaired *t*-test, $P=.044$) and *c-fos* (Figure 3, Table 5; unpaired *t*-test, $P=.043$) in the DG area. This result suggests that the expression of *Dnmt3a* and *c-fos* in the DG area is correlated with different emotional memories.

Increasing *Dnmt3a* Expression in the Dentate Gyrus of Mice Enhances Reward Memory

To further investigate the relationship between *Dnmt3a* expression in the DG of mice and emotional memory, we used a virus overexpressing *Dnmt3a*²⁴ in the DG and then tested reward memory. The location of the viral injection is shown in Figure 4A. We employed a conditional place preference test, as shown in Figure 4A (Green fluorescent protein(GFP), $n=12$; *Dnmt3a*, $n=12$). The percentage of time spent in the white is shown in Figure 4B and Table 6. The Preference Changed With Female part was not different between the 2 groups (Figure 4C right, Table 7; Mann-Whitney U-test, $P=1.000$). However, the preference changed significantly between the 2 groups for the After Female part. The number of times that the preference changed was significantly higher in the *Dnmt3a* group compared with the GFP group (Figure 4C left, Table 7; Mann-Whitney U-test, $P=.024$). These results indicate that increasing *Dnmt3a* expression in the DG of mice significantly enhances reward memory.

Discussion

In this study, we investigated the impact of different types of memories on gene expression in the DG region of the hippocampus by establishing fear and reward memories in the same context. The results showed that different types of memories caused different gene expression changes in the DG region. Fear memory led to increased expression of *Dnmt3a* and *c-fos*. Furthermore, to validate the correlation between *Dnmt3a* and reward memory, we used an recombinant Adeno-Associated Virus (AAV) virus to increase *Dnmt3a* expression in the DG region and found that *Dnmt3a* expression promoted reward memory formation and delayed its decline.

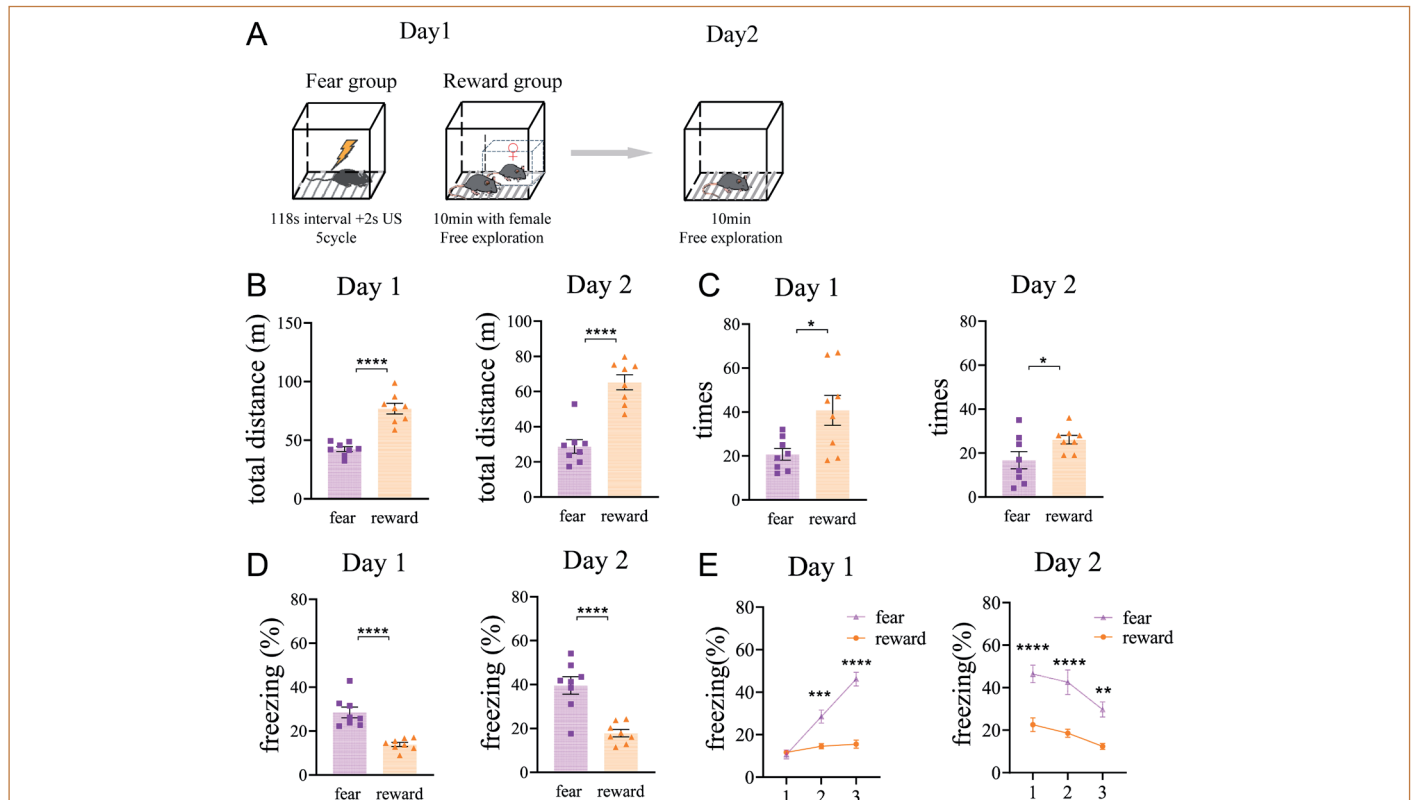


Figure 1. Fear and reward test behavioral results. (A) Experimental procedures. Fear, $n=8$; Reward, $n=8$. (B) The total distance traveled by the Fear group was significantly lower than that by the Reward group on the first day of training and the second day of testing (Day 1, unpaired t -test, $P < .001$; Day 2, unpaired t -test, $P < .001$). (C) The number of head probes of mice in the 2 groups was recorded. On the first day, the number of head probes in the Reward group was significantly lower than that in the Fear group (unpaired t -test, $P = .016$). The difference between the 2 groups decreased on the second day, but the number in the Reward group was still significantly higher than in the Fear group (unpaired t -test, $P = .049$). (D) The freezing time percentage of mice in the 2 groups was recorded; on the first day, it was significantly higher in the Fear group than in the Reward group (Day 1, unpaired t -test, $P < .001$; Day 2, unpaired t -test, $P < .001$). (E) The total percentage of freezing time was divided into segments of 180 seconds each (Day 1, 2-way RM ANOVA, $P < .001$; Day 2, 2-way RM ANOVA, $P < .001$). Results are presented as mean \pm SEM; US, Unconditioned Stimulus; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. RM ANOVA, Repeated Measures ANOVA.

Dnmt3a is a de novo DNA methyltransferase.²⁶ In previous studies, we found that *Dnmt3a* expression in the DG region is correlated with fear memory renewal, and high expression of *Dnmt3a* can inhibit fear memory renewal.^{24,25} Additionally, we found a positive correlation between *Dnmt3a* expression and *c-fos*.²⁴ In this study, we also found that *Dnmt3a* and *c-fos* expression simultaneously increased in the fear memory group, further indicating a correlation between

Dnmt3a and *c-fos* expression. But why does fear memory evoke more *Dnmt3a* and *c-fos* expression?

Fear memory activates more *c-fos* expression, indicating that fear memory is more intense compared with reward memory.²⁷ As *Dnmt3a* is positively correlated with *c-fos*, the Fear group showed higher levels of *c-fos* and *Dnmt3a* expression. Increasing *Dnmt3a* expression using the AAV made the reward memory more stable. This suggests that the DG region must be activated regardless of whether fear or reward memory is formed, and increasing *Dnmt3a* expression can enhance both fear and reward memories,²⁸ indicating that *Dnmt3a* is only correlated with the strength of memory, not its type. More *Dnmt3a* formation leads to more stable corresponding memories. Fear and reward memories may be achieved by activating cells in different locations within the DG.

As *Dnmt3a* correlates with *c-fos*, it may lead to more stable corresponding memories by increasing neural activity. LaPlant et al²⁹ discovered that the DNA methyltransferase inhibitor RG108 can reduce dendritic spine density in NAc neurons induced by cocaine, resulting in a significant decrease in functional synapses and a reduction in

Table 1. Statistical Details of Figure 1B, 1C, and 1D

Comparison of Fear and Reward Between Groups (mean \pm SEM)—Unpaired t -test					
Group		Fear (n=8)	Reward (n=8)	t	P
Total distance (m)	Day 1	42.40 \pm 2.054	76.96 \pm 4.467	7.029	<.001
	Day 2	28.79 \pm 3.865	65.25 \pm 4.262	6.336	<.001
Times	Day 1	20.75 \pm 2.624	40.75 \pm 6.813	2.739	.016
	Day 2	16.75 \pm 3.899	26.13 \pm 1.968	2.146	.049
Freezing (%)	Day 1	28.47 \pm 2.467	13.94 \pm 0.930	5.513	<.001
	Day 2	39.59 \pm 3.953	17.84 \pm 1.645	5.079	<.001

Results are presented as mean \pm SEM. SEM, standard error of the mean.

Table 2. Statistical Details of Figure 1E, Day 1

Comparison of Fear and Reward Between Groups (mean ± SEM)—2-way ANOVA, $F(1, 14) = 30.28, P < .001$, Bonferroni's Multiple Comparisons Test Results Below

Group		Fear (n=8)	Reward (n=8)	t	P
Freezing (%)	Day 1				
	1 (0~180 s)	10.72 ± 2.040	11.68 ± 0.938	0.304	1.000
	2 (180~360 s)	28.51 ± 3.069	14.60 ± 1.239	4.415	<.001
	3 (360~540 s)	46.19 ± 3.204	15.57 ± 1.878	9.717	<.001

Results are presented as mean ± SEM.

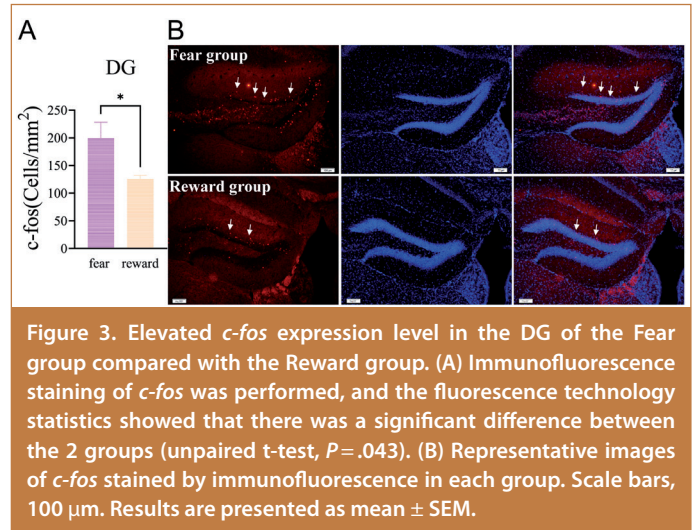
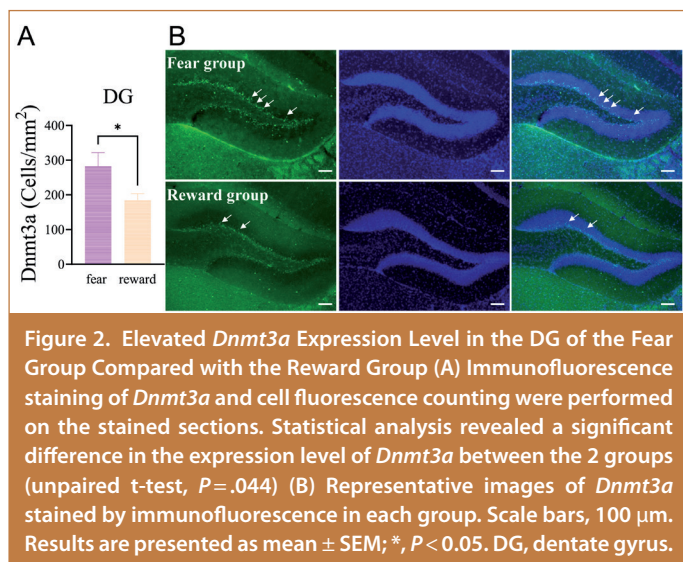
Table 3. Statistical Details of Figure 1E, Day 2

Comparison of Fear and Reward Between Groups (mean ± SEM)—2-way ANOVA, $F(1, 14) = 25.80, P < .001$, Bonferroni's Multiple Comparisons Test Results Below

Group		Fear (n=8)	Reward (n=8)	t	P
Freezing (%)	Day 2				
	1 (0~180 s)	46.46 ± 4.113	22.54 ± 3.238	4.682	<.001
	2 (180~360 s)	42.57 ± 5.706	18.56 ± 1.901	4.699	<.001
	3 (360~540 s)	29.73 ± 3.586	12.43 ± 1.378	3.386	.005

Results are presented as mean ± SEM.

the frequency of mEPSC. Moreover, overexpression of *Dnmt3a* in the NAc region can increase dendritic spine density.²⁹ Studies on cultured neurons found that DNA methyltransferase regulates glutamatergic synaptic homeostasis by modulating the expression of downstream genes.³⁰ These results indicate that *Dnmt3a* may regulate neural activity by modulating neural plasticity and downstream genes, thereby making the memory more stable. Research on how *Dnmt3a* regulates neural activity by modulating downstream genes may be important for future studies.



In human evolution, fear memories are more easily remembered than reward memories,³¹ which may also be why fear memories lead to more *c-fos* expression. Many studies have shown a correlation between *Dnmt3a* and long-term changes in the brain.³² DNA methyltransferase inhibitors such as RG108 can inhibit memory formation,³³ and to some extent, *Dnmt3a* can promote memory stability.²⁸ This may also explain why fear memories lead to more *Dnmt3a* expression, as fear memories must be remembered more stably to protect against predators.³⁴ Using an AAV to promote *Dnmt3a* expression can promote the stability of reward memories.

Conclusion

In conclusion, our study demonstrates that *Dnmt3a* expression in the DG region is correlated with fear and reward memories, and it can regulate neural activity and memory formation in the DG, while overexpression of *Dnmt3a* can promote the formation of reward memories. Our research suggests that certain methylation-enhancing strategies, such as injecting B vitamins, may improve reward memory and promote the extinction of fear memory, potentially playing a positive role in the treatment of depression and PTSD.

Table 4. Statistical Details of Figure 2A

Comparison of *Dnmt3a* Expression Between Groups (mean ± SEM)—Unpaired t-test

Group	Fear (n=6)	Reward (n=6)	t	P
<i>Dnmt3a</i> Day 2	283.3 ± 38.37	185.3 ± 18.10	2.311	.044

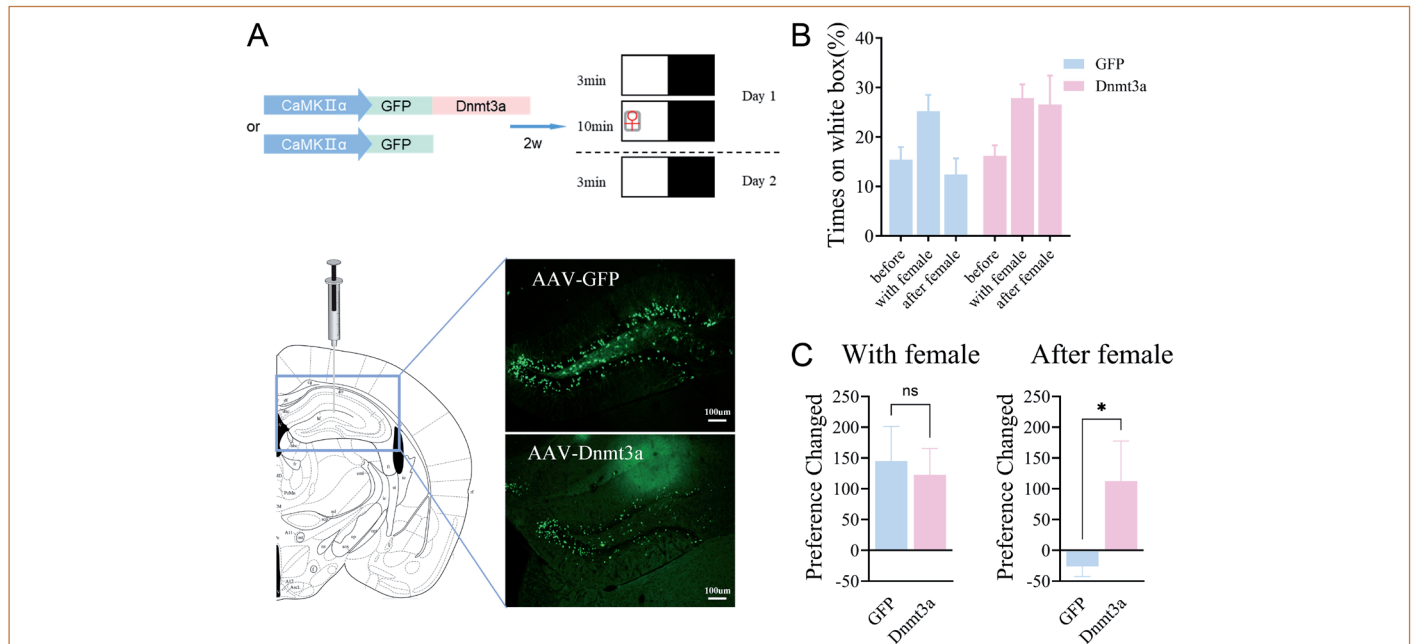
Results are presented as mean ± SEM.

Table 5. Statistical Details of Figure 3A

Comparison of *c-fos* Expression Between Groups (mean ± SEM)—Unpaired t-test

Group	Fear (n=4)	Reward (n=4)	t	P
<i>c-fos</i> Day 2	200.0 ± 28.32	126.0 ± 6.241	2.552	.043

Conditional Results are presented as mean ± SEM.

**Table 6.** Statistical Details of Figure 4B**Comparison of GFP and Dnmt3a Between Groups (mean \pm SEM)—2-way ANOVA**

Group		With Female			GFP vs Dnmt3a	
		Before	Female	After Female	t	P
Time in white box (%)	GFP (n = 12)	15.41 \pm 2.532	25.25 \pm 3.241	12.41 \pm 3.260	2.547	.125
	Dnmt3a (n = 12)	16.14 \pm 2.184	27.86 \pm 2.749	26.57 \pm 5.843		

Results are presented as mean \pm SEM.**Table 7.** Statistical Details of Figure 4C**Comparison of Preference Changed Between Groups [median (min-max)]—Mann–Whitney U-test**

Group	GFP (n = 12)	Dnmt3a (n = 12)	P
With female	82.98 (–36.11-675.1)	65.21 (–25.51-489.4)	1.000
After female	–30.79 (–100-64.69)	29.95 (–67.59-608.8)	.024

Preference changed:

With female; Time in white box (Day 1 (with female) – Day 1 (before))/Day 1 (before) \times 100%.After female; Time in white box (Day 2 (no female) – Day 1 (before))/Day 1 (before) \times 100%.

Results are presented as median (min-max).

Data Availability Statement: All data generated or analyzed during this study are included in this published article.**Ethics Committee Approval:** This study was approved by the Ethics Committee of Dali University (Approval No: 2022-P2-119, Date: 07/01/2022).**Informed Consent:** N/A.**Peer-review:** Externally peer reviewed.**Author Contributions:** Concept – Xi.Z., Yi.L., Z.G.; Design – Xi.Z., Yi.L., Z.G.; Supervision – Z.G.; Resources – Yi.L., Z.G.; Materials – Xi.Z., R.M., E.H., X.P., W.M., Xu.Z., Yi.L., H.L.; Data Collection and/or Processing – Xi.Z., R.M., E.H., X.P., W.M., Xu.Z., Yi.L., H.L.; Analysis and/or Interpretation – Xi.Z., R.M., Yi.L., Z.G.; Literature Search – Xi.Z., R.M., E.H., X.P., W.M., Xu.Z., Yi.L., H.L., Ya.L., Z.G.; Writing – Xi.Z., R.M., E.H., X.P., W.M., Xu.Z., Yi.L., H.L., Ya.L., Z.G.; Critical Review – Xi.Z., R.M., E.H., X.P., W.M., Xu.Z., Yi.L., H.L., Ya.L., Z.G.**Acknowledgments:** The authors would like to thank the peer reviewers for their comments and suggestions on this study; the people who provided technical assistance and help with the histological work; and all those who have helped the authors in the process of writing this manuscript.**Declaration of Interests:** The authors have no conflicts of interest to declare.**Funding:** This research was funded by (1) National Natural Science Foundation of China (NSFC) (82360231), (2) Yunnan Basic Research Program General Project (202401AT070075), (3) Basic Research Project of the Education Department of Yunnan Province—Teacher Project (2023J0880), and (4) Youth Special Project for Basic Research of Local Universities in Yunnan Province (202301BA070001-127).**References**

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