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ORIGINAL RESEARCH

Valproate Reverses Mania-Like Behavior of *Clock*[delta19] Mouse and Alters Monoamine Neurotransmitters Metabolism in the Hippocampus

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Background: Mice with a deletion at exon 19 of the circadian locomotor output cycles Kaput gene (*Clock*[delta19]) exhibit mania-like behavior and have been one of the most common animal models for bipolar disorder (BD). The predictive validity of the *Clock*[delta19] was investigated via studies using lithium previously. Determination of effects of other mood stabilizers on *Clock*[delta19] mouse would be helpful for better understanding of the mechanism underlined.

Methods: Wildtype (WT) and *Clock*[delta19] mice were treated with saline (n = 10 for WT and n=10 for *Clock*[delta19]) or valproate (VPA) (n = 10 for WT and n=10 for *Clock*[delta19]) for 10 days. The hyperactivity, anxiety-like behaviors and depression-like behaviors were tested. The concentration of monoamine neurotransmitters and their metabolites in the hippocampus of saline or VPA treated WT and *Clock*[delta19] mouse (n = 8 for each) were also determined.

Results: VPA can reverse hyperactivity, lower level of anxiety-like and depression-like behaviors of the *Clock*[delta19] mouse. *Clock*[delta19] mouse exhibited lower levels of serotonin (5-HT) and dopamine (DA) in right hippocampus compared to WT mouse. Chronic VPA treatment did not affect the levels of 5-HT and DA, but can reduce the level of levodopa (L-DOPA) in the right hippocampus of *Clock*[delta19] mouse.

Conclusion: Our results indicated that chronic VPA treatment can reverse the mania-like behaviors of the *Clock*[delta19] mouse and further consolidate the validity of the *Clock*[delta19] mouse as a model of BD. Monoamine neurotransmitters and their metabolites in the hippocampus are partly regulated by mutation of the *Clock* gene or VPA treatment.

Keywords: bipolar disorder, *Clock*[delta19] mouse, valproate, mania-like behavior, neurotransmitter

Introduction

Bipolar disorder (BD) is a common psychiatric disorder that affects about 1–3% of the population globally and is one of the leading causes of disability worldwide.[1] As a severe psychiatric disorder, BD contributes to dominate the global burden of mental disorders associated with great morbidity and high risk of suicide.[2,3] BD is characterized by suffering cycling through manic and depressive periods with euthymic or normal mood states between episodes.[4,5] The manic state is associated with hyperactivity, little need for sleep, impulsivity, increased risk-taking behavior and increased reward-seeking; whereas the depressive state usually consists of

sadness, increased sleep, guilt, and anhedonia. The underlying etiology of BD is unknown, both genetic and environmental factors are involved.[6]

Previous studies indicated that mice carrying a deletion of exon 19 of the circadian locomotor output cycles Kaput (*Clock*) gene (*Clock*delta19) display behaviors that resemble mania in human.[7,8] The *Clock*delta19 mouse exhibit hyperactivity, decreased anxiety-like and decreased depression-like behavior, which are strikingly similar to patients of BD in the manic state.[9–14] In addition, neurobiological and neurophysiological alterations that appeared on bipolar patients were also observed in the *Clock*delta19 mouse. A series of studies have indicated that *Clock*delta19 mouse are hyperdopaminergic, as demonstrated by elevated dopamine (DA) neuron activity in the ventral tegmental area (VTA) and increased DA signaling in the nucleus accumbens (NAc).[10,15,16] Moreover, the manic-like behavioral traits and neurobiological and neurophysiological changes of the *Clock*delta19 mouse could be ameliorated by lithium.[2,11,13,14,17,18]

Valproate (VPA) was first introduced as an anticonvulsant drug and has been commonly prescribed mood stabilizer in the treatment of BD.[19] VPA is used as the typical mood stabilizer with a broad spectrum of antimanic activity both in acute mania, cyclothymia, mixed state and rapid cycling in the treatment of BD.[20] In addition, VPA is comparable with lithium in the treatment of acute mania, with relatively mild adverse reactions and somewhat better tolerated.[21,22] The mechanism of effects of VPA on BD remains largely elusive. A series of studies suggested that the action of VPA on central nervous system neurons[4,23,24] and neurotransmitters transmission[25–27] may be relevant to its actions in BD, but these need to be further investigated. Application of *Clock*delta19 mouse on the studies of mood-stabilizing effects of VPA would facilitate exploration of the mechanism of treatment effects of VPA that may be relevant to its actions in BD.

In this study, we examined the mood-stabilizing effect of VPA on the *Clock*delta19 mouse. Moreover, we explored the association between neurotransmitters in brain regions and the effects of VPA on the mania-like behavior of the *Clock*delta19 mouse.

Materials and Methods
Animals
*Clock*delta19 mutant mice were created by *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis and produce a dominant-negative *CLOCK* protein defective in transcriptional activity as previously described.[28] All experiments using *Clock*delta19 mutants were maintained on a BALB/cJ genetic background without disruption of circadian rhythm and purchased from the Jackson Laboratory. *Clock*delta19 and wildtype (WT) littermates used in the study were 8 to12 week old adult male mice (weighing 19–35 g) and were group-housed in sets of 2–5 per cage with food and water ad libitum under standard conditions (22 ± 1°C; 12/12 light/dark cycle, ZT 0 = lights on 7:00 AM; ZT 12 = lights off 7:00 PM). WT and *Clock*delta19 mouse treated with saline (n = 10 for WT and n = 10 for *Clock*delta19) or VPA (n = 10 for WT and n = 10 for *Clock*delta19) were used for behavioral studies and the determination of monoamines and their metabolites (n = 8 for each). All animal procedures were carried out in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85 23, revised 1985), and all animal protocols were approved by the Institutional Animal Care and Use Committee at West China Hospital, Sichuan University.

Drug Treatments
VPA used in this study was purchased from Sigma–Aldrich. VPA was dissolved in saline and administered in intraperitoneal (i.p.) injection at a volume of 400mg/kg. Animals were received one daily i.p. injection of VPA for 10 days before behavioral testing. VPA was freshly prepared before experiments. The control group was treated with the saline solution following the same schedule described to treatment groups. The doses of VPA employed in our study have been previously reported in the literature to prevent mania-like behavior in rodents.[29,30] All injections were performed between ZT3 and ZT4 (10:00–11:00 AM).

Preparation of Behavioral Assays
Animals habituated to testing rooms for at least 30 minutes before the behavior tests. Testing occurred early in the light phase (ZT6-ZT10) (13:00–17:00). The open field test, elevated plus maze test and portlet forced swim paradigms test were performed as described previously.[31]

Open Field Test
The mouse was placed in the periphery of a novel open field environment in a dimly lit room and allowed to explore for 10 minutes. The apparatus is a 40 × 40-cm open field surrounded by 40-cm-high walls. The animals were monitored from above by a video camera connected to a computer running

video tracking software (Ethovision XT12, Noldus, Leesburg, VA) to determine the time in the center arena, total distance moved, and the number of entries into two areas: the periphery (15 cm from the walls) and the center (10 ×10 cm). Total distance moved in the open field is correlated with hyperactivity. The open field arenas were wiped with water-alcohol (75%) solution and allowed to dry between mice.

Elevated Plus Maze Test

Mice were placed in the center of an elevated plus-maze (each arm is 30-cm long and 5-cm wide with two opposite arms closed by 10-cm high walls) elevated 60cm off the floor in a dimly lit room and allowed to explore for 10 minutes. The animals were monitored from above by a video camera connected to a computer running video tracking software (Ethovision XT12, Noldus, Leesburg, VA) to determine time spent in the open and closed arms, time spent in the middle, as well as the number of entries into the open and closed arms. Since rodents have an innate fear of height and openness, time spent on the open arm and the number of entries in the open arm are both correlated with anxiety-like behavior. An increase in open arm activity (duration and/or entries) reflects less anxiety behavior. The apparatus was wiped with water-alcohol (75%) solution and allowed to dry between mice.

Forced Swim Test

The forced swim test was performed as described in Krishnan et al.[32] Mice were placed in a 3 litters Pyrex glass beaker containing 2 liters of water at 24 ± 1°C. The mice remained in the water for 6 min and were then removed and allowed to dry in a clean dry cage before returning to their home cage. The water was changed between each subject. The animals were monitored from above by a video camera connected to a computer running video tracking software (Ethovision XT12, Noldus, Leesburg, VA) to determine the immobile time in the water. The last 4 minutes of the test was scored for latency to the first immobility and total time spent immobile. Immobility was defined as no motion, except for single limb paddling to maintain flotation.

Preparation of Brain Tissue Samples

Mice were anesthetized with chloral hydrate (50 mg/kg body weight) and sacrificed by cervical dislocation. Brains were removed and rinsed in ice-cold isotonic saline. The hippocampus was quickly dissected out and stored at −80°C until use.

Extraction of Neurotransmitters

The tissue samples were weighed and ground in liquid nitrogen. 200μL of pre-cooled water and 800μL of methanol (Merck, 144,282)/acetonitrile (Merck, 1,499,230–935) (1:1, V/V/) were added to each sample and mixed by the vortex. The ultrasonic was used for 60 seconds in an ice bath. Then, the mixed samples were incubated at −20°C to precipitate the protein and centrifuged at 14,000 × g at 4°C for 20 minutes. The supernatants that contained mixture of neurotransmitters were taken and vacuum dried.

Analysis of Neurotransmitters

The samples were separated by Shimadzu Nexera X2 LC-30AD ultra-high pressure liquid chromatography (HPLC) (Shimadzu). The operating conditions for HPLC were: Mobile phase: Solution A was 5% acetonitrile with 20mM ammonium acetate (Sigma, 70,221), PH 9.45, solution B was 100% acetonitrile. The sample was placed in the automatic sampler at 4°C, the column temperature was 40°C, the flow rate was 300μL/min, the injection volume was 5μL. A quality control (QC) sample is set for a certain number of experimental samples at every interval in a sample queue to test and evaluate the stability and repeatability of the system. Mass spectrometry (MS) analysis was performed using a 5500 QTRAP mass spectrometer (AB SCIEX) in positive ion mode. During the mass spectrometric detection, acetonitrile-aqueous solutions (1:1, V/V) of different volumes (50–200μL) were added, and the same amount of the re-standard Glutamate-D3 was added.

Statistical Analyses

The data of behavioral tests and monoamines and their metabolites were analyzed by two-way analysis of variance (ANOVA) followed by Fisher's LSD test for post hoc analysis. Bonferroni correction was employed to adjust p values of main effects and interaction in ANOVA. All statistical analyses were performed using the software Prism version 5.0 (Graphpad Software Inc, San Diego, USA). Adjusted p values less than 0.05 were considered statistically significant.

Results

VPA Reversed the Hyperactivity of $Clock^{delta19}$ Mouse

We first accessed the locomotor activity of $Clock^{delta19}$ and WT mouse treated with saline or VPA by analyzing the total distance moved in the open field test. The result

showed that there is no statistically significant interaction between the effect of the *Clock* gene and drug treatment (F (1,36) = 0.99, p = 0.33) on total distance moved (Table S1). The main effects analysis indicated that both *Clock* gene (*F* (1, 36) = 22.63, p < 0.0001) and drug treatment (*F* (1,36) = 5.08, p = 0.03) had significant effects on total distance moved (Table S1). The Fisher's LSD test for post hoc analysis revealed that when treated with saline, *Clock*^{delta19} mouse exhibit a significant increase in the total distance moved compared to the WT mouse (p<0.0001) (Table S2, Figure 1A). VPA treatment significantly decreased the total distance moved of *Clock*^{delta19} mouse in the open field test (p = 0.028) (Table S2, Figure 1A). There is no significant difference in the total distance moved between saline-treated WT mouse and VPA-treated *Clock*^{delta19} mouse (p = 0.09) (Table S2, Figure 1A). We examined the total distance moved of *Clock*^{delta19} mouse and WT mouse treated with saline or VPA for every minute over 10 minutes in the open field. The results also indicated that *Clock*^{delta19} mouse exhibit a significant increase in the total distance moved for every minute compared to the WT mouse, VPA treatment prevents the increased total distance moved in *Clock*^{delta19} mouse (Figure 1B). These results suggested that the hyperactivity presented in *Clock*^{delta19} mouse can be reversed by chronic VPA treatment.

VPA Reversed the Level of Anxiety-Like Behavior of *Clock*^{delta19} Mouse

We then examined anxiety-like behavior in *Clock*^{delta19} and WT mouse treated with saline or VPA by analyzing time in open arms and the number of entries in open arms in the elevated plus-maze test. There is a statistically significant interaction between the effect of the *Clock* gene and drug treatment (F (1, 34) = 6.84, p = 0.013) on time in open arms (Table S1). The main effects analysis indicated that both *Clock* gene (F (1, 34) = 9.20, p = 0.005) and drug treatment (F (1,34) = 4.86, p = 0.034) had significant effects on time in the open arms (Table S1). The Fisher's LSD test for post hoc analysis revealed that when treated with saline, *Clock* ^{delta19} mouse exhibited a significant increase in the time in open arms compared to the WT mouse (p<0.0001) (Table S2, Figure 2A). In *Clock* ^{delta19} mouse, VPA treatment significantly decreased the time in the open arms (p = 0.002) (Table S2, Figure 2A). There is no significant difference in the time in the open arms between saline-treated WT mouse and VPA-treated *Clock*^{delta19} mouse (p = 0.55) (Table S2, Figure 2A). There is a statistically significant interaction between the effect of the *Clock* gene and drug treatment (*F* (1, 34) = 7.01, p = 0.012) on the number of entries in the open arms (Table S1). The main effects analysis indicated that both *Clock* gene (F (1, 34) = 11.12, p = 0.002) and drug
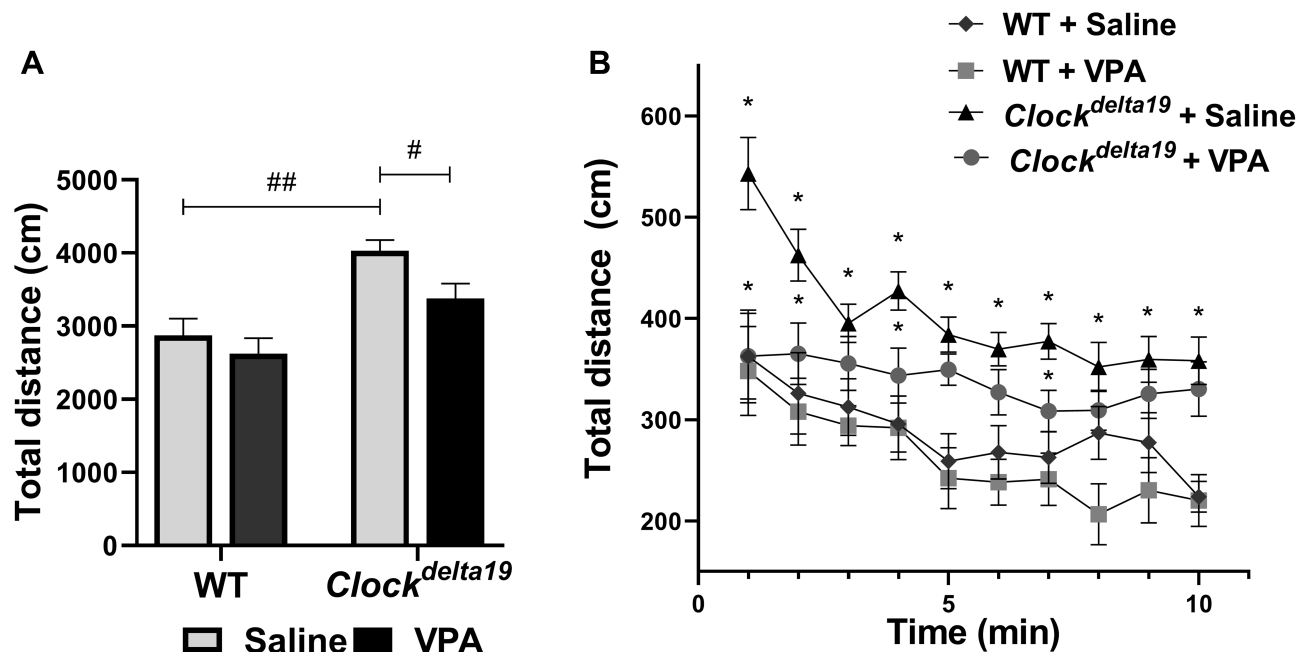
Figure 1 Effect of VPA on the *Clock*^{delta19} mice in the open field test. Hyperactivity was assessed in mice using the open field test. Total distance moved in the open field test was measured in mice. (**A**) Total distance moved in the open field test for 10 minutes. (**B**) Distance moved in the open field for every minute over a 10 minutes period (#p < 0.05, ##p < 0.01; *p < 0.05, n = 10). In all panels error bars show S.E.M.
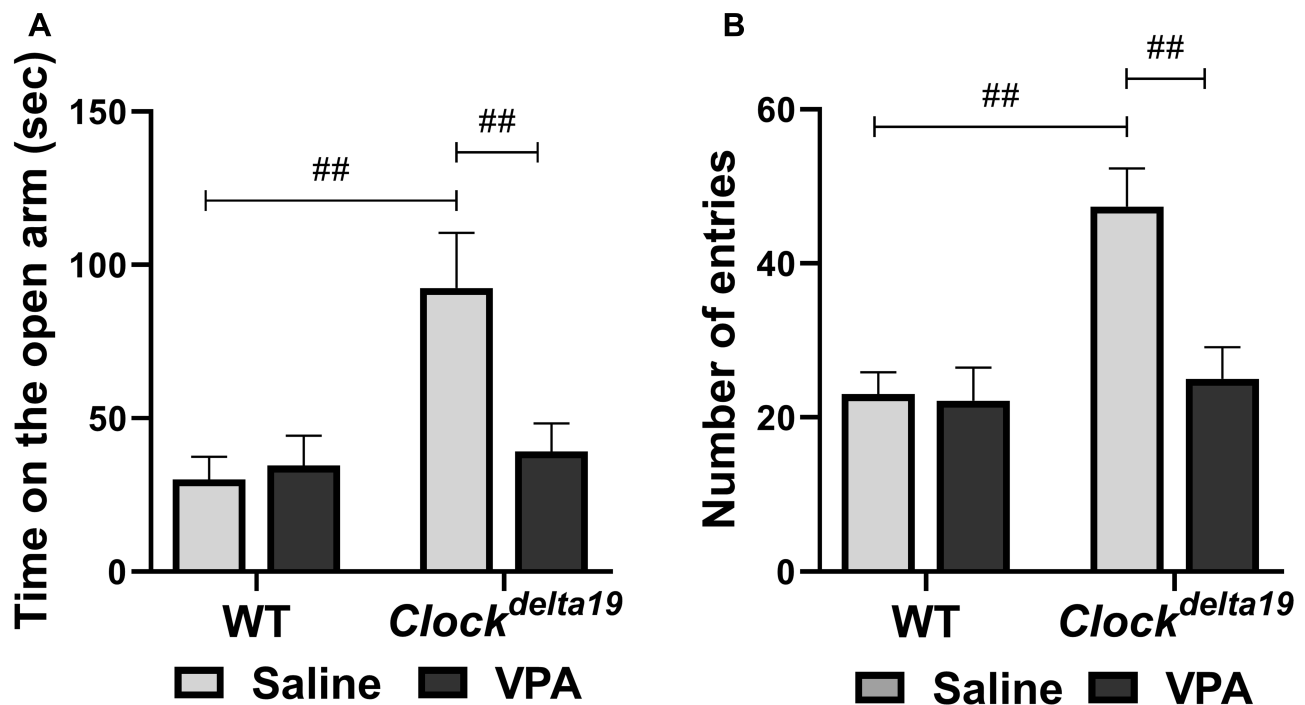
Figure 2 Effect of VPA on the *Clock*[delta19] mice in the elevated plus maze test. Anxiety-like behavior was assessed in mice in the elevated plus maze test. The time on the open arms and number of entries into the open arms were recorded. (**A**) Time on the open arms in 10 minutes. (**B**) Number of entries into the open arms in 10 minutes (##p < 0.01, n = 8–10). In all panels error bars show S.E.M.

treatment ($F (1, 34) = 8.09$, $p = 0.007$) had significant effects on number of entries in the open arms (Table S1). The Fisher's LSD test for post hoc analysis revealed that when treated with saline, *Clock*[delta19] mouse exhibit a significant increase in the number of entries in the open arms compared to the WT mouse ($p < 0.0001$) (Table S2, Figure 2B). In *Clock*[delta19] mouse, VPA treatment significantly decreased the number of entries in the open arms ($p = 0.001$) (Table S2, Figure 2B). There is no significant difference in the number of entries between saline-treated WT mouse and VPA-treated *Clock*[delta19] mouse ($p = 0.72$) (Table S2, Figure 2B). These results suggested that decreased anxiety-like behavior in *Clock*[delta19] mouse can be reversed by chronic VPA treatment.

VPA Reversed the Level of Depression-Like Behavior of *Clock*[delta19] Mouse

We examined the depression-like behavior of *Clock*[delta19] and WT mouse treated with saline or VPA by analyzing immobile time in the forced swim test. The result showed that there is a statistically significant interaction between the effect of the *Clock* gene and drug treatment ($F (1,30) = 5.67$,

$p = 0.024$) on immobile time (Table S1). The main effects analysis indicated that both *Clock* gene ($F (1, 30) = 7.42$, $p = 0.011$) and drug treatment ($F (1, 30) = 6.38$, $p = 0.017$) had significant effects on immobile time (Table S1). The Fisher's LSD test for post hoc analysis revealed that when treated with saline, *Clock*[delta19] mouse exhibit a significant decrease in immobile time compared to the WT mouse ($p = 0.001$) (Table S2, Figure 3). In *Clock*[delta19] mouse, VPA treatment significantly increased the immobile time ($p = 0.002$) (Table S2, Figure 3). There is no significant difference in immobile time between saline-treated WT mouse and VPA-treated *Clock*[delta19] mouse ($p = 0.90$) (Table S2, Figure 3). These results suggested decrease depression-like behavior in *Clock*[delta19] mouse can be reversed by chronic VPA treatment.

Monoamines and Their Metabolites in the Hippocampus

To explore the potential roles of neurotransmitters in the effect of VPA on mania-like behavior of *Clock*[delta19] mouse, we determined levels of dopamine (DA), serotonin (5-HT), levodopa (L-DOPA) and 5-hydroxy-L-tryptophan (L-5-HTP) in the hippocampus of *Clock*[delta19] and WT mouse treated with saline or VPA. There were no statistically significant interactions between the effect of the
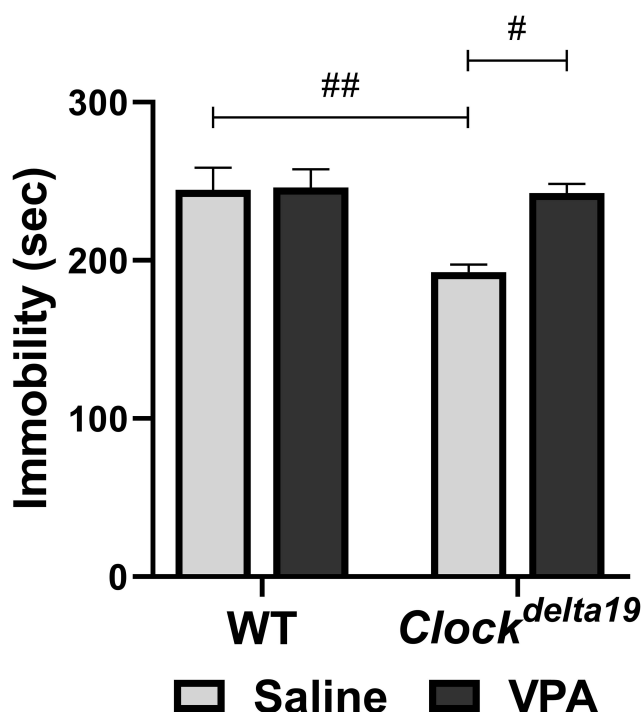
Figure 3 Effect of VPA on the *Clock*[delta19] mice in the forced swim test. Depression-like behavior was assessed in mice using the forced swim test (#p < 0.05, ##p < 0.01, n = 8–10). Immobility time in a 6 minutes test was recorded. In all panels error bars show S.E.M.

Clock gene and drug treatment on levels of the four neurotransmitters tested in this study (Table S3). The main effects analysis indicated *Clock* gene had significant effects on the levels of 5-HT (F = 13.02, p = 0.001) and DA (F = 13.35, p = 0.001) in right hippocampus (Table S3), drug treatment had significant effects on the levels of L-DOPA in right hippocampus (F = 10.32, p = 0.003) (Table S3). The Fisher's LSD test for post hoc analysis revealed that *Clock*[delta19] mouse exhibit a significant decrease in levels of 5-HT (p = 0.013 when treated with saline; p = 0.02 when treated with VPA) and DA (p = 0.023 when treated with saline; p = 0.011 when treated with VPA) in right hippocampus compared to the WT mouse (Table S4, Figure 4B and F). There was no significant difference in the level of 5-HT and DA in left hippocampus between *Clock*[delta19] mouse and WT mouse treated with saline or VPA (Table S4, Figure 4A and E). VPA treatment significantly decreased levels of L-DOPA (p = 0.004) in right hippocampus in *Clock*[delta19] mouse (Table S4, Figure 4H). In left hippocampus, no significant difference in the level of L-DOPA was observed between *Clock*[delta19] mouse and WT treated with saline or VPA (Table S4, Figure 4G). There was no significant difference in L-5-HTP level in the hippocampus between *Clock*[delta19]

mouse and WT treated with saline or VPA (Table S4, Figure 4C and D).

Discussion

In this study, we examined the effects of VPA on the mania-like behaviors in *Clock*[delta19] mouse. We also explored the potential roles of VPA on DA and 5-HT and their metabolites in brain regions. We found that VPA can reverse the mania-like behavior observed in the *Clock*[delta19] mouse. Our results also showed that there were significant differences in the level of monoamines and their metabolites in the left and right hippocampus of *Clock*[delta19] mouse compared with WT mouse. Administration of VPA partially altered the level of neurotransmitters in the right hippocampus of *Clock*[delta19] mouse. These results suggest that the mania-like behaviors of *Clock*[delta19] mouse may be related to the changed level of DA and 5-HT systems and the treatment effects of VPA on *Clock*[delta19] mouse may be related to DA and 5-HT metabolism.

Clock[delta19] mouse have been proved to be an animal model for BD with substantial face and predictive validity. *Clock*[delta19] mouse exhibited hyperactivity, decreased anxiety-related behavior, and decreased depression-related behavior that similar to the manic state of patients with BD.[9–11,14,15,17,33–36] Chronic lithium treatment could reverse behavioral abnormalities[9,11,15,17,18] and neurobiological abnormalities observed in the *Clock*[delta19] mouse.[3,15,18,37] In this study, we indicated that chronic VPA treatment can reverse the mania-like behavior of the *Clock*[delta19] mouse. This result further proved the well predictive validity of the *Clock*[delta19] mouse as the model of BD underly the previous studies. It would be interesting in the future to examine the effect of other medication treatments frequently used in BD on the *Clock*[delta19] mouse, such as lamotrigine, carbamazepine, oxcarbazepine, antipsychotics, and electroconvulsive therapy. In addition, our results identified that WT mouse are not altered behaviorally by administrated with VPA. This can against the possibility that VPA is causing the *Clock*[delta19] mouse to not feel well and thus simply lowers their activity and behavior on all measures, which further consolidates the credibility of our study results.

VPA was first introduced as an anticonvulsant and has been widely used as a mood stabilizer in the treatment of BD for many years.[38] VPA is effective for the treatment of acute mania and the maintenance therapy for mania prevention.[39,40] Our study identified that chronic VPA
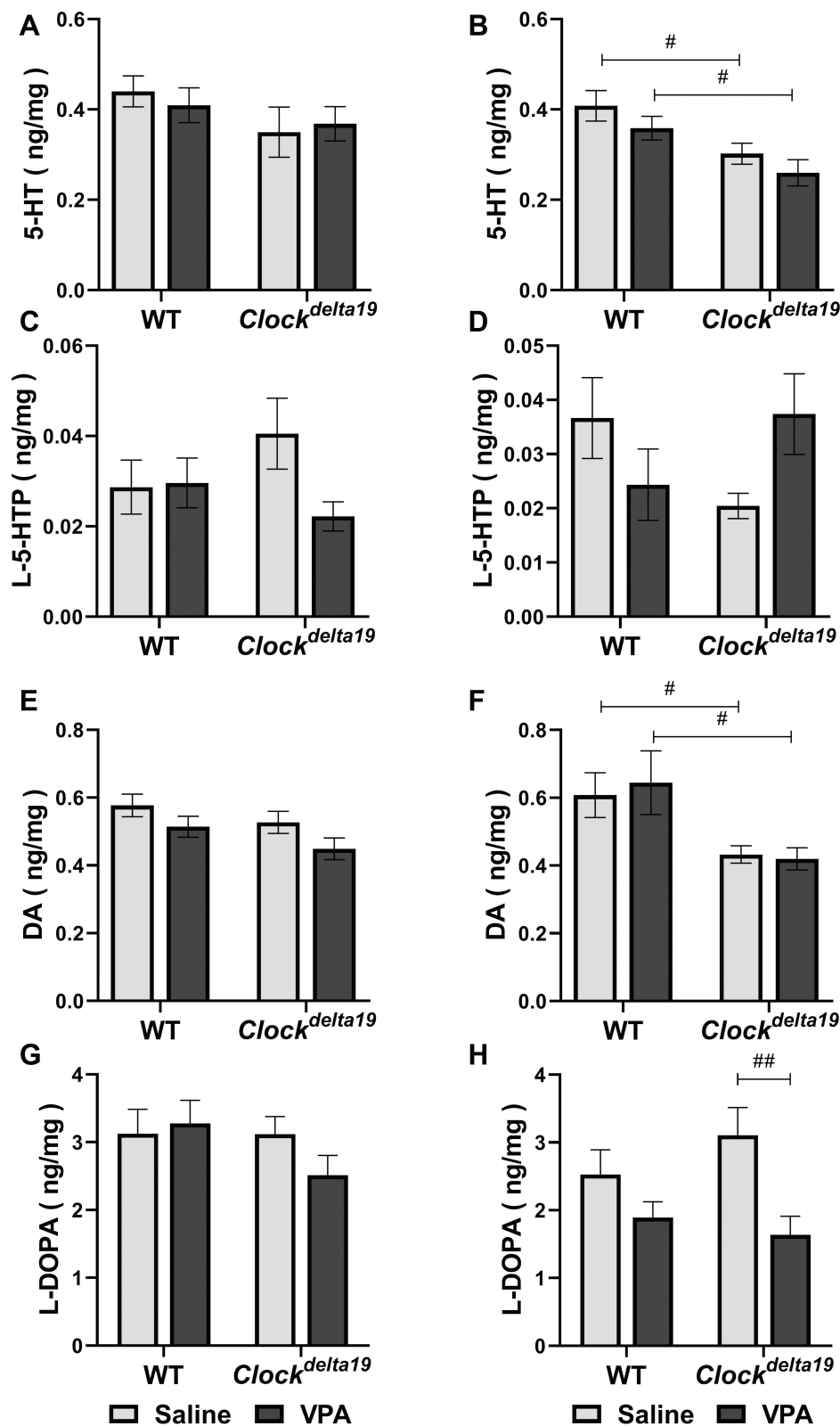
Figure 4 Levels of monoamines and their metabolites in the hippocampus. The levels of monoamines and their metabolites in the hippocampus were determined. (**A**) Levels of 5-HT in left hippocampus. (**B**) Levels of 5-HT in right hippocampus. (**C**) Levels of L-5-HTP in left hippocampus. (**D**) Levels of L-5-HTP in right hippocampus. (**E**) Levels of DA in left hippocampus. (**F**) Levels of DA in right hippocampus. (**G**) Levels of L-DOPA in left hippocampus. (**H**) Levels of L-DOPA in right hippocampus. (#$p < 0.05$, ##$p <$ 0.01, n = 5–8). In all panels error bars show S.E.M.
Abbreviations: 5-HT, serotonin; L-5-HTP, 5-hydroxy-L-tryptophan; DA, dopamine; L-DOPA, levodopa.

treatment can reverse the mania-like behavior of the *Clock*[delta19] mouse. This is consistent with the clinical treatment effect of VPA on BD patients. However, some important aspects of VPA known from human studies remain to be investigated. Studies suggesting that VPA causes users tremors, which may be related to its antic-onvulsant effect. To further consolidate the predictive validity of the *Clock*[delta19] mouse as an animal model for BD, it would be of interest to investigate whether VPA also causes to develop tremors in this mouse strain. Similarly, cosmetic side effects such as hair loss, color changes and curling of hair have also been reported in VPA-treated patients.[41] To strengthen the predictive valid-ity of the *Clock*[delta19] mouse as an animal model for BD, future studies should examine whether the *Clock*[delta19] mouse also emerges cosmetic side effects following treat-ment with VPA. In addition, the mechanisms of action of VPA are still not fully understood. The application of the *Clock*[delta19] mouse on the studies of mood-stabilizing effects of VPA would facilitate exploration of the patho-physiology underlying.

Previous studies have indicated that dopaminergic abnormalities were observed in several brain regions of the *Clock*[delta19] mouse, such as the ventral tegmental area (VTA),[11] striatum[16] and nucleus accumbens (NAC).[15] In this study, we found that the *Clock*[delta19] mouse exhibited a reduction of DA compared with the WT mouse in the right hippocampus. Hippocampus is a critical brain region that has been associated with depression and anxiety. The expression of the *Clock* gene in the hippocampus is important in regulating mood-related behaviors. The antidepressant treatment has been shown to increase *Clock* expression in the hippocampus.[42] *Clock*[delta19] mouse display mania-like behavior and exhibit a lower level of DA compared with the WT mouse in the right hippocampus in our study. It can be suspected that there may be underline relationship between mania-like behavior phenotype and the alteration on level of DA. However, the lower level of DA was observed in right hippocampus of *Clock*[delta19] mouse in our study, which is inconsistent with the elevated dopaminergic activity in VTA of the *Clock*[delta19] mouse.[15] It is maybe relevant to the expres-sion of the *Clock* gene in the hippocampus in the *Clock*[delta19] mouse. Study had indicated that the *Clock* gene has the potential to regulate the second group of genes including the genes related to the dopamine system.[43] Thus, we could suspect that the discrepancy

on the level of DA in the *Clock*[delta19] mouse may be relevant to the regulation of the *Clock* gene in the hippocampus. It is also likely that the *Clock* gene in other brain regions is important in regulating mood-related behaviors. The ventral and orbital part of the medial prefrontal cortex (mPFC) has been suggested to contribute to the emotional regulation impairment in bipolar disorder.[44] It is a limitation that the present study did not explore the function of the *Clock* gene in mPFC.

Simultaneously, we found that VPA could reduce the level of L-DOPA in the right hippocampus of the *Clock*[delta19] mouse. As the major metabolites of DA, the level of L-DOPA in the brain indicates the turnover of DA. The effect of VPA on L-DOPA in right hippocampus of the *Clock*[delta19] mouse is consistent with the effect of lithium on DA and its metabolisms in VTA and NAC of *Clock*[delta19] mouse.[15,18] Thus, the effects of VPA on the mania-like behavior of the *Clock*[delta19] mouse may be related to the dopaminergic activity in the hippocampus. Further experiments are needed to imple-ment and to explore the underlying mechanism in the future.

The central 5-HT system is known to be involved in emotion, learning, and memory.[45] The pathophysiology of anxiety and depression involves dysfunctions in serotoner-gic activity.[46] Our results showed that there is a lower level of 5-HT in right hippocampus of the *Clock*[delta19] mouse compared with WT mouse. Studies explicated that defi-ciency of 5-HT is thought to cause depression and anxiety.[47,48] Yun-Fang Jia et al[49] showed that the anxiety-like behaviors of the central 5-HT-deficient mouse were decreased and the depression-like behaviors were similar to the control mouse. We supposed that the alteration of anxiety and depression-like behavior in the *Clock*[delta19] mouse may be related to the serotonergic activity in the hippocampus. Besides, we could not exclude the possibility that the *Clock* gene influenced the behavioral performances of the *Clock*[delta19] mouse by possible regulatory mechan-isms that are associated with the 5-HT transmitter or ser-otonergic neurons. Our current investigation provides suggestive research data on the relationship between sero-tonergic and psychiatric disorders.

In conclusion, our study indicated that chronic VPA treatment can reverse the mania-like behavior of the *Clock*[delta19] mouse, which further consolidated the predic-tive validity of the *Clock*[delta19] mouse as an animal model for BD. Monoamine neurotransmitters and their metabo-lites in the hippocampus are partly regulated by mutation of the *Clock* gene or VPA treatment.

Ethics Approval and Consent to Participate

Acknowledgments

Disclosure

All authors declare no personal or financial conflicts of interest in this work.

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