pCREB-Per2 pathway in hippocampal CA1 region mediates the transition of depression and mania-like behaviors in rats

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Highlights

1. Knockdown of *Per2* in CA1 produces mania-like behavior.
2. Over-expression of *Per2* in CA1 induces depression-like behavior.
3. Over-expression of *Per2* in CA1 can rescue the ouabin-induced mania-like behavior.
4. Knockdown of *CREB* in CA1 leads to mania-like behavior, which can be rescued by over-expression of *Per2* in this region.
5. Regulation of *Per2* expressions in CA1 provides a novel animal model of bipolar disorder.

Abstract

Bipolar disorder (BD) is a severe psychiatric disorder characterized by alternating manic/hypomanic and depressive episodes, with rare reports on the mechanism of manic/depressive behavior switching. Our study revealed that knockdown of *Per2* in the hippocampal CA1 brain region induced manic-like behavior in rats, in contrast, over-expression of the *Per2* gene in the same brain region results in depression-like behavior, indicating that Per2 mediates the manic/depressive phenotype switch. It is known that pCREB is the transcription factor of *Per2*. We found that pCREB regulates the expression of Per2 and pCREB-Per2 pathway mediates the manic/depressive behavioral switch. This provides a novel animal model of bipolar disorder, which may be the first animal model that presents both mania and depression-like behaviors, and may promote the progress of research into bipolar disorder.

Keywords: pCREB, Per2, mania, depression, bipolar

Introduction

Bipolar disorder (BD) is a chronic, severe mental disorder, characterized by manic or hypomanic episodes and depressive episodes. It ranks 17th among all disabling diseases in the world [1]. The World Mental Health Survey reported that the lifetime prevalence and 12-month prevalence of BD were 2.4 percent and 1.5 percent, respectively [2]. Throughout the course of BD, the manic or hypomanic episodes can be converted to depressive episodes or normal intermittent periods. Manic episodes and depressive episodes are two symptom groups with opposite manifestations of BD. Some antidepressants have the risk of turning the patients into mania episodes in clinic. Therefore, preventing and treating the inversion of mania and depression is an urgent problem to be solved clinically. Researches into the mechanism of manic and depressive behavior transition have important clinical values for the treatment of BD, especially for the prevention of manic/depressive course transition.

The clock gene *Period* (*Per*) includes three subtypes of *Per1*, *Per2* and *Per3*. Among them, there are many reports about the involvement of *Per1* and *Per2* genes in affective disorders. The latest study reported that *Per1* knockout mice developed depressive-like behaviors, and the depressive-like behaviors of mice could be reversed by inducing the increased expression of *Per1* in the lateral stiff nucleus [3]; Knockout of *Per2* in glial cells also reversed depression-like behavior in mice [4]. In consistent with their reports, our previous study found that knockdown of the *Per1* gene in the hippocampal CA1 brain region resulted in depression-like behaviors, while the phenotype of *Per2* was the opposite [5]. In this study, we aimed to explore the role of Per2 in mood-related behaviors, including mania- and depression-like behaviors. We found that knockdown of *Per2* in hippocampal CA1 region results in mania-like behaviors in rats, on contrary, over-expression of *Per2* in this region produces depression-like behaviors. What's more, knockdown of CREB in hippocampal CA1 produces mania-like behavior, which can be reversed by over-expression of *Per2*, demonstrating that pCREB-Per2 pathway mediates the transition of mania and depression-like behaviors.

Materials and Methods

Animals

Male Sprague Dawley (SD) rats (220-240 g) were bought from the Ji'nan Pengyue Laboratory Animal Breeding Company and housed in groups of three under a reverse 12 h/12 h light/dark cycle (8:00 light on and 20:00 light off) with free access to food and water. The room temperature was maintained 23±1°C and humidity was 50±5% controlled.

All of the animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of Shandong University School of Basic Medicine (Ethical approval number: ECSBMSSDU2022-2-51).

Drugs

Intracranial microinjections

After the rats were anaesthetized by isoflurane gas, we microinjected AAV-shPer2/shCREB (1ul per side) or AAV-Scramble (1ul per side) bilaterally into CA1 (anterior/posterior, -4.3 mm; medial/lateral, ±2.0 mm; and dorsal/ventral, -2.0 mm with Hamilton syringes connected to 30-gauge injectors. We injected within 5 minutes and kept the injector in place for an additional 3 minutes to allow diffusion.

Ouabin was administrated according to a former study [6].

Sucrose preference test (SPT), forced swim test (FST), Open field test (OFT) and elevated plus maze (EPM)

The procedures were based on our previous studies [5].

Statistical analysis

The data were expressed as mean±SEM. Statistical analyses were performed using Prism 5 software (GraphPad). The statistical analyses of data were done using unpaired two-tailed Student's t-test, two-way ANOVA followed by Bonferroni's multiple tests as appropriate, which can be referred to the figure legends. Values of $p < 0.05$ were considered statistically significant.

Results

1. Knockdown of *Per2* in CA1 produces mania-like behavior

In order to explore the role of Per2 in CA1 region in mood, we performed knockdown of *Per2* in this region and tested the behaviors. After rats were habituated for seven days, we did brain micro-injection to infuse the AAV-sh*Per2* into the rats' CA1 region, which could knockdown the expression of Per2. 21 days later, we tested the expression levels of Per2 in CA1 and the mood-related behaviors of rats, including sucrose preference test, forced swimming test, open field test and elevated plus maze test. Western Blot analysis showed that Per2 protein levels were significantly down-regulated in CA1 in the AAV-shPer1 infusion group (Fig. 1 b, c, $p < 0.05$). In sucrose preference test, there were not significant difference between the two groups (Fig. 1d). However, in the forced swimming test, the immobility time of *Per2*-knockdown group was significantly reduced (Fig. 1e, $p < 0.05$), revealing a potency in mania-like behavior. In the elevated plus maze test, the *Per2* knockdown group spent more time in the open arms (Fig. 1f, $p < 0.01$), more peaks of heading into open arms (Fig. 1f, $p < 0.01$), and they ran longer distances than the control group significantly (Fig. 1f, $p < 0.05$), showing more exploratory behaviors and mania-like behaviors. In the open field test, this group rats also showed significantly larger activity quantities and stayed longer in the center zone, demonstrating more exploratory behaviors and mania-like behaviors. In summary, knockdown of *Per2* in CA1 induces mania-like behaviors in rats.

2. Over-expression of *Per2* in CA1 induces depression-like behavior

In order to explore the role of Per2 in mood, we also performed over-expression of *Per2* in CA1 by micro-injection of LV-*Per2* into this region and did behavioral tests. After 12 days of infusion of LV, we also tested the Per2 protein levels and found it was significantly elevated in CA1, compared with the control group (Fig. 2b, $p < 0.05$). As expected, we found that the sucrose preference values were significantly down-regulated (Fig. 2c, $p < 0.05$), demonstrating a depression-like behavior. Besides,

in the open field test and elevated plus maze test, we found that the *Per2* over-expression group spent less time in the center zone and less time in the open arms (Fig. 2d, e, $p < 0.05$), exhibiting anxiety-like behaviors. Overall, over-expression of *Per2* in CA1 may lead to depression-like behaviors, opposite to that of knockdown of it.

3. Ouabin-induced mania-like behaviors can be rescued by the over-expression of *Per2* in CA1

To further confirm the role of Per2 in the pathogenesis of bipolar disorder, we investigated it in the bipolar disorder model. Infusion of ouabin in the lateral cerebral ventrical is a commonly used model of mania. We used this model to induce mania-like behaviors in rats, and explored whether over-expression of *Per2* in CA1 can rescue them. After injected csf/ouabin in lateral cerebral ventrical and LV in CA1 5 days, we performed behavioral tests, including sucrose preference test, open field test and elevated plus maze test (Fig. 3a). We did not find any significant difference among groups in the sucrose preference values (Fig. 3b, $p > 0.05$). In the open field test and elevated plus maze test, LV-*Per2* can significantly rescue the ouabin-induced increases in center time and open arms (Fig. 3c, d, $p < 0.05$), showing an anti-manic-like effect.

In order to explore the upstream factor that affect the *Per2* expression and mood, we aimed at pCREB. Although *Per2* has a lot of transcription factors, of which pCREB is one of the most important that regulate mood. As is known to all, CREB is the convergent point of various pathways in the cytoplasm. After activated, it is phosphorylated and translocates into cell nucleus and regulate hundreds of genes' expressions as a transcription factor. Therefore, we aimed to explore the role of pCREB in regulating *Per2* expression and mood-related behaviors, and conducted the following research.

4. Knockdown of CREB in CA1 induces mania-like behaviors, which can be rescued by the over-expression of *Per2* in this region

We conducted knockdown of CREB by infusing AAV-scramble or AAV-shCREB into CA1 and tested the mood-related behaviors 21 days later. After that, we infused lentivirus into this region to over-express *Per2* and also did behavioral tests 12 days after this surgery (Fig.4a). In the sucrose preference test, we found that the sucrose preference values were significantly increased in the *CREB* knockdown group, compared with the scramble group (Fig.4c, $p < 0.05$), showing a mania-like behavior. This can be reversed by lentivirus that over-express *Per2*. The sucrose preference values were significantly down-regulated after infusion of LV-*Per2* (Fig. 4c, $p < 0.05$). Besides, we found that the AAV-shCREB group also increased in the center time significantly (Fig. 4d right panel, $p < 0.001$) and the running distances (Fig. 4d left panel, $p < 0.05$) in open field test, demonstrating mania-like behaviors. Furthermore, the time at center zones can also be decreased by LV-*Per2* significantly (Fig. 4d right panel, $p < 0.05$), revealing an anti-manic like effect of LV-*Per2*. In the elevated plus maze test, we also find the same trend. The number of entrances into open arms was significantly increased in the *CREB* knockdown group (Fig. 4e left panel, $p < 0.01$), which can also be down-regulated by over-expression of *Per2* significantly (Fig. 4e left panel, $p < 0.05$). In parallel, the time that AAV-shCREB group spent in open arms was also increased significantly, compared with the control group (Fig. 4e right panel, $p < 0.05$), which can be down-regulated by LV-*Per2*. Therefore, knockdown of *CREB* in CA1 shows pro- manic-like effect, which can be mitigated by over-expression of *Per2* in this region. In other words, over-expression of *Per2* in CA1 produces an anti-mania like effect.

Discussion

In this paper, we reveal that *Per2* in CA1 mediates the transition of depression- and mania-like behaviors. Knockdown of *Per2* in this region induces mania-like behaviors, in contrast, over-expression of it in the same region leads to depression-like behaviors. Besides, over-expression of *Per2* produces an anti-mania like effect in ouabin model, demonstrating an involvement of *Per2* in the pathogenesis of bipolar disorder. pCREB is the transcription factor of *Per2*, also involves in the modulation of mania-like

behavior. Knockdown of CREB in CA1 also leads to mania-like behaviors, which could be remitted by over-expression of *Per2*.

Our results were consistent with former studies. Gaspar et al. found that the elevated levels of CREB/pCREB were involved in the pathogenesis of bipolar disorder, which was in line with us [7]. Alda et al. also reported that pCREB levels were significantly increased in the lymphoblasts from peripheral blood samples in bipolar patients in clinical studies [8]. However, most of the previous studies demonstrated the role of *Per2* in bipolar disorder mainly from peripheral blood or tissues, limited by clinical research limitations or animal models of bipolar disorder. To this extent, this study may be the first to demonstrate a certain pathway in a certain brain region that mediates the transition of mania and depression-like behaviors.

Until now, there is not a commonly accepted animal model that presents both depression-like phenotype and mania-like phenotype [9]. This study also provides a novel animal model showing both phenotypes to research into bipolar disorder. We can use knockdown of *Per2* in CA1 to induce mania-like phenotype and over-expression of it in the same region to induce depression-like phenotype. To our knowledge, this is the first animal model of bipolar disorder that involves both phenotypes and involves interventions of only one gene, avoiding disturbances of two factors. This models two phases of bipolar disorder from only one pathophysiologic process and may be the most suitable animal model of it.

However, this paper mainly shows the behavioral results, we need to do further studies to reveal the underlying molecular pathogenesis.

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Author Contribution

Xin-Ling Wang contributed to the study conception, design and performed the experiments herself. Besides, the manuscript was written by Xin-Ling Wang.

Acknowledgement

This study was supported by the National Natural Science Foundation of China (NSFC82201682), Natural Science Foundation of Shandong Province (ZR2021QH282) and the Fundamental Research Funds of Shandong University (2020GN095) to Xin-Ling Wang. The author thanks her husband Fuchao Jiao for his encouragement.

Conflict of Interests

The author declares that there are no conflict of interests.

Figure Legends

Figure 1 Knockdown of *Per2* in CA1 produces mania-like behavior

a. Experimental timeline. b. Immunofluorescence showed the infusion site of AAV in hippocampal CA1 region. c. Westernblot analysis of knockdown of Per2 in CA1. d. Sucrose preference test results. e. Forced swimming test. f. elevated plus maze test. g. open field test. Unpaired *t* test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, compared with the AAV-scramble group.

Figure 2 Over-expression of *Per2* in CA1 induces depression-like behavior

a. Experimental timeline. b. Western blot analysis of Per2 over-expression in CA1. c. Sucrose preference test. d. Open field test. e. Elevated plus maze test. Unpaired *t* test, $*p < 0.05$, compared with the LV-control group.

Figure 3 Over-expression of *Per2* in CA1 can rescue the ouabin induced mania-like behavior

a. Experimental timeline. b. Sucrose preference test. c. Open field test. d. Elevated plus maze test. Two-way ANOVA, $*p < 0.05$, compared with the control+LV-control group. $\#p < 0.05$, compared with the ouabin+LV-Control group.

Figure 4 Knockdown of *CREB* in CA1 leads to mania-like behaviors, which can be rescued by over-expression of *Per2*.

a. Experimental timeline. b. Western blot analysis of *CREB* knockdown in CA1. c. Sucrose preference test. d. Open field test. e. Elevated plus maze test. Unpaired *t* test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, compared with the AAV-scramble group; $\#p < 0.05$, compared with the AAV-sh*CREB* group.

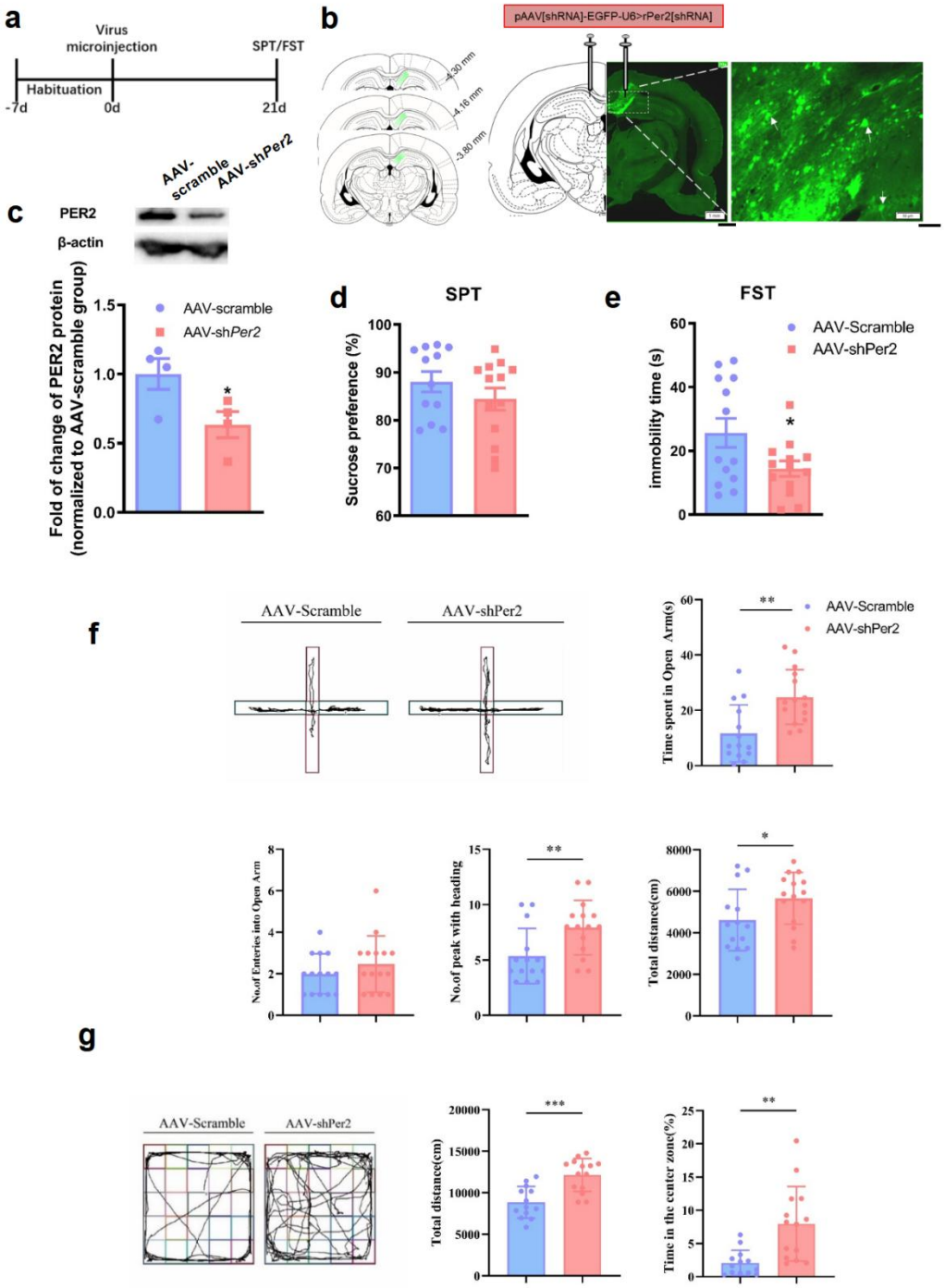


Figure 1 Knockdown of Per2 in CA1 produces mania-like behavior

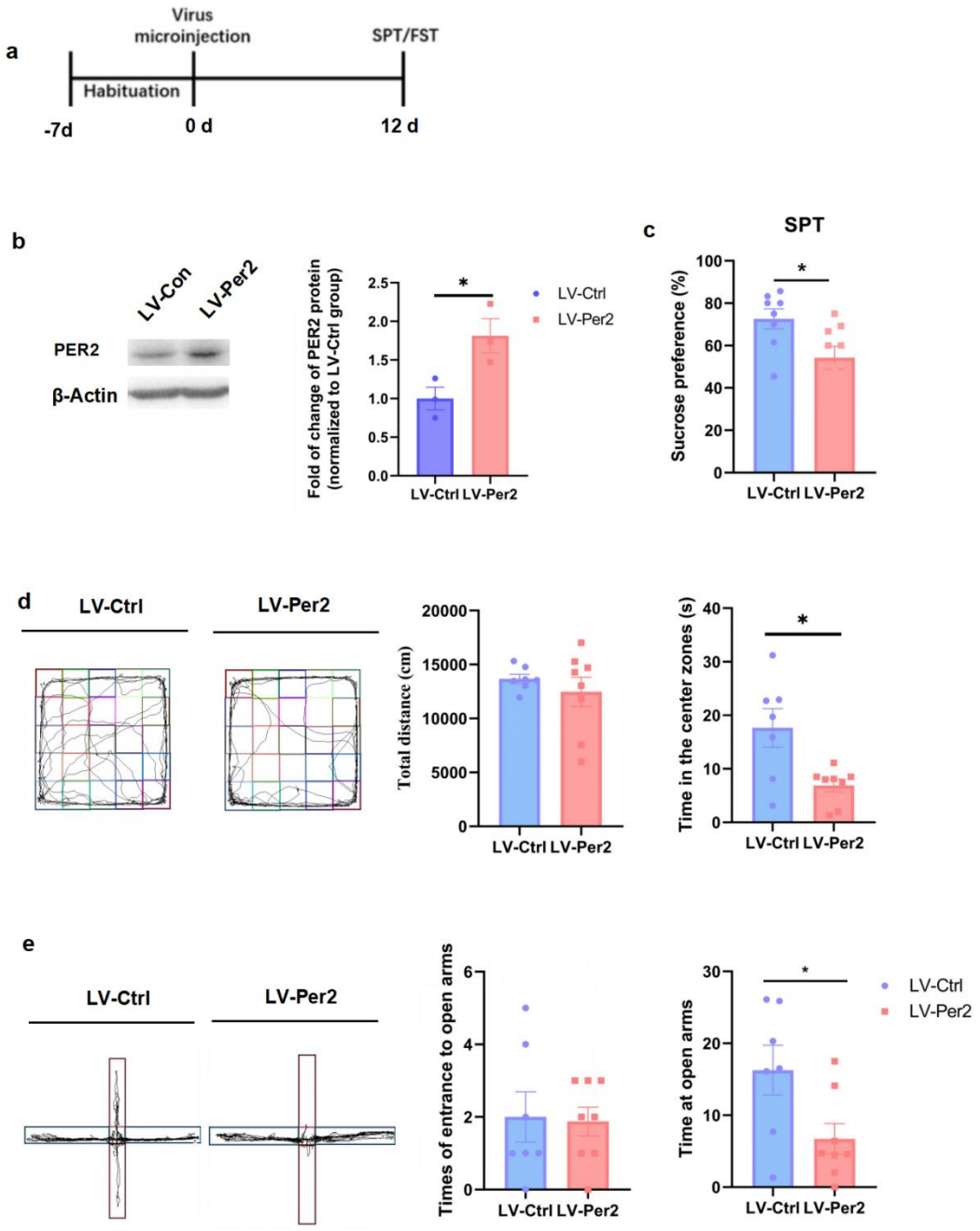


Figure 2 Over-expression of Per2 in CA1 induces depression-like behavior

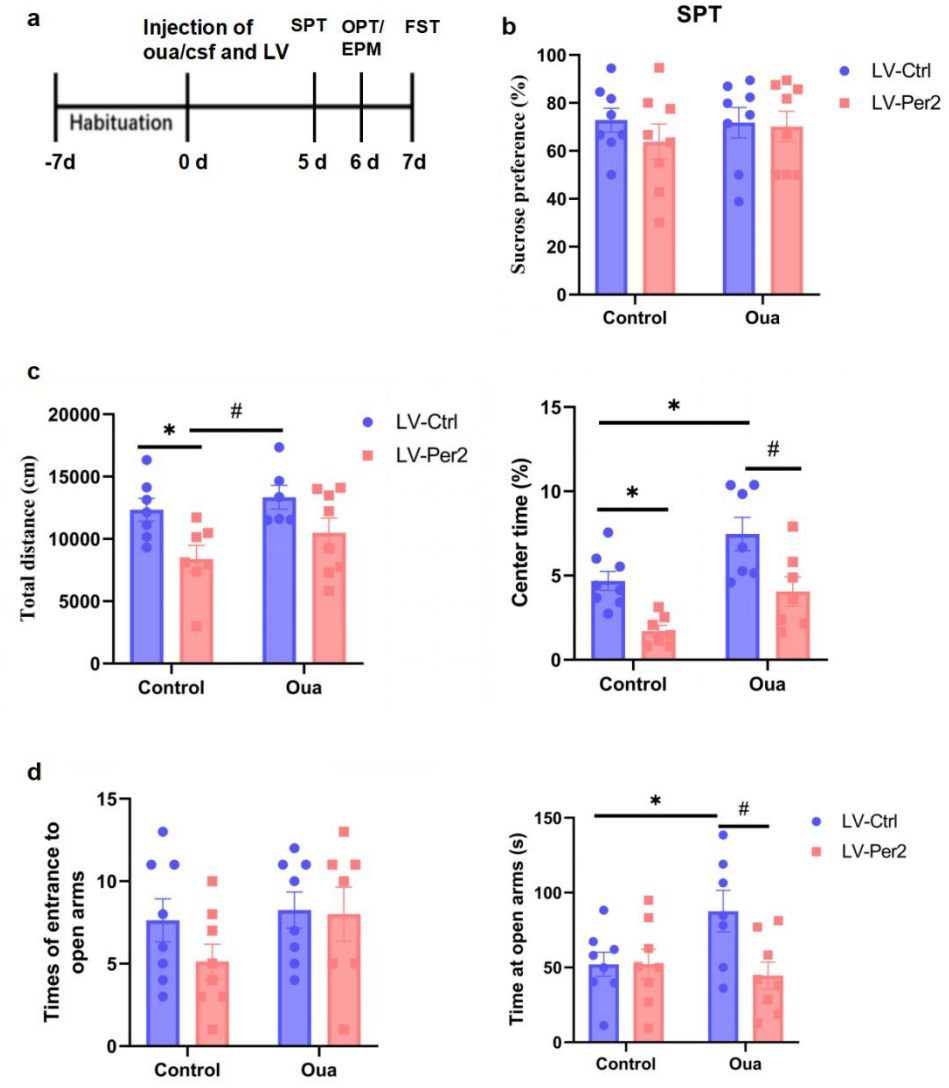


Figure 3 Over-expression of Per2 in CA1 can rescue the ouabin induced mania-like behavior

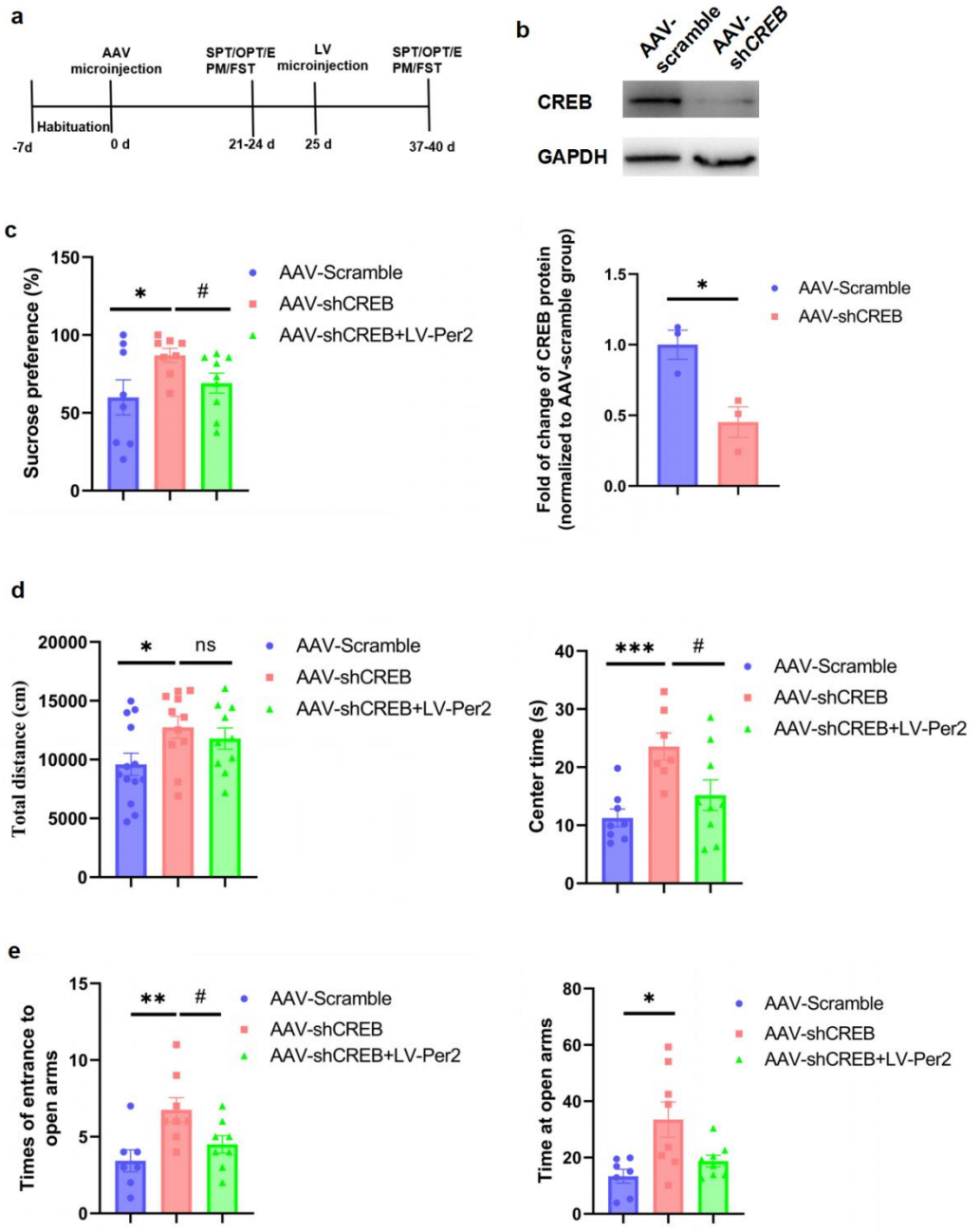
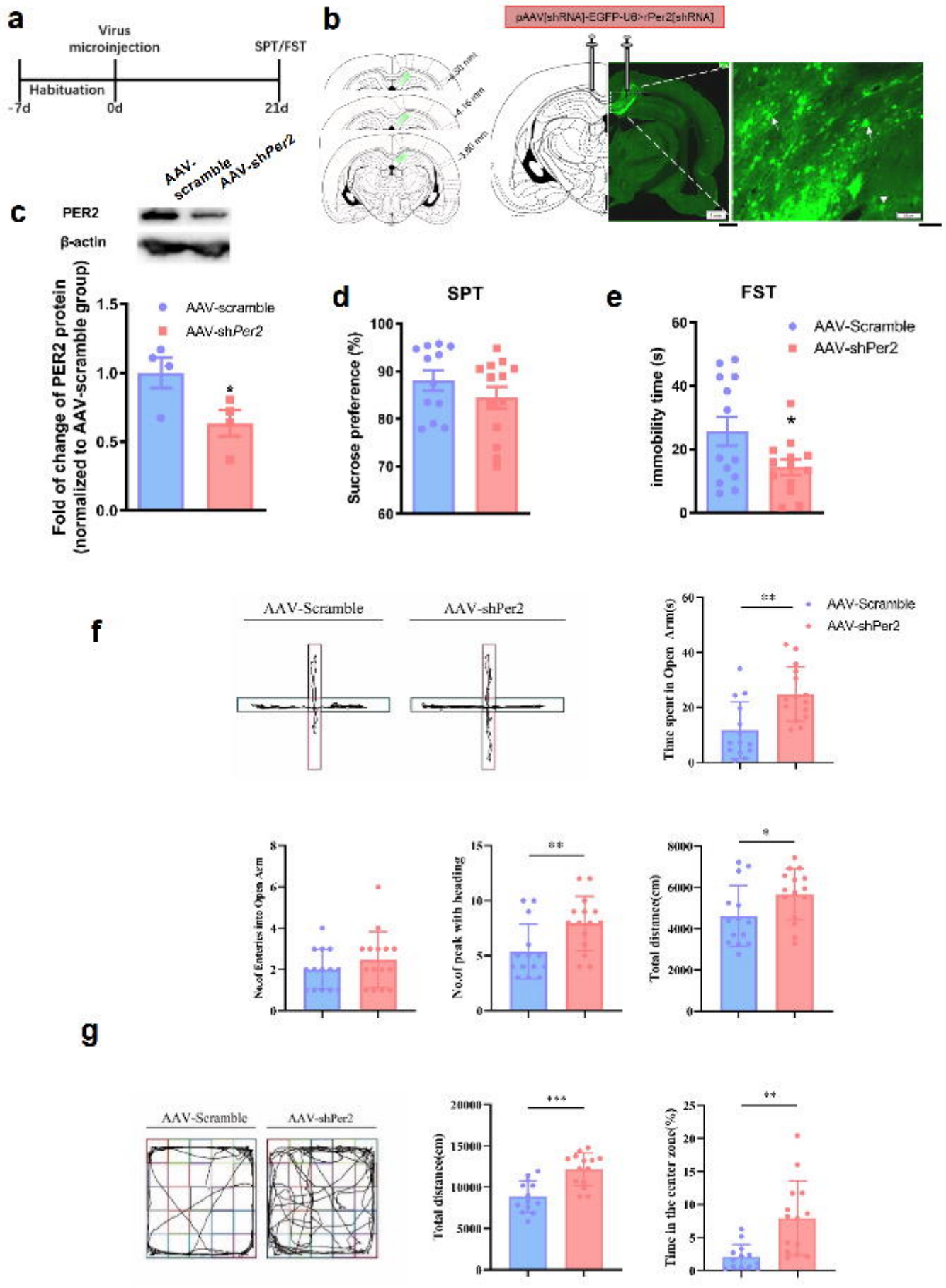


Figure 4 Knockdown of CREB in CA1 leads to mania-like behaviors, which can be rescued by over-expression of Per2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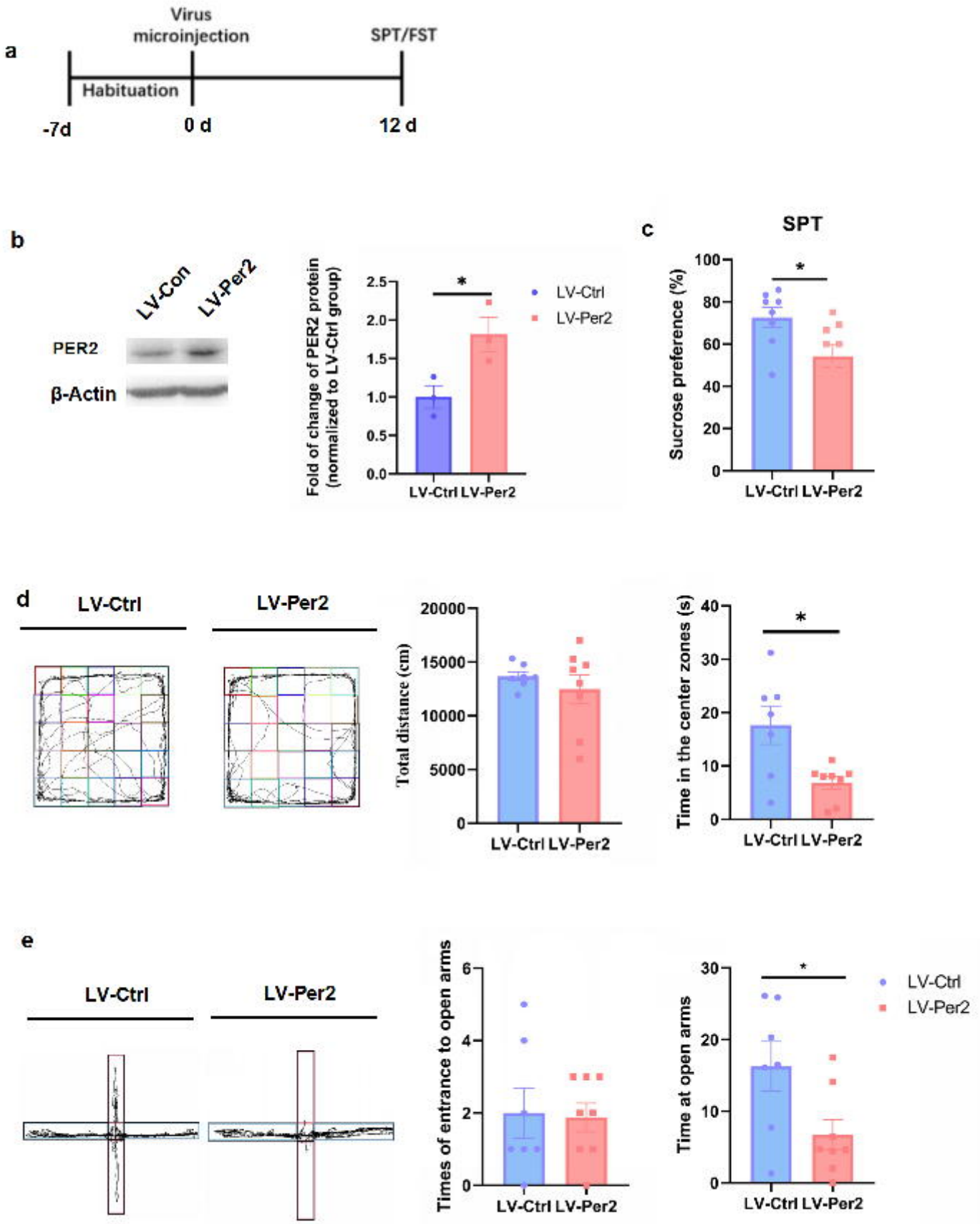
Virus microinjection
SPT/FST
Habituation
-7d
0d
21d
b
pAAV[shRNA]-EGFP-U6>rPer2[shRNA]
c
AAV-scramble
AAV-shPer2
PER2
β-actin
Fold of change of PER2 protein (normalized to AAV-scramble group)
AAV-scramble
AAV-shPer2
d
SPT
Sucrose preference (%)
e
FST
immobility time (s)
AAV-Scramble
AAV-shPer2
f
AAV-Scramble
AAV-shPer2
Time spent in Open Arm(s)
No.of Entries into Open Arm
No.of peak with heading
Total distance(cm)
g
AAV-Scramble
AAV-shPer2
Total distance(cm)
Time in the center zone(%)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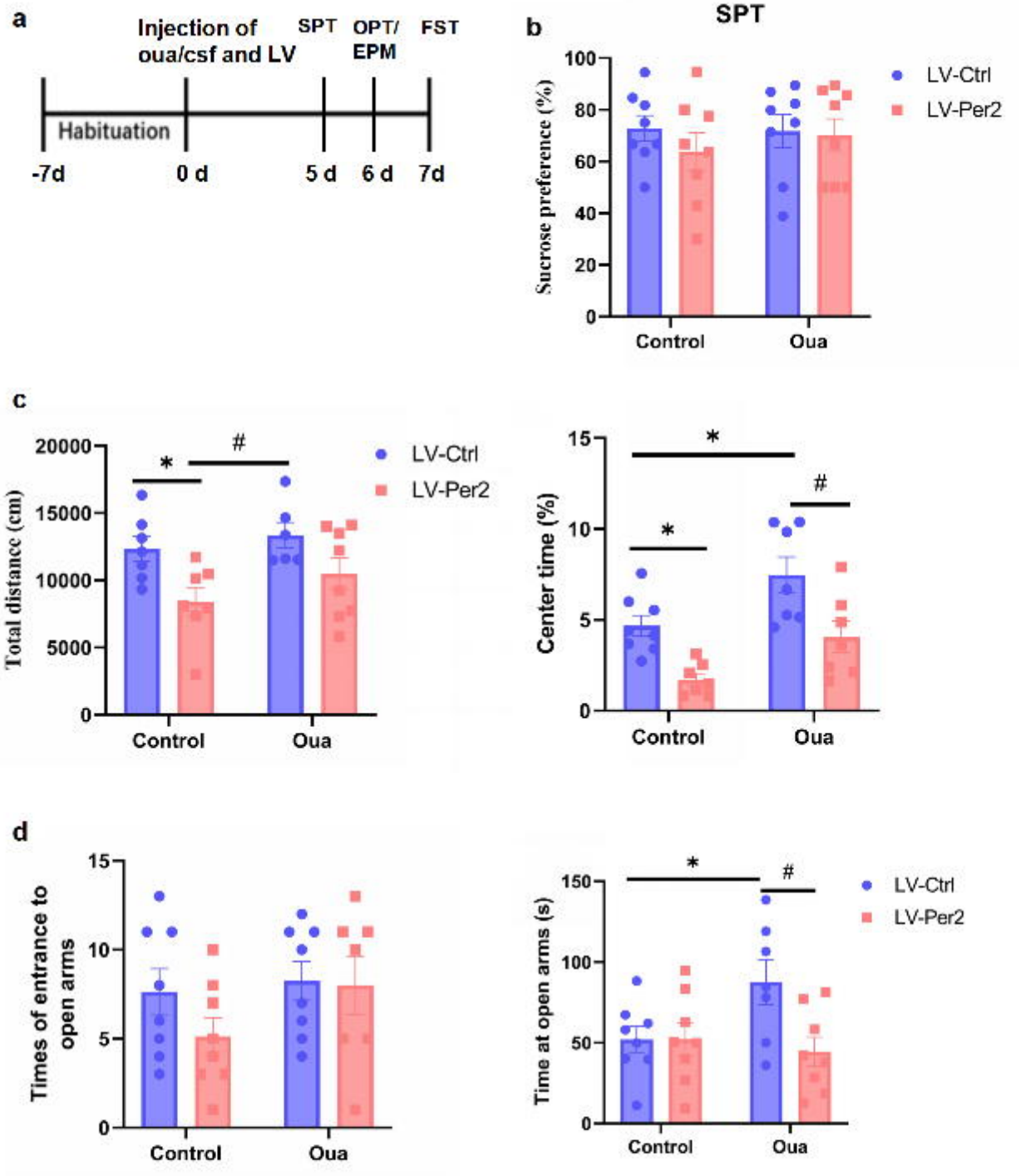
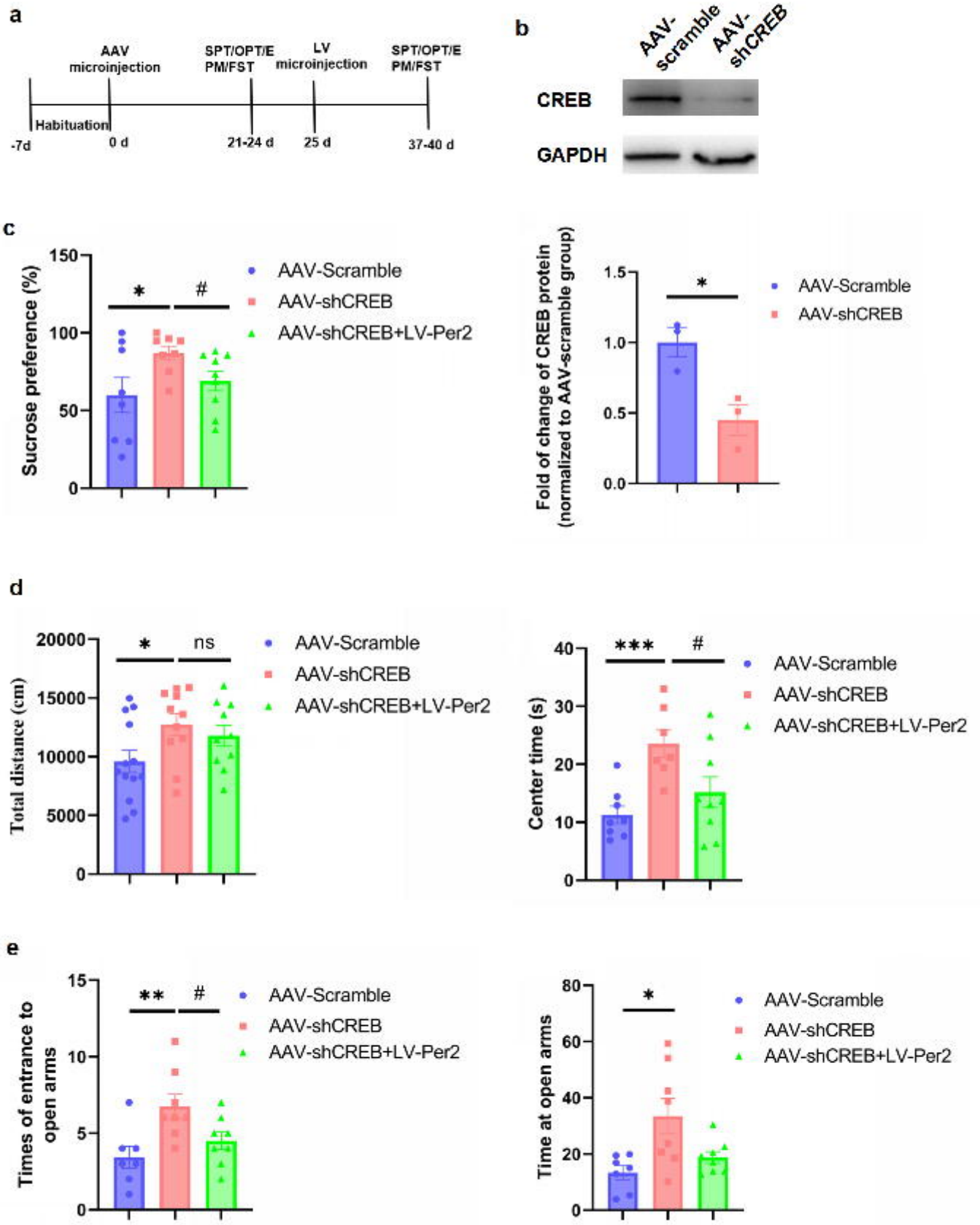
Virus microinjection
SPT/FST
Habituation
-7d
0 d
12 d
b
LV-Con
LV-Per2
PER2
β-Actin
Fold of change of PER2 protein (normalized to LV-Ctrl group)
LV-Ctrl
LV-Per2
c
SPT
Sucrose preference (%)
d
LV-Ctrl
LV-Per2
Total distance (cm)
Time in the center zones (s)
e
Times of entrance to open arms
Time at open arms


a
Injection of oua/csf and LV
SPT
OPT/ EPM
FST
Habituation
-7d
0 d
5 d
6 d
7d
b
SPT
Sucrose preference (%)
LV-Ctrl
LV-Per2
Control
Oua
c
Total distance (cm)
Center time (%)
d
Times of entrance to open arms
Time at open arms (s)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AAV microinjection
SPT/OPT/EPM/FST
LV microinjection
SPT/OPT/EPM/FST
Habituation
-7d
0 d
21-24 d
25 d
37-40 d
b
AAV-scramble
AAV-shCREB
CREB
GAPDH
c
Sucrose preference (%)
AAV-Scramble
AAV-shCREB
AAV-shCREB+LV-Per2
Fold of change of CREB protein (normalized to AAV-scramble group)
d
Total distance (cm)
ns
Center time (s)
e
Times of entrance to open arms
Time at open arms