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RESEARCH ARTICLE

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Optogenetic stimulation of ventral tegmental area dopaminergic neurons in a female rodent model of depression: The effect of different stimulation patterns

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Abstract

Major depressive disorder is one of the most common mental disorders, and more than 300 million of people suffer from depression worldwide. Recent clinical trials indicate that deep brain stimulation of the superolateral medial forebrain bundle (mfb) can have rapid and long-term antidepressant effects in patients with treatment-resistant depression. However, the mechanisms of action are elusive. In this study, using female rats, we demonstrate the antidepressant effects of selective optogenetic stimulation of the ventral tegmental area's dopaminergic (DA) neurons passing through the mfb and compare different stimulation patterns. Chronic mild unpredictable stress (CMUS) induced depressive-like, but not anxiety-like phenotype. Short-term and long-term stimulation demonstrated antidepressant effect (OSST) and improved anxiolytic effect (EPM), while long-term stimulation during CMUS induction prevented depressive-like behavior (OSST and USV) and improved anxiolytic effect (EPM). The results highlight that long-term accumulative stimulation on DA pathways is required for antidepressant and anxiolytic effect.

KEYWORDS
depression, medial forebrain bundle, optogenetics, RRID:AB_143157, RRID:AB_221568, RRID:AB_2534069, RRID:AB_291659, RRID:SCR_019096, ventral tegmental area

1 | INTRODUCTION

Major depressive disorder (MDD, or depression) has become a global disease, and is considered to be the leading psychiatric disorder today, impacting on quality of life, public health, and multiple other socioeconomic domains (DiLuca & Olesen, 2014). Women suffer from depression nearly twice as much as men (Malhi & Mann, 2018; Michaud, 2001). Conventional antidepressant treatments, including psychotherapy or pharmacotherapy, are effective in around 70%–80% of the patients, but the significant numbers who fail to respond are classified as having treatment-resistant depression (TRD) (Holtzheimer & Mayberg, 2011; Rush et al., 2006). In clinical trials

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with patients with TRD, deep brain stimulation (DBS) has been used to target several brain structures associated with depression (for a review see Dandekar et al., 2018; Drobisz & Damborská, 2019). Clinical studies of bilateral and chronic DBS of the superolateral medial forebrain bundle (slMFB, or more precisely the projection pathway linking forebrain and the ventral tegmental area, "VTApp") produced fast and long-term antidepressant effects with 75% responders, and sustained remission in 50% of the patients even after the 4 years follow-up (Bewernick et al., 2017; Coenen et al., 2012, 2019; Schlaepfer et al., 2013). In the following "mfb" will refer to experimental (rodent) and "MFB" to clinical (human) work.

MDD is both biologically and symptomatically diverse with certainly multiple networks and transmitters implicated to different levels in subgroups of patients with depression. The two principle symptoms necessary for clinical diagnosis of depression are reduced motivation and anhedonia. It has been postulated that it is a dysfunction of the dopaminergic projections running via the MFB on the limbic circuitry that is in part responsible for these seminal symptoms observed in MDD and in other psychiatric disorders (Heshmati & Russo, 2015; Li et al., 2018). VTA dopaminergic neurons are controlled by numerous hubs, such as the lateral habenulla or the rostromedial tegmental nucleus, and the structure contributes to different functions, including aversive behaviors (Bourdy & Barrot, 2012; Yang et al., 2018). Preclinical data also strongly suggest that the A10 midbrain mesocorticolimbic dopaminergic neurons projecting on the frontal striatal network from the VTA via the mfb to the nucleus accumbens (NAC) and dorsolateral prefrontal cortex (PFC) contribute to positive emotional and euphoric behaviors that support exploration, motivation, and control appetitive learning (Alcaro & Panksepp, 2011; Berridge, 2019; Berridge & Kringelbach, 2015; Howe et al., 2013; Ikemoto & Panksepp, 1999; Panksepp, 1998; Wise & McDevitt, 2018).

The antidepressant mechanisms of action of the clinical slMFB DBS are not understood, but it has been hypothesized that it could be a consequence of the modulation of the activity of the mesocorticolimbic dopaminergic pathway implicated in motivation, but also in the stress response (Ashouri Vajari et al., 2020; Coenen et al., 2012, 2020; Furlanetti et al., 2016; Holly et al., 2015; Holly & Miczek, 2016; Thiele et al., 2018, 2020). Electrical high-frequency stimulation is nondiscriminatory and cannot manipulate a single transmitter system since the heterogeneous constituent neural populations (such as VTA glutamatergic and GABAergic neurons) project across rodent mfb (Nair-Roberts et al., 2008). Optogenetics for selective manipulation of neuronal function has been widely used to investigate the connectivity and function of dopaminergic neurons within complex brain circuits (Cohen et al., 2012; Engelhard et al., 2019; Parker et al., 2016; Tye et al., 2013). Furthermore, optogenetic methods have been used to demonstrate A10 VTA dopamine's contribution to key depressive-like symptoms, and to the antidepressant effects of selective dopaminergic manipulation using experimental models (Furlanetti et al., 2016; Heshmati & Russo, 2015; Lobo et al., 2012; Tye et al., 2013).

In this study, we hypothesized that stimulating VTA DA neurons at the mfb cause anxiolytic and antidepressant effects. In addition, we investigated the importance of timing of the stimulation. By using chronic mild unpredictable stress (CMUS) as rodent model of depression, the antianxiety and antidepressant qualities of short-term stimulation pattern (ss-ChR2) or long-term stimulation during (Prev-ChR2) or after (Post-ChR2) the CMUS paradigm and their capacity to interfere or reverse the CMUS induced behavioral phenotype were tested.

Significance

Major depressive disorder (MDD) is one of the most common mental disorders in over 300 million of people worldwide. Women are twice as likely, compared with men, to suffer from depression. Over 20% of MDD patients failed to respond to conventional antidepressant treatments. Mesolimbic and mesocortical dopaminergic (DA) pathways—implicated in motivation, reward-orientated, and aversive learning behaviors that are part of key symptoms of depression—have been understudied in preclinical research. Understanding the mechanism for the abnormal dopaminergic system in MDD is clinically significant. Here, we selectively stimulate DA pathways through medial forebrain bundle (mfb) in rodent models of depression. Our results suggest that long-term accumulative stimulation on mfb DA pathways is essential for antidepressant and anxiolytic effect.

2 | METHODS

2.1 | Animals and experimental design

TH:Cre BAC transgenic rats were bred by mating Cre-positive founders to wild-type Long Evans rats to produce TH:Cre heterozygous transgenic rats (Witten et al., 2011). Female rats ($n = 46$) aged 4 to 8 months were used for optogenetic stimulation and behavioral experiments. The stimulation parameter and behavioral designs are demonstrated in Figure 1. Animals had two behavioral testing blocks with the CMUS induction protocol sandwiched in between "Baseline" and "CMUS+", except the poststimulation group had an additional testing after the stimulation ("Stim"). Three different acute or chronic stimulation patterns were employed in this study. The short-term stimulation pattern animals received stimulation 30 min prior to every behavioral test. The preventive pattern animals received stimulation every other day during the CMUS protocol, and the postpattern animals were stimulated every other day once the CMUS induction ended. All rats were sacrificed immediately after the last behavioral test. All rats were single housed throughout the study with a 12 hr standard light–dark cycle (lights on between 07h00 and 19h00; off between 19h00 and 07h00). Water and food were available *ad libitum* except when rats underwent water and food deprivation or restraint stress during CMUS protocol (Table S2). Experimental protocols were approved by the local ethics committee and followed the ethical guidelines set by the Regierungspräsidium Freiburg (Tierversuchsantrag G14/40).

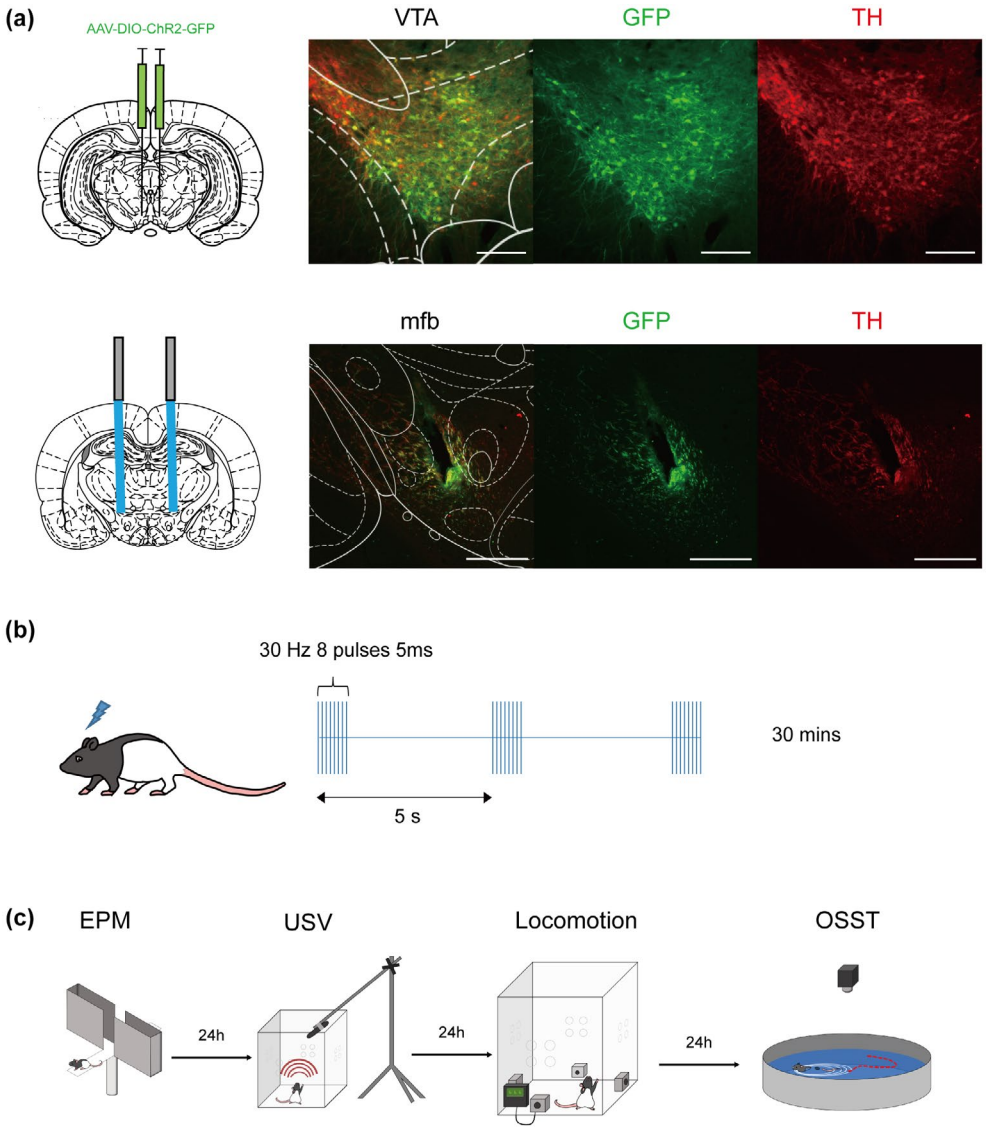

FIGURE 1 Histology and experimental design. (a) AAV injection into VTA (scale bar 200 μm) and optic fibers implantation above mfb (scale bar 500 μm). The graphs AAV labeled neurons in VTA delimited by TH immunostaining (red). (b) Laser stimulation parameters. (c) Time line of the behavioral sessions

2.2 | Stereotactic injection and optic fiber implantation

Anesthesia was established by placing the animal in an induction box with 4% isoflurane (with 2 L/min O_2 as carrying gas), and then transferring them to a stereotactic frame (Stoelting, USA) where the anesthesia was maintained between 1.5% and 2%. All coordinates used in the surgery were taken from a "flat skull" position. The experimental AAV2-EF1a-DIO-ChR2-eYFP virus (titer 4.2 * 10^{12} particles per ml) and the control AAV2-EF1a-DIO-eYFP virus (titer 4.6 * 10^{12} particles per ml) were obtained from the University of North Carolina, USA. The virus was injected bilaterally at two sites and at two depths in the ventral tegmental area (VTA) using a 2 μl Hamilton syringe controlled by an injection pump at 100 nl/min at the following coordinates and volumes: Site 1: 1 μl injected at AP: −5.4/−6.0 Ml: ±0.7 DV: −8.0; Site 2:0.8 μl at AP: −5.4/−6.0 Ml: ±0.7 DV: −7.5. The needle was left in place after injection for 8 min before slowly withdrawing it. All rats received bilateral mfb (AP: −2.8 Ml: −1.8 DV: −7.7) fiber optic light guide implants consisting of a metal ferrule, 2.5 mm in diameter with a 200-mm thick, 9-mm long cleaved bare optic fiber (Doric Lenses, Canada). Instant adhesive (Loctite 401, Germany) was placed between the base of the optic fiber and the skull, and bone cement ("Palacos," Heraeus, Germany) was used to adhere the fiber to the skull.

2.3 | Chronic mild unpredictable stress (CMUS) protocol

The CMUS protocol was used to induce depression-like phenotype in rodents (Willner, 1997; Willner et al., 1987). Over a period of 6 weeks, the diverse mild stressors were pseudo randomly scheduled and imposed on the animals on a daily basis: white noise (http://www.simplynoise.com) for 2 hr; paired housing (alternating between

being the normal resident or an intruder) for 4 hr; light cycle (continuous illumination) for 24 to 36 hr; damp bedding (200 ml water poured onto 100 g sawdust bedding) for 14 hr; cage tilt on a 45° angle for 16 hr; food deprivation for 17 hr followed by 1 hr of restricted access to food (5 micropellets); water deprivation for 17 hr immediately following exposure to empty bottles for 1 hr (Table S2).

2.4 | Laser delivery and protocols

A 200-μm patch cord (0.48 NA, Doric Lenses) was connected to the external portion of the chronically implantable optical fiber with a zirconia sleeve. Patch cords were attached through a 2FC-FC Fiber optic rotary joint (Doric Lenses) to a 473-nm blue laser diode ("LuxX," Omicron-Laserage Laserprodukte GmbH, Rodgau, Germany), and light pulses were generated through a stimulator (Doric Lenses). For rats expressing ChR2 and their eYFP controls, the light paradigm was 8 light pulses at 30 Hz every 5 s for 30 min in all experiments (Figure 1b). Optical-fiber light power from the patch cord was measured using a light sensor (PM100D, Thorlab) and intensity calculated using a model based on empirical measurements from mammalian brain tissue for predicting irradiance values (http://www.stanford.edu/group/dlab/cgi-bin/graph/chart.php). For ChR2-transduced rats or controls, estimated light intensity at 0.5 mm from fiber tip ranged from 3 to 10 mW/mm^2.

2.5 | Behavioral testing schedule

There were two or three behavioral blocks in each group, but additional weight information was collected more frequently. The behavior sessions contained elevated plus maze (EPM), ultrasonic vocalization (USV), locomotion test, and open space swimming test (OSST) (Figure 1c). All behavioral tests were conducted between 09h00 and 14h00 and were monitored by Biobserve Viewer 2 software (Biobserve GmbH, Bonn, Germany).

2.6 | Open space swimming test (OSST)

OSST measures coping ability to stressful situations (Sun & Alkon, 2003). The rats were placed into a pool with a diameter of 132 cm and a height of 60 cm. The pool was filled with water (22 ± 1°C) to a depth of 40 cm, which was rendered white with odorless, innocuous paint to improve tracking of the animals. No escape platform was provided. The subjects were allowed to swim (or not to swim) freely for 15 min on four consecutive days. Total track length during this period in the pool was recorded for analysis.

2.7 | The home cage locomotor activity

Exploratory behavior was evaluated in the home cage at different time points during the study. Home cage was equipped with two pair of light beams, splitting the cage into four equal virtual quadrants. Crossing of the beams was counted automatically, and used as a measure to reflect general activity. The monitoring session lasted 30 min.

2.8 | Ultrasonic vocalization (USV)

Rodent vocalization has been shown to vary according to their affective state, with low-frequency ultrasonic vocalization (USV) (around 22 kHz) reflecting negative affective or aversive behavior, while high USV (around 50 kHz) associated with pleasurable or rewarding experiences (Portfors, 2007; Wöhr et al., 2008). Animals were placed into individual cages with recording microphones placed 60 cm above them connected to a Sonotrack Ultrasound recording and analysis system (Sonotrack, Metris, Netherlands). Vocalization was monitored in two frequency bands: a low band of 18–32 kHz, associated with negative affect; and a high band of 47–53 kHz, associated with positive affect. USV was recorded for 20 min. The number of events that occurred in each frequency range during the experiment was assessed. Sessions where the animals emitted few vocalizations (<5 calls/min) were excluded from the analysis.

2.9 | Elevated plus maze (EPM)

EPM performance reflects the animals' anxiety levels. The testing apparatus was made of dark gray PVC consisting of two opposite open arms (50 × 12 cm) and two opposite closed arms surrounded by 50 cm high walls of the same dimensions. The middle section that allows the animal to transit from arm to arm consisted of a square with dimensions of 12 × 12 cm. The maze was elevated 1 m above ground and the open arms were equipped with 0.5 × 0.5 cm ledges to ensure that no animals would fall off the maze. Each rat was placed in the same open arm of the maze always facing away from the experimenter. Trials lasted 5 min each, allowing the rat to freely explore the EPM. Between each animal the maze was thoroughly disinfected to remove odors. Time spent in open arm of the EPM was measured.

2.10 | Adrenal weight

At the end of the experiment, rats were perfused, the adrenals were dissected, weighed, and the adrenal weights calculated as a percentage of body weight.

2.11 | Immunohistochemistry

Following the last behavioral test, animals were terminally anesthetized by an overdose of 10% ketamine (Bela-Pharm GmbH & Co., KG, Germany) and 2% xylazine (Rompun, Bayer-Leverkusen, Germany) and intracardially perfused with ice-cold solution containing 4%

paraformaldehyde (PFA) and 0.05% glutaraldehyde in 0.1 M phosphate buffered saline (PBS) at pH 7.4. The brains were removed from the skull, kept in 30% sucrose at 4°C until they sunk, and cut into 40 μm coronal sections. Slices were then incubated in a blocking solution (5% Bovine Serum Albumin in 0.3% Triton X-100 in PBS) for 1 hr. Sections were then washed and incubated overnight with mouse monoclonal antibody against GFP (Invitrogen, A11120, RRID:AB_221568, made in mouse, 1:500), rabbit polyclonal antibody against TH (Covance, PRB515P, RRID:AB_291659, made in rabbit, 1:600). Following several washes, sections were incubated for 4 hr in Alexa Fluor 488 goat anti-mouse (Life Tech., A11001, RRID:AB_2534069, 1:200) and Alexa Fluor 568 goat anti-rabbit (Life Tech., A11011, RRID:AB_143157, 1:200). High-resolution images were captured by a LSM-I-Duo-Live laser scanning confocal microscope and analyzed using ZEN 2.5 software (Carl Zeiss).

2.12 | Statistics

Statistical analyses were performed with IBM SPSS Statistics V21.0 software (RRID:SCR_019096). After confirmation of homogeneity or Mauchly's test for Sphericity, *t* test and repeated measures ANOVA were used to analyze the behavior results when appropriate. Factors compared were Groups and Sessions. Statistically significant main effects were further assessed by post hoc analysis, using Bonferroni correction. Statistical significance was accepted for $p < 0.05$. Results are expressed as mean ± *SEM*. Detailed statistics are listed in Table S1.

3 | RESULTS

The behavioral data are summarized in Table 1.

TABLE 1 Summary of behavior data

	Weight	EPM	USV	OSST	Locomotion
CMUS	↓↓↓	–	↓	↓↓↓	–
ss-ChR2	–	↑↑	–	↑↑	–
Prev-ChR2	↑↑↑	↑	↑	↑	–
Post-ChR2	–	↑↑↑	–	↑	–

Note: The table shows the impact of the chronic mild stress protocol and the different stimulation conditions targeting the dopaminergic pathway had on the key read-outs. Each condition is compared to its appropriate control group. Arrow represents direction of change: increase (↑) or decrease (↓). ↑, $p < 0.05$; ↑↑, $p < 0.01$; ↑↑↑, $p < 0.001$; –, no change.

3.1 | CMUS induced depressive-like behaviors but not anxiety-like behavior

In order to investigate the anxiolytic and antidepressant effects, we first evaluated the phenotype induced by the CMUS protocol on rodents. Two groups of animals were recruited in this part. The CMUS group received 5 weeks CMUS protocol (Table S2), with a baseline behavior block before and one block after the CMUS protocol. CMUS Ctrl group did not receive any CMUS, but had two behavior assessments according to the timeline of CMUS group (Figure 2a). A two-way ANOVA (group × week) was conducted on the weight growth of the rodents under CMUS or control. There was a significant interaction effect on weight data (group × week, $F_{(5,50)} = 8.919$ $p < 0.0001$, Figure 2b). Post hoc analysis revealed that the CMUS prohibited weight growth from week 1 to week 5 compared to its baseline ($p < 0.010$, Figure 2b, Table S1), while CMUS control group had significant weight growth over time ($p \leq 0.0003$, Figure 2b, Table S1). For anxiety-like behavior, both CMUS and CMUS control groups showed a reduction of percentage time spent in open arm compared to the baseline, and there was no significant difference between groups ($t_{(10)} = 0.380$, $p = 0.7120$, Figure 2c). However, CMUS generated depressive-like behavior in USV and OSST. In USV, positive mood-associated 50-kHz USV calls reduced significantly after CMUS induction ($t_{(10)} = 2.263$, $p = 0.0471$, Figure 2d, Table S1). CMUS had no impact on rodent's general motor activity since there was no statistical difference in the home cage locomotion test ($t_{(10)} = 1.031$, $p = 0.3269$, Figure 2e). A depressive-like phenotype was observed in the OSST paradigm: the swimming distance in OSST significantly decreased after CMUS protocol (group × session × day, $F_{(3,30)} = 6.866$, $p = 0.001$, Figure 2f). Post hoc analysis presented that significant effects reduction of swimming distance between CMUS− session (control group) and CMUS+ session (CMUS group) ($p < 0.0001$, Figure 2f, Table S1); and within CMUS group after the CMUS protocol ($p < 0.0001$, Figure 2f, Table S1).

Overall, our behavior analyses showed CMUS was able to induce and depressive-like but not anxiety-like behaviors in rodents.

3.2 | Short-term stimulation of VTA DA-projection elicits anxiolytic and antidepressant effects

Given that CMUS prohibited the weight growth, reduced 50-kHz USV, and the swimming distance in OSST, we assessed the effect of short-term (ss-ChR2) VTA DA pathway stimulation. For short-term stimulation, animals received optogenetic stimulation at the last week of the experiment and within 30 min before each behavior session (Figure 3a). Short-term stimulation could not "rescue" the weight stagnation caused by CMUS (group × week, $F_{(5,50)} = 1.322$, $p = 0.270$, Figure 3b). In EPM, short-term stimulation showed significant increased percentage time spent in open arm compared with controls ($t_{(10)} = 3.424$, $p = 0.007$, Figure 3c). ss-ChR2 demonstrated a strong but nonsignificant tendency in 50-kHz USV calls compared to controls ($t_{(10)} = 1.976$, $p = 0.076$, Figure 3d). No impairment of motor activity was detected in either groups ($t_{(10)} = 0.449$, $p = 0.663$, Figure 3e), but a significant lower swimming distance was found in OSST (group × session × day, $F_{(3,30)} = 20.033$, $p = 0.000$, Figure 3f). Post hoc analysis showed that the significant decreased swimming distance was observed only in ss-Ctrl group ($p = 0.003$, Figure 3f,

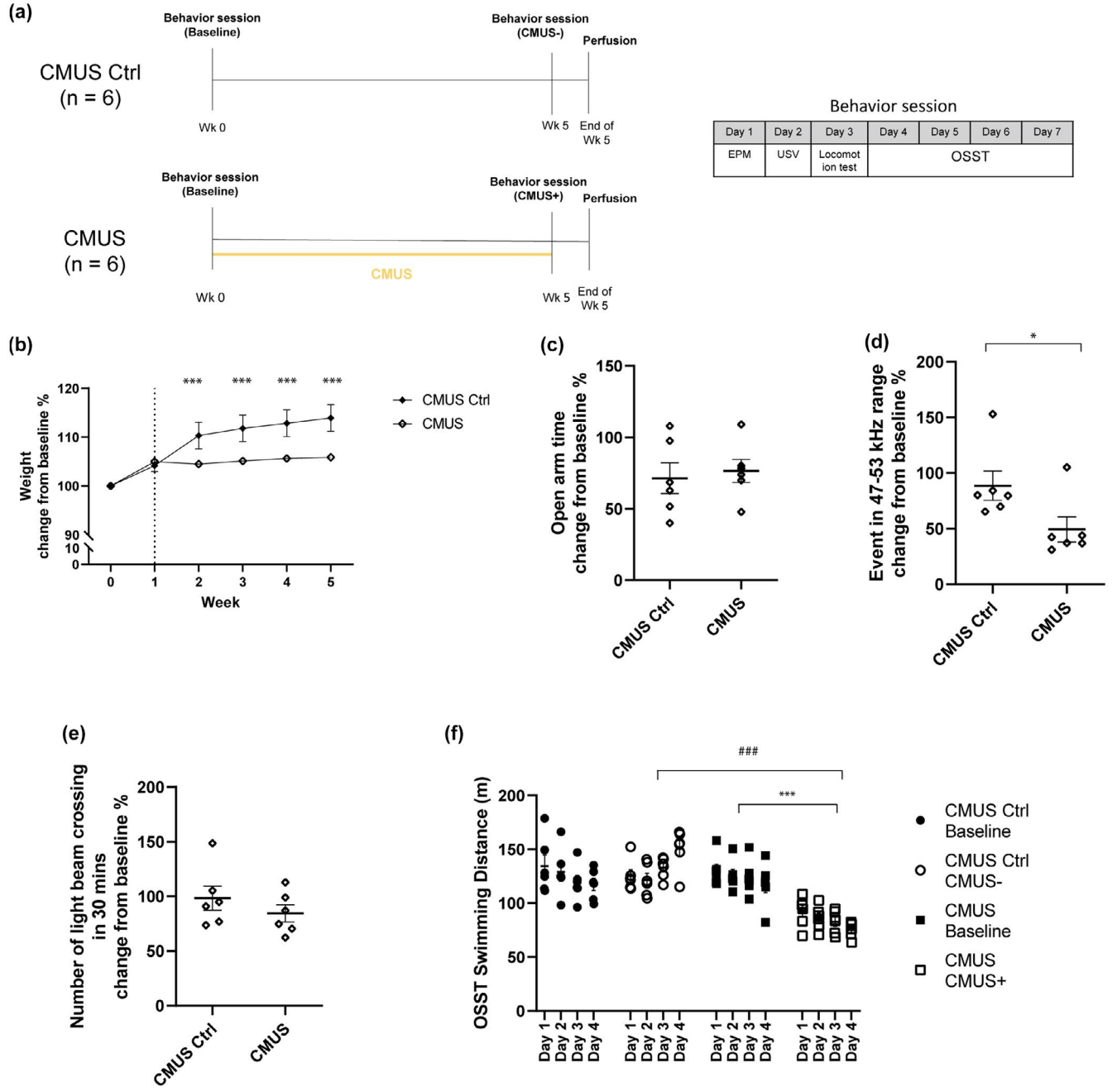

FIGURE 2 The depressive-like and anxiety-like behaviors induced by CMUS. (a) Experimental designs. (b) Trends in the change in percentage body weight along CMUS. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to week 1. (c) The percentage time spent in open arm in EPM after CMUS compared to baseline. (d) The percentage change of the number of USV between 47 and 53 kHz after CMUS compared to baseline. $^{*}p < 0.05$ compared to control group. (e) The locomotion activity assesses explorative behavior compared to baseline. (f) OSST swimming distance moved (mobility) before and after CMUS. $^{***}p < 0.001$ compared to baseline. $^{\#\#\#}p < 0.001$ when the CMUS+/− behavior session was compared between CMUS Ctrl and CMUS groups

Table S1), but not in ss-ChR2 group ($p = 0.456$, Figure 3f, Table S1). This indicates that the chronic stress protocol induced a phenotype reflected by the reduction of swimming distance only in the control group, but that this was rescued by the selective dopaminergic short-term stimulation.

Overall, short-term VTA DA pathway stimulation impacted behavior on EPM, OSST but not on weight, USV, nor general locomotion.

3.3 | Long-term stimulation pattern on VTA DA mfb pathway during the CMUS paradigm has antidepressant and anxiolytic effects

For long-term preventive stimulation, animals received optogenetic stimulation 30 min every other day during the CMUS protocol, and the behavior sessions were tested at least an hour after the stimulation (Figure 4a). In contrast to constraint of weight growth under

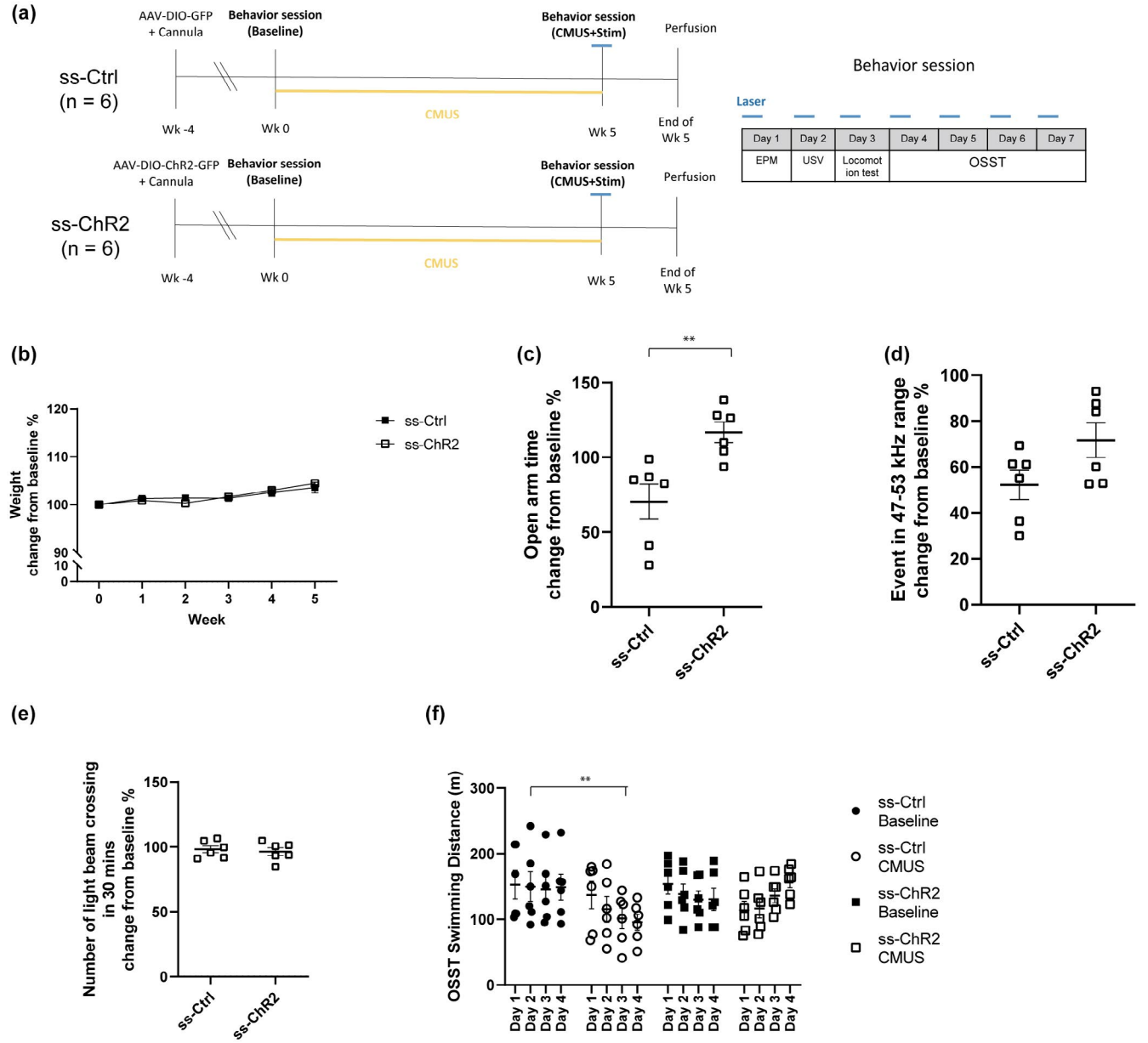

FIGURE 3 The antidepressant and anxyolitic effect by short-term stimulation. (a) Experimental and stimulation designs. (b) Trends in the change in percentage body weight along CMUS and stimulation (on week 5). (c) The percentage time spent in open arm in EPM compared to baseline. $^{*}p < 0.05$, $^{**}p < 0.01$. (d) The percentage change of the number of USV between 47 and 53 kHz compared to baseline. (e) The locomotion activity assesses explorative behavior compared to baseline. (f) OSST swimming distance moved (mobility) before and after CMUS+Stim. $^{**}p < 0.01$ compared to baseline

CMUS induction and short-term stimulation, long-term stimulation during CMUS was able to 'prevent' the weight stagnation from week 4 to week 6 of CMUS (group × session, $F_{(6,60)} = 8.893$ $p < 0.0001$, Figure 4b, Table S1). Stimulation caused significant increase in percentage time spent in open arm ($t_{(10)} = 2.386$ $p = 0.0382$, Figure 4c) and significant increase of 50-kHz USV calls ($t_{(10)} = 3.103$ $p = 0.0112$, Figure 4d). No statistical difference was found in locomotion test in either groups ($t_{(10)} = 0.342$ $p = 0.739$, Figure 4e, Table S1). The adrenal glands were dissected from the two groups: Prev-ChR2 group had significant smaller adrenal glands than controls ($t_{(10)} = 3.805$. $p = 0.003$, Figure 4f). A significant interaction effect was found in OSST (group × session × day, $F_{(3,30)} = 3.222$ $p = 0.037$, Figure 4g): Post hoc analysis demonstrated a significant lower swimming distance in Prev-Ctrl group after CMUS induction ($p = 0.003$, Figure 4g, Table S1). There was a significant "prevent" effect between groups after CMUS (CMUS+Stim session) ($p = 0.039$, Figure 4g, Table S1), and post hoc analysis showed the preventive stimulation effect was on OSST day 1 ($p = 0.001$, Figure 4g, Table S1), but not day 2–4 ($p > 0.088$, Figure 4g, Table S1).

Overall, long-term preventive VTA DA pathway stimulation has impact on weight growth, EPM, USV, adrenal gland weight, and OSST but not on general activity/locomotion.

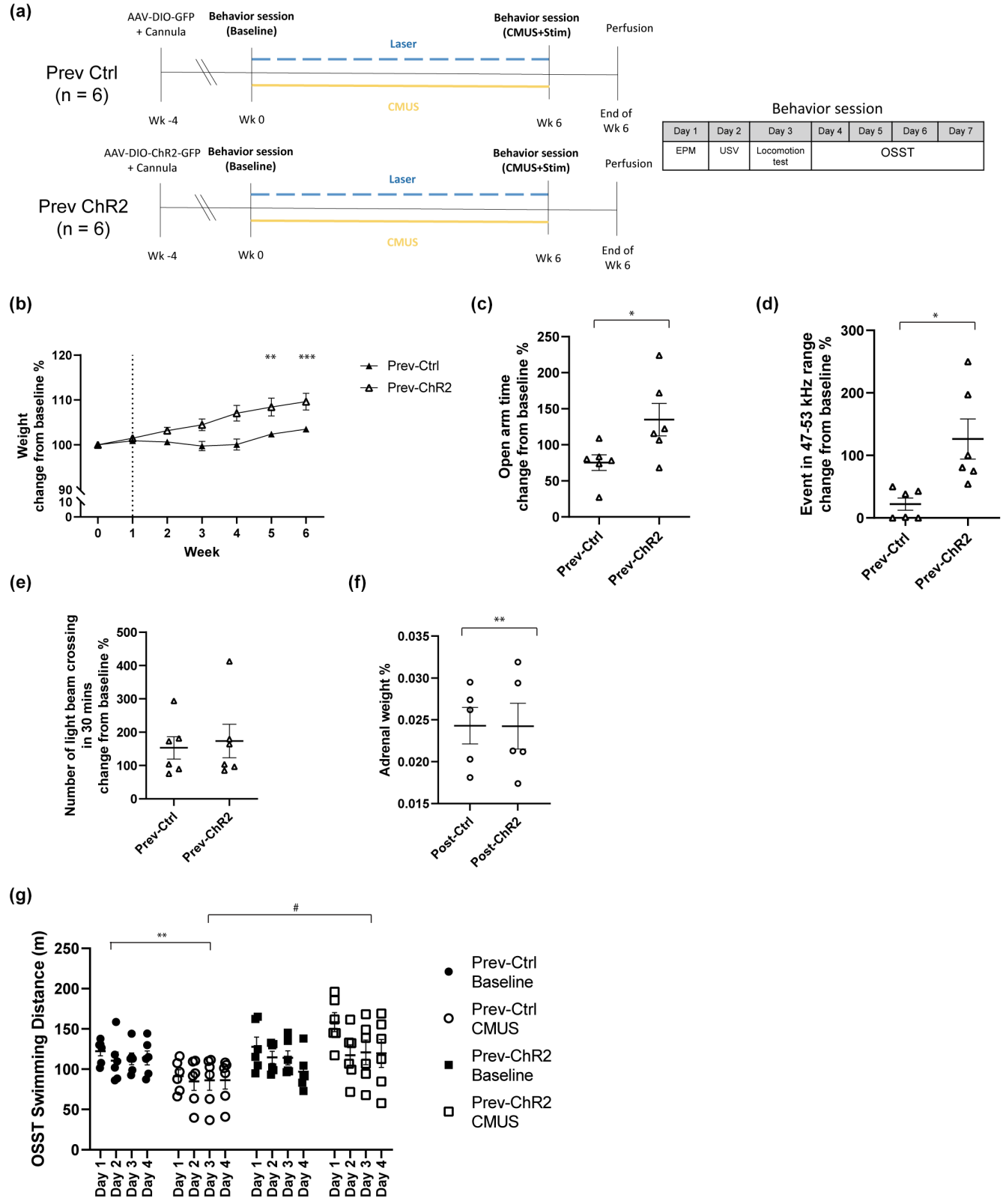

FIGURE 4 The antidepressant and anxiolytic effect by long-term preventive stimulation. (a) Experimental and stimulation designs. (b) Trends in the percentage change in body weight along CMUS and stimulation compared to week 1. $^{*}p < 0.05$, $^{**}p < 0.01$. (c) The percentage time spent in open arm in EPM compared to baseline. * indicates $p < 0.05$. (d) The percentage change of the number of USV between 47 and 53 kHz compared to baseline. $^{*}p < 0.05$. (e) The locomotion activity assesses explorative behavior compared to baseline. (f) Effects of long-term preventive stimulation on the percentage of adrenal weight. $^{**}p < 0.01$ compared to the controls. (g) OSST swimming distance moved (mobility) before and after CMUS+Stim. $^{**}p < 0.01$ compared to baseline. $^{\#}p < 0.05$ when the CMUS+Stim behavior session was compared between Prev-Ctrl and Prev-ChR2 groups

3.4 | Long-term poststimulation pattern on VTA DA mfb pathway has anxyolitic and antidepressant effect

For the long-term poststimulation group, the CMUS induction phase (week 0 to week 6) was followed by the stimulation phase (week 6 to week 9) (Figure 5a). Weight gain dynamics were comparable across the control and the long-term poststimulation groups, and post hoc analysis showed both Post-ChR2 and Post-Ctrl animals were significantly heavier on week 9 compared to week 1 (group × week $F_{(9,72)} = 2.545$, $p = 0.014$, Figure 5b). Poststimulation impacted anxiety-like behavior: a significant interaction effect (group × session, $F_{(2,16)} = 11.72$ $p = 0.009$, Figure 5c) was found in EPM. Post hoc analysis showed a significant decrease in percentage time spent in open arm after CMUS induction in Post-Ctrl group ($p = 0.027$, Figure 5c, Table S1). Post-ChR2 presented a "rescued" effect on percentage time spent in open arm compared to the controls ($p = 0.001$, Figure 5c, Table S1). There were no statistical differences either in the 50-kHz USV calls between groups (group × session, $F_{(2,16)} = 1.799$ $p = 0.217$, Figure 5d), or in the general activity measurement (group × session, $F_{(2,16)} = 0.419$ $p = 0.536$, Figure 5e, Table S1). Adrenal weights were similar between groups ($t_{(8)} = 0.017$. $p = 0.987$, Figure 5f). In OSST, there was no three-factor interaction (group × session × day, $F_{(6,48)} = 0.464$ $p = 0.831$, Figure 5g), but a significant two-factor interaction (group × session, $F_{(2,16)} = 4.749$, $p = 0.024$, Figure 5g). Post hoc analysis showed (a) a significant lower swimming distance impact under CMUS induction phase (between baseline and CMUS sessions) on Post-Ctrl ($p = 0.003$, Figure 5g) and Post-ChR2 ($p = 0.032$, Figure 5g); (b) a long-lasting lower swimming distance impact by CMUS (between baseline and stim sessions) on Post-Ctrl ($p = 0.027$, Figure 5g); (c) a significant poststimulation effect on OSST (stim session between two groups, $p = 0.021$); and (d) a stimulation induced rescue effect in the Post-ChR2 animals (between CMUS and stim sessions, $p = 0.026$, Figure 5g).

Overall, long-term post-VTA DA pathway stimulation demonstrated impact on EPM, and OSST but not on weight growth, USV, adrenal gland weight, nor on the general locomotor activity.

4 | DISCUSSION

Depression affects almost twice as many women than men (Malhi & Mann, 2018), and daily stressors are vital components of the etiology of depression for many patients (Hammen, 2005). DA neurons originating in the VTA are regulated by reward and aversive stimuli, and have been associated with depression-related behavior (Belujon & Grace, 2017; Felger, 2017; Tye et al., 2013). Using a CMUS induced rodent depression model, the study demonstrated that distinct types of selective optogenetic stimulation of the mfb DA pathway can mitigate or reverse the stress induced depressive phenotype. Both short-term (ss-ChR2) and long-term stimulation of DA neurons (Prev-ChR2 and Post-ChR2) reduced the depressive-like phenotype induced by CMUS. In addition, although CMUS did not induce anxiety-like behavior, stimulation of DA neurons reduced anxiolytic phenotype.

4.1 | Chronic stress as a preclinical depression model

Chronic stress can trigger clinical depression in many patients, and its effects have been widely investigated in preclinical research (Willner, 1997, 2017). Several chronic stress paradigms have been applied as models of depression, as either repeated or persistent stress, in the form of chronic social defeat stress, chronic isolation stress, chronic restraint stress, and CMUS (Chiba et al., 2012; Venzala et al., 2012; Willner, 1997; Yorgason et al., 2016). These paradigms induce changes in the behavior of the experimental animals that are considered as "depressive-like" and "anxiety-like" phenotype. In our experiment, we found CMUS prohibited weight growth already starting 1 week after the onset of the induction. The exposure of CMUS induced depressive-like behavior such as decreased swimming distance in OSST. The OSST can detect "depressive-like" phenotypes, or changes in coping strategies, in rodents, and under certain conditions the paradigm itself can be considered a stressor (Sun & Alkon, 2003). For example, 24 min/day of OSST for 3 days was sufficient to induce reduction of swimming distance (Alkon et al., 2017). However, this was not the case in the current study that used 15 min daily OSST and where the rodents did not develop decreased swimming distance across the daily trials. Moreover, CMUS induced change in swimming distance was not due to the motor deficit since the locomotor activity remained intact in all groups. High USV (around 50 kHz) is associated with appetitive stimuli or rewarding experiences (Burgdorf et al., 2000; Portfors, 2007; Wöhr et al., 2008; Wöhr & Schwarting, 2007): CMUS produced a significant decrease in calls in this range indicating a decrease in positive mood.

CMUS has shown both decreasing (D'Aquila et al., 1994; Kopp et al., 1999) and increasing (Griebel et al., 2002; Monteiro et al., 2015) anxiety-like behavior as measured on the EPM. The current study suggests that CMUS failed to trigger anxiety-like behavior compared to the unstressed control animals.

4.2 | The effect of short- and long-term stimulation patterns

The study looked at the antidepressant and anxiolytic effect of mfb VTA DA stimulation with short-term (ss-ChR2), long-term preventive pattern (Prev-ChR2), and long-term post-CMUS stimulation strategies. We report antidepressant and anxiolytic effects in all stimulation patterns. However, ss-ChR2 and Post-ChR2 stimulation patterns only had antidepressant effects in OSST, and failed to reverse the weight stagnation and the decreased 50-kHz USV calls caused by CMUS. In addition, a reduction of adrenal weight under Prev-ChR2 indicated that repetitive stimulation can have

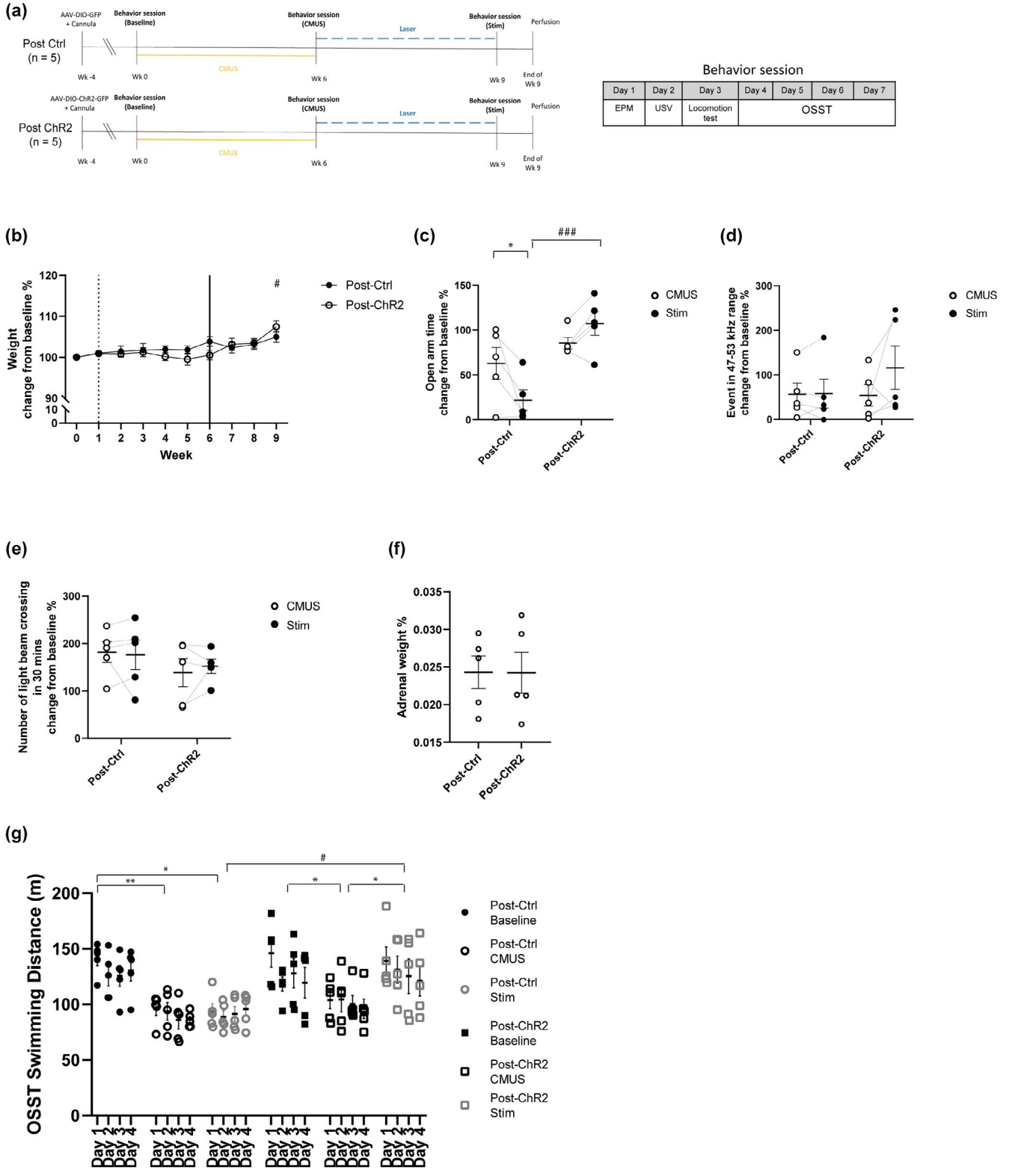

FIGURE 5 The antidepressant and anxyolitic effect by long-term poststimulation. (a) Experimental and stimulation designs. (b) Trends in the percentage change in body weight along CMUS and stimulation compared to week 1. $^{\#}p < 0.05$ in both groups. (c) The percentage time spent in open arm in EPM after CMUS and after stimulation compared to baseline. $^{*}p < 0.05$ between behavior sessions, $^{\#\#\#}p < 0.001$ between group. (d) The percentage change number of USV between 47 and 53 kHz after CMUS and after stimulation compared to baseline. (e) The locomotion activity assesses explorative behavior after CMUS and after stimulation compared to baseline. (f) Effects of long-term post stimulation on the percentage of adrenal weight. (g) OSST swimming distance moved (mobility) before and after CMUS and after stimulation. $^{*}p < 0.05$, $^{**}p < 0.01$ compared between behavior sessions within group. $^{\#}p < 0.05$ when the after stimulation (Stim) behavior session was compared between Post-Ctrl and Post-ChR2 groups

chronic, long-lasting antidepressant impact. Previous research that selectively targeted midbrain dopaminergic transmission projecting through the mfb showed acute "antidepressant" and "antianxiety" effects (Tye et al., 2013). Tye and colleagues stimulated during the actual behavioral testing and showed that it modulated etiological circuits firing and the depressive-like phenotype returned when the stimulation was off. However, in our study stimulation took place either within 30 min to (ss-ChR2) or more than an hour prior to (Prev-ChR2 and Post-ChR2) behavioral testing indicating that under these stimulation parameters etiological circuits do not return immediately to previous activity levels but may take same time to re-establish. The different results might be due to the accumulative nature of the long-term stimulation, but the mechanism of this effect is unclear.

Chronic stress can lead to weight loss (Kiecolt-Glaser, 2010), but Prev-ChR2 was able to rescue the weight stagnation induced by CMUS. However, ss-ChR2 was not sufficient to normalize the weight dynamics. A previous electrophysiological study (Razzoli et al., 2011) showed that the stress-induced increase of VTA DA firing lasted 3 weeks after the social defeat protocol, which indicated that the chronic stress protocol could have long-term effects. In our study, both Post-ChR2 and control group had significant weight growth by week 9 compared to week 1. This indicated that CMUS (weeks 1–6) had little impact on weight dynamics 3 weeks after the termination of the stress conditions.

Although the CMUS protocol did not induce anxiety-like behavior (as the phenotype in the EPM was similar to the control group), all stimulation patterns tested in the study were anxiolytic. This effect could relate to the activation of VTA DA neurons that regulate anxiety: optogenetic stimulation of LHb terminals in VTA (Stamatakis & Stuber, 2012) or activated D1 and D2 receptors via VTA amygdala projections induced robust real-time anxiety-like behavior (Bananej et al., 2012; Zarrindast et al., 2011).

Overall, the data suggest that long-term and accumulative stimulation of midbrain DA pathways contribute to mediating a stress-induced depressive phenotype.

4.3 | Consequence of VTA DA stimulation

VTA DA neurons are functionally and anatomically diverse which could explain the contradictory observations concerning DA release in the projection areas following reward/reward predictive stimuli and aversive/stressful events (Holly et al., 2015; Holly & Miczek, 2016). There is electrophysiological and neurochemical evidence that VTA DA neurons are strongly activated by reward and reward predictive cues evoking increased DA release along the mesocorticolimbic pathways, and conversely, there is suppression of VTA DA neuronal firing during stress or aversive stimulus presentation (Guarraci & Kapp, 1999; Mantz et al., 1989; Mirenowicz & Schultz, 1996; Schultz & Romo, 1987; Ungless et al., 2004). However, acute aversive and stressful stimuli can also rapidly excite a subset of VTA DA neurons increasing their tonic and phasic firing rate (Anstrom & Woodward, 2005; Cao et al., 2010; Holly & Miczek, 2016; Krishnan et al., 2007). Contrary to acute stress, the impact of chronic stress, as used in the study, on VTA DA neurons remains uncertain. Few *in vivo* studies indicate that repeated exposure to the stressors can alter DA release in VTA projection targets, such as NAC and mPFC. For example, chronic social defeat stress reduced DA level in NAC (Mangiavacchi et al., 2001; Miczek et al., 2011) and mPFC (Cuadra et al., 2001; Watt et al., 2014).

Our results demonstrated antidepressant effects under all three stimulation profiles, underlying the vital role of VTA DA pathway in mood regulation. The stimulation patterns likely promoted the "normalization" of VTA DA firing patterns more consistent with regular function: the strengthening of these patterns in the case of the "preventive" stimulation, and the re-establishment of the patterns in the case of the "post" stimulation conditions. Dopamine release after electrical and optogenetic stimulation of VTA DA neurons projecting to NAC in short-term studies has been investigated (Ashouri Vajari et al., 2020; Bass et al., 2013; Klanker et al., 2017; Lu et al., 2015; Melchior et al., 2015; Settell et al., 2017), but it is less understood how the DA release patterns evolve following long-term and repetitive stimulation.

Heterogeneous constituent neural populations project across rodent mfb, including fine, unmyelinated axons of midbrain DA neurons and other well-myelinated fibers (Nieuwenhuys et al., 1982). Some authors propose that a key mechanism of mfb DBS is the antidromic activation of the highly excitable myelinated glutamatergic inputs into the midbrain DA neurons. The dopaminergic projections in turn modulate numerous up-stream antidepressant effects in the NAC and PFC (Schlaepfer et al., 2014) (Trujillo-Pisanty et al., 2020). Furthermore, it is not known how optogenetic stimulation of the DA system impacts on the noradrenergic system, although it is recognized that the cross-talk between the transmitters in stress regulation is important (Isingrini et al., 2016). Further studies are needed looking into how long-term optogenetic stimulation impacts the DA concentration and microcircuits activities at mfb terminals.

5 | CONCLUSIONS

In conclusion, our data demonstrated that CMUS in female rats induces a broad range of behavioral changes that are considered important correlates of depressive symptoms in humans. Moreover, for the first time, different optogenetic stimulation patterns targeting VTA DA system rescued "depressive-like" phenotypes induced by CMUS and improved anxiolytic behavior. The current study, which only used female rats, demonstrates the importance of the stimulation strategy used and provides a new approach to understanding the neurobiology and treatment of depression.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript

presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

All the authors take responsibility for the integrity of the data and accuracy of the data analysis. All the authors have access to the full data set of the study. *Conceptualization*, Y.T., L.P., and M.D.D.; *Methodology*, Y.T., L.P., and M.D.D.; *Validation*, M.D.D. and V.A.C.; *Investigation*, Y.T. and L.P.; *Formal Analysis*, Y.T. and M.D.D.; *Resources*, V.A.C. and M.D.D.; *Writing – Original Draft*, Y.T. and M.D.D.; *Writing – Review & Editing*, Y.T., T.S., V.A.C., and M.D.D.; *Visualization*, Y.T., T.S., and M.D.D.; *Supervision*, M.D.D. and V.A.C.; *Project Administration*, M.D.D.; *Funding Acquisition*, M.D.D. and V.A.C.

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DATA AVAILABILITY STATEMENT

The data presented in this study and custom written analysis codes are available from the corresponding authors upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

TABLE S1 Statistical analysis results

TABLE S2 Chronic mild unpredictable stress paradigm for different stimulation patterns

TABLE S3 Key resources table

Transparent Science Questionnaire for Authors

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