



Optogenetic activation of GABAergic neurons in the nucleus accumbens decreases the activity of the ventral pallidum and the expression of cocaine-context-associated memory

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Abstract

GABAergic medium-sized spiny neurons (MSNs) in the nucleus accumbens (NAc) differentially express D1 and D2 dopamine receptors. Both D2- and D1-MSNs in the NAc form projections into the ventral pallidum, whereas only D1-MSNs directly project into midbrain neurons. They are critical in rewarding and aversive learning, and understanding the function of these NAc efferents and the alteration of their targeted brain regions in responding to a reward-associated context is important. In this study, we activated the GABAergic neurons in the NAc of mice expressing channelrhodopsin-2 under the control of the vesicular GABA transporter promoter by an optogenetic approach, and examined its effects on the expression of cocaine-context-associated memory. *In vivo* optogenetic activation of the NAc GABAergic neurons inhibited the expression of cocaine-conditioned place preference (CPP). When tested 24 h later, these mice exhibited normal cocaine-induced CPP, indicating that the inhibitory effect on the expression of CPP was transient and reversible. Activation of the NAc GABAergic neurons also attenuated the learning of cocaine-induced reinforcement, as indicated by the results of behavioural sensitization. To explore how the cocaine-context-associated information was processed and integrated, we assessed the activity of NAc MSN-targeted brain nuclei and found that the activation of NAc GABAergic neurons during CPP expression resulted in a decrease of c-Fos⁺ cells in the ventral pallidum. Our data suggested that the NAc GABAergic efferents inhibit the ventral pallidum activity and negatively regulate the expression of motivational effects induced by cocaine-context-associated cues.

Received 2 July 2013; Reviewed 23 July 2013; Revised 8 November 2013; Accepted 26 November 2013;
First published online 23 January 2014

Key words: Cocaine, conditioned place preference, GABA, nucleus accumbens, optogenetics.

Introduction

The nucleus accumbens (NAc), as a major efferent target of dopaminergic neurons, integrates sensory and cognitive inputs that drive motivationally relevant behaviours in response to psychomotor stimulation of drugs of abuse and environmental stimuli (Everitt and Robbins, 2005; Hyman et al., 2006). The function of the NAc is regulated by excitatory inputs from the medial prefrontal cortex (mPFC), the basolateral amygdala (BLA) (Kalivas et al., 2005), the hippocampus and the thalamus, as well as dopaminergic and GABAergic afferents from the ventral tegmental area (VTA) (Gerfen and Surmeier, 2011). More than 95% of NAc output neurons are GABAergic

medium spiny neurons (MSNs), which express the D1 dopamine receptor (D1-MSN) or the D2 dopamine receptor (D2-MSN) (Graybiel, 2000; Surmeier et al., 2007). Recent studies show that both D1- and D2-MSNs in the NAc project into the ventral pallidum (VP), which functions as both an intrinsic and output structure of the basal ganglia (Kalivas and Volkow, 2011; Smith et al., 2013; Stefanik et al., 2013). D1-MSNs also project into ventral midbrain output structures (the substantia nigra (SNr) and the VTA) directly. D1-MSNs in the NAc positively regulate cocaine-conditioned place preference and behavioural sensitization, whereas D2-MSNs have the opposite function (Lobo et al., 2010). This reveals that a balance between the two subpopulations is likely necessary for addictive behaviour.

The development of optogenetic technology facilitates cell type- and projection-specific manipulation of neural activity with precise temporal control for understanding the complicated mechanisms of reward seeking (Chen et al., 2012; Stuber et al., 2012). Optogenetic control of specific neuronal afferents and efferents in the NAc to

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manipulate behaviours in free-moving mice have been reported. Optical stimulation of BLA-to-NAc glutamatergic fibres, which requires intra-NAc D1 receptors, facilitates a behavioural response to earn additional optical stimulation of these synaptic inputs (Stuber et al., 2011). Silencing cocaine-induced activation of cholinergic interneurons (which represent only <1% of neurons in the NAc) during cocaine exposure by optogenetic manipulation attenuates the acquisition of cocaine conditioned place preference (CPP) (Witten et al., 2010). VTA GABA projection neurons, through their selective targeting of the NAc, inhibit accumbal cholinergic interneurons enhancing stimulus–outcome learning (Brown et al., 2012). Direct optogenetic activation of D1- or D2-MSNs in the NAc does not induce CPP in the absence of cocaine, but exerts bidirectional control over cocaine conditioning (Tsai et al., 2009; Lobo et al., 2010). These studies elucidate functions of specific neurons in the NAc in the learning of cocaine rewarding. However, addiction is not simply a result of the direct neurobiological effects of drugs, but also the consequence of exposure to drug and drug-associated environmental backgrounds (Piazza and Le Moal, 1996; Deroche-Gamonet et al., 2004; Swendsen and Le Moal, 2011). The role of NAc GABAergic neurons in responding to a cocaine-associated context, as well as alternation of NAc efferents-targeted brain nuclei, needs to be further investigated. Addressing such a question requires a selective and temporally precise control of GABAergic neurons during exposure to drug-related environmental stimuli (for example, after CPP training).

In the current study, we used an optogenetic technique in conjunction with VGAT-ChR2 transgenic mice to activate GABAergic neurons in the NAc, and investigated their role in the modulation of the expression of cocaine-context-associated memory. The activity of NAc efferent-targeted brain nuclei was also examined when NAc GABAergic neurons were optogenetically stimulated during cocaine-context-associated memory expression.

Materials and methods

Animals and housing

VGAT-ChR2/EYFP transgenic C57 mice expressing CHR2 fused to Enhanced Yellow Fluorescent Protein (ChR2-EYFP) under the control of the mouse vesicular GABA transporter (VGAT) promoter (Zhao et al., 2011) were provided by Professor G. P. Feng (Massachusetts Institute of Technology, USA). VGAT-ChR2-EYFP C57 hemizygous mice and their wild-type (WT) littermates were obtained from self-crossing of VGAT-ChR2-EYFP C57 hemizygous mice. Genotypes were determined by PCR of mouse tail DNA samples (Zhao et al., 2011). Male mice were used and housed in groups on a 12 h light/dark cycle with food and water available *ad libitum*. All animal treatments were strictly in accordance with the

National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Immunohistochemistry and cell counting

Mice were anesthetized with choral hydrate and perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). The brains were removed, fixed in 4% paraformaldehyde overnight and subjected to dehydration in increasing sucrose solutions (20–30%) at 4 °C. The frozen coronal slices of 20 μ m thickness were prepared and stored at –20 °C in 20% ethanediol PBS solution containing 20% sucrose. Brain sections were incubated in 3% normal goat serum and 0.2% Triton-X for 1 h. Then they were incubated in mouse anti-GAD67 (Millipore, USA) or mouse anti-c-Fos (Santa Cruz, USA) antibody overnight at 4 °C. Slices were rinsed in PBS then incubated in donkey anti-rabbit Cy3 (Jackson ImmunoResearch, USA) for 1 h and DAPI for 10 min, then mounted after rinsing. Images were acquired on a scanning laser confocal microscope using a 20 \times air objective or a 63 \times oil immersion objective (Zeiss 510; Carl Zeiss Jena, Germany). The images performed on a 63 \times oil immersion objective were used for c-Fos positive cell quantification, and identical light intensity and threshold conditions were employed for all sections. Cells were counted using Image-Pro Plus software.

Electrophysiology

Acute brain slices at the NAc level were prepared from 3 wk-old VGAT-ChR2-EYFP mice. The ChR2-expressing neurons were identified by membrane-associated EYFP fluorescence. The whole-cell current clamp recordings were carried out at 34 °C. Patch pipettes (3–5 M Ω) were filled with an internal solution containing 10 mM HEPES (pH 7.2), 130 mM K-gluconate, 6 mM KCl, 2 mM MgCl₂, 0.2 mM EGTA, 2.5 mM Na₂ATP, 0.5 mM Na₂GTP, 10 mM K-phosphocreatine, 0.5% mM Neurobiotin and 0.3% Alexa Fluor 568 hydrazide (285 mOsm). The action potentials of NAc VGAT neurons were recorded in current-clamp mode using an Axopatch 700B amplifier (Molecular Devices, USA) and data acquisition was realized with pCLAMP10 software (Molecular Devices). Series resistance was monitored during the experiments. Laser pulses were generated by a UGA-40 programmable spot illumination system with a DL-473 blue diode laser (100 mW, Rapp OptoElectronic, Germany). The slices were fixed in 4% paraformaldehyde (PFA) in PBS (pH 7.4) after the recordings were completed. The morphology of recorded neurons loaded with neurobiotin were later visualized with Alexa Fluor 568-conjugated streptavidin (Invitrogen, USA) and reconstructed in 3D with a NeuroLucida system (MicroBrightField, USA).

Stereotaxic surgery and laser stimulation

Male mice of 8–12 wk age anesthetized with choral hydrate (40 mg/kg, i.p.) were placed in a stereotactic

instrument, which was followed by a craniotomy; cannula guides (Plastics one, USA) were implanted over the bilateral NAC and secured with dental cement. The intended stereotaxic coordinates were: AP+1.4 mm; ML \pm 1.3 mm (with an angle of 10° from the middle to the lateral); DV−4.2 mm (Zhao et al., 2011). Cannula placements were confirmed by histology (Fig. S1a). All mice were given at least 7 d to recover before the behavioural experiments. To manipulate neuronal activity during the behavioural experiments, two 200 μ m diameter optical fibres were inserted through the cannula to bilaterally deliver laser to the NAC. Optical fibres were attached through an FC/PC adaptor to a 473 nm blue laser diode (Brain-King, China), light pulses were generated through a stimulator (AMPI, Israel), and the light intensity at the fibre tip was measured before implantation as 8–10 mW using a light sensor (Thorlabs, USA). The laser stimulation parameters used in all the following experiments referring to the related papers (Lobo et al., 2010; Witten et al., 2010) were: 10 Hz frequency, 20 ms duration delivered at 2 min duration with a 2 min interval.

Locomotor activity

Open field test chambers (27.3 cm \times 27.3 cm \times 20.3 cm, Med-Associates, USA) equipped with infrared source and detector beam strips to detect lateral and vertical movement of the mice were used. The mice were individually placed in the centre of the chamber, and their horizontal locomotion and spatial placement were recorded by the infrared-tracking system.

Cocaine-induced conditioned place preference

Conditioned place preference induced by cocaine hydrochloride (Qinghai Pharmaceutical Firm, China) was assessed using a two-chamber apparatus (Med-Associates, USA) with distinct tactile environments to maximize contextual differences. One chamber of the box had a wire mesh floor while the other chamber had a grid rod floor. A manual guillotine door separated the two chambers. On Day 1, mice were placed in one of the chambers and allowed to freely explore the entire apparatus for 20 min (pre-test). On Days 2 and 3, mice were given an i.p. injection of saline (an equivalent volume to that of cocaine) in the morning and confined to one of the chambers for 20 min, and in the afternoon they received an i.p. injections of cocaine (15 mg/kg) then confined to the other (drug-paired) chamber for 20 min. On Day 4, mice were allowed to freely explore the entire apparatus for 20 min (test). The time spent in each chamber was recorded during the pre-test and test sections. The CPP score was defined as the time (s) spent in the cocaine-paired chamber minus the time spent in the saline-paired chamber.

For testing the effect of optogenetic manipulation on the expression of cocaine-induced CPP, on Day 4, 15 min before the experiment, optical fibres were attached

to the cocaine-conditioned mice through the implanted cannulas. Mice were allowed free exploration of the entire apparatus for 20 min before the laser was turned on for another 20 min. In another experiment, on Day 4, cocaine conditioned mice were tested for place preference while the laser was turned on throughout the 20 min test period. On Day 5, mice were tested again for place preference. The time that mice spent in each chamber before and after laser stimulation was recorded.

Cocaine-induced behavioural sensitization

The effect of cocaine injections on locomotor activity was evaluated in open field test chambers. On Days 1 and 2, mice received an i.p. injection of saline and were placed in an open field arena to habituate for 30 min, during which locomotor activity was recorded. On Days 3–7, these mice were placed in an open field chamber and 30 min later an i.p. injection of cocaine (15 mg/kg) was given, and locomotor activity was monitored for another 60 min. On Day 9, mice with the optical fibres secured to their head-mounts received a challenge injection of cocaine (5 mg/kg) 30 min after they were placed in an open field arena, and then locomotor activity was recorded for 60 min with the laser turned on. The horizontal locomotion of each mouse in the open field arena was recorded.

Statistical analysis

Data are expressed as mean \pm s.e.m., and analysed by Sigma Stat (Systat Software, USA). Student's *t* test was used to compare the pretest and test CPP scores, and the number of c-Fos⁺ neuron within each genotype. Two-way ANOVA with Tukey *post-hoc* test was performed when assessing the effects of laser stimulation on accumulative CPP scores and locomotor activity within each genotype.

Results

Optogenetic activation of GABAergic neurons in the NAC increases neuronal activity

VGAT is specifically expressed in GABAergic neurons (Sagne et al., 1997; Gasnier, 2000). VGAT-ChR2-EYFP mice express the ChR2-EYFP fusion protein in GABAergic populations of neurons directed by the mouse VGAT promoter/enhancer (Zhao et al., 2011). As shown by immunohistochemistry data, a moderate level of ChR2-EYFP expression was detected in the NAC in VGAT-ChR2-EYFP mice. The localization of ChR2-EYFP fluorescence in GAD67-positive cells could be observed throughout the NAC (Fig. 1a, b), indicating these ChR2-EYFP expressing cells are mostly GABAergic neurons.

To confirm that the ChR2 expressed in the GABAergic neurons of VGAT-ChR2-EYFP mice is functional, we performed electrophysiological recordings in acute brain

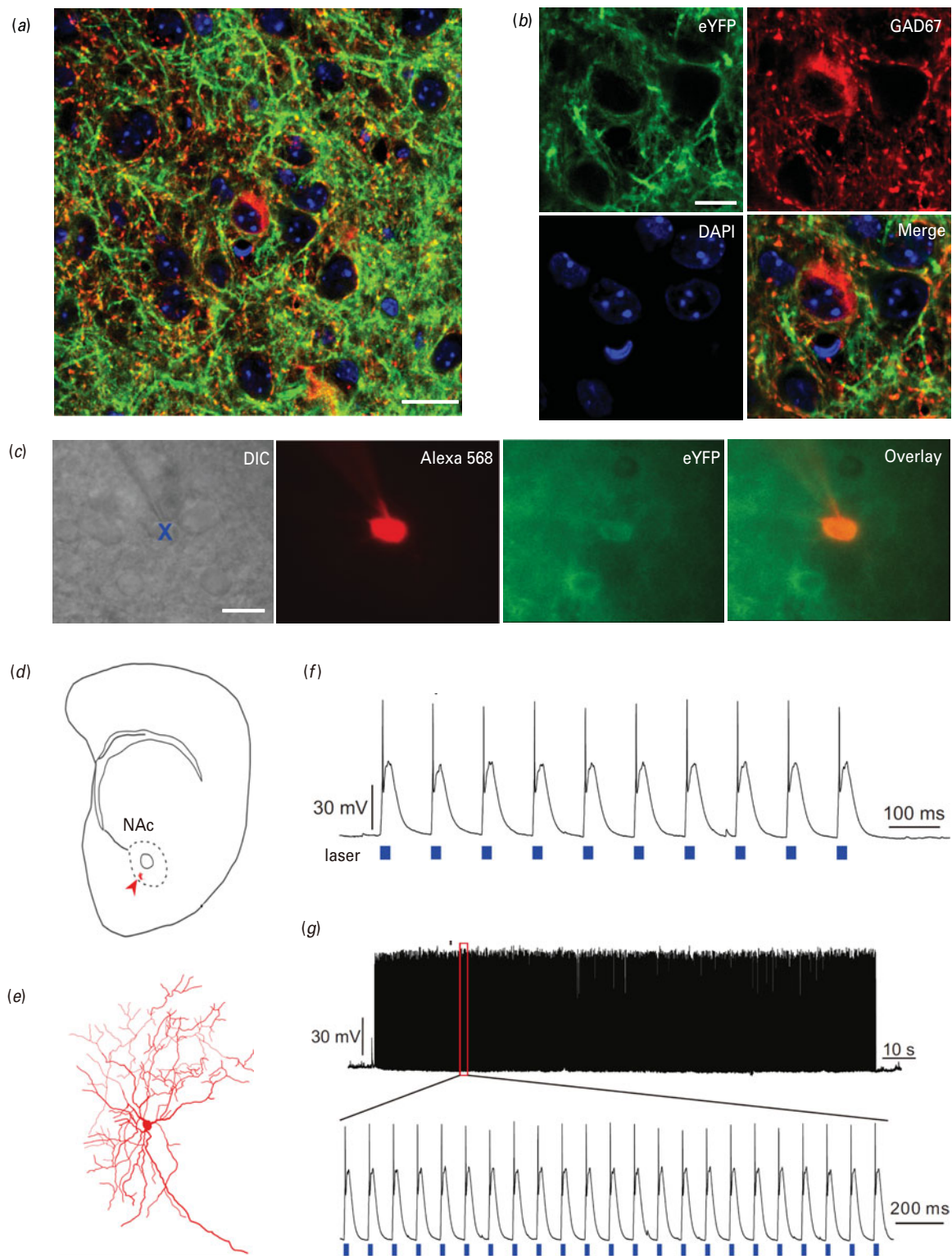


Fig. 1. Optogenetic activation of nucleus accumbens (NAc) GABAergic neurons in VGAT-ChR2-EYFP mice. (a) Confocal images showing expression of ChR2-EYFP (green) in the neuronal membrane around the soma, as well as in the dendrites of NAc neurons. Scale bar, 20 μ m. (b) ChR2-EYFP (green) is expressed in GAD67-positive neurons (red) of VGAT-ChR2-EYFP mice. Scale bar, 10 μ m. (c) Image of a ChR2-EYFP (green) neuron filled with Alexa Fluor 568 dye (red) through the recording pipette. The position of 473 nm laser stimulation is marked with 'X' in the IR-DIC image of an acute brain slice. Scale bar, 30 μ m. (d and e) Diagram of coronal brain slice in which the NAc neuron recorded in B is indicated by the red arrow head (d), and the morphological reconstruction of the neuron recorded (e). (f and g) Current clamp mode recording showing activation of a ChR2-EYFP-expressing neuron by a laser pulse train (473 nm, 10 Hz, 200 ms) lasting for 1 s (f) and 2 min (g).

slices from VGAT-ChR2-EYFP mice. NAc GABAergic neurons, as recognized by membrane-associated EYFP fluorescence, were selected for recording (Fig. 1c–e). The GABAergic neurons expressing ChR2 in the NAc responded with high fidelity and sustainability to prolonged blue laser up to 10 Hz, in strings of light flashes lasting for 1 s (Fig. 1f) and 2 min (Fig. 1g).

Optogenetic activation of NAc GABAergic neurons inhibits the expression of cocaine-associated context memory

The CPP paradigm is a classic measure of reward properties of drugs (Tzschentke, 1998). The effect of precisely timed activation of NAc GABAergic neurons on the expression of cocaine-induced CPP was examined. The wild type and VGAT-ChR2-EYFP mice were given cocaine injections and trained to associate the drug with one chamber daily for 2 d (Day 2 and Day 3). On Day 4, two optical fibres connected to a laser diode were secured to the cannulas implanted into the bilateral NAc of these mice, and their preference for the cocaine-paired chamber was tested with or without laser stimulation (Fig. 2a). Under the condition of no laser delivery, both WT and VGAT-ChR2-EYFP mice showed significant preference for the cocaine-paired chamber, indicating the successful retrieval and expression of the acquired cocaine-associated context memory. However, laser delivery to the bilateral NAc abolished the cocaine CPP in VGAT-ChR2-EYFP mice, but had no effect on WT mice (Fig. 2b) (laser off: $t(11)=1.256$, $p=0.235$; laser on: $t(11)=4.231$, $p<0.001$). As indicated by the accumulative preference score, the cocaine-induced CPP in VGAT-ChR2-EYFP mice was attenuated almost as soon as the laser was turned on (Fig. 2c) ($F(1,49)=11.973$, $p=0.002$). No tissue damage to the brain was observed after laser delivery into the NAc of VGAT-ChR2-EYFP mice (Fig. S1b and S1c). Stimulation of NAc GABAergic transmission by laser during cocaine CPP training did not influence the motor function of either WT or VGAT-ChR2-EYFP mice, as indicated by the traveling distance and velocity (data not shown). These results suggest that optogenetic activation of GABAergic neurons in the NAc produces a rapid inhibitory effect on the expression of cocaine-context-associated memory.

To examine the long-term effect of the activation of NAc GABAergic neurons, mice that acquired cocaine-CPP were tested for drug-induced CPP with laser delivered into bilateral NAc (Test 1), and a second CPP test (Test 2) was carried out 24 h later without laser stimulation (Fig. 2d). The optogenetic activation of GABAergic neuron significantly reduced the cocaine preference score in VGAT-ChR2-EYFP mice as compared with WT mice. However, when tested 24 h later, no significant difference in the expression of cocaine-induced CPP was observed between the VGAT-ChR2-EYFP and WT mice, as shown by both preference scores (Fig. 2e, $t(12)=5.260$, $p<0.001$

for Test 1 and $t(12)=0.151$, $p=0.882$ for Test 2) and accumulative preference scores (Fig. 2f, $F_{1,71}=204.027$, $p<0.001$ for Test 1 and $F_{1,71}=0.204$, $p=0.653$ for Test 2). These results demonstrate the validity of the optogenetic activation of GABAergic transmission in the NAc, and indicate that the activation of GABAergic transmission in the NAc upon re-exposure to reward-associated context transiently and reversibly inhibits the expression of cocaine-induced CPP.

Optogenetic activation of GABAergic neurons in the NAc attenuates the expression of cocaine-induced behavioural sensitization

Behavioural sensitization, a progressive and long-lasting enhancement of locomotor response to repeated drug exposure, provides a simple model of drug incentive learning underlying the development of addiction. Such learning also depends upon neuronal plasticity in the NAc. We next carried out experiments using the optogenetic technique to establish a causal link between NAc GABAergic neuron activity and the expression of chronic cocaine-induced behavioural sensitization. As shown in Fig. 3a, after being given a cocaine injection, both VGAT-ChR2-EYFP and WT mice showed robust enhancement in locomotor activity, as demonstrated by the increased velocity (Fig. 3a) and distance (Fig. 3b) traveled in the open field test chamber, with no significant difference between WT and VGAT-ChR2-EYFP mice. Moreover, no difference in the distance traveled was found between WT and VGAT-ChR2-EYFP mice when laser stimulation was delivered into the NAc, indicating that optogenetic manipulation and activation of NAc GABAergic neurons does not have a significant effect on basal locomotor activity (Fig. 3c). On the challenge day (Day 9), the mice which had previously received daily consecutive cocaine injection were placed in the open field chamber and injected with a challenge dose of cocaine (5 mg/kg) plus bilateral NAc laser delivery. The low-dose cocaine-induced behavioural sensitization was significantly decreased by laser stimulation of ChR2-expressing GABAergic neurons in the NAc of VGAT-ChR2-EYFP mice, as compared with WT mice (Fig. 3a, left; $t(9)=3.236$, $p<0.01$). As shown in Fig. 3d, cocaine-induced locomotor sensitization in VGAT-ChR2-EYFP mice was inhibited as soon as the laser was turned on ($F(1,57)=21.351$, $p<0.001$). The above data suggest that NAc GABAergic neurons positively regulate the learning of cocaine-induced reinforcement.

Optogenetic stimulation of NAc GABAergic neurons during CPP expression down-regulates ventral pallidum activity

The projections from NAc target different brain regions, such as the ventral pallidum (VP), substantia nigra

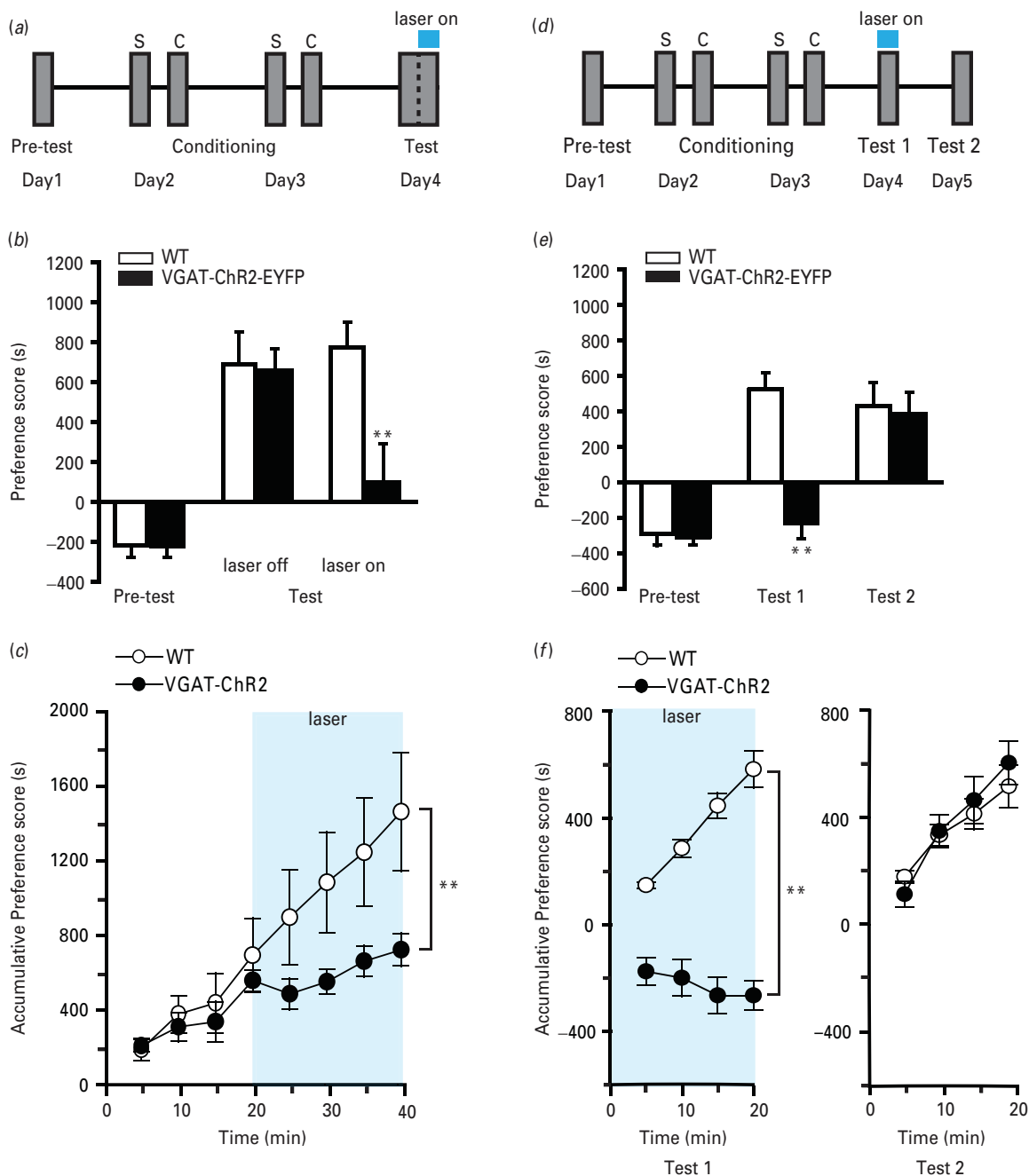


Fig. 2. Optogenetic activation of GABAergic neurons in the nucleus accumbens (NAc) of cocaine-conditioned mice upon re-exposure to drug-associated context inhibits the expression of conditioned place preference (CPP). Wild-type (WT) and VGAT-ChR2-EYFP mice were tested for place preference on Day 1, and received cocaine-conditioning on Days 2 and 3. (a–c) On Day 4, mice were allowed to freely explore the entire apparatus for 20 min without laser stimulation (laser-off) and then the laser was delivered (laser-on) for the next 20 min. (d–f) On Day 4, mice were allowed to freely explore the entire apparatus for 20 min with laser delivery (Test 1), and on Day 5, mice were allowed to freely explore the entire apparatus again without laser delivery (Test 2). Time spent in each chamber was recorded. Illustration of the experiment (a and d), place preference scores of 20 min (b and e), accumulative preference score (c and f) are shown. Blue shadow indicates the duration of laser stimulation. Data plotted are mean \pm s.e.m., $**p < 0.01$ vs. WT.

(SNr) and ventral tegmental area (VTA) of the midbrain (Wilson et al., 2005; Bock et al., 2013). To better understand the neuroanatomical basis of NAc GABAergic efferents in the expression of cocaine context-associated memory, we carried out immunofluorescence assay to

capture the activation patterns in NAc MSNs targeted brain nuclei after opto-activation of NAc GABAergic neurons in saline control or cocaine conditioned mice during the post-conditioning session (Fig. S2 and Fig. 4). There were hardly any c-Fos⁺ cells in the VP, SNr or VTA of

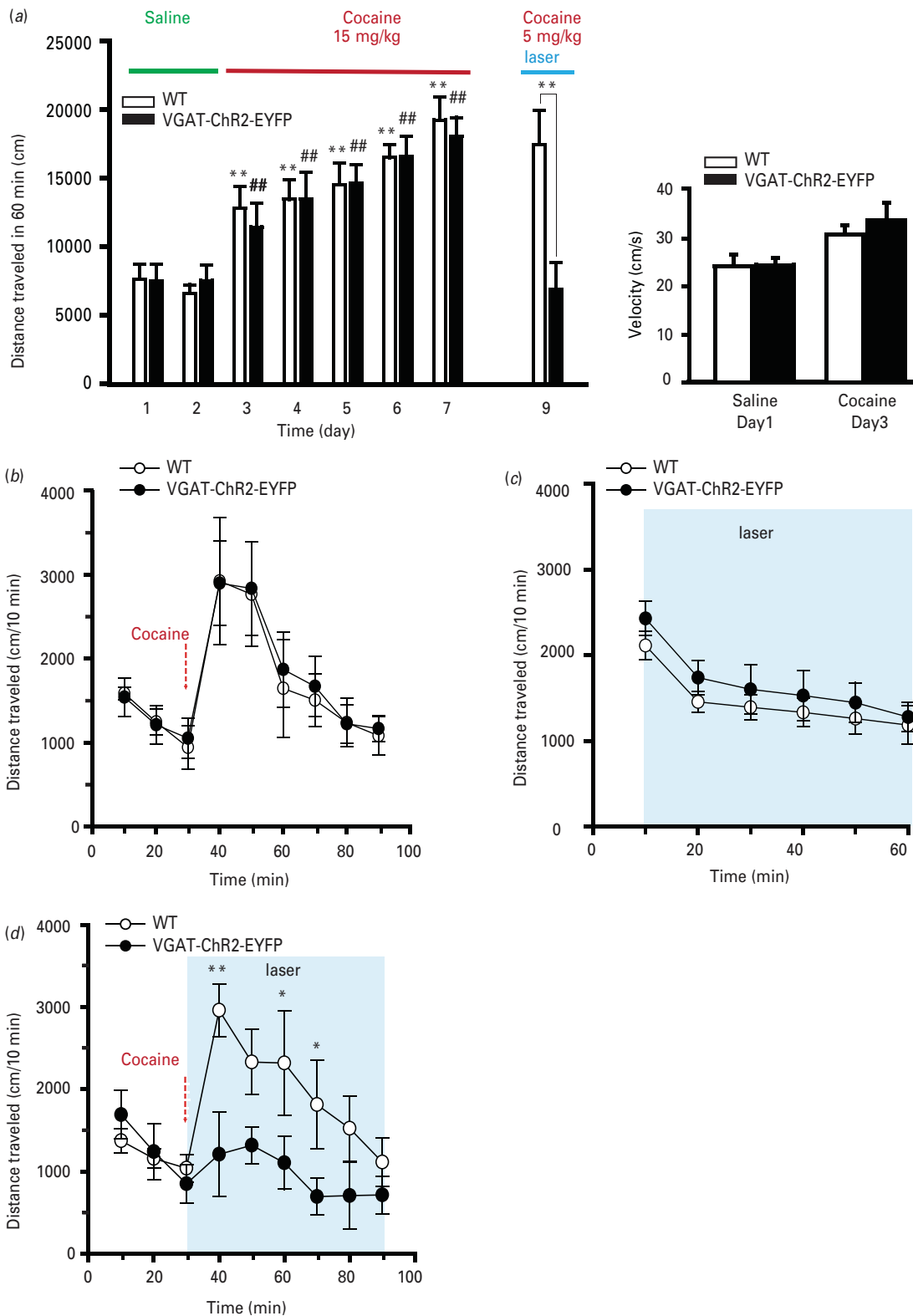


Fig. 3. Optogenetic activation of GABAergic neurons in the nucleus accumbens (NAc) attenuates the expression of cocaine-induced behavioural sensitization. Locomotor activity of wild-type (WT) and VGAT-ChR2-EYFP mice were measured after they were placed in an open field environment. $n=6$ per group. Data are mean \pm s.e.m. (a) Left: Total distance traveled in a 60 min recording section after receiving saline, 15 mg/kg cocaine or 5 mg/kg cocaine plus laser delivery. Right: average velocity measured in a 60 min recording session after receiving saline on Day 1 or 15 mg/kg cocaine on Day 3. $**p < 0.01$ (WT), $##p < 0.01$ (Vgat-ChR2) vs. saline group. (b) Average distance traveled per 10 min interval measured on Day 4. Mice were given an injection of 15 mg/kg cocaine 30 min after being placed in the open field. (c) Average distance traveled per 10 min interval measured on Day 8 after mice were given laser stimulation. (d) Average distance traveled per 10 min interval measured on Day 9 before and after mice were given an injection of 5 mg/kg cocaine plus laser stimulation. Blue shadow indicates the laser on. $**p < 0.01$ vs. WT.

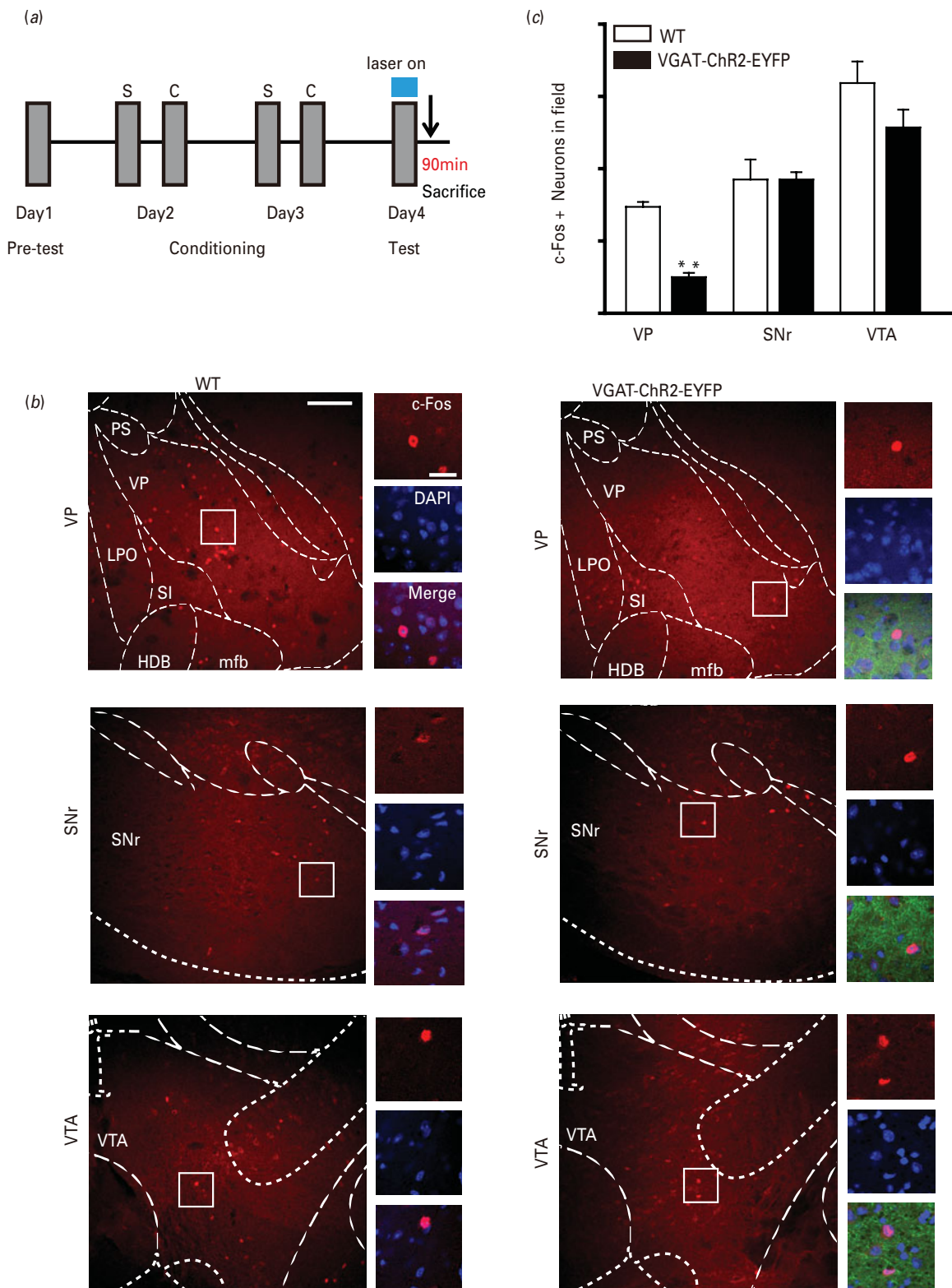


Fig. 4. Optogenetic activation of nucleus accumbens (NAc) GABAergic neurons during cocaine conditioned place preference (CPP) expression decreases the number of c-Fos⁺ cells in the ventral pallidum (VP). (a) Timeline of the experimental procedure. The wild-type (WT) and VGAT-ChR2-EYFP mice were trained for cocaine conditioning and tested for cocaine CPP under blue light delivery. The brains were collected 90 min later. (b) c-Fos⁺ fluorescence (red) in the VP, substantia nigra and ventral tegmental area. Ps, parastriatal nucleus; LPO, lateral preoptic area; SI, substantia innominata; HDB, nucleus of the horizontal limb of the diagonal band; mfb: medial forebrain bundle. Scale bars, 100 μm (low-power images) and 20 μm (high-power images). (c) Quantification of c-Fos⁺ cells after CPP conditioning. Data plotted are mean ± S.E.M., ***p* < 0.01 vs. WT.

the saline-treated WT and VGAT-ChR2-EYFP mice (Fig. S2b). Instead, cocaine induced a remarkable increase of the c-Fos⁺ cells in the VP, SNr, and VTA (Fig. 4b). The optogenetic activation of NAC GABAergic neurons significantly decreased c-Fos⁺ activation of VP, but not SNr or VTA neurons (Fig. 4b, c, $t(11)=10.675$, $p<0.001$). These data suggest that VP activity is down-regulated in cocaine conditioned mice following the activation of NAC GABAergic neurons, and that it is positively correlated with the expression of drug-context-associated memory.

Discussion

In this study, we used an optogenetic approach, a method for isolating the action of a specific subtype of neuron activity in a region with heterogeneous anatomical connectivity (Tye and Deisseroth, 2012), to examine the role of NAC GABAergic neurons and their efferents in the regulation of cocaine-context-associated memory. We found that optogenetic activation of GABAergic neurons in the NAC of VGAT-ChR2-EYFP mice was sufficient to impair the expression of cocaine-context-associated place preference and cocaine-induced locomotor sensitization. These results suggest that NAC GABAergic transmission negatively regulates reward-seeking behaviour induced by cocaine and cocaine-predictive cues.

Previous research has shown that optogenetic activation of D1- and D2-MSNs during cocaine exposure enhances and diminishes the development of cocaine-induced CPP, respectively (Lobo et al., 2010). Our study showed that optogenetic activation of the NAC GABAergic transmission after a cocaine conditioning session impaired the expression of cocaine CPP transiently and reversibly. The potential neuronal mechanism by which optogenetic activation of GABAergic transmission in the NAC modulates the cocaine-context associated memory could be attributed to alterations of neuronal activity in reward-related brain nuclei. Previous studies have shown that selective VP lesions abolish 'liking', 'wanting' and 'learning' of food rewards, while excitation or disinhibition of the VP strongly promotes food wanting (Smith and Berridge, 2005; Shimura et al., 2006; Smith et al., 2013). In the current study, we observed that during optogenetic stimulation of NAC GABAergic neurons, the reduced expression of cocaine CPP was accompanied by a decreased number of c-Fos⁺ cells in the VP, indicating that the overlapping projections of both NAC D1- and D2-MSNs into the VP may directly inhibit VP activation, and this may contribute to decreased drug seeking. However, the number of c-Fos⁺ cells in the SNr and VTA of cocaine-conditioned mice did not change after the optogenetic activation of NAC GABAergic neurons. This may be because the SNr and VTA are regulated by two afferents: the D1-MSNs afferent from the NAC and the afferent from the VP. The activation of NAC GABAergic neurons probably inhibits the

activity of the SNr and VTA by the D1-MSNs afferent from the NAC, whereas the D1- and D2-MSNs efferents from the NAC decrease the activity of the VP, thus attenuating VP's inhibition of the SNr and VTA.

Under optogenetic stimulation of the NAC, GABAergic interneurons and MSNs, as well as GABAergic inputs to the NAC were activated. The activation and function of these different GABAergic transmissions in responding to drug-associated cues, the mechanism by which GABAergic efferents from the NAC regulate VP activity during the expression of cocaine CPP, and the subregion and subpopulation of neurons in the VP affected need to be further investigated. Glutamatergic inputs from multiple cortical and sub-cortical regions are integrated within NAC sub-territories to regulate the motivational state and govern goal-directed behaviours (Wise and Bozarth, 1985; Groenewegen et al., 1999; Asher and Lodge, 2012; Tukey et al., 2013). The medial regions of the NAC are innervated by afferents arising in the ventral medial regions of the prefrontal cortex, and the lateral regions of the NAC receive preferential innervation from the dorsal mPFC and orbitofrontal cortices (Berendse et al., 1992; Brog et al., 1993). These glutamatergic terminals might also be at the action site of NAC GABAergic interneurons, and their function on the NAC might be disrupted by optogenetic activation of NAC GABAergic transmission. Thus, the behavioural changes in CPP and sensitization might be a result of cooperative alteration of the NAC input and output pathways. The relevant interactions of NAC GABAergic neurons with other neuronal transmission circuits, including dopaminergic afferents from VTA and glutamatergic afferents from the prefrontal cortex, should also be assessed in the future.

Supplementary material

For supplementary material accompanying this paper, visit <http://dx.doi.org/10.1017/S1461145713001570>.

Acknowledgments

This research was supported by grants from the Ministry of Science and Technology (2014CB942801 to LM and FW, 2013CB835102 to YC and XL), the Natural Science Foundation of China (31121061 & 91232307 to LM, and 31270027 to FW), and Science and Technology Commission of Shanghai Municipality (12JC1401000 to LM).

Statement of interest

None.

Authors' contribution

LM and FW were responsible for the conception and design of the study and analysis of the data. LW, MS and YT contributed to the acquisition of animal data. YY

carried out the electrophysiology experiment. LW and MS assisted with data analysis. LW drafted the manuscript. LM, PZ and FW provided critical revision of the manuscript for important intellectual content. All authors critically reviewed the content and approved final version for publication.

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