Cyproheptadine Regulates Pyramidal Neuron Excitability in Mouse Medial Prefrontal Cortex

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Abstract Cyproheptadine (CPH), a first-generation anti-histamine, enhances the delayed rectifier outward K^+ current (I_K) in mouse cortical neurons through a sigma-1 receptor-mediated protein kinase A pathway. In this study, we aimed to determine the effects of CPH on neuronal excitability in current-clamped pyramidal neurons in mouse medial prefrontal cortex slices. CPH (10 $\mu\text{mol/L}$) significantly reduced the current density required to generate action potentials (APs) and increased the instantaneous frequency evoked by a depolarizing current. CPH also depolarized the resting membrane potential (RMP), decreased the delay time to elicit an AP, and reduced the spike threshold potential. This effect of CPH was mimicked by a sigma-1 receptor agonist and eliminated by an antagonist. Application of tetraethylammonium (TEA) to block I_K channels hyperpolarized the RMP and reduced the instantaneous frequency of APs. TEA eliminated the effects of CPH on AP frequency and delay time, but had no effect on spike threshold or RMP. The current-voltage relationship showed that CPH increased the membrane depolarization in response to positive current pulses and hyperpolarization in response to negative current pulses, suggesting that other types of membrane ion channels might also be affected by CPH. These results suggest that CPH increases the excitability of medial prefrontal cortex

neurons by regulating TEA-sensitive I_K channels as well as other TEA-insensitive K^+ channels, probably I_D and inward-rectifier K_{ir} channels. This effect of CPH may explain its apparent clinical efficacy as an antidepressant and antipsychotic.

Keywords Cyproheptadine · Neuronal excitability · Tetraethylammonium-sensitive I_K · cortical neurons · Sigma-1 receptor

Introduction

Cyproheptadine (CPH) is a first-generation, typical anti-histamine that is widely used as an antagonist of histamine H1 and 5-hydroxytryptamine receptors for the treatment of allergic reactions, migraine prophylaxis, and for the symptomatic treatment of metastatic carcinoid syndrome [1, 2]. Notably, the structure of CPH is similar to that of antidepressants such as amitriptyline, imipramine, and N-methylamitriptyline [3], while the receptor-binding profile and neuron adaptation of CPH are thought to resemble those of clozapine, indicating a possible use in the treatment of schizophrenia [4]. Although CPH has neurological side-effects, such as sedation and reduced cognitive function [2], it has recently been reported to control seizures, improve survival, reduce seizure duration, and reduce the number of dying cells in the rat brain following exposure to soman [5]. These data suggest that CPH plays a role in the central nervous system, though few studies have explored its possible neurological role and the underlying mechanisms.

CPH has multiple pharmacological activities and also acts as an antagonist for serotonergic, dopaminergic, histaminergic, adrenergic, and muscarinic receptors, due

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to its relatively high binding affinity [6]. In addition to the above receptor-dependent effects, CPH also directly inhibits ion channels, including K^+ , Na^+ , and N - and L -type Ca^{2+} channels in cardiac cells [3, 6]. CPH has also been reported to induce myeloma cell apoptosis and inhibit the proliferation of hepatocellular carcinoma cells *via* inhibition of the phosphatidylinositol-3-kinase/Akts signaling pathway, independent of its known activity as a histamine and serotonin receptor antagonist [7, 8]. We previously demonstrated that CPH enhances the delayed rectifier outward K^+ current (I_K) in cultured mouse cortical neurons by modulating the activity of protein kinase A, possibly also involving activation of the sigma-1 receptor ($\sigma 1R$)/Gi-protein pathway [9]. This suggested that CPH may modify the activity of neuronal voltage-gated K^+ channels (VGKCs) *via* a mechanism unrelated to its serotonergic and histaminergic properties. I_K is composed of $Kv2 \alpha$ -subunits and contributes to action potential (AP) repolarization and firing patterns under physiological conditions [10]. Thus, whether the CPH-induced increase of I_K enhances neuronal excitability, and whether $\sigma 1R$ activation by CPH has universal significance in the brain as well as in cultured neurons, need further study.

The prefrontal cortex (PFC) is critically involved in many higher brain functions, including working memory, attention, behavioral planning, and behavioral flexibility [11, 12]. Both electrophysiological and behavioral studies have suggested that the PFC and the medial prefrontal cortex (mPFC) may be involved in recognition memory and working memory [13]. Pharmacological modulation of the excitability and synaptic transmission of PFC or mPFC neurons by voltage-activated ion channels has been reported [14, 15]. The mPFC is also involved in several aspects of drug addiction, including the primary rewarding effects of cocaine and the mechanisms underlying addiction and craving [16]. CPH has been reported to have neurological side-effects such as sedation and reduced cognitive function [2], but its structure is similar to that of antidepressants, and its receptor binding profile, neuron adaptation, and structure suggest potential uses in the treatment of schizophrenia or as an antidepressant [3, 4]. However, the ability of CPH to modify neuronal excitability in the mPFC remains unclear.

In this study, we determined the effects of CPH on neuronal excitability in the mPFC using whole-cell current-clamp recordings. We demonstrated a previously uncharacterized effect of CPH on neuronal excitability through both tetraethylammonium (TEA)-sensitive I_K and TEA-insensitive VGKCs. These results confirm involvement of the $\sigma 1R$, and provide novel insights into the mechanisms of CPH function in neurophysiology.

Materials and Methods

Ethics and Animal Use Statement

This study was conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experimentation at Fudan University (permit no. 20090614-001). All surgery was performed under sodium pentobarbital anesthesia (50 mg/kg, intraperitoneal) and all efforts were made to minimize animal suffering.

Animals

Female C57/LB mice 3–4 weeks old (SLAC Laboratory Animal Co. Ltd., Shanghai, China) were housed in plastic cages at room temperature (23°C–25°C) with *ad libitum* access to standard food pellets and water. No specific dietary supplements were provided. The light-dark cycle was set at 12 h (lights on from 08:00 to 20:00).

Brain-Slice Preparation

The mice were deeply anesthetized with pentobarbital sodium before rapid decapitation and removal of the entire brain into ice-cold, oxygenated cutting solution (in mmol/L: 220 sucrose, 3 KCl, 5 $MgCl_2$, 1 $CaCl_2$, 1.25 NaH_2PO_4 , 26 $NaHCO_3$, and 10 glucose; 315 mOsm/L, 95% O_2 /5% CO_2 and pH 7.3). Coronal slices (200 μm) of the prefrontal cortex (bregma 3.6 mm–2.5 mm) were cut on a vibrating microtome (VT1200S; Leica, Wetzlar, Germany), and then incubated in artificial cerebral spinal fluid (in mmol/L: 125 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.5 $MgSO_4$, 1 NaH_2PO_4 , 26.2 $NaHCO_3$, and 11 glucose; 305 mOsm/L and pH 7.3) for 1 h at 34°C. Slices were stored at room temperature until use.

Whole-Cell Patch-Clamp Recording

APs were recorded from pyramidal neurons in layers III and IV of the cortical slices in current-clamp mode. Prior to AP recording, the artificial cerebral spinal fluid was replaced with a bath solution containing (in mmol/L) 140 NaCl, 2.5 KCl, 10 N -2-hydroxyethylpiperazine- N' -2-ethanesulfonic acid (HEPES), and 1 $MgCl_2$ (pH adjusted to 7.4 using NaOH), or 119 NaCl, 2.5 KCl, 1 Na_2HPO_4 , 26.2 $NaHCO_3$, 3 $MgSO_4$, 1 $CaCl_2$, and 11 glucose. The internal pipette solution contained (in mmol/L) 150 K^+ gluconate, 0.4 ethylene glycol bisaminoethyl ether tetraacetate (EGTA), 8 NaCl, 2 ATP·Mg, 0.1 GTP· Na_3 , and 10 HEPES (pH adjusted to 7.4 using KOH). In the depolarization and hyperpolarization experiments, the pipette

solution contained (in mmol/L) 150 K⁺ gluconate, 2 EGTA, 8 NaCl, 2 ATP·Mg, 2 GTP·Na₃, and 10 HEPES. TTX (1 μmol/L) was added in the bath solution to block the sodium channel. Resting membrane potential (RMP) and APs were recorded in the current-clamp mode. The series resistance (Rs) was 20 MΩ–30 MΩ. Data were discarded if the Rs of the recorded cells varied by > 15%. Recordings from cortical neurons were performed at 23°C–25°C.

Data Acquisition and Analysis

All currents were recorded using an Axopatch 200B amplifier (Molecular Devices, Foster City, CA) operated in voltage-clamp mode. A Pentium computer was connected to the recording equipment using a Digidata 1300 analog-to-digital interface. The current was sampled digitally at 100 μs (10 kHz). The current signals were filtered using a 5-kHz, five-pole Bessel filter. The currents were corrected online for leak and residual capacitance transients by a P/4 protocol. Data acquisition and analysis were performed using pClamp 8.01 software (Molecular Devices) and/or Origin6.1 (MicroCal, Northampton, MA). Statistical analysis was performed using Student's *t*-tests with non-paired or paired comparisons, as appropriate. The values are given as mean ± SEM, with *n* representing the number of cells tested. *P* < 0.05 was considered to be statistically significant.

Chemicals

CPH, BD-1063, TEA, and 2-(4-morpholino ethyl)-1-phenyl cyclohexanecarboxylate (PRE-084) were from Sigma-Aldrich (St. Louis, MO). CPH was dissolved in methanol and then diluted in the previously-described bath solution to a final methanol concentration of < 0.1%.

Results

CPH Enhanced the Excitability of mPFC Neurons by Activation of Sigma-1 Receptors

We previously demonstrated that both intracellular and extracellular application of CPH enhances the *I_K* [9]. Therefore the effects of intracellular or extracellular CPH on the excitability of mPFC pyramidal neurons were investigated in cortical layers III–IV injected with a current to evoke AP firing. Neurons were clamped at a holding potential of −80 mV, which was close to the neuronal RMP. The injected current was 100 pA with a 500 ms duration. Perfusion of CPH *via* the bath solution or intracellular application *via* the internal pipette solution increased the AP frequency from 8.6 Hz ± 1.01 Hz

(control, *n* = 12) to 12.46 Hz ± 2.01 Hz (*n* = 20) in bath solution and to 14.1 Hz ± 2.41 Hz (*n* = 10) with intracellular application, suggesting similar effects of the two approaches (Fig. 1A). We therefore used intracellular application of CPH in subsequent experiments to obtain a stable concentration and a rapid effect. We investigated the influence of current on the effect of CPH on AP frequency by injecting currents of 30 pA–330 pA, with a 500 ms duration and increments of 10 pA. We found that CPH significantly increased the number of spikes evoked by injection of depolarizing current in the range 60 pA–200 pA, and significantly decreased the current needed to generate multiple APs from 60 pA to 40 pA (Fig. 1B). CPH at 10 μmol/L significantly increased the instantaneous spike frequency evoked by an injected current of 100 pA from 7.85 Hz ± 0.50 Hz (control, *n* = 12) to 10.54 Hz ± 0.26 Hz (*n* = 10, *P* < 0.05). CPH also increased the instantaneous spike frequency evoked by an injected 200-pA current from 16.12 Hz ± 0.55 Hz (control, *n* = 12) to 20.76 Hz ± 1.40 Hz (*n* = 10, *P* < 0.05) (Fig. 1C, D). In addition to increasing the AP frequency, CPH at 10 μmol/L also significantly reduced the delay time to elicit a spike from 21.92 ms ± 1.06 ms (control, *n* = 12) to 16.08 ms ± 1.89 ms (*n* = 10, *P* < 0.05) when a 200-pA current was injected (Fig. 2A). The threshold was significantly decreased by 10 μmol/L CPH from −35.03 mV ± 1.02 mV (control, *n* = 12) to −38.81 mV ± 1.05 mV (*n* = 10, *P* < 0.05) (Fig. 2B). In addition, CPH at 10 μmol/L depolarized the RMP from −70.25 mV ± 1.28 mV (control, *n* = 12) to −67.56 mV ± 0.75 mV (*n* = 10, *P* < 0.05) (Fig. 2C), while having no significant effect on AP duration (Fig. 2D).

A previous study showed that CPH increases *I_K* *via* activation of σ1Rs in cultured cortical neurons. We therefore used the σ1R agonist PRE-084 and the antagonist BD-1063 to investigate whether the activation of σ1Rs is required for CPH-induced enhancement of neuronal excitability. Application of 10 μmol/L PRE-084 mimicked the effect of CPH and increased the AP frequency elicited by an injection of 100 pA from 8.6 Hz ± 1.01 Hz (control, *n* = 12) to 12.05 ± 1.14 Hz (*n* = 12, *P* < 0.05). PRE-084 also showed characteristics similar to CPH in terms of reducing the threshold and the delay time (Fig. 3A, C). In contrast, blocking σ1Rs with 60 μmol/L BD-1063 significantly inhibited the CPH-induced increase in AP frequency, while 10 μmol/L BD-1063 had no such effect (Fig. 3B, C). The instantaneous frequency of APs in the presence of 60 μmol/L BD-1063 was 5.22 Hz ± 0.51 Hz, and the application of CPH caused no further significant increase (6.29 ± 1.24, *n* = 10, *P* > 0.05) compared with BD-1063 alone, but a significant difference from CPH alone (Fig. 3C). These data suggested that activation of σ1Rs is necessary for the CPH-induced change in neuronal excitability.

Fig. 1 CPH enhanced AP frequency in mPFC pyramidal neurons. **A** Intracellular (in) or extracellular (out) application of 10 $\mu\text{mol/L}$ CPH increased the spike numbers in mPFC pyramidal neurons. Neurons were clamped at a holding potential of -80 mV and APs were evoked by injection of a 100-pA current. **B** Current–spike number response curves showing that the number of evoked spikes in response to depolarizing currents from 30–330 pA was significantly

increased by CPH (* $P < 0.05$ vs corresponding control, two-sample t -test). **C** Representative traces showing APs evoked by a depolarizing current pulse of 100 or 200-pA in control and CPH-treated neurons. **D** Statistical analysis of the effect of 10 $\mu\text{mol/L}$ CPH on instantaneous AP frequency in mPFC pyramidal neurons. Data are mean $\pm$ SEM (* $P < 0.05$, two-sample t -test).

CPH Enhanced the AP Frequency in mPFC Neurons via TEA-Sensitive I_K

A previous study has shown that the TEA-sensitive I_K is associated with AP frequency in mPFC neurons [17], and that CPH significantly enhances the I_K in cultured cortical neurons [9]. We therefore applied TEA to determine whether CPH enhances the neuronal excitability of mPFC neurons via activation of TEA-sensitive I_K . Application of 20 mmol/L TEA in the bath solution significantly reduced the AP frequency evoked by a 200-pA current from $16.12 \text{ Hz} \pm 0.55 \text{ Hz}$ to $11.93 \text{ Hz} \pm 1.03 \text{ Hz}$ ($n = 12$ and 14, $P < 0.05$) (Fig. 4A, B), hyperpolarized the RMP from $-70 \text{ mV} \pm 1.28 \text{ mV}$ to $-73.99 \text{ mV} \pm 1.18 \text{ mV}$ ($n = 12$ and 14, $P < 0.05$) (Fig. 4C), and increased the delay time from $16.08 \text{ ms} \pm 1.89 \text{ ms}$ to $27.67 \text{ ms} \pm 1.93 \text{ ms}$ ($n = 12$

and 14, $P < 0.05$) (Fig. 4D). However, TEA had no effect on the firing threshold (Fig. 4E). TEA eliminated the CPH-induced increase in AP frequency and delay time: the AP frequency changed from $11.93 \text{ Hz} \pm 1.03 \text{ Hz}$ in TEA alone ($n = 10$) to $11.39 \text{ Hz} \pm 1.04 \text{ Hz}$ in TEA plus CPH ($P > 0.05$) (Fig. 4B), and the delay time changed from $27.67 \text{ ms} \pm 1.93 \text{ ms}$ in TEA alone ($n = 12$) to $25.98 \text{ ms} \pm 1.07 \text{ ms}$ in TEA plus CPH ($n = 14$) ($P > 0.05$) (Fig. 4D). TEA did not eliminate the effects of CPH on depolarization of the RMP and threshold (Fig. 4C, E).

CPH Altered the Current–Voltage (I – V) Relationship in mPFC Neurons

In addition to the TEA-sensitive I_K , we also investigated the effect of CPH on the outward and inward I –

Fig. 2 Effect of CPH on membrane potential properties of mPFC pyramidal neurons. **A** Representative current-clamp recording traces and statistical analyses of delay to first AP elicited by 100-pA current injection. **B**, **C** Statistical analyses of effect of CPH on spike threshold

potential (**B**) and RMP (**C**). **D** Representative example and statistical analyses showing the effect of CPH on the half-width of APs. Data are mean $\pm$ SEM (* $P < 0.05$, two-sample *t*-test).

V relationship in mPFC neurons, based on the alterations in the membrane potential in response to applied current pulses, to determine whether CPH affects other ion channels. Injection of depolarizing current pulses into control mPFC pyramidal neurons increased the depolarized membrane potential, and significantly increased the depolarized membrane potential responses to step-pulse injection of a range of depolarizing current pulses (Fig. 5A). The *I-V* curve was therefore shifted significantly upward to more depolarized levels following CPH treatment (Fig. 5B). CPH also significantly changed the inward *I-V* relationship during membrane hyperpolarization (Fig. 6A). Application of CPH significantly enhanced the hyperpolarized membrane potential responses to step-pulse injection of a range of hyperpolarizing current pulses, and the *I-V* curve was thus shifted significantly downward to more hyperpolarized levels in CPH-treated neurons (Fig. 6B). These results suggested that CPH affects other inward and outward currents in addition to the TEA-sensitive I_K .

Discussion

The results of the present study demonstrated that CPH administration significantly altered the membrane properties and excitability of mouse mPFC pyramidal neurons, at least partly as a result of increased activity of the TEA-sensitive I_K , as well as potentially other TEA-insensitive voltage-gated K^+ currents.

A major finding was that CPH significantly increased the firing frequency of APs in pyramidal neurons evoked by a suprathreshold current injection, with no increase in AP duration. We previously demonstrated that CPH increases the TEA-sensitive I_K current, regulated by Kv2 α -subunits including Kv2.1 and Kv2.2 [9, 17]. Kv2 channels activate relatively slowly, and thus contribute to AP repolarization under physiological conditions [10]. As expected, blocking the I_K with TEA substantially reduced the trough voltage after the first spike before depolarization to the second spike began, and depressed the AP frequency. Similar changes have been found in superior cervical ganglion neurons and hippocampal CA1 pyramidal neurons using the highly specific Kv2 channel blocker Guangxitoxin-1E [18]. Moreover, TEA eliminated the effect of

Fig. 3 Effect of sigma-1 receptor agonist or antagonist on the CPH-induced increase in instantaneous AP frequency in mPFC pyramidal neurons.

A Representative traces showing the effect of PRE-084 (σ 1R agonist) on APs evoked by a depolarizing current pulse (100 nA, 500 ms). **B** Representative traces showing the effect of CPH on AP instantaneous frequency in the absence or presence of BD1063 (σ 1R antagonist). **C** Statistical analyses of the effects of PRE-084 and BD1063 on the CPH-induced increase in instantaneous AP frequency (data are mean $\pm$ SEM; $*P < 0.05$, two-sample t -test).

CPH on AP frequency. Overall, these results indicate that the CPH-induced increase in neuronal excitability, at least in terms of enhanced AP frequency, involves activation of the TEA-sensitive I_K .

In addition to AP frequency, CPH also reduced the AP spike threshold and delayed the onset of spiking, resulting in easier induction of APs and improved neuronal excitability. We noted that TEA treatment had no

significant effect on the AP voltage threshold or on the CPH-induced decrease in AP voltage threshold, suggesting the involvement of TEA-insensitive ion channels. A recent study of hippocampal and neocortical pyramidal neurons has shown that the D-current (I_D) plays an important role in regulating neuronal excitability by delaying the onset of spiking, regulating the spike threshold, and causing temporal integration of multiple inputs due to their activation

Fig. 4 Effects of TEA on CPH-induced alteration of instantaneous AP frequency and membrane potential properties of mPFC pyramidal neurons. **A, B** Representative traces and statistical analyses showing APs evoked by a depolarizing current pulse (200 nA, 500 ms) in the

absence (Control) or presence of TEA (TEA). **C–E** Statistical analyses showing the effects of CPH on RMP, spike threshold potential, and delay time in the absence or presence of TEA. Data are mean $\pm$ SEM (* P < 0.05, two-sample t -test).

in the subthreshold range and slow inactivation [19]. I_D channels are Kv1 family K^+ channels that are specifically blocked by α -dendrotoxin [20]. Furthermore, an upward shift in the I - V curve was also found in the presence of CPH during membrane depolarization following blockade of voltage-sensitive Na^+ channels with tetrodotoxin. The more depolarized membrane potential in response to positive current pulses revealed a decrease in outward rectification, suggesting the reduction of a voltage-dependent K^+ current that does not participate in AP repolarization. Further studies are needed to determine whether CPH activates the TEA-sensitive I_K while suppressing I_D .

Our results also demonstrated that CPH induced a more hyperpolarized membrane potential response (downward shift) to negative current pulses in mPFC neurons, suggesting the involvement of other types of membrane ion channels in addition to TEA-sensitive Kv2 or TEA-insensitive Kv1 channels. The RMP is known to be regulated dynamically and maintained by inward rectifiers,

such as Kir, which carries some outward current in the membrane potential range slightly more positive than the equilibrium potential of K [21, 22]. Such changes in Kir activity could shift the I - V curve downwards during membrane hyperpolarization, and depolarize the membrane potential from the RMP level. In addition to Kir, previous investigations have shown that hyperpolarization-activated cation currents (Ih) also participate in maintaining the RMP and regulating the membrane potential at more hyperpolarizing levels in various cells, including heart, neurons, retina, and taste buds [23, 24]. However, whether Kir or Ih is associated with the effect of CPH on RMP and membrane properties remains to be clarified.

CPH is known as a typical, first-generation antihistamine [25] with powerful anticholinergic effects [5, 26]; however our previous study in cultured mouse cortical neurons demonstrated that CPH enhances I_K by activating the $\sigma 1R$ /Gi-protein/PKA pathway [9]. In the current study, using a pharmacological antagonist and agonist of $\sigma 1R$ s

Fig. 5 Effect of CPH on outward rectification during membrane depolarization in mPFC pyramidal neurons in the presence of TTX (1 $\mu\text{mol/L}$).

A Representative traces showing changes in membrane voltage responses to depolarizing current pulses in the absence or presence of CPH. **B** I - V curves indicate a significant shift towards more depolarized potential levels by CPH (mean $\pm$ SEM; $*P < 0.05$ compared with corresponding control, two-sample t -test).

we confirmed that the effect of CPH on neuronal excitability was also mediated by σ 1Rs. We also noted a significant decrease in instantaneous frequency when the σ 1R antagonist Bd1063 was used, suggesting that Bd1063 has a direct inhibitory effect on frequency *via* an unknown mechanism in the mPFC. However, this effect did not influence the role of Bd1063 in inhibiting the CPH/ σ 1R-induced increase in instantaneous frequency. As an interorganelle signaling modulator, σ 1Rs directly or indirectly regulate a variety of functional molecules in a G protein-dependent manner to activate the PKA and PKC signaling pathways [9, 27, 28]. Activation of σ 1Rs has been reported to change neuronal excitability through the regulation of voltage-gated K^+ currents [29, 30]. The diverse actions of σ 1Rs on function and signaling pathways help to explain the CPH-induced effects demonstrated in the current study. The results also suggest that CPH affects multiple ion

channels, and that the CPH-induced changes in neuronal excitability may be caused by its comprehensive effects on these ion channels, rather than by its effect on the TEA-sensitive I_K alone.

In summary, the results of this study provide evidence for a CPH-induced increase in the membrane excitability of mPFC pyramidal neurons. This increased excitability is modulated primarily *via* the regulation of the TEA-sensitive I_K and other TEA-insensitive VGKCs, probably I_D and Kir. Given that the transmission of information in the nervous system and synaptic efficiency are encoded by the AP firing frequency, CPH-induced increases in firing frequency and/or membrane excitability would not only facilitate the responsiveness of mPFC pyramidal neurons to excitatory stimuli, but also enhance the mPFC neuronal glutamate output to subcortical areas. These changes may help to explain the neurological role and mechanisms

Fig. 6 Effect of CPH on inward rectification during membrane hyperpolarization in mPFC pyramidal neurons. **A** Representative traces showing changes in the membrane voltage responses to injection of hyperpolarizing current pulses in the absence or presence of CPH in mPFC neurons. **B** *I*-*V* curves show that the significantly hyperpolarized membrane potential in response to negative currents was significantly enhanced by CPH (mean $\pm$ SEM; $*P < 0.05$ compared with corresponding control, two-sample *t*-test).

responsible for the therapeutic effects of CPH. These alterations in mPFC neuronal excitability induced by CPH may also help to explain its apparent clinical efficacy as an antidepressant and antipsychotic.

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Compliance with Ethical Standards

Conflict of interest All authors claim that there are no conflicts of interest.

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