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Research article

Ginsenoside Rg1 modulates medial prefrontal cortical firing and suppresses the hippocampo-medial prefrontal cortical long-term potentiation

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ABSTRACT

Background: Panax ginseng is one of the most commonly used medicinal herbs worldwide for a variety of therapeutic properties including neurocognitive effects. Ginsenoside Rg1 is one of the most abundant active chemical constituents of this herb with known neuroprotective, anxiolytic, and cognition improving effects.
Methods: We investigated the effects of Rg1 on the medial prefrontal cortex (mPFC), a key brain region involved in cognition, information processing, working memory, and decision making. In this study, the effects of systemic administration of Rg1 (1 mg/kg, 3 mg/kg, or 10 mg/kg) on (1) spontaneous firing of the medial prefrontal cortical neurons and (2) long-term potentiation (LTP) in the hippocampal−medial prefrontal cortical (HP−mPFC) pathway were investigated in male Sprague−Dawley rats.
Results: The spontaneous neuronal activity of approximately 50% the recorded pyramidal cells in the mPFC was suppressed by Rg1. In addition, Rg1 attenuated LTP in the HP−mPFC pathway. These effects were not dose-dependent.
Conclusion: This report suggests that acute treatment of Rg1 impairs LTP in the HP−mPFC pathway, perhaps by suppressing the firing of a subset of mPFC neurons that may contribute to the neurocognitive effects of Rg1.

1. Introduction

The neuropyschopharmacology of *Panax ginseng*, one of the most famous traditional herbs, has been extensively explored by both preclinical and clinical studies. *P. ginseng* and its pharmacologically active constituents, ginsenosides, have found their use in various neuropsychiatric and neurodegenerative conditions such as depression, ischemic stroke, Alzheimer's disease, and Parkinson's disease [1−3]. One of the most abundant constituents among these ginsenosides is Rg1 [4], which is structurally classified under the panaxitriol group [2]. Many preclinical studies delineate the neuroprotective and procognitive effects of Rg1 in various animal models. Behavioral investigations in mice showed that Rg1 enhances spatial memory in naïve [5] and Tg-mAPP overexpressing mice [6] and cognitive performance of senescence-accelerated mouse prone 8 (SAMP8), a model of Alzheimer's disease [7]. Furthermore, Rg1 treatment ameliorates learning and memory impairments, induced by morphine [8], chronic restraint stress [9], scopolamine [10,11], and beta-amyloid peptide (25−35) [12]. In rats, Rg1 was shown to reverse the cognitive impairments ensuing

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electrical injury of the hippocampus [13], bilateral fimbria fornix transection [14], ovariectomy followed by D-galactose treatment [15] and lipopolysaccharide-induced neuroinflammation [16]. It is noteworthy that the aforesaid reports substantiated the procognitive behavioral effects with data on anatomical, electrophysiological, protein, and neurotransmitter level changes in the rodent brain.

This *in vivo* electrophysiological investigation will draw attention to the effects of Rg1 focusing on the changes in the medial prefrontal cortex (mPFC). The mPFC is bilateral brain loci that receives neuronal projection from different parts of the brain [17–19]. The mPFC integrates complex information from various brain regions such as cortex, hippocampus (HP), midbrain, and brainstem to maintain and modulate emotion, cognition, and reward processing. Long-term potentiation (LTP) in the HP–mPFC pathway is a reliable model to study pharmacological and behavioral manipulations that could influence the aforesaid processes [20–22]. The electrophysiological studies on Rg1 that were published to date focused on its modulatory effects on cognitive behavior mediated by the hippocampus. To mention a few, systemic administration of Rg1 increased the synaptic plasticity in the perforant path–dentate gyrus of conscious rats [23], and central administration Rg1 or its metabolites (Rh1 or Ppt) increased hippocampal excitability in unconscious rats [11,23,24]. Rg1 induced LTP in the hippocampus mediated by calcium dependent *N*-methyl-D-aspartate (NMDA) receptor [24] and reversed the chronic morphine-induced impairment of LTP in the CA1-Schaffer collateral [8]. Ginseng dose-dependently reversed the deficits in T-shaped water maze performance (errors) due to prefrontal cortical lesioning in rats [25]. Although this study did not specifically examine the effects of Rg1, it stands as a good representation to accentuate the role of prefrontal cortex underlying the effects of ginsenosides Rg1 and Rb1, taken together. It is noteworthy that another ginsenoside (Re) with reported procognitive effects, belonging to the same group of ginsenoside, dose-dependently increases the extracellular levels of acetylcholine and dopamine in the hippocampus and mPFC with the effect being prominent in the former structure [26]. The present study, first of its kind, has been designed to examine the effects of acute treatment of Rg1 on the changes in the firing rate of mPFC neurons and LTP in the HP–mPFC pathway in unconscious rats.

2. Material and methods

2.1. Animals

Adult male Sprague–Dawley rats (250–380 g) obtained from InVivos Pte. Ltd. (Singapore) were housed in pairs in the animal housing facility of the National University of Singapore for at least 48 h prior to the start of experiments. All cages were individually ventilated in temperature-controlled (range, 22–24°C) rooms with 12-h cycles of day/night light (07:00–19:00). Animals had free access to food and water. All experimental procedures were conducted in accordance with National Institutes of Health Guide for Care and Use of Animals following the approval by the Institutional Animal Care and Use Committee of the National University of Singapore, Singapore.

2.2. Drugs and chemicals

The 7% w/v solution of chloral hydrate (Sigma Aldrich, St. Louis, MO, USA) and 1 mg/ml, 3 mg/ml, or 10 mg/mL solutions of ginsenoside Rg1 (95%; Nature Standard, Shanghai, China) were prepared in sterile normal saline (B Braun, Bayan Lepas Pulau Pinang, Malaysia). Pentobarbital (Valabarb) was purchased from Jurox Pty Ltd. (Rutherford, NSW, Australia). A 2% w/v solution of Pontamine

sky blue (Alfa Aesar, Karlsruhe, Germany) in 2M NaCl (Schedelco, Penang, Malaysia) filled the glass electrode that was used for single unit recording. Solutions of 0.9% w/v sodium chloride (Schedelco) and 4% w/v paraformaldehyde (PFA; Sigma Aldrich) in phosphate buffer (Na_2PO_4 and $NaH_2PO_4 \cdot 2H_2O$; Merck, Darmstadt, Germany) were used for perfusion. The 30% w/v sucrose (Fisher Chemicals, Loughborough, UK) in 10% phosphate buffer saline (1st BASE, Singapore) was used for saturating the harvested brain prior to cryosectioning.

2.3. Surgery

Rats were acclimatized to the electrophysiology procedure room for 30 min, after which they were anesthetized via a single intraperitoneal injection of chloral hydrate (400 mg/kg). Typically, the anesthetized rat was depilated at the head region and mounted on a stereotaxic frame. The body temperature was maintained at 37°C by a homeothermic blanket with rectal temperature probe. The level of anesthesia was maintained by supplemental doses of chloral hydrate administered through the cannulated lateral tail vein. A single sagittal incision on the scalp exposed the bare skull, and burr holes were drilled to target the infralimbic medial prefrontal cortical area (anterior-posterior (AP): 3.3 mm, medial-lateral (ML): ±0.8 mm) for single unit (Fig. 1A) or evoked potential (Fig. 1B) recording, and the ventral hippocampal area (AP: −6.3 mm, ML: ±5.5 mm) for evoked potential stimulation (Fig. 1C), based on the standard coordinates [27].

2.3.1. Extracellular single unit recording of the mPFC neurons

Glass electrodes were pulled from Starbore glass capillaries (Radnoti, Monrovia, CA, USA) using a micropipette puller (PE-21; Narishige Instruments, Tokyo, Japan) and were filled with Pontamine sky blue dye (2% w/v in 2M NaCl). The impedance was adjusted to 20–40 MΩ. The glass electrode was gradually lowered (1–100 μm steps) into the brain via the burr hole on the skull using
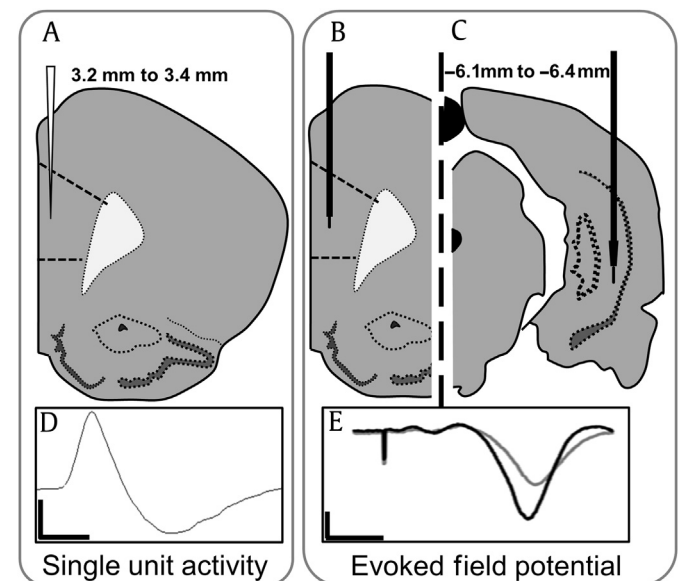

Fig. 1. Schematic representation of sites of intervention at specified distances from bregma. (A) The glass recording electrodes for single unit recording in the medial prefrontal cortex (mPFC). (B) The monopolar electrode in the mPFC for recording evoked field potentials in response to (C) the concentric bipolar stimulating electrode at the CA1/vH. (D) A representative spike from an mPFC neuron (scale bar: 2 mV and 0.5 ms). (E) Representative evoked potential waveforms during baseline (gray) and after high frequency (black) stimulation (scale bar: 0.2 mV and 10 ms).

the single axis motorized micromanipulator (IVM Scientifica, Uck-field, East Sussex, UK) to a region 3—5 mm below the skull surface (Fig. 1A). Once the characteristic medial prefrontal cortical neuron (Biphasic shape, amplitude: 0.5—5 mV, Frequency: 0.1—4 Hz, duration: >1.2 ms, Fig. 1D) was encountered as reported earlier [22], the recording was stabilized for 15—30 min prior to the commencement of the saline and Rg1 treatment [intravenous (i.v.)]. In single dose studies, following 3-min recordings (each for base-line and with sterile normal saline infusion), Rg1 solution (1 mg/kg, 3 mg/kg, or 10 mg/kg) was infused and the recording was continued for 21 min. In cumulative dose—response studies, following base-line and saline recordings, Rg1 (1.25 mg/kg, 1.25 mg/kg, 2.5 mg/kg, and 5 mg/kg) was sequentially infused, and the recording was continued for 12 min. The volume of injections was 0.1 mL, and chloral hydrate supplements did not intersperse with saline/Rg1 injections. The real-time capture of the spontaneous firing of the medial prefrontal cortical neurons was achieved by an ELC-03XS preamplifier (NPI Electronics, Tamm, Germany). The signal was filtered via a Humbug (Quest Scientific, Vancouver, Canada) to remove 50—60 Hz noise, digitized using Power 1401 MK2 interface (CED, Cambridge, UK), and viewed with Spike2 (version 7.12; CED). The sweeps of spike activity were sorted to remove artifacts using the offline forced clustering and principal component analysis module of the Spike2, and firing rate was calculated.

2.3.2. Evoked field potential recording

The procedure published earlier [20,22] was adopted with slight modifications. Briefly, the dorsoventral positions of the bipolar stimulating (50 mm shaft, 250 μm diameter, and 500 μm tip sep-aration; SNE-100; Kopf Instruments, Tujunga, CA, USA) at the CA1/vH (4—7.2 mm below the skull level; Fig. 1C) and the monopolar recording electrode (50 mm shaft, 100 μm diameter, 250 μm recording tip length, SNE-300; Kopf Instruments) at mPFC (4.2—4.7 below the skull level; Fig. 1B) were adjusted to maximize the negative going evoked field potential response in the mPFC (Fig. 1E). An input—output curve was constructed (100—400 μA), and the current producing 60% of maximal response was used for the entire recording procedure. The experimental protocol con-sisted of four steps: (1) a baseline recording with stimulation every 30 s for a period of 30 min; (2) vehicle/Rg1 (1 mg/mL, 3 mg/mL, or 10 mg/mL) administrations (1 mL/kg, i.v.) over 15 s, followed by recording for 30 min; (3) high-frequency stimulation (HFS; 10 trains, 50 pulses, 250 Hz) to produce LTP; and (4) post-HFS recording with stimuli every 30 s for 90 min. Electrical stimula-tion (S88X; Grass Technologies, Warwick, RI, USA) was integrated with data acquisition and analysis system with preamplifier (Dagan), Humbug (Quest Scientific) to remove 50—60 Hz noise, Digitizer (Micro 1401 mk II, CED), and the Signal software (version 5, CED). The field-evoked postsynaptic potentials were expressed as mean percentage $\pm$ standard error of the mean normalized to the baseline for each group.

2.3.3. Perfusion and harvest

The rats subjected to single unit or evoked potential recordings were perfused with isotonic saline followed by 4% PFA in 0.1M phosphate buffer, and the brain was harvested. The harvested brains were sequentially postfixed in PFA and saturated in 30% sucrose. The brain was then cryosectioned for locating the tracks of the electrodes. Animals with incorrect electrode positions were excluded from analysis.

2.4. Statistical analysis

Data were expressed as mean $\pm$ standard error of the mean. The evoked potential data (5-min epochs) and the firing rate data (3-min epochs) were subjected to repeated-measures analysis of variance with planned contrasts corrected for multiple compari-sons. To aid in clarity, the treatment effects (data averaged across 30-min epochs) on the evoked potential data were subjected to one-way analysis of variance with Bonferroni corrected *post hoc* tests. The level of statistical significance was fixed at $p < 0.05$. The analysis was performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Extracellular single unit recording of mPFC neurons

Data from 40 rats were used for analysis. The mean baseline firing rate of the recorded spikes ranged from 0.77 Hz to 1.76 Hz. At least three different populations of medial prefrontal cortical neurons were observed that increased firing, decreased firing, or were not affected by Rg1 (1—10 mg/kg) treatment (Fig. 2A), and hence the three groups were separately subjected to statistical analyses. Six rats (1 mg/kg), four rats (3 mg/kg), and three rats (10 mg/kg) receiving the described doses of Rg1 showed significant ($F_{8,80} = 6.846$, $p < 0.001$) increase in firing rate (Fig. 1B). The dif-ferences among the Rg1 dose levels approached statistical signifi-cance ($F_{2,10} = 3.680$, $p = 0.063$). In total, spikes from 20 animals receiving a single dose of Rg1 showed a decrease in firing rate including six rats (1 mg/kg), 10 rats (3 mg/kg), and four rats (10 mg/kg) (Fig. 1C). The observed decrease in different groups was sta-tistically significant ($F_{8,136} = 23.646$, $p < 0.001$). There was also a significant difference between groups ($F_{2,17} = 4.389$, $p = 0.029$). The difference was mainly between the 1 mg/kg and 3 mg/kg groups. In some of the rats, Rg1 (3 mg/kg and 10 mg/kg) did not affect the firing rate ($F_{8,40} = 1.888$, $p = 0.090$), and there was no difference between treatment groups ($F_{1,5} = 1.168$, $p = 0.329$). The effects of cumulative doses of Rg1 from 0.125 mg/kg to 10 mg/kg were studied in 16 medial prefrontal cortical neurons (Fig. 2D). Individ-ual neurons were classified according to whether they showed an increase ($n = 6$ neurons), decrease ($n = 6$ neurons), or no change ($n = 4$ neurons) in firing rate. The change in firing rate in response to the cumulative doses of Rg1 was statistically significant in the group of neurons classified as showing decreased firing rate ($F_{8,53} = 27.71$, $p < 0.0001$) but not in the group of neurons classified as showing increased firing rate ($F_{8,53} = 1.756$, not significant).

3.2. Evoked field potential recording

Based on the histological verification, data from 25 rats were included for analysis. Systemic administration of Rg1 (1 mg/kg, 3 mg/kg, or 10 mg/kg) did not alter the baseline evoked field po-tential, indicating no long-lasting potentiation effects (Fig. 3). HFS induced LTP in all experimental groups, manifested by the abrupt increase in the amplitude of the negative going wave following the HFS and lasting for at least 90 min ($F_{30,690} = 29.125$, $p < 0.001$). Rg1 (1 mg/kg, 3 mg/kg, or 10 mg/kg) treatment prior to HFS significantly prevented the induction of LTP ($F_{3,21} = 5.747$, $p = 0.005$). Analysis of 30-min epochs of the data showed that Rg1 treatment significantly prevented the increase of post-HFS evoked potential ($F_{3,21} = 6.026$, $p = 0.004$). *Post hoc* analysis showed that all three doses of Rg1 were significantly different from saline treatment ($p < 0.005$). However, there was no statistically significant difference among the doses of Rg1.

4. Discussion

We report a neuroinhibitory effect of Rg1 observed from the suppression of mPFC firing and attenuation of LTP in the HP—mPFC
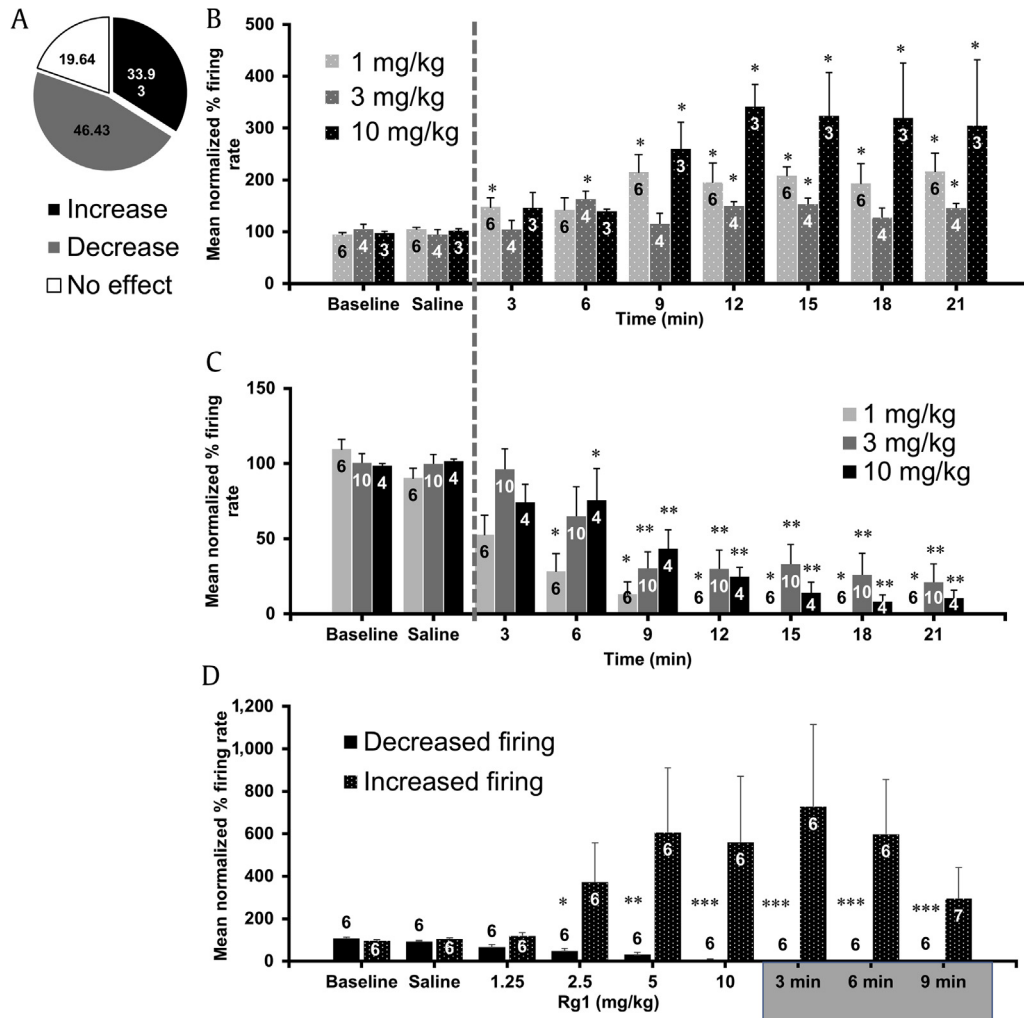
Fig. 2. Effect of Rg1 on firing rate of medial prefrontal cortical (mPFC) neurons. (A) Percentage of neurons that showed increased, decreased or nil effect in firing in response to single dose (1 mg/kg, 3 mg/kg, or 10 mg/kg) or cumulative Rg1 treatment. Columns represent the mean firing rate of neurons that (B) increased or (C) decreased firing in response to single dose of Rg1 (1 mg/kg, 3 mg/kg, or 10 mg/kg) or (D) cumulative doses. The gray broken line in (B) and (C) indicates the time of Rg1 administration. The gray box on the x axis of (D) indicates the posttreatment recording after the last cumulative dose of Rg1. The column data labels represent the number of neurons analyzed. Data from neurons that did not respond to Rg1 treatment are not shown. All statistical comparisons were made against the baseline of the corresponding treatment. Error bars represent SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (repeated-measures analysis of variance planned comparisons). HFS, high-frequency stimulation; SEM, standard error of the mean.

pathway following acute i.v. injections of 1 mg/kg, 3 mg/kg, and 10 mg/kg doses. The modulatory effects of Rg1 on the hippocampus have been attributed to the procognitive effects observed especially in water maze test, a rodent model that putatively reflects hippocampal-dependent spatial function. Chronic treatment with Rg1 (10 mg/kg for 3 mo) was reported to reverse deficits in water maze performance and reduced the Aβ1−40 and Aβ1−42 in the hippocampus of mAPP mice, a model Alzheimer's disease [6]. Likewise, a 28-d treatment regimen of Rg1 (20 mg/kg) reversed D-galactose-induced deficits in the performance of rats in the water maze, presumed to be mediated by the changes in senescence-related markers and hippocampal neurogenesis [28]. Finally, administration of Rg1 (30 mg/kg for 10 d) to rats reversed morphine-induced (1) spatial learning deficits in water maze and (2) impairment in LTP in the CA1-Schaffer collaterals [8].

LTP in the HP−mPFC pathway has been a reliable model that has been regularly used in our laboratory to understand the effects of test compounds or stress and to examine the role of particular neuronal structures in cognitive processing [20−22]. We thus sought to examine the effects of Rg1 in this established *in vivo* model. Several preclinical investigations of Rg1 on the *in vivo*

electrophysiological models sustained the claims of procognitive effects. Increases in synaptic plasticity indices, namely, increased sensitivity of population spike and amplitude, and induction of long-lasting potentiation in the perforant path−dentate gyrus synapse, were reported following a 12-d treatment regimen with Rg1 (10 mg/kg and 30 mg/kg). These effects along, with the findings of increased expression of GAP-43 in the granular layer of the dentate gyrus and increased mossy fiber sprouting, were proposed to underlie the nootropic effects [23]. The contribution of neuronal nitric oxide in the plasticity effects of Rg1 was highlighted by a study that showed that Rg1-mediated [10 nmol and 100 nmol, intracerebroventricularly (i.c.v)] enhancement in the LTP (post-HFS) in the perforant path−dentate gyrus pathway was inhibited by i.c.v. infusion of 7-nitroindazole, a selective neuronal nitric oxide synthase inhibitor, an effect that was reversed by intraperitoneal pretreatment with L-arginine [29]. Furthermore, the Rg1 (100 nmol, i.c.v) induced LTP at perforant path−dentate gyrus synapses in unconscious rats was prevented by pretreatment with 2-amino-5-phosphonovaleric acid but not by nimodipine, indicating the role of NMDA receptors [24]. A recent report showed that administration of Rg1 (0.1−10 mg/kg, for 30 d) enhanced long-term memory (fear
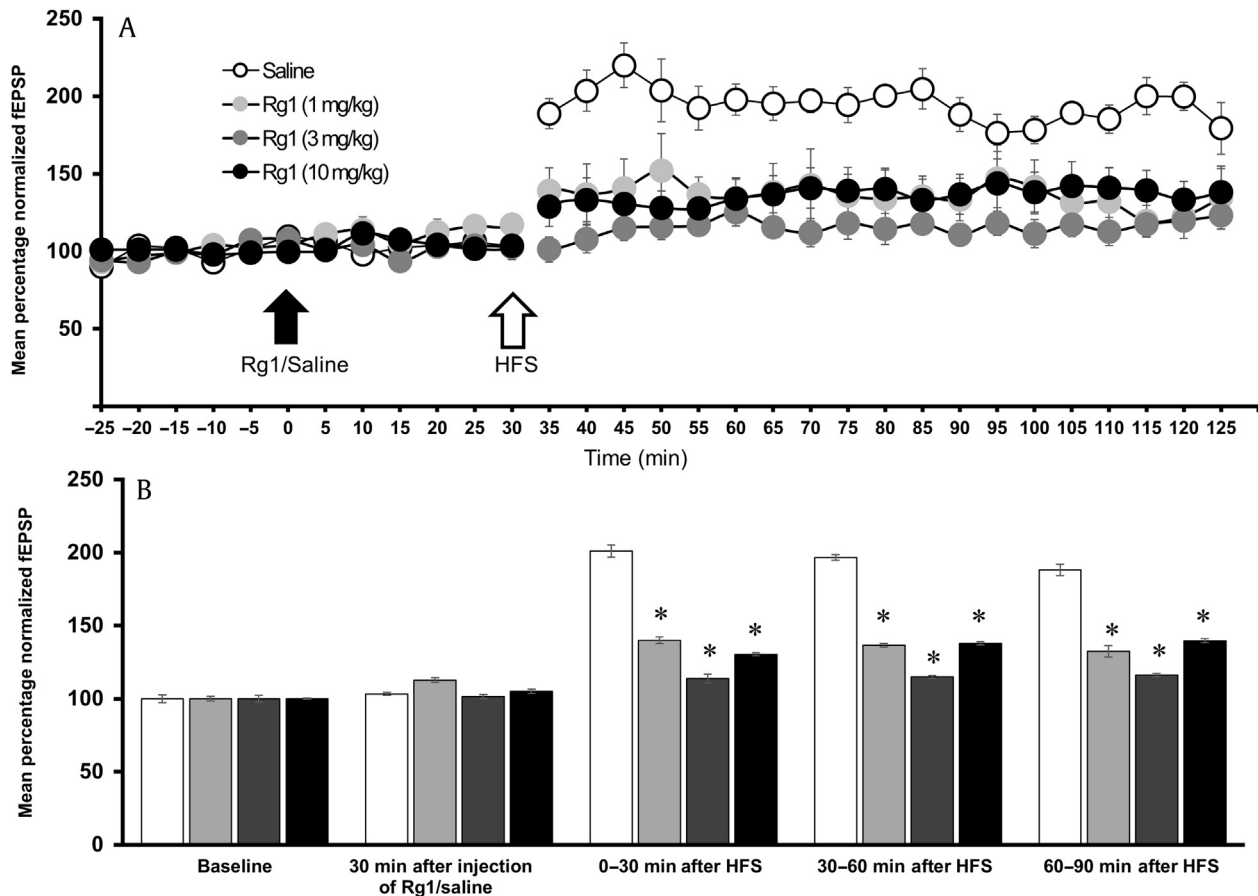
Fig. 3. Evoked field potential recording in the hippocampo−medial prefrontal cortical (HP−mPFC) pathway. (A) The baseline-normalized average potential recorded every 5 min showing no significant change immediately after drug or saline treatment (black arrow). After high-frequency stimulation (HFS; white arrow), induction of a persistent step-up can be observed in the saline treatment group (empty circles) but Rg1 treatment (filled circles) attenuates this long-term potentiation (LTP). (B) Thirty-minute averages of evoked field potentials in different treatment groups showing the suppression of the LTP by Rg1 treatment. Error bars represent standard error of the mean (SEM). $*p < 0.005$ compared to respective normal saline treatment group (one-way analysis of variance and Bonferroni corrected *post hoc* tests). fEPSP, field excitatory post-synaptic potential.

conditioning) in middle-aged mice that was supported by data on facilitation of theta bursts induced LTP in hippocampal slices, increased dendritic apical spine numbers in CA1 region, upregulation of hippocampal p-AKT, brain-derived neurotrophic factor (BDNF), proBDNF, and glutamate receptor, indicating its use in reversal of age-related impairment in learning and memory [30]. The present study is in contrast to aforementioned reports, by demonstrating the suppression of LTP in the HP−mPFC pathway, which may be explained by the acute dosing regimen and the dose levels adopted in this study. Future studies must aim to assess the effects of LTP in the HP−mPFC pathway in response to chronic Rg1 treatment regimen to clarify if they are distinct from the observed acute treatment effects.

The observed impairment of LTP by Rg1 treatment might be mediated by the effects on hippocampus and/or the effect on mPFC. We propose that the effects on the latter are feasible. The inhibitory effect of Rg1 on the LTP in the HP−mPFC is very likely attributable to its effect on the mPFC, because ICV administration of Rg1 (5 nmol) did not affect the LTP at perforant path−dentate gyrus synapses in anesthetized rats [31]. The present study shows that doses that suppressed LTP in the HP−mPFC pathway had differential effects on mPFC neuron firing. In addition, the effects (increase or decrease) of Rg1 are irreversible at least at the tested dose levels and duration. This lack of reversal may be explained by the pharmacokinetics of Rg1 as illustrated by a recent report that highlighted the idea that mPFC is the putative site of action of Rg1.

In that recent report, analysis of dialysates by LC-MS/MS showed that following a single subcutaneous injection of Rg1 (40 mg/kg), the elimination of this ginsenoside was lower in the mPFC with a significantly higher area under the curve value as compared to the hippocampus and the lateral ventricle [32]. However, the lack of reversal might also be attributable to the duration of our recordings. Longer recording times can be achieved by recording in the awake animals or in anesthetized animals with a longer-acting anesthetic agent such as urethane [33,34].

This set of data suggests that there might be different populations of pyramidal cells in the mPFC that respond differently to Rg1 treatment. Similar differences in neuronal activity were reported for the populations of cells in the rat mPFC during working memory tasks [35,36], in response to stressful conditions [37] or amphetamine administration [38], or in mouse mPFC during spontaneous oscillation [39]. These differences in characteristics of different populations of pyramidal cells in the mPFC have been suggested to be involved in the tolerance and adaptive responses, which are important in time-dependent behavioral modulation [37].

In summary, the present study shows that acute treatment of Rg1 has varied effects on different neuron groups in the mPFC neurons that may underlie suppression of LTP in the HP−mPFC pathway. Our current results suggest the need for further investigation of the effects of Rg1 on the mPFC to characterize the neuronal subgroups that differentially respond to Rg1 treatment.

Considering the cardinal role of the mPFC in anxiety, memory, and cognition, the present study draws attention to the mPFC in understanding the effects of Rg1.

Conflicts of interest

There was no financial or other conflicts of interest in designing, performing, or drafting this work.

Acknowledgments

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