NEURAL REGENERATION RESEARCH
June 2015, Volume 10, Issue 6
www.nrronline.org

RESEARCH ARTICLE

Curcumin improves synaptic plasticity impairment induced by HIV-1gp120 V3 loop

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***doi:**10.4103/1673-5374.158358*
***http:**//www.nrronline.org/*

***Accepted:** 2015-03-13*

Abstract

Curcumin has been shown to significantly improve spatial memory impairment induced by HIV-1 gp120 V3 in rats, but the electrophysiological mechanism remains unknown. Using extracellular microelectrode recording techniques, this study confirmed that the gp120 V3 loop could suppress long-term potentiation in the rat hippocampal CA1 region and synaptic plasticity, and that curcumin could antagonize these inhibitory effects. Using a Fura-2/AM calcium ion probe, we found that curcumin resisted the effects of the gp120 V3 loop on hippocampal synaptosomes and decreased Ca^{2+} concentration in synaptosomes. This effect of curcumin was identical to nimodipine, suggesting that curcumin improved the inhibitory effects of gp120 on synaptic plasticity, ameliorated damage caused to the central nervous system, and might be a potential neuroprotective drug.

***Key Words:** nerve regeneration; curcumin; neurons; HIV-1 gp120 V3 loop; plasticity; HIV-associated neurocognitive disorders; output/input curve; long-term potentiation; excitatory postsynaptic potential; paired-pulse facilitation; Ca^{2+}; synaptosome; NSFC grants; neural regeneration*

***Funding:** This study was supported by the National Natural Science Foundation of China, No. 81171134 and 81471235; a grant from the Introducing Talents of Discipline to Universities, No. B14036; a grant from the College Students' Extracurricular Scientific Innovation and Entrepreneurial Activity Research Topic of Jinan University Challenge Cup, No. (2013)27 and (2014)16; a grant from the College Students' Extracurricular Scientific Innovation and Entrepreneurial Activity Research Topic of Jinan University in China, No. 201410559079, 1055914162 and CX14261; a grant from the National Basic Research Program of China (973 Program), No. 2011CB707501; the Science and Technology Foundation of Guangdong Province in China, No. 2010B030700016; the Natural Science Foundation of Guangdong Province in China, No. 2014A030313360.*

Shen LL, Jiang ML, Liu SS, Cai MC, Hong ZQ, Lin LQ, Xing YY, Chen GL, Pan R, Yang LJ, Xu Y, Dong J (2015) Curcumin improves synaptic plasticity impairment induced by HIV-1gp120 V3 loop. Neural Regen Res 10(6):925-931.

Introduction

HIV-associated neurocognitive disorders (HAND) are a series of neurological conditions induced by HIV (Antinori et al., 2007). Extensive data suggest that the cognitive impairments seen in acquired immunodeficiency syndrome (AIDS) patients are associated with the brain infiltration of HIV-1 infected mononuclear phagocytes and the release of soluble viral and cellular factors (Koutsilieri et al., 2002; Kaul and Lipton, 2006; Hult et al., 2008). These soluble factors induce neuronal apoptosis, reduce synaptic and dendrite densities, and cause selective neuronal loss (Arendt et al., 1994; Bachis et al., 2006; Ghafouri et al., 2006; Samuelsson et al., 2006). However, the molecular and cellular pathogenesis of HAND is unclear (Borjabad and Volsky, 2012; Gelman et al., 2012; Morgan et al., 2012; Fields et al., 2013; Trevillyan et al., 2013; Atluri et al., 2014; Bagashev et al., 2014; Purohit et al., 2014; Vassallo et al., 2014).

gp120 is a viral protein released from HIV-infected mononuclear phagocytes. The neurotoxic activity of this protein occurs by interactions with uninfected mononuclear phagocytes to release cellular neurotoxins such as glutamate, pro-inflammatory cytokines and chemokines (Xiong et al., 2000). Animal studies have revealed that gp120 causes amnesia and memory dysfunction (Xiong et al., 2000; Merino et al., 2011). Further findings have shown that the released neurotoxins can activate N-methyl-D-aspartate receptors and induce calcium influx, disrupting calcium homeostasis and triggering the pathway for intrinsic neuronal apoptosis (Louboutin et al., 2007; Silverstein et al., 2012). The V3 loop, a signal peptide of gp120, is the principal neutralizing determinant of HIV and the main target for antibody and cellular immunity (Ghiara et al., 1994).

Curcumin, a monomer of Chinese traditional medicine, has unique neuroprotection properties (Li and Wang, 2009; Xie et al., 2011; Zhao et al., 2012; Xu and Li, 2014; Zhao et al., 2014). Many previous studies have reported the protective effect of

curcumin on the central nervous system, which improves damaged neurons by anti-oxidant and anti-inflammatory mechanisms as well as inhibiting the expression of nuclear factor-kappaB (NF-κB) (Cashman et al., 2008; He et al., 2010; Maher et al., 2010). However, there have been limited studies regarding the neuroprotective effects of curcumin for HAND. Our laboratory has been involved in this field for many years. Previous studies in our laboratory (Tang et al., 2009, 2013) confirmed that curcumin improved spatial memory impairment induced by gp120. However, the effects on electrophysiological mechanisms such as synaptic plasticity are still unclear (Dong et al., 2008; Tang et al., 2009; Deng et al., 2011; Gong et al., 2012; Guo et al., 2013; Tang et al., 2013).

Therefore, we hypothesized that curcumin might be useful to treat or prevent HAND by ameliorating synaptic plasticity. To test this hypothesis, we evaluated the neuroprotective effect of curcumin in the hippocampus CA1 with long-term potentiation induced by the HIV-1gp120 V3 loop and measured the Ca^{2+} concentration in synaptosomes.

Materials and Methods

Animals

A total of 110 neonatal male and female Sprague-Dawley rats aged 9–11 days were purchased from the Experimental Animal Center of Guangdong Province in China (Experimental Animal Certificate SCXK (Yue) 2008-002). Of these, 14 rats were excluded because of processing accidents. The animals were housed at 25°C with humidity of 50% and free feeding with regular illumination (on at 7:00 a.m. and off at 5:00 p.m.) for 3 days. The animal protocol was strictly reviewed by the Institutional Animal Care Committee of Jinan University in China.

Preparation of isolated rat hippocampus

Briefly, 36 rats were anesthetized and decapitated, and their brains were quickly removed from the cranial cavity. The brains were placed into ice-cold (4°C) oxygenated artificial cerebrospinal fluid (solute per liter of solution contained: NaCl 124 mM, KCl 3 mM, $NaHCO_3$ 26 mM, NaH_2PO_4 1.25 mM, $CaCl_2$ 2 mM, $MgCl_2$ 2 mM, glucose 10 mM, and double-distilled water, pH between 7.30–7.42). The hippocampi were dissected and sliced into 400 μm-thick coronal hippocampal sections using a vibrating microtome (World Precision Instruments, Sarasota, FL, USA). The slices were kept in a humidified and oxygenated chamber at room temperature (33°C) for 30 minutes before incubating with the treatment drug of each group for 1 hour and then were transferred into a recording chamber.

Experimental groups and drug treatment of brain slices

The previously prepared brain slices were randomly divided into six groups: control, curcumin, nimodipine, gp120 V3 loop, curcumin + gp120 V3 loop and nimodipine + gp120 V3 loop groups. Slices in the control group were cultured normally. The brain slices in the curcumin, nimodipine, gp120 V3 loop, curcumin + gp120 V3 loop, and nimodipine + gp120 V3 loop groups were incubated with 1 μM curcumin (Fluka, Buchs, Switzerland), 10 μM nimodipine (Sigma, St. Louis, MO, USA), 1 nM gp120 V3 loop (Sigma), 1 μM curcumin and 1 nM gp120 V3 loop, and 10 μM nimodipine and 1 nM gp120 V3 loop, respectively, at room temperature for 1 hour. Subsequently, the electrophysiological signs were measured.

Recording input/output curves

After treatment, the brain slices were transferred into the recording chamber (Axon, Union, CA, USA). In the recording chamber, single hippocampal slices were fully submerged and continuously perfused with artificial cerebrospinal fluid, equilibrating with 95% O_2 and 5% CO_2, at a constant flow rate of 1.5 mL/min with the use of a peristaltic pump (World Precision Instruments). The temperature of perfusion was maintained at 30–34°C. A bipolar concentric electrode (Sutter, Novato, CA, USA) (50–100 μm between each tip, insulated except at the tips) was used to stimulate the Schaffer collateral commissural axons in the dendritic field of hippocampal CA1 with a dissecting microscope (Olympus, Japan). The stimulator provided a square wave (a waviness width of 0.2 ms, frequency of 0.05 Hz, initial strength of 0.1 mA, duration of 0.1 ms, separation of 30 seconds, progressive increasing strength of 0.1 mA) to induce the field excitatory postsynaptic potential (fEPSP). A glass microelectrode (Sutter) (filled with artificial cerebrospinal fluid, 2–5 MΩ in resistance), acting as the unipolar recording electrode, was placed at the CA1 pyramidal cell layer. The recorded electric signals were processed by a Digital to Analog Converter (Axon) and Amplifier (Axon) and recorded by a computer. The data were shown as fEPSP initial slopes and the mean of triplicate signals recorded at each level of strength was considered the final result. The output/input curve was used to assess the release of neurotransmitters from presynaptic neurons and the excitability of postsynaptic neurons. Values of fEPSP initial slopes were recorded at 15 minutes after stimulation with high frequency.

Inducing and recording long-term potentiation

Using the same instrument and hippocampus slice as for the above experiment, the basis strength was determined to be 30% of the maximum amplitude of fEPSP induced by the progressive increasing stimulated square wave. The brain slices were stimulated by a square wave (at this base strength, duration of 0.1 ms, and separation of 30 seconds). The base line was recorded for different treatment factors and was used to reflect the basal transfer of synapsis. After recording the steady base line for 30 minutes, long-term potentiation was induced by high frequency stimulation (frequency of 100 Hz, duration of 1 second, and separation of 20 seconds). Following stimulation by high frequency stimulation, the long-term potentiation was recorded continuously for 60 minutes. When the fEPSP initial slopes increased more than 50% at range and continued this level for at least 60 minutes, this indicated the successful induction of long-term potentiation.

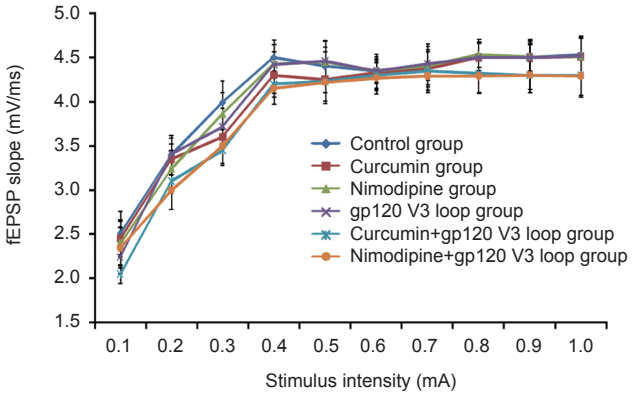

Figure 1 Effects of curcumin on gp120 V3 loop peptide-induced input/output curves of rat hippocampal slices in the CA1 region.
The output/input curve was used to assess the release of neurotransmitters from presynaptic neurons and the excitability of postsynaptic neurons. Data are expressed as the mean ± SD. Statistical analysis between groups was performed using one-way analysis of variance, independent-samples *t*-test, and multiple comparisons using the least significant difference test. The experiment was repeated six times. fEPSP: Field excitatory postsynaptic potential.

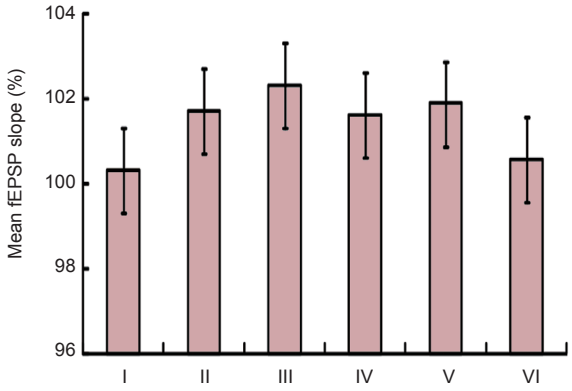

Figure 2 Effects of curcumin on HIV-1 gp120-induced synaptic transmission in Schaffer collateral to CA1 neuronal synapses.
Values of fEPSP initial slopes were recorded at 15 minutes after stimulation with high frequency. Data are expressed as the mean ± SD. Statistical analysis between groups was performed using one-way analysis of variance, independent-samples *t*-test, and multiple comparisons using the least significant differenc test. The experiment was repeated six times. fEPSP: Field excitatory postsynaptic potential. I: Control group; II: curcumin group; III: nimodipine group; IV: gp120 V3 loop group; V: curcumin + gp120 V3 loop group; VI: nimodipine + gp120 V3 loop group.

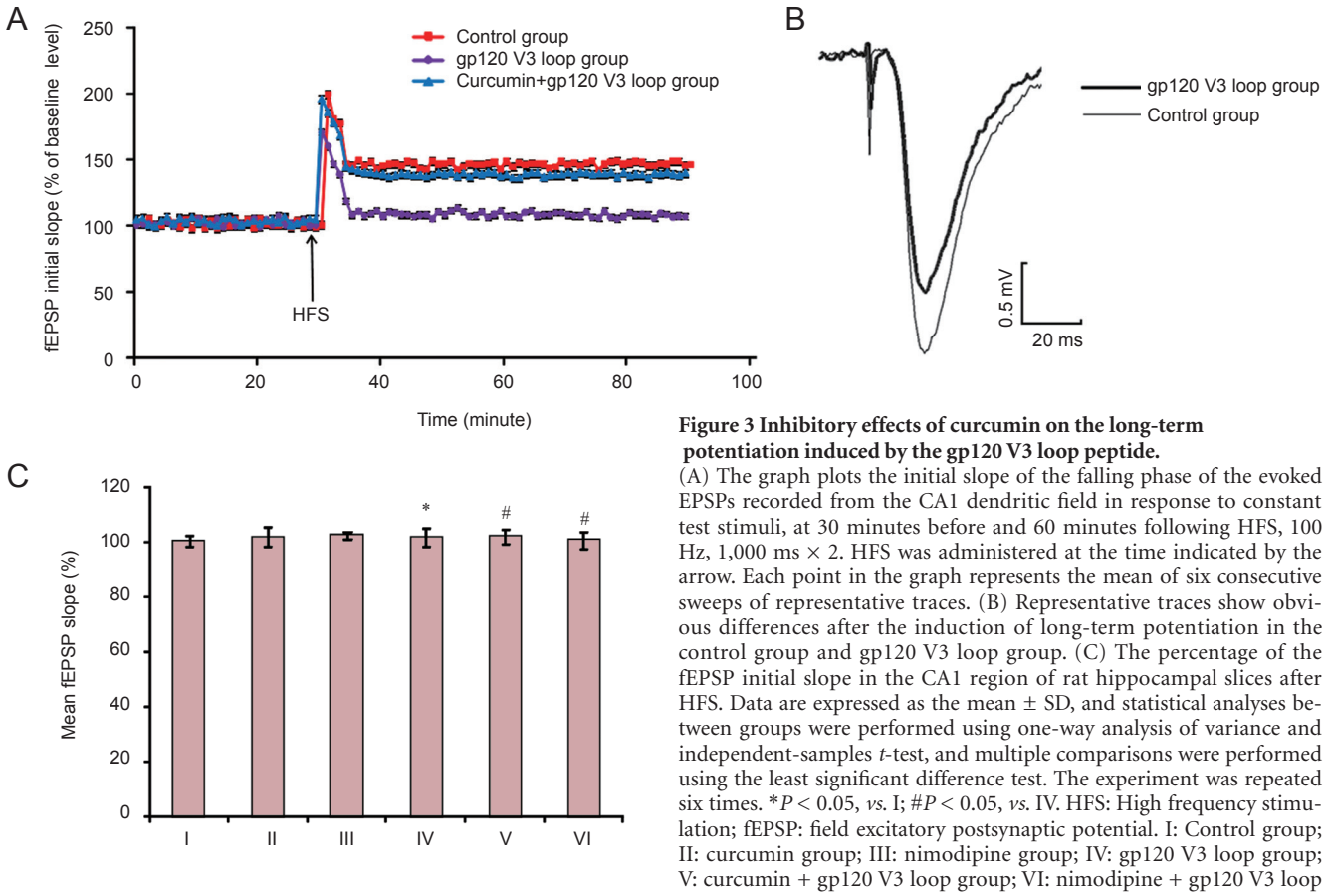

Figure 3 Inhibitory effects of curcumin on the long-term potentiation induced by the gp120 V3 loop peptide.
(A) The graph plots the initial slope of the falling phase of the evoked EPSPs recorded from the CA1 dendritic field in response to constant test stimuli, at 30 minutes before and 60 minutes following HFS, 100 Hz, 1,000 ms × 2. HFS was administered at the time indicated by the arrow. Each point in the graph represents the mean of six consecutive sweeps of representative traces. (B) Representative traces show obvious differences after the induction of long-term potentiation in the control group and gp120 V3 loop group. (C) The percentage of the fEPSP initial slope in the CA1 region of rat hippocampal slices after HFS. Data are expressed as the mean ± SD, and statistical analyses between groups were performed using one-way analysis of variance and independent-samples *t*-test, and multiple comparisons were performed using the least significant difference test. The experiment was repeated six times. **P* < 0.05, *vs.* I; #*P* < 0.05, *vs.* IV. HFS: High frequency stimulation; fEPSP: field excitatory postsynaptic potential. I: Control group; II: curcumin group; III: nimodipine group; IV: gp120 V3 loop group; V: curcumin + gp120 V3 loop group; VI: nimodipine + gp120 V3 loop group.

The fEPSP initial slopes recorded at 45 minutes were used to compare the range of induced long-term potentiation in different groups. The range of long-term potentiation is a significant and recognized factor for the assessment of the level of synaptic plasticity.

Inducing and recording paired-pulse facilitation

Using the same instruments and hippocampus slices as the above experiment, the brain slices were stimulated by paired-pulse stimulation, 30% maximum stimulating intensity and durations of 25, 50, 100, 150, 200 ms respectively, to induce

paired-pulse facilitation. The fEPSPs recorded before and after dosing were denoted as fEPSP1 and fEPSP2. The paired-pulse ratio was denoted as fEPSP1/fEPSP2. A value of paired-pulse ratio > 1 indicated paired-pulse facilitation, and the contrary value was paired-pulse depression.

Preparation and intervention of synaptosome

Briefly, 60 rats were anesthetized and decapitated, and their hippocampus was quickly removed from the cranial cavity. The hippocampus was mixed into an ice-cold (4°C) 0.32 M isotonic sucrose solution of 1 g/10 mL to make a homogenate. This was centrifuged at 1,500 r/min for 10 minutes at a low temperature and the liquid supernate was collected. The supernate was then centrifuged at 9,000 r/min for 20 minutes to collect the P2 component, containing crude synaptosomes. The P2 component was suspended slowly in a 0.32 M sucrose solution and placed into a 10 mL 0.8 M sucrose solution carefully. The solution was centrifuged at 9,000 r/min for 25 minutes. The 0.32 M sucrose solution liquid phase and sucrose solution interface band between 0.32 M and 0.8 M was discarded. The 0.8 M sucrose solution liquid phase was added dropwise into an isometric precool medium for 5 minutes. The mixed solution was centrifuged at 20,000 r/min, at 4°C for 10 minutes and precipitated to collect the synaptosome. The protein level of synaptosomes was measured using Lowry's method (Lowry et al., 1951) and diluted to a protein level (1 mg/mL) using nutrient solution in an ice-bath for further use. The group and drug doses were the same as for the examination of brain slices.

Fura-2 /AM load

Fura-2/AM 0.5 M in dimethyl sulfoxide was placed into the synaptosome suspension. The mixed solution was incubated at 37°C for 45 minutes, then centrifuged twice at 15,700 × *g* for 5 minutes. The precipitated synaptosome was resuspended in artificial cerebrospinal fluid without $MgSO_4$ and NaH_2PO_4 and placed into an enzyme linked immunosorbent assay plate. The mixed solution was added to 0.1% Triton X-100 to obtain the R_{max} (the maximum of 340/380 nm fluorescence intensity specific value) and 7.5 mM glycol-bis-(2-aminoethylether)-N,N,N′,N′-tetraacetic acid chelated calcium ion to obtain the R_{min} (the minimum of 340/380 nm fluorescence intensity specific value) using a fluorescence microplate reader (Bio-Rad, USA) (510 nm at emission wavelength, 340 and 380 nm at excitation wavelength). The Grynkiewicz equation: $[Ca^{2+}]_i$ (nM) = $Kd•[(R–R_{min})/(R_{max}–R)]•(Sb1/Sb2)$ (Kd = 224, R = 340/380 nm fluorescence intensity specific value, Sb1 = 380 nm fluorescence intensity without calcium, Sb2 = 380 nm fluorescence intensity with saturated calcium) was used to calculate the value and the background autofluorescence value was deleted from this to determine the acquired synaptosome $[Ca^{2+}]_i$.

Statistical analysis

Data were analyzed with SPSS 13.0 software (SPSS, Chicago, IL, USA), Original 7.0 software (OriginLab Corporation, Northampton, MA, USA) and p Clamp 9.0 software (Axon Instruments). Data are expressed as the mean ± SD. Statistical analysis between groups was performed using one-way analysis of variance, independent-samples *t*-test, and multiple comparisons using the least significant difference. Differences were considered significant when $P < 0.05$.

Results

Effects of curcumin on the gp120 V3 loop peptide-induced input/output curves of rat hippocampal slices in the CA1 region

Compared with the control group, input/output curves of rat hippocampal slices in the CA1 region showed no significant change in the curcumin, nimodipine, curcumin + gp120 V3 loop and nimodipine + gp120 V3 loop groups ($P > 0.05$), which indicate that curcumin and nimodipine had no effects on the basic transmission of excitatory synapses of the hippocampal CA1 region in rats (**Figure 1**).

Effects of curcumin on HIV-1 gp120-induced synaptic transmission in Schaffer collateral to CA1 neuronal synapses

Compared with the control group, there was no statistically significant difference in fEPSP slopes in the curcumin, nimodipine, gp120 V3 loop, curcumin + gp120 V3 loop, nimodipine + gp120 V3 loop groups ($P > 0.05$; $n = 6$; **Figure 2**).

Inhibitory effects of curcumin on long-term potentiation induced by gp120 V3 loop peptide

While the fEPSP initial slope did not increase or return to baseline quickly in the gp120 V3 loop group, the difference of the fEPSP initial slope between the gp120 V3 loop group and the control group was statistically significant ($P < 0.05$). Compared with the gp120 V3 loop group, the fEPSP initial slope was increased significantly in the curcumin + gp120 V3 loop group and nimodipine + gp120 V3 loop group ($P < 0.05$). fEPSP initial slopes in the curcumin group and nimodipine group were similar to the control group ($P > 0.05$; **Figure 3**).

Effects of curcumin on the paired-pulse ratio of the hippocampal CA1 region in rats

No significant differences in paired-pulse ratio were observed before or after the treatment of the rat hippocampal CA1 region in each group ($P > 0.05$). Moreover, the paired-pulse ratio was similar between all groups ($P > 0.05$; **Figure 4**).

Effects of curcumin on Ca^{2+} concentration induced by the gp120 V3 loop peptide in hippocampal synaptosomes

Compared with the control group, the concentration of free Ca^{2+} was significantly higher in the gp120 V3 loop group ($P < 0.01$). Compared with the gp120 V3 loop group, the concentration of free Ca^{2+} was significantly lower in the curcumin + gp120 V3 loop group and nimodipine + gp120 V3 loop group ($P < 0.01$). No significant difference in the concentration of free Ca^{2+} was observed between the curcumin

and nimodipine groups and the control group ($P > 0.05$; **Figure 5**).

Discussion

Curcumin inhibits integrases and proteases that are necessary for the replication of HIV-1, as well as possessing anti-inflammatory and antineoplastic effects. Curcumin has a protective effect in cerebrovascular disease, cerebral trauma, and neurodegenerative disease (He et al., 2010; Maher et al., 2010). In addition, curcumin, a plant based medicine, is well tolerated and has a low incidence rate of side effects. No previous studies have shown that high-dose curcumin causes toxic effects on humans or animals (Shankar et al., 1980; Qureshi et al., 1992; No author, 1996; Adams and Ferraro, 1997; Barks et al., 1997; Koutsilieri et al., 2002; Albright et al., 2003; von Giesen et al., 2005; Bachis et al., 2006). Previous studies (Dong et al., 2008; Tang et al., 2009; Deng et al., 2011; Gong et al., 2012; Guo et al., 2013) in our laboratory indicate that curcumin protected rat hippocampal neurons from apoptosis caused by the gp120 V3 loop. Our previous experiments (Tang H et al., 2009) showed that curcumin improved the learning ability of gp120 impaired rats through anti-oxidation, and ameliorated tumor necrosis factor-α-induced neuronal damage *in vivo*.

The current study highlighted the neuroprotective effect of curcumin on HAND from the aspect of synaptic plasticity by observing the input/output curve, fEPSP, long-term potentiation, double-pulse facilitation, by using extracellular microelectrode recording techniques. Furthermore, the electrophysiological mechanism of this effect was demonstrated by observing Ca^{2+} concentrations using a Fura-2/AM calcium ion probe.

In this study, we observed that the gp120 V3 loop had no significant effect on the release of neurotransmitters from the presynaptic membrane of rat hippocampal slices as there was no significant change in the input/output curve, fEPSP or paired-pulse facilitation. Therefore, the target of the gp120 V3 loop might be located in the postsynaptic membrane. L-type Ca^{2+} channels are important ion channels present in the postsynaptic membrane, playing a major role in synaptic plasticity. Nimodipine is an L-type Ca^{2+} channel-blocking agent. Long-term potentiation in the rat hippocampal CA1 region was increased in the nimodipine and gp120 V3 loop groups, which indicated that the gp120 V3 loop plays a role in long-term potentiation by influencing L-type Ca^{2+} channels. There was a similar phenomenon in the curcumin and gp120 V3 loop groups, suggesting that the neuroprotective effect of curcumin is associated with antagonizing the gp120 V3 loop-induced inhibitory effect on long-term potentiation in hippocampal CA1 region. To verify this, we observed changes in the synaptic Ca^{2+} concentration using a Fura-2/AM Ca^{2+} probe technique. The results suggested that the gp120 V3 loop raised the synaptic Ca^{2+} concentration, which could be reduced by either curcumin or nimodipine. Thus, curcumin ameliorated the neuronal impairment induced by the gp120 V3 loop, which inhibited L-type Ca^{2+} channels and reduced the intracellular Ca^{2+} concentration in rat hippocampal synapses. Therefore, the present study demonstrated that the neuroprotective effect of curcumin is associated with antagonizing the inhibitory effect on long-term potentiation in the hippocampal CA1 region and the increased Ca^{2+} concentration in hippocampal synaptosomes induced by the gp120 V3 loop.

Compared with the current treatments for HAND, curcumin has significant advantages, including its greater ability to cross the blood-brain barrier compared with HAART, lower toxicity and side effects and reduced financial cost. Thus, curcumin may be a potential treatment to prevent and cure HAND.

Author contributions: *JD and YX designed this study. LLS, MLJ, SSL, MCC, and ZQH performed experiments. YYX, GLC, RP, and LJY analyzed the data. LLS, MLJ and SSL wrote the paper. MCC and ZQH did the translation. JD and LLS did the modification. All authors approved the final version of the paper.*

Conflicts of interest: *None declared.*

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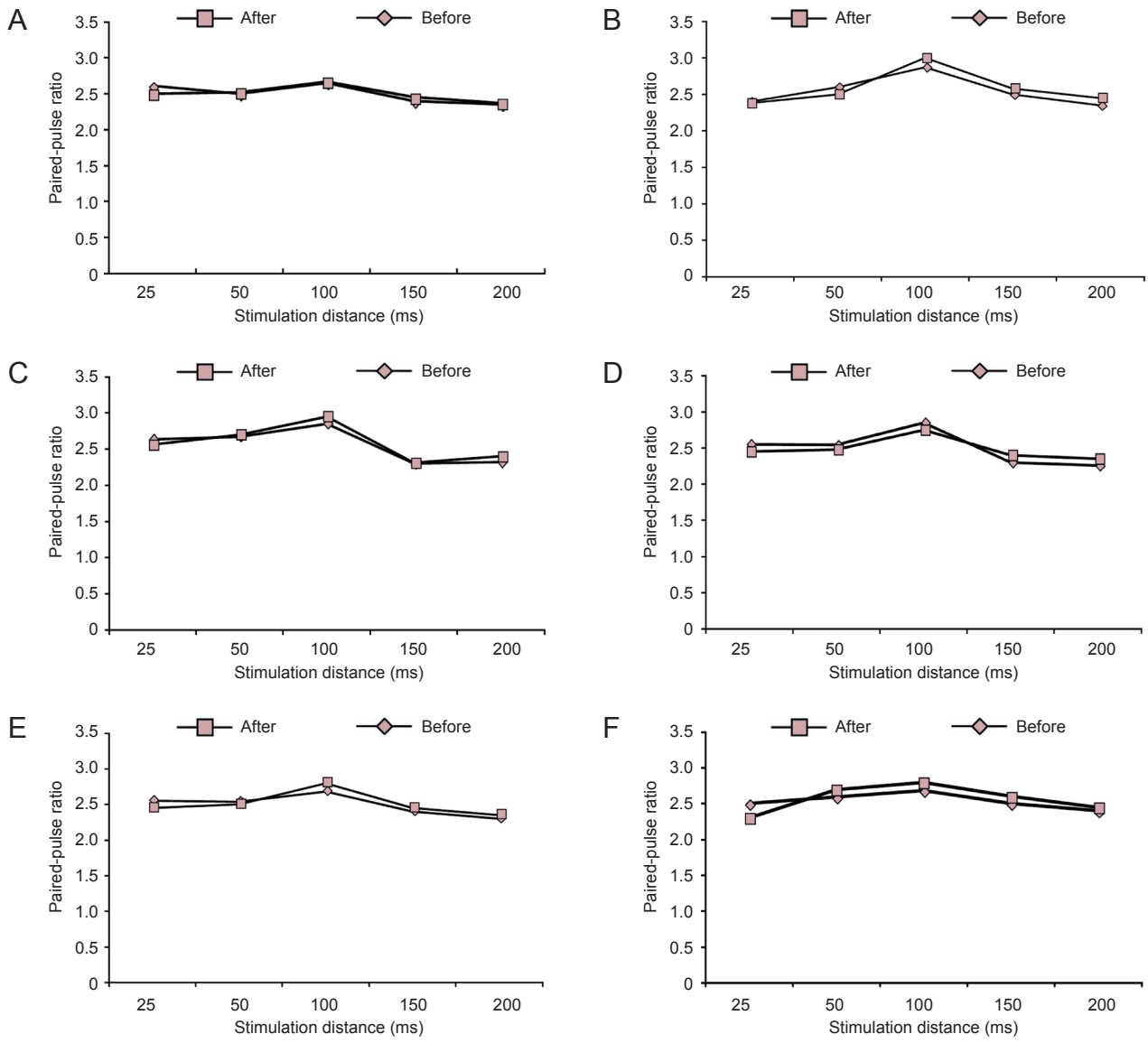

Figure 4 Changes of paired-pulse stimulation of the rat hippocampal CA1 region.
(A) Control group; (B) curcumin group; (C) nimodipine group; (D) gp120 V3 loop group; (E) curcumin + gp120 V3 loop group; (F) nimodipine + gp120 V3 loop group. Statistical analyses between groups were performed using one-way analysis of variance and independent-samples *t*-test, and multiple comparisons were performed using the least significant difference test. The experiment was repeated six times.

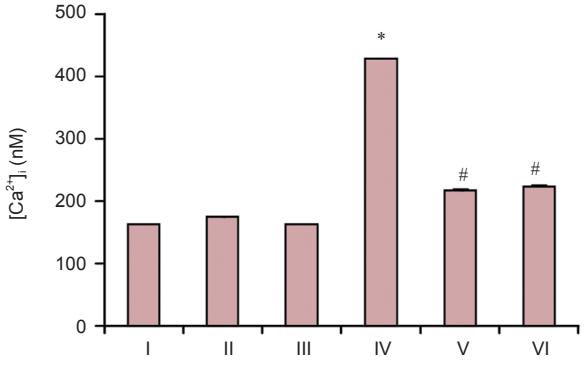

Figure 5 Effects of curcumin on Ca^{2+} concentration induced by the gp120 V3 loop peptide in hippocampal synaptosomes.
$[Ca^{2+}]_i$ (nM) = Kd•[(R–R_{min})/(R_{max}–R)]•(Sb1/Sb2) (Kd = 224, R = 340/380 nm fluorescence intensity specific value, Sb1 = 380 nm fluorescence intensity without calcium, Sb2 = 380 nm fluorescence intensity with saturated calcium, R = fluorescence value, R_{max} = the maximum fluorescence value, R_{min} = the minimum fluorescence value). Data are expressed as the mean ± SD. Statistical analyses between groups were performed using one-way analysis of variance and independent-samples *t*-test, and multiple comparisons were performed using the least significant difference test. The experiment was repeated ten times. **P* < 0.05, *vs*. I; #*P* < 0.05, *vs*. IV. I: Control group; II: curcumin group; III: nimodipine group; IV: gp120 V3 loop group; V: curcumin + gp120 V3 loop group; VI: nimodipine + gp120 V3 loop group.

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Copyedited by Croxford L, Norman C, Yu J, Qiu Y, Li CH, Song LP, Zhao M