HIV-1 Inhibits Long-Term Potentiation and Attenuates Spatial Learning

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Although memory deficits have been clearly documented in patients with human immunodeficiency virus type-1 (HIV-1) infection, the physiological basis of this dysfunction is poorly understood. We focused on Tat, a viral protein released from HIV-1–infected cells and investigated its effect on spatial learning in adult mice. An intracerebroventricular injection of Tat leads to attenuation of spatial learning accompanied by suppression of long-term potentiation (LTP), the cellular basis of spatial learning, in hippocampal cornu ammonis 1 pyramidal neurons. Tat facilitates extrasynaptic but not synaptic N-methyl-D-aspartate (NMDA) receptor activity. Taken together, these data provide strong evidence that the Tat pathway underlies the development of memory dysfunction in patients with HIV-1 infection and suggest a causal relationship between Tat, the facilitation of extrasynaptic NMDA receptor activity, inhibition of LTP, and attenuation of spatial learning.


Infection with human immunodeficiency virus type-1 (HIV-1) frequently results in behavioral and cognitive changes that range in intensity from memory dysfunction to global dementia.1,2 Immunocytochemical analysis of HIV-1–infected adult patient brains showed HIV-1 antigen immunoreactivity of 78%,3 whereas the virus has not been detected in neuronal cells. Thus, it is considered that viral products released from HIV-1–infected or HIV-1–activated macrophages and glia cells mediate HIV neurotoxicity.4,5 One such product is HIV-1 Tat, a transactivator of viral transcription that, as shown by in vitro studies, is released extracellularly by HIV-1–infected lymphocytes. The presence of Tat in brain tissue was demonstrated by immunohistochemistry, Western blot, and mRNA analysis in HIV encephalitis.6 Several studies have shown that Tat causes loss of primary cultured neurons,7–11 presumably caused by cell membrane depolarization,7,12 increases in the intracellular Ca2+ concentration,5,8,13,14 or promoted aggregation.15,16 Infusion of Tat into the lateral ventricle or gray matter also resulted in neuronal cell loss, behavioral neurotoxic symptoms, and/or led to death within 2 hours after injection.17–19 On the other hand, it also has been reported that the injection of Tat alone does not induce neuronal cell death in vivo.20 In addition, there has been little direct evidence showing which pathway of Tat-associated neurotoxicity underlies the development of memory dysfunction in patients with HIV-1 infection.

Prompted by the contradictory findings of past studies concerning the neurotoxic effect of Tat, and to verify precisely whether Tat accumulation undergoes functional aberrations in central nervous system by HIV-1 infection, we examined neuronal injury and spatial learning in vivo, and the induction of long-term potentiation (LTP), a phenomenon considered to be the cellular mechanism for spatial learning in vitro. We demonstrate here that Tat attenuates spatial learning without, or at least before, leading to neuronal cell loss, accompanied with disrupted LTP induction in hippocampal cornu ammonis 1 (CA1) pyramidal neurons. We also investigated the mechanism underlying these neurotoxic effects of Tat and demonstrate that Tat facilitates the extrasynaptic, but not synaptic, N-methyl-D-aspartate (NMDA) receptor function, via the basic arginine-rich domain.

Materials and Methods

Construction of TAT Vectors

The following oligonucleotides (GENESET, Kyoto, Japan) were synthesized: Oligo TAT-S, TAT-AS corresponds to the complements of the coding strand of HIV-TAT coding se-
Expression and Purification of Recombinant Protein

BL21 (DE3) cells containing each expression plasmid were grown at 37°C to an OD600 of 0.8. Isopropylthiogalactoside was added to a final concentration of 0.2mM and each culture was incubated for 12 hours at 37°C. Cells were harvested and resuspended in 100ml of lysis buffer containing 20mM HEPES (pH 8.0), 100mM NaCl, 8M urea, and 20mM imidazol. Subsequently, the cells were sonicated, and the supernatants were recovered and applied to a column of Ni-NTA agarose (Invitrogen, La Jolla, CA). Fractions containing expressed protein were pooled and applied to a second MONO-S column (AKTA system; Pharmacia, Gaithersburg, MD). Protein purity was greater than 95% based on analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and staining with Coomassie Brilliant Blue. Protein concentrations were determined using a BCA kit (Pierce, Rockford, IL). Glycerol was added to 20% (v/v), and aliquots were stored at 80°C.

Synthesis of Peptides

Peptides were synthesized by Sigma Genosis Japan (Hokkaido, Japan). The peptides were purified by preparative reversed-phase high-performance liquid chromatography, were greater than 95% pure as analyzed by high-performance liquid chromatography, and had the expected amino acid composition and mass spectra.

Intracerebroventricular Injection and Histological Assessment

All animal use procedures were approved by Okayama University Animal Care Committee and were in strict accordance with the Japan Physiological Society of Guiding Principles for the Care and Use of Animals in the field of physiological science. All the injections were made stereotaxically (Model 900; David Kopf Instrument, Tujunga, CA) under pentobarbital anesthesia into the right ventricle of male C57BL6 mice aged 7 to 8 weeks. The coordinates were 1.2mm and 2.5mm under the surface of the skull. Each injection was performed over 5 minutes 0.7mm posterior to bregma lateral to the midline; through a Hamilton needle (26 gauge), and the needle was left in place for an additional 2 minutes. After recovering for 2 days, the mice then were used in an eight-arm maze task. For histological assessment, mice were transcardially perfused with saline followed by 4% paraformaldehyde and anesthesia. The extirpated brains were embedded in paraffin and coronally cut into sections of 6μm thickness. The sections then were stained with cresyl violet.

Radial Maze Task

The eight-arm radial maze consisted of eight equally spaced arms radiating from an octagonal central platform. Each arm was 56.2cm long by 7.9cm wide. After recovery from surgery, the mice were semistarved for 48 hours to reduce their body weight to 85% of their original body weight. The mice were allowed to familiarize themselves with the radial maze. Before each trial, five of the eight arms were baited with food pellets. The mice were placed in the center of the maze and allowed to freely explore the maze. The trial was terminated when the animal had taken the food reward from all five arms or after 10 minutes if all five arms were not visited. During the trial, the researcher stood at a marked location in the room and monitored the animal’s performance, recording the number of entries into the arms and the total time required to visit all eight arms. Reentry into an already visited arm was considered an error. Each animal was given one trial daily. All tests were conducted between 3 and 6 PM in a temperature-controlled (25–26°C) room.

Slice Preparation and Primary Cell Culture

Hippocampal slices were prepared as described previously and were maintained for at least 2 hours in artificial cerebrospinal fluid (ACSF) containing 124mM NaCl, 3mM KCl, 2mM CaCl₂, 2mM MgSO₄, 26mM NaHCO₃, and 10mM glucose saturated with a mixed gas (95% O₂, 5% CO₂) at 25–26°C. Before each experiment, individual slices were transferred to a submersion-recording chamber, where they were superfused continuously (2ml/minute) with ACSF at 28°C. Dissociated neuronal culture was prepared from the brain of E17-18 fetal C57BL6 mice as previously described. The plated cell density was approximately 25,000/cm². Cultures on days 9 to 14 after plating were used for calcium measurement and other experiments.

Electrophysiology

Stimulating and recording procedures were as previously described. The amplitudes of the field excitatory postsynaptic potentials (EPSPs) were calculated as the initial slope of the EPSP. Whole-cell patch-clamp recordings were made from CA1 pyramidal cell bodies using an upright microscope (Olympus BX50WI). The patch electrodes (glass with a fillament, 5–8 MΩ resistance; WPI, Berlin, Germany) were filled with an internal solution containing 130mM cesium gluconate, 4mM NaCl, 2mM MgCl₂, 0.2 EGTA, 2mM ATP, and 10mM HEPES (pH 7.25, adjusted with gluconic acid). Data were acquired with an Axopatch-200B amplifier (Axon Instruments, Burlingame, CA) and analyzed (filtered at 2kHz, sampled at 5kHz) on a personal computer using PClamp 8 (Axon Instruments).

Fluorescence Measurement

Intracellular Ca²⁺ imaging was performed using the Ca²⁺-specific fluorescent probe fura-2 AM. Cells were incubated for 30 minutes at 25°C in ACSF containing 2μM fura-2 AM, washed with ACSF to remove extracellular fura-2 AM and incubated at 37°C for 30 minutes to allow complete de-esterification of the probe. Cells were excited at 340 and 380nm, and emission was recorded at 510nm with a video-
based imaging system (Aquacosmos Imaging System; Hamamatsu Photonics, Shizuoka, Japan).

Statistical Analysis
Data were calculated as the mean ± SEM. The unpaired t test was applied to the electrophysiological and behavioral studies to determine significant difference in the means of the two sets of data.

Results
Tat Attenuates Spatial Learning and Inhibits Long-term Potentiation Induction
To examine the effect of Tat on learning and memory in vivo, we microinjected Tat into the right ventricle of mice. Because Tat penetrates across the plasma membrane,23 we synthesized and injected Cy3-conjugated Tat (1.6 µl, 200 µM) and analyzed the fluorescence in brain slices prepared 3 hours after injection (Fig 1A) by confocal microscopy. The Cy3 signal was most obvious in hippocampal neurons near the injection trace, whereas the signal was less or not detected in the hippocampus at the contralateral site and in those cortical neurons outside the hippocampi. This was probably because of the small amount (0.32 nmol) of Tat and ease of access to the hippocampal neurons close to the injected point. In accordance with a previous report,20 this small amount of Tat did not induce any detectable neuronal cell loss until 7 days after the injection in all three mice examined (see Fig 1B, C). To investigate the influence of Tat on learning and memory, we performed an eight-arm radial maze task from 2 days after the injection. On the third and fourth days of the test, Tat-injected mice showed a significantly higher number of errors (20.7 ± 4.5 on third day, p < 0.01, and 15.7 ± 4.2 on fourth day, p < 0.001; n = 6) than the ACSF-injected control mice (6.7 ± 0.8 on third day and 5.2 ± 0.8 on fourth day; n = 6), although this gap decreased on the fifth day (5.2 ± 1.4 in ACSF-injected mice, n = 6; and 12.0 ± 4.3 in Tat injected mice, n = 6; p > 0.1; Fig 2A). We further examined LTP induction in mice that finished the radial maze task (10 days after the injection). Applying high-frequency stimulation (HFS, 100Hz, 1 second) induced typical LTP in ACSF-injected control mice (68.7 ± 6.5%, n = 5). In Tat-injected mice, however, the LTP induction was significantly inhibited (19.8 ± 7.1%, n = 5; p < 0.001; see Fig 2B).

Because several laboratories previously have reported the neurotoxicity of Tat, including neuronal cell death,7–11 membrane depolarization,7,32 and increased intracellular Ca2+ concentration5,8,13,14 in primary cultured neurons, it is important to verify whether the basic synaptic transmission is influenced by Tat. We examined this issue by the in vitro application of Tat to hippocampal slices. Bath application of Tat (1 µM) up to 1 hour did not alter the initial slope of field EPSP (n = 5; Fig 3A). The induction of LTP, however, was significantly suppressed in Tat-incubated slices (18.1 ± 4.6%, n = 10; p < 0.001) compared with naive control slices (53.6 ± 3.5%, n = 8; see Fig 3B). Taken together, these results demonstrated that both the in vivo and in vitro application of Tat inhibits LTP induction in hippocampal CA1 neurons.

Essential Role of the Arginine-Rich Domain
The arginine-rich region of Tat is reportedly important in cytotoxicity19,24 and cell membrane transduction.25 Thus, we synthesized a peptide containing this arginine-rich domain (Tat peptide, position 47–57; Fig 4A) and examined its effect on LTP induction. After incubating slices in an fluorescein isothiocyanate (FITC)--conjugated Tat peptide (1 µM) for 1 hour, the
FITC signal was detected in either pyramidal neurons or granule neurons (Fig 4B). In accordance with Tat protein, LTP was largely inhibited in the Tat peptide–treated (16.2 ± 8.6%, n = 5; p > 0.2) slices compared with naive control slices (see Fig 4C). In contrast, a mutant peptide that replaced Q with R to disrupt the amphipathic helix showed no inhibition of LTP (52.8 ± 7.3%, n = 5; p = 0.001), although the membrane transduction is similar to the Tat peptide (see Fig 4A–C). Therefore, the Tat peptide comprising 11 amino acids is both essential and sufficient for induction of LTP.
hibiting LTP induction. To further investigate whether or not the Tat peptide influences spatial learning, we next injected either the Tat peptide (1.6 μl, 1mM) or mutant peptide (1.6 μl, 1mM) into the right ventricle and performed the eight-arm radial maze task. In accordance with Tat, the Tat peptide–injected mice show impaired spatial learning (25.5 ± 2.2 on third day, \( p < 0.001 \), 15.8 ± 0.9 on fourth day, \( p < 0.001 \), 12.0 ± 1.6 on fifth day, \( p < 0.01 \); \( n = 4 \)) compared with mutant peptide–injected mice (8.0 ± 1.6 on third day, 6.3 ± 1.3 on fourth day, 4.3 ± 1.0 on fifth day; \( n = 4 \); see Fig 4D).

Facilitation of Repeated Stimuli but Not Single Stimulus-evoked N-methyl-D-aspartate Current by Tat

We next examined paired pulse facilitation (PPF) to assess whether Tat affects the presynaptic release mechanisms. The ratio of PPF was not significantly different at any interpulse intervals between the naïve control (\( n = 12 \)) and the Tat peptide–treated (\( n = 15 \)) slices (all, \( p > 0.2 \); Fig 5A), suggesting that Tat does not affect presynaptic release probability. Two types of glutamate receptors respond to transmitter release in hippocampal CA1 neurons: NMDA receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. We calculated and compared the baseline input/output relationships between the control (\( n = 9 \)) and Tat peptide–incubated (\( n = 10 \)) slices and found these to be very similar, with no significant differences in the mean at any level of stimulation (all, \( p > 0.3 \), see Fig 5B). Because extracellular Mg\(^{2+}\) blocks NMDA receptor channel activity under physiological conditions, the field EPSP recorded in our experimental conditions represents non-NMDA receptor–dependent EPSP. Thus, considering the results in Figure 3A, these data demonstrate that both Tat and the Tat peptide do not affect the activities of non-NMDA receptors.

We next examined the effect of the Tat peptide on the NMDA receptor–mediated synaptic response using

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**Fig 4.** The essential roles of the arginine-rich domain on long-term potentiation (LTP) induction and spatial learning. (A) Schematic representation of the Tat peptide (left) and the point mutant of the Tat peptide in which Q has been replaced with R (right). (B) Transduction of FITC-conjugated Tat peptide (left) and mutant peptide (right) into hippocampal neurons. Peptides were incubated with slices for 30 minutes, after which the medium was changed, incubated for another 30 minutes, and washed three times with PBS. The slices were analyzed by confocal microscopy. There are no detectable differences between Tat peptide– and mutant peptide–incubated slices. Scale bars = 100 μm. (C) Averaged field potential recordings. Tat peptide (open circles; \( n = 5 \)) but not mutant peptide (filled circles; \( n = 5 \)) inhibited LTP induction. Hippocampal slices were incubated in the Tat peptide or mutant peptide containing artificial cerebrospinal fluid (ACSF) for 1 hour and then washed with normal ACSF for another 1 hour. HFS was applied at the time indicated by the arrow. (D) The number of errors in an eight-arm radial maze task over 5 days’ training. Note that the mean errors were significantly different between Tat peptide–injected (filled circles; \( n = 4 \)) and mutant peptide–injected mice (open circles; \( n = 4 \)) on third, fourth, and fifth day (\( p < 0.01 \); *\( p < 0.001 \)).
the whole-cell patch-clamp recording technique. Both the rise time (Tat peptide, 15.6 ± 0.4 milliseconds, n = 5; control, 15.1 ± 0.3 milliseconds, n = 6; p > 0.2) and the decay time constant (Tat peptide, 59.6 ± 4.6 milliseconds, n = 5; control, 58.9 ± 4.7 milliseconds, n = 6; p > 0.2) of the NMDA receptor-mediated excitatory postsynaptic current (EPSC) currents were similar between the control and the Tat peptide–treated slices. The I-V curves of NMDA in these two groups were almost identical (Tat peptide, n = 5; control, n = 6; all, p > 0.3; see Fig 5D). The ratio of the NMDA to AMPA current was not significantly changed (p > 0.1) in Tat peptide–treated slices (85.5 ± 12.7%; n = 5) compared with that of naive

![Fig 5. Effect of Tat on synaptic current. (A, B) Averaged field potential recordings. (A) The ratio of the field EPSP slopes evoked by the second and the first stimulus is plotted at interpulse intervals from 25 to 800 milliseconds. No differences in the mean ratios were significant between Tat peptide–treated slices (n = 15) and naive control slices (n = 12) at any interval examined (all, p > 0.2). (B) Comparison of baseline input/output relationships between naive control slices (n = 9) and Tat peptide–treated (n = 10) slices. No differences in mean initial slope were significant at any stimulus level examined (all, p > 0.3). (C, D) Whole-cell patch-clamp recordings. In these experiments, bicuculline (50 μM), a specific antagonist of the GABAA receptor, was added to artificial cerebrospinal fluid (ACSF). (C) (left) AMPA receptor–mediated EPSCs (downward traces) and NMDA receptor–mediated EPSCs (upward traces) were recorded at membrane potentials of −80 and +50 mV, respectively. When NMDA EPSCs were recorded, 20 μM CNQX (6-cyano-7-nitroquininaline-2,3-dione), a non-NMDA receptor antagonist, was present to block AMPA EPSCs. (right) The ratio of amplitude of the NMDA EPSC to that of AMPA EPSC was calculated and compared between Tat peptide–treated (n = 5) and naive control (n = 6) slices. The mean ratio was not significantly (p > 0.1) different between the two groups. (D) The current-voltage relationships of synaptic NMDA currents in naive control (open circles; n = 6) and Tat peptide–treated (filled circles; n = 5) slices. Current amplitudes were normalized to the value obtained at +50 mV for easier comparison. Before each recording, slices were incubated in Tat peptide containing ACSF for 1 hour and then washed with normal ACSF for 1 hour. Control slices were incubated in normal ACSF for the same period.](image-url)
control slices (61.8 ± 11.8%; n = 6; see Fig 5C). The mean relative amplitude of the HFS-evoked (100Hz, 100 millisecond) NMDA current, however, was significantly enhanced in Tat peptide–treated slices (603.5 ± 12.6%, n = 5; p < 0.001), but not in mutant peptide–treated slices (472.3 ± 9.1%, n = 5; p > 0.2), compared with naive control slices (429.2 ± 20.3%, n = 6; Fig 6A, B). Taken together, these results demonstrate that the Tat peptide facilitates the HFS-evoked NMDA current but has no effect on the synaptic NMDA current.

Enhanced Extrasynaptic N-methyl-D-aspartate Receptor Response by TAT Peptide

HFS facilitates glutamate release and saturates the glutamate uptake mechanisms, thereby subsequently activating the extrasynaptic NMDA receptors.\textsuperscript{28,29} Therefore, one possible explanation for the enhancement of the HFS-evoked NMDA current but not the single stimulus-evoked current by the Tat peptide is the selective acceleration of extrasynaptic but not synaptic NMDA receptors. To address this hypothesis, we examined the activities of extrasynaptic NMDA receptors using a Ca\textsuperscript{2+} imaging technique.\textsuperscript{30} In primary cultured hippocampal neurons, up to approximately 10% of the inhibitory interneurons that imposed a tonic inhibition on the neuronal network were contained. Thus, blocking the GABA\textsubscript{A} receptor function with bicuculline (50\(\mu\)M) causes the neurons to fire synchronous bursts of action potential and release glutamate from presynaptic sites, which, in turn, activates synaptic NMDA receptors and causes Ca\textsuperscript{2+} influx (Fig 7A). Subsequently, the synaptic NMDA receptors were selectively inactivated using MK801. Because MK801 is an open channel blocker and binds only to activated NMDA receptors, the population of NMDA receptors not stimulated by bicuculline-induced firing (those not located at synapses) was left unblocked. This population of extrasynaptic NMDA receptors was then activated by the simultaneous bath application of NMDA (500\(\mu\)M) and glycine (200\(\mu\)M; see Fig 7B). The mean peak Ca\textsuperscript{2+} influx through synaptic NMDA receptors was not significantly different among the control (22.8 ± 2.9%, n = 39), Tat peptide–treated (25.4 ± 3.1%, n = 54; p > 0.2), or mutant peptide–treated (21.9 ± 3.5%, n = 53; p > 0.2) neurons (see Fig 7A). In contrast, the extrasynaptic NMDA receptor–dependent Ca\textsuperscript{2+} influx was significantly facilitated by the Tat peptide (44.9 ± 1.8%, n = 54, p < 0.001) but not by the mutant peptide (41.4 ± 3.5%, n = 53, p > 0.1) compared with that of naive control neurons (36.0 ± 2.1%, n = 39 see Fig 7B). Taken together, these data demonstrate that the Tat peptide promotes extrasynaptic but not synaptic NMDA receptor activity.

Discussion

Although several clinical and laboratory features that are risk factors for the development of HIV dementia have been identified, it remains difficult to predict which HIV-associated pathway underlies this dysfunction.\textsuperscript{3} In this study, we showed that an intracerebroventricular injection of Tat leads to the attenuation of spatial learning accompanied by LTP suppression, an
We demonstrated here that Tat facilitates extrasynaptic but not synaptic NMDA receptor activity, suggesting that there is a causal relationship between Tat, the facilitation of extrasynaptic NMDA receptor activity, LTP inhibition, and the attenuation of spatial learning.

Previous observations suggest that Tat may act on central nervous system in a dose-dependent manner. Sabatier and colleagues first observed that an intracerebroventricular injection of Tat led to murine death with a 50% lethal dose of 8.1nmol.19 Wang and colleagues reported that an intrahippocampal injection of up to 31pmol of Tat did not elicit overt hippocampal injury, but increased the severity of NMDA-induced injury in neonatal rats.20 We showed in this study that neuronal cell loss and basic synaptic transmission are not induced by the in vivo (0.32nmol) or in vitro (1μM) application of Tat, respectively, thereby suggesting that Tat induces neuronal dysfunction before leading to neuronal cell death. Several reports have previously implicated the importance of the arginine-rich region as a mediator of neuronal cell death, although whether the region is sufficient to induce a

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**Fig 7.** Comparison of synaptic and extrasynaptic NMDA receptor–mediated calcium signals. (A) Imaging of global calcium transients in primary cultured hippocampal neurons after the indicated treatments. Bicuculline, 50μM; MK801, 10μM. (B) The mean peak synaptic NMDA receptor–mediated calcium influx is not significantly different (p > 0.2) between these groups (Tat peptide, n = 54; mutant peptide, n = 53; naive control, n = 39). (C) Imaging of extrasynaptic NMDA receptor–dependent Ca2+ influx. Ten minutes after washing out bicuculline and MK801, NMDA (500μM) was applied with glycine (200μM) as indicated by the arrow. (D) The extrasynaptic NMDA receptor–mediated Ca2+ influx is significantly higher in the Tat peptide–treated (n = 54), but not in mutant peptide–treated (n = 53) neurons, than naive control neurons (n = 39). Before each recording, cells were incubated in Tat peptide or mutant peptide containing medium for 1 hour and then washed with normal medium. (*p < 0.001).
neurotoxic effect remains controversial. We showed here that this arginine-rich domain suppresses both LTP induction and spatial learning and facilitates extrasynaptic NMDA receptor–mediated Ca\(^{2+}\) influx, whereas a point mutant peptide does not, thereby demonstrating that the arginine-rich region is both essential and sufficient for Tat-induced neuronal dysfunction.

An important finding of this study is that Tat acts on extrasynaptic but not synaptic NMDA receptors. The accumulated evidence previously has implicated the involvement of NMDA receptor activity in Tat-induced neuronal damage. Tat increases phosphorylation of the NMDA receptor subunits NR2A and NR2B in a tyrosine kinase–dependent manner, thereby facilitating Ca\(^{2+}\) influx by the extracellular application of NMDA in primary cultured hippocampal neurons.\(^{14}\) Tat injection into the hippocampus induces neuronal injury only when coinjected with NMDA.\(^{20}\)

On the other hand, NMDA receptor antagonists prevent Tat-induced neuronal damage in primary cultured neurons.\(^{11}\) Using the patch-clamp recording and Ca\(^{2+}\) imaging techniques, we demonstrated here that Tat facilitates extrasynaptic but not synaptic NMDA receptor activity. One possible explanation is the variation in the composition of subunits between synaptic and extrasynaptic NMDA receptors.\(^{32}\) Indeed, It recently has been reported that the synaptic and extrasynaptic NMDA receptors are differentially regulated, possibly resulting from their differential interactions with tyrosine kinases and phosphatases and/or proteins enriched in the postsynaptic density, such as PSD-95.\(^{33}\)

How does enhanced Ca\(^{2+}\) influx through extrasynaptic NMDA receptors inhibit LTP induction? Currently, a straightforward examination of this issue is problematic because it is difficult to activate or inactivate extrasynaptic NMDA receptors specifically without affecting synaptic NMDA receptor activity. However, the accumulated evidence has shown that synaptic and extrasynaptic NMDA receptors play contrasting roles in various physiological pathways: the activation of synaptic NMDA receptors is required for LTP induction,\(^{31,34}\) whereas the activation of both synaptic and extrasynaptic NMDA receptors by bath application of NMDA inhibits LTP induction,\(^{35}\) causing long-term depression.\(^{29,36}\) Ca\(^{2+}\) entry through synaptic NMDA receptors induces cAMP response element binding protein (CREB) activity, and has antiapoptotic activity, whereas Ca\(^{2+}\) entry through extrasynaptic NMDA receptors activates a general and dominant CREB shut-off pathway.\(^{30}\) It is well established that CREB phosphorylation is required for both learning and memory, and long-lasting LTP induction.\(^{37–39}\)

Taking these reports together with our findings, we conclude that HIV-1 Tat, possibly through the facilitation of extrasynaptic NMDA receptor–dependent Ca\(^{2+}\) influx, inhibits LTP induction in hippocampal CA1 neurons and attenuates spatial learning. Because antiretroviral therapies and treatment for opportunistic infections in AIDS patients have lengthened survival but not eradicated the virus from the central nervous system, the incidence of neurological dysfunction, including HIV-1 dementia, will increase.\(^{40}\) Our findings provide strong evidence that the Tat pathway underlies the development of memory dysfunction in patients with HIV-1 infection and may offer important clues toward the identification of a cure for HIV dementia.

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