Posttetanic enhancement of striato-pallidal synaptic transmission

Juhyon Kim and Hitoshi Kita
Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee Health Science Center, Memphis, Tennessee

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Kim J, Kita H. Posttetanic enhancement of striato-pallidal synaptic transmission. J Neurophysiol 114: 447–454, 2015. First published May 20, 2015; doi:10.1152/jn.00241.2015.—The striato (Str)-globus pallidus external segment (GPe) projection plays major roles in the control of neuronal activity in the basal ganglia under both normal and pathological conditions. The present study used rat brain slice preparations to characterize the enhancement of Str-GPe synapses observed after repetitive conditioning stimuli (CS) of Str with the whole cell patch-clamp recording technique. The results show that 1) the Str-GPe synapses have a posttetanic enhancement (PTE) mechanism, which is considered to be a combination of an augmentation and a posttetanic potentiation; 2) the degree of PTE observed in GPe neurons had a wide range and was positively correlated with a wide range of paired-pulse ratios assessed before application of CS; 3) a wide range of CS, from frequencies as low as 2 Hz with as few as 5 pulses to as high as 100 Hz with 100 pulses, could induce PTE; 4) the decay time constant of PTE was dependent on the strength of CS and was prolonged greatly, up to 120 s, when strong CS were applied; and 5) the level of postsynaptic Cl⁻ became a limiting factor for the degree of PTE when strong CS were applied. These results imply that Str-GPe synapses transmit inhibitions in a nonlinear activity-weighted manner, which may be suited for scaling timing and force of repeated or sequential body movements. Other possible factors controlling the induction of PTE and functional implications are also discussed.

Striatum; globus pallidus; inhibitory postsynaptic currents; short-term plasticity

The striato-globus pallidus external segment (Str-GPe) projection is the most massive of the basal ganglia circuits, and GPe outputs may play major roles in controlling neuronal activity of all nuclei in the basal ganglia under both normal and pathological conditions. Kita and Kita (2013) and few others (Kita and Kita 2011b; Surmeier et al. 2010; Wu et al. 2011) showed that Str-GPe synapses show very strong enhancement during repetitive activation, and thus the efficacy of the transmission is greatly dependent on the firing patterns of the Str neurons. In this study, we explored more fundamental aspects of PTE including whether or not all GPe neurons that evoke inhibitory postsynaptic currents (IPSCs) to Str stimulation can induce PTE, how many activation pulses in CS are required for the induction of PTE, comparison of the magnitude and decay times of PTE induced by CS with different intensities and lengths of pulse trains, and the possible involvement of a postsynaptic mechanism. We also aimed to identify presynaptic Ca²⁺-channels involved in synaptic transmission and enhancement. In many central nervous system (CNS) synapses the P/Q type is the dominant Ca²⁺-channel, while in some GABAergic CNS synapses the N type is dominant (Catterall 1997). This information will be valuable for the development of future strategies for in vivo and in vitro studies to interpret the GPe activity of normal and pathological animals.

METHODS

Slice preparations. Animal handling and all procedures were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all experiments received approval by the University of Tennessee Health Science Center Animal Care and Use Committee. Sprague-Dawley juvenile rats (16–21 days old) were used. Previous studies suggested that the physiological features of Str-GPe and Str-substantia nigra pars reticulata (SNr) synapses in rodents become adultlike by about postnatal day 16 (Connelly et al. 2010; Ogura and Kita 2000). Rats were anesthetized with intraperitoneal injection of a mixture of ketamine (85 mg/kg) and xylazine (15 mg/kg) and perfused through the heart with cold oxy-
generated (95% O2-5% CO2 gas mixture) cutting solution containing (in mM) 252 sucrose, 3 KCl, 1.24 NaH2PO4, 26 NaHCO3, 0.5 CaCl2, 6.3 MgSO4, 0.2 thiourea, 0.2 ascorbic acid, and 20 glucose. After decapsulation, the brains were rapidly removed, and blocks containing the GPe were obtained. Slices (350 μm in thickness) were cut from the blocks on a vibrating blade microtome (Leica VT1000S; Leica Microsystems, Nussloch, Germany) with the cut plane tilted approximately −8° from the midsagittal plane in the dorsal view in ice-cold oxygenated cutting solution. The slices were incubated in oxygenated standard artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 3 KCl, 1.24 NaH2PO4, 26 NaHCO3, 2.4 CaCl2, 1.3 MgSO4, 0.2 thiourea, 0.2 ascorbic acid, and 10 glucose at 34°C for 30 min. Recording was performed after incubation at room temperature for at least 30 min.

Electrophysiological recordings. The slices were transferred to a recording chamber with oxygenated ACSF continuously perfused at a flow rate of 1 ml/min. The temperature of the recording chamber was gradually increased from room temperature and maintained at 32°C after placing of the slice. Whole cell patch-clamp recording pipettes with a tip diameter of −1.5 μm were pulled from 1.5-mm, thin-wall, borosilicate glass capillaries on a horizontal electrode puller (P-97; Sutter Instrument, Novato, CA). The whole cell recording pipettes contained (in mM) 135 K-gluconate, 5 KCl, 10 HEPES, 0.1 CaCl2, 1 EGTA, 2 Mg-ATP, 0.2 Na-GTP, and 10 phosphocreatine, and the pH was adjusted to 7.3 with KOH. Pipette resistance was 3–6 MΩ. GPe neurons having spontaneous firing of 0–20 Hz and spike amplitudes >60 mV were recorded. Unless otherwise noted, recordings were performed in voltage-clamp mode at −50 mV to analyze IPSCs. Whole cell liquid junction potentials were calculated to be −11.6 mV for our internal solutions, and membrane potentials were not corrected. Short −10-mV pulses were applied every 15 s to monitor the input impedance of the neuron. Neurons and recording pipettes were visualized with an infrared-differential interference contrast microscope (BX50WI; Olympus, Tokyo, Japan) with a ×40 water immersion objective and a CCD camera (4990 series; Cohu Electronics, San Diego, CA). Data were corrected and digitized with a sampling rate of 20 kHz with a Multiclamp 700B amplifier and a Digidata 1322A A/D converter board (Molecular Devices, Foster City, CA). Signals were filtered at 3 kHz and recorded on a hard disk with data acquisition and analysis software pCLAMP 10 (Molecular Devices).

Electrical stimuli (each pulse 200 μs in duration and ≤200 μA) to activate striato-pallidal (Str-GPe) fibers were applied with a bipolar electrode (tip distance of 0.2–0.5 mm) placed on the Str and another bipolar electrode on the internal capsule (IC) to activate other GABAergic fibers (Fig. 1A). GPe neurons that evoked 30- to 50-pA IPSCs to single stimulation of Str with stimulus current of <60 μA were selected for this study. The parameters of Str-CS to induce PTE are described in RESULTS. To monitor IPSCs before and after Str-CS, test pulses (single or paired-pulse stimulation with 40-ms interstimulus interval) were applied to the Str and IC stimulating electrodes every 15 s with a 5-s delay on each IC stimulation (Fig. 1B). GPe neurons that responded to Str stimulation but failed to respond to IC

Fig. 1. A and B: diagrams show the setup of experiments and stimulus (stim) schedules used in this study. Stimulus electrodes were placed in the striatum (Str) and the internal capsule (IC). A hyperpolarizing pulse (−15 mV, 100 ms) to monitor the input impedance and single or double test stimuli were given every 15 s. Test stimulus intensity was adjusted to evoke 30- to 50-pA inhibitory postsynaptic currents (IPSCs) unless otherwise noted. For all recordings, ACSF contained 10 μM NBQX and 30 μM CPP to block AMPA/kainate and NMDA responses. CS, conditioning stimuli; GPe, globus pallidus external segment. Ca: example of posttetanic enhancement (PTE) of Str-GPe IPSCs. Open arrowheads indicate baseline IPSC (i.e., an average of 4 IPSCs recorded before application of CS), and filled arrowheads point to a single IPSC recorded 5 s after the CS. Cb: Str-CS did not potentiate IPSCs to IC stimulation. D: group plots of changes of the amplitudes of IPSCs to test stimuli recorded from 43 GPe neurons. Fifteen of the 43 neurons also responded to IC stimulation. IPSC amplitudes were normalized with baseline IPSCs. E: 9 GPe neurons were tested with 3 Str-CS spaced 5 min apart, and the amplitudes of IPSCs to test Str stimuli were plotted. The repeated Str-CS evoked similar degrees of PTE.
stimulation were included in the analysis. The mean amplitude of four test IPSCs recorded prior to CS was used as the baseline amplitude to normalize the amplitudes of test stimulus-induced IPSCs after application of CS. The amplitude of the IPSC to the first test stimulation divided by the baseline amplitude is called the PTE ratio.

To isolate IPSCs, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzof[1]quinoxaline-7-sulfonamide (NBQX, 10 μM) and the NMDA receptor antagonist 3-(carboxybenzyl)-propyl-1-phosphonic acid (CPP, 30 μM) were included in the ACSF superfused during recording. Previous reports in our lab demonstrated that application of the GABA<sub>A</sub> receptor antagonist gabazine (SR-95531, 10 μM) abolished Str stimulation-evoked IPSCs (Kim and Kita 2013; Ogura and Kita 2000). To block Ca<sup>2+</sup> channels, nimodipine (20 μM), ω-agatoxin TK (200 nM), or ω-conotoxin GVIA (2 μM) was applied to the bath. Cytochrome c (0.1%) was added with ω-agatoxin TK or ω-conotoxin GVIA to prevent nonspecific protein binding. To deplete intracellular Ca<sup>2+</sup> stores, cyclopiazonic acid (CPA, 10 μM) was applied to the bath.

**Statistics.** All group data are expressed as means ± SE. For comparison of two groups, Student’s paired or unpaired t-test was used. Wilcoxon signed-rank test was used for values normalized to control (195.7 ± 20 –30 min after CS was significantly larger than the pre-CS level the mean amplitude of test IPSCs evoked after application of CS with low or high stimulus intensity to evoke 30–50 pA IPSCs, degree of paired-pulse ratios (PPRs), input resistances, degrees of membrane sags to hyperpolarizing current pulses, and spontaneous firing rate of recorded neurons. However, these investigations yielded no clear association with the long-term changes.

**RESULTS**

**Induction of posttetanic enhancement.** The first stage of the experiments was to test whether all GPe neurons evoking an IPSC to Str stimulation induce PTE and to assess the maximum strength and decay time of the PTE. For the experiments, 46 GPe neurons that evoked an IPSC > 30 pA to a single Str stimulation with <50–μA pulse were tested for an induction of PTE with a high-frequency (100 Hz) and 100-pulse CS, which mimics strong behavioral event-related Str activity as described above. In 43 of the 46 neurons, the amplitude of the first test IPSC evoked 5 s after CS (post-CS IPSC hereafter) was larger than the baseline amplitude (i.e., mean of 4 pre-CS IPSCs). The mean PTE ratio of the 43 neurons was 577.5 ± 46.9% (Fig. 1, Ca and E). The remaining three (6.5%) neurons evoked a smaller post-CS IPSC. IC stimulation evoked IPSCs in 15 of the 46 neurons, and the IPSCs were unaffected by Str-CS (n = 15; Fig. 1Ch).

The enhanced IPSCs evoked in 9 of the 43 neurons did not return to pre-CS level. Thus the decay time constant of PTE was estimated from the remaining 34 neurons. The estimate using an exponential regression to the responses to test stimuli applied every 15 s was 79.5 ± 11.4 s. Nine of the 34 neurons were tested three times with CS with an interval of 5 min. All five neurons repeatedly induced PTE with a similar ratio (Fig. 1E).

In five of the nine neurons with IPSCs that did not return to the pre-CS level the mean amplitude of test IPSCs evoked 20–30 min after CS was significantly larger than the pre-CS control (195.7 ± 26.7% increase), and in the remaining four neurons the mean IPSC amplitude obtained from the same sampling periods was significantly smaller (50.1 ± 15.0% of baseline). We spent some effort to find the factors that might cause the induction of these long-term potentiation (LTP)-like and long-term depression (LTD)-like changes, including association with low or high stimulus intensity to evoke 30–50 pA IPSCs, degree of paired-pulse ratios (PPRs), input resistances, degrees of membrane sags to hyperpolarizing current pulses, and spontaneous firing rate of recorded neurons. However, these investigations yielded no clear association with these long-term changes.

**Dependence on number of CS pulses to induce PTE.** To determine the minimum number of synaptic activations to induce detectable PTE and the number of synaptic activations that induces the strongest PTE, experiments with repeated (every 5 min) application of CS having various numbers of pulses, from 2 to 100, at 100 Hz were performed. The results show that 5 or more pulses induced statistically significant PTE, 10 or 20 pulses induced the highest PTE ratio, and the degree of PTE appeared slightly decreased with 50 and 100 pulses, but the changes were insignificant (Fig. 2A). The mean decay time constant of the PTE increased with an increase in pulses (Fig. 2B). The effects of the frequency of CS on the induction of the enhancement during stimuli and PTE have been described in one of our previous reports (Kim and Kita 2013).

**Dependence on CS intensity to induce PTE.** We assumed that CS with moderate-intensity current pulses would not reliably activate neurons and axons after every pulse, and therefore an increase in the current intensity of CS would increase activation reliability and increase PTE ratio more than described above. This assumption was tested on five GPe neurons. For each recording neuron, the intensity of a single stimulation to induce a nearly saturated amplitude of IPSCs (called 100% intensity) was determined by applying stimuli with a graded increase in the intensity. It should be noted that further increases in the stimulus intensity resulted in a stepwise shortening of the latency and evoked much larger IPSCs, which we attributed to the involvement of activated GPe collateral axons (Ogura and Kita 2000). Using test stimulus intensity to evoke 30- to 50-pA IPSCs, Str-CS were repeatedly applied every 5 min with progressively increasing intensity. The results show that the ratio and the decay time constant of PTE increased with an increase in CS up to 25% intensity (Fig. 2, C and D). However, stronger CS decreased the amplitude of PTE (Fig. 2, C–F). Although the amplitude was decreased, the decay time constant was further increased (Fig. 2D). We hypothesized that an accumulation of intracellular Cl<sup>−</sup> reduced the amplitude of IPSCs. To analyze this possibility, three CS with 25%, 100%, and 25% intensity were tested with the recording neurons held at normal −50 mV for the first two CS and at −70 mV for the last. The PTE ratio induced by the last CS was significantly larger than that induced by the second CS, suggesting that the reduction of PTE amplitude by a strong CS is at least in part due to an accumulation of intracellular Cl<sup>−</sup> (Fig. 2, G and H).

**Relationship between paired-pulse ratio and PTE.** The development of short-term plasticity is often associated with an alteration of the PPR, which is inversely related to the probability of vesicular release at the synapse (Dobrunz and Stevens 1997). Figure 3 shows the results of 62 GPe neurons subjected to Str-CS with 100 pulses, 100 Hz, and 25% intensity. The PPRs measured from IPSCs evoked by 40-ms interstimulus interval paired-pulse stimuli applied before application of CS (pre-CS PPR) were distributed from 0.5 to ~3 (Fig. 3A). CS...
Fig. 2. A and B: induction of PTE depended on the number of pulses in CS. A: changes of test IPSCs to CS with 2, 5, 10, 20, 50, and 100 pulses at 100 Hz. The amplitude of the IPSC was normalized by the baseline IPSC. B: mean PTE and the decay time constant recorded after CS with 2, 5, 10, 20, 50, and 100 pulses. CS with 10 or 20 pulses induced the largest PTE. Differences between 20 pulse- and 50 or 100 pulse-induced PTE were not significant (P > 0.05, repeated-measures ANOVA, n = 5). The decay time constant increased with the number of CS, C: IPSC amplitude recorded from 5 neurons before and after CS with 5%, 10%, 15%, 25%, 50%, 75%, and 100% of intensity to induce a saturated amplitude of IPSC (see text for details). IPSC amplitude was normalized to baseline IPSC. D: mean PTE rate and decay time constants recorded after CS with different intensities. The PTE rate was largest with 25% intensity CS and significantly decreased with 50%, 75%, and 100% CS (repeated-measures ANOVA and post hoc Newman-Keuls test, n = 5). On the other hand, the decay time constant increased with the increase of CS intensity. E and F: to demonstrate that 100% CS did not damage the stimulus site, 3 CS, 1st with 25% intensity, 2nd with 100%, and 3rd again with 25%, were given at 5-min intervals. The PTE ratio for the 2nd CS was significantly smaller than the 1st (P < 0.01, paired t-test, n = 5), and the PTE ratio for the 3rd was similar to the 1st. G and H: to examine the effects of CI⁻ accumulation in postsynaptic neurons, 3 CS, 1st with 25% intensity, 2nd with 100%, and 3rd again with 100%, were given at 5-min intervals. Holding potential (MP) during the 3rd CS was lowered from −50 to −70 mV to reduce CI⁻ entry and accumulation. The 3rd CS induced significantly larger PTE than the 2nd CS.

resulted in >100% PTE in 60 of 62 neurons, and neurons having a higher pre-CS PPR had a stronger PTE (linear regression R² = 0.46; Fig. 3A). The distribution was not related to response latencies (data not shown). In most of the neurons, the PPR obtained from the first post-CS test stimulus (post-CS PPR) decreased compared with pre-CS PPR, although some of the neurons that had <1 pre-CS PPR showed slightly increased post-CS PPR (Fig. 3B). Most of the post-CS PPRs were centered around 1, and they were only weakly correlated with pre-CS PPRs (linear regression R² = 0.11; Fig. 3B). Figure 3C shows examples of recordings from 2 GPe neurons marked in Fig. 3, A and B, one with a high (2.8) and another with a low (0.7) pre-CS PPR. The mean of the pre-CS PPRs was 1.50 ± 0.08, and the mean of the post-CS PPRs decreased to 0.87 ± 0.02 (P < 0.001, paired t-test, n = 62). These results indicate that an increase in the presynaptic releasing efficiency underlies the development of PTE.

Effect of low extracellular Ca²⁺ and Ca²⁺ channel blockers on induction of PTE. Reducing extracellular Ca²⁺ from 2.4 to 1.2 mM, with the latter mimicking Ca²⁺ in CSF in vivo, significantly decreased Str stimulation-induced IPSC amplitudes to test stimuli from 33.8 ± 7.7 pA to 11.4 ± 2.4 pA (P < 0.01, paired t-test, n = 10; Fig. 4, Aa, Ba, and Ca). However, the PTE ratios were similar (534.3 ± 91.0% in 2.4 mM Ca²⁺
and 433.4 ± 122.6% in 1.2 mM Ca2+; P > 0.05, paired t-test, n = 10; Fig. 4Ca). The pre- and post-CS PPRs were also similar (pre-CS 1.58 ± 0.18 in 2.4 mM Ca2+ and 1.4 ± 0.19 in 1.2 mM Ca2+; post-CS 0.95 ± 0.09 in 2.4 mM Ca2+ and 1.2 ± 0.16 in 1.2 mM Ca2+).

In many presynaptic boutons in the CNS the P/Q type is the dominant Ca2+ channel, while in some CNS boutons the N type is dominant (Catterall and Few 2008; Poncer et al. 1997). Bath application of the P/Q-type Ca2+ channel blocker ω-agatoxin TK (200 nM) significantly decreased the amplitude of single stimulation-induced IPSCs (from 42.6 ± 8.2 to 12.5 ± 2.6 pA, n = 7, P < 0.01, paired t-test; Fig. 4, Ab, Bb, and Cb). The N-type blocker ω-conotoxin GVI A (2 μM) also significantly decreased the amplitude of baseline IPSCs but to a lesser degree than ω-agatoxin (from 32.0 ± 5.8 to 19.0 ± 4.0 pA, n = 9, P < 0.05, paired t-test; Fig. 4Cc). The L-type Ca2+ channel blocker nimodipine (20 μM) did not affect Str-GPe IPSCs (Fig. 4Cd). The results demonstrated that the P/Q type was dominant in Str-GPe boutons. However, ω-agatoxin and ω-conotoxin did not change the mean PTE rates (in ω-agatoxin: from 538.2 ± 104.6% in control to 404.8 ± 86.1%, n = 7, P > 0.05; in ω-conotoxin; from 622.5 ± 128.1% in control to 559.7 ± 118.9%, n = 9, P > 0.05, paired t-test). The PPRs before and after CS were also unchanged by ω-agatoxin TK and ω-conotoxin, similar to the low-Ca2+ treatment described above. These results indicate that P/Q- and N-type Ca2+ channels mediate Ca2+ influx to Str-GPe synaptic boutons and that the degree of channel activation does not affect PPRs and ratios of PTE induction.

Ca2+ released from intracellular stores may contribute to CS-induced vesicle mobilization and induction of PTE (Shakiryanova et al. 2007; Tang and Zucker 1997). The specific inhibitor of the endoplasmic reticulum Ca2+-ATPase CPA depletes internal Ca2+ stores (Seidler et al. 1989). Bath application of CPA (10 μM) did not change baseline IPSCs (38.0 ± 8.4 to 38.9 ± 6.9 pA, n = 8; Fig. 4, Ac, Bc, and Ce). However, the mean PTE ratio decreased from 519.5 ± 53.6% in control to 369.0 ± 24.2% in CPA (P < 0.05, n = 8, paired t-test; Fig. 4Ce). The pre-CS PPRs decreased from 1.95 ± 0.18 to 1.69 ± 0.12 (P < 0.05, n = 8, paired t-test). The post-CS PPRs were similar. These results suggested that intracellular Ca2+ stores contribute to the PPR and the induction of PTE.

DISCUSSION

PTE is a common feature of Str-GPe synapses. The present results show that PTE is a common feature of Str-GPe synapses. We found that a wide range of CS, from frequencies as low as 2 Hz with as few as 5 pulses to as high as 100 Hz with 100 pulses, could induce PTE. The range of CS used in this study mimics that of behavioral event-related activity of Str projection neurons observed in unit recordings from task-performing monkeys and rodents (Chen et al. 2001; Kimura et al. 1990; Kubota et al. 2009).

Str-CS induced PTE in IPSCs to Str stimuli but not in IPSCs to IC stimuli. Our interpretation of this result is that PTE develops only in synapses activated by Str-CS. Str-substantia nigra synapses induced PTE (Connelly et al. 2010), and IC-CS could induce PTE to IPSCs to IC test stimuli (Kita H, unpublished observation), suggesting that GPe collaterals of Str-nigral axons also induce PTE. Thus the present result was probably due to the condition that most of the descending axons stimulated at the Str did not reach the IC stimulus site because of the way the slices were prepared. This may also explain why only 15 of 46 Str responding neurons evoked IPSCs to IC stimulation.

Relationship between PPRs and degrees of PTE. PPRs recorded from GPe neurons before application of CS were distributed from 0.5 to near 3, with nearly 24% of neurons below 1. This observation suggests that paired-pulse depression and enhancement mechanisms coexist in Str-GPe synapses. The main enhancement mechanism may be an accumulation of influxed Ca2+ in the boutons. The small reduction of PPRs by CPA, which reversibly depletes intracellular Ca2+ stores (Seidler et al. 1989), suggested that Ca2+-induced Ca2+ release from internal storage sites also contributed to the enhancement. Possible depression mechanisms are activation of GABA and other receptors located on both pre- and perisynaptic sites of Str-GPe synapses (Hashimoto and Kita 2008; Kaneda and Kita 2005; Matsumi and Kita 2003; Ogura and Kita 2000). Assuming that Str stimulation activated a large number of Str synapses, possible mechanisms for some neurons that show depression and other enhancement are that two types of Str synapses, one with depression and the other with enhancement, somewhat selectively terminate on some GPe neurons or...
that some postsynaptic neurons alter presynaptic release for a long period through retrograde signaling mechanisms.

This study revealed that Str-CS induced a wide range of degree, some \( >1,000\% \), of PTE in GPe neurons and that the degree of PTE was positively correlated with the wide range of pre-CS PPRs. Thus pre-CS PPR predicted the degree of PTE, as reported in hippocampal synapses (Emptage et al. 2001). CS shifted high PPRs very close to 1. Based on the assumption that PPR is inversely related to the initial release probability (Dobrunz and Stevens 1997), this observation suggests that Str-GPe synapses have a wide range of release probability and that CS increased those having low probability to near saturation level.

Tecuapetla et al. (2009) showed that intra-Str collaterals of Str-GPe neurons synapsing onto other medium spiny neurons had an average PPR <1 and induced PTE. Their observation together with the present observations suggest that the Str collateral synapses have PPR suppression mechanisms and that synapses belonging to the same neuron can show different PPRs at different synaptic sites.

\( Ca^{2+} \) in synaptic boutons. The vesicle release probability is dependent on the \( Ca^{2+} \) level in the synaptic boutons; therefore, an increase in the internal \( Ca^{2+} \) concentration is the most likely factor of PTE (Catterall and Few 2008; Humeau et al. 2007; Jensen et al. 1999; Korogod et al. 2005; Xu et al. 2007; Zucker and Regehr 2002). The present study revealed that \( Ca^{2+} \) influx into Str-GPe boutons is mediated principally by P/Q-type and then by N-type but not L-type channels, as are many other CNS synapses (see Catterall and Few 2008 for review). P/Q- or N-type \( Ca^{2+} \) channel blockers significantly decreased the amplitude of IPSCs, but strong PTE could still develop, indicating that \( Ca^{2+} \) entering boutons through either of these channels can contribute to the development of PTE and that even a low influx of \( Ca^{2+} \) under certain conditions, such as a lower extracellular \( Ca^{2+} \) environment or a partial \( Ca^{2+} \) channel blockade by modulators such as D2 agonists, can cause \( Ca^{2+} \) accumulation in the boutons and induce PTE. The effects of CPA on pre-CS PPRs and development of PTE suggest that intracellular \( Ca^{2+} \) storage and release mechanisms are also involved in the elevation of presynaptic \( Ca^{2+} \) levels.
Details of the Ca\(^{2+}\) clearance mechanism of Str-GPe synaptic boutons are unknown. The present results suggest that the clearance rate is slow enough to develop an enhanced PPR and PTE even under low Ca\(^{2+}\) influx conditions and that the decay constant slows down even further to \(\sim 120\) s when a large elevation in Ca\(^{2+}\) levels occurs after strong CS.

**Postsynaptic Cl\(^{-}\).** We found that elevation of postsynaptic Cl\(^{-}\) level became a significant factor in controlling the amplitude of IPSCs in Str-GPe synapses activated by strong CS with a high frequency and a large number of pulses and can dampen the degree of PTE. Other possible reasons for the decrease of PTE amplitude with strong CS may be activation of GABA and other receptors located on both pre- and perisynaptic sites of Str-GPe synapses (Hashimoto and Kita 2008; Kaneda and Kita 2005; Matsui and Kita 2003; Ogura and Kita 2000). However, the decay time increased along with the strength of CS, suggesting the presence of some presynaptic mechanisms, including Ca\(^{2+}\) accumulation, for the continued increase in PTE.

**Possible long-term plasticity.** Small numbers of neurons showed LTP- and LTD-like changes of IPSCs after CS of Str, suggesting the existence of some mechanisms for long-term plasticity. However, we could not find any specific physiological or morphological features of GPe neurons that were linked to these long-term changes. The most commonly used CS for the induction of LTD, two or three repetitive applications of 1 s of 100-Hz stimuli, were tested on five neurons and failed to reliably induce LTD (unpublished data).

**Summary and functional implications.** The main findings of the present study are as follows: 1) PTE of IPSCs is a general feature of Str-GPe synapses, since \(>90\)% of GPe neurons tested with Str-CS developed the PTE. 2) The degree of PTE observed in GPe neurons had a wide range and was positively correlated with a wide range of PPRs. 3) A wide range of CS, from frequencies as low as 2 Hz with as few as 5 pulses to as high as 100 Hz with 100 pulses, could induce PTE. 4) The decay time constant of PTE was dependent on the strength of CS and was prolonged greatly when strong CS were applied. 5) The level of postsynaptic Cl\(^{-}\) became a limiting factor for the degree of PTE when strong CS were applied.

These results imply that Str-GPe synapses transmit inhibitions in a nonlinear, activity-weighted manner, which may be well suited for scaling the timing and controlling the force of repeated or sequential body movements but not for incidental decision making or for rapid stimulation-triggered initiation of movement. The present results suggest that the Str-GPe components of behaviorally related activity observed in previous studies were induced by a highly enhanced state of synapses because most task-related GPe unit activity studies used repeated trials with trial intervals shorter than 30 s, which are well within the PTE decay time (e.g., DeLong 1971; Hamada et al. 1990; Jaeger et al. 1993). The present observations could also explain, at least in part, why the behavioral event-related changes of GPe activity reported in these studies often had a wide range of onset times and strengths, since the recent activity history of the Str-GPe synapse modulates incoming synaptic transmission.

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PRESENT ADDRESS OF J. KIM: Div. of Bio-Information Engineering, Faculty of Engineering, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: J.K. performed experiments; J.K. and H.K. analyzed data; J.K. and H.K. interpreted results of experiments; J.K. and H.K. prepared figures; J.K. drafted manuscript; J.K. and H.K. approved final version of manuscript; H.K. conception and design of research; H.K. edited and revised manuscript.

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