Exp Brain Res (2002) 144:238–246
DOI 10.1007/s00221-002-1039-3

RESEARCH ARTICLE

Shigeki Moriguchi · Shigenori Watanabe
Hitoshi Kita · Hiroshi Nakanishi

Enhancement of *N*-methyl-D-aspartate receptor-mediated excitatory postsynaptic potentials in the neostriatum after methamphetamine sensitization

An in vitro slice study

Received: 10 July 2001 / Accepted: 9 January 2002 / Published online: 15 March 2002
© Springer-Verlag 2002

Abstract It has been suggested that behavioral methamphetamine sensitization involves changes in cortical excitatory synaptic inputs to neostriatal (Str) projection neurons. To test this, we performed blind whole-cell recording of medium spiny neurons in Str slice preparations. In Str neurons of naive rats, the amplitude of the subcortical white matter stimulation-induced *N*-methyl-D-aspartate receptor-mediated excitatory postsynaptic potentials (NMDA-EPSPs) was decreased upon hyperpolarization, owing to the voltage-dependent Mg^{2+} blockade of NMDA receptor channels. In contrast, the amplitude of the NMDA-EPSPs in Str neurons of rats undergoing methamphetamine withdrawal (MW) did not show the Mg^{2+} blockade and was nearly voltage independent over the membrane potential range of –70 to –110 mV. Application of the specific protein kinase C (PKC) activator, phorbol 12, 13-DL-acetate, blocked the voltage-dependent Mg^{2+} blockade of NMDA receptor channels in Str neurons of naive rats. Application of the specific activator of cAMP-dependent protein kinase A (PKA), Sp-cAMPS-triethylamine salt, increased the amplitude of the NMDA receptor-mediated EPSPs at the rest but not at hyperpolarized potentials. Coapplication of the PKC and PKA activators yielded NMDA-EPSPs similar to those seen in Str neurons of MW rats. In Str slices of naive rats, tetanic subcortical white matter stimulation induced long-term depression of field potentials. In Str slices treated with the PKC and/or PKA, the same stimulation induced long-term potentiation of field potentials similar to those observed in slices obtained from MW rats. These results suggest that the enhancement of the NMDA receptor-mediated corticostriatal synaptic transmission plays an important role in behavioral methamphetamine sensitization. This enhancement is probably associated with phosphorylation of NMDA receptors mediated by the simultaneous activation of PKC and PKA.

Keywords Methamphetamine · Behavioral sensitization · NMDA receptor-mediated excitatory postsynaptic potential · Corticostriatal transmission · Phosphorylation · Slice patch · Rat

Abbreviations *AMPA* α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate · *BMI* Bicuculline methiodide · *CPP* 3-(2-Carboxypiperzin-4-yl)-propyl-1-phosphonic acid · *DA* Dopamine · *DPSPs* Depolarizing postsynaptic potentials · *EPSPs* Excitatory post synaptic potentials · *KA* Kainate · *LTD* Long-term depression · *LTP* Long-term potentiation · *MW* Methamphetamine withdrawal · *NBQX* 3-Dihydroxy-6-nitro-7-sulfamonybenzo[f]quinoxaline · *NMDA N*-Methyl-D-aspartate · *PDA* Phorbol 12, 13-DL-acetate · *PKA* Cyclic adenosine monophosphate-dependent protein kinase · *PKC* Protein kinase C · *Rp-cAMPS* Rp-cAMPS-triethylamine salt · *Sp-cAMPS* Sp-cAMPS-triethylamine salt · *Str* Neostriatum

S. Moriguchi · S. Watanabe
Graduate School of Pharmacology,
Faculty of Pharmaceutical Sciences, Kyushu University,
Fukuoka 812–8582, Japan

H. Kita
Department of Anatomy and Neurobiology, College of Medicine,
University of Tennessee Memphis, Memphis, TN 38163, USA

H. Nakanishi (✉)
Laboratory of Oral Aging Science,
Division of Oral Biological Sciences, Faculty of Dental Sciences,
Kyushu University, Fukuoka 812–8582, Japan
e-mail: nakandeg@mbox.nc.kyushu-u.ac.jp
Tel.: +81-92-6426413, Fax: +81-92-6426632

Introduction

The neostriatum (Str) is the major structure of the basal ganglia and is involved in the planning and control of motor activity (Alexander and Crucher 1990). The Str receives glutamatergic inputs from the cerebral cortex

and the thalamus, and dopaminergic inputs from the substantia nigra. These glutamatergic and dopaminergic inputs play crucial roles in the regulation of the motor function of Str.

Str neurons possess four major subtypes of glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate (KA), *N*-methyl-D-aspartate (NMDA) receptors, and metabotropic glutamate receptors (Mori et al. 1994; Calabresi et al. 1996; Kita 1996; Götz et al. 1997). When the membrane potential of the Str neuron is near its resting membrane potential, corticostriatal glutamatergic synaptic transmission is mainly mediated by AMPA/KA receptors. When the cortical inputs are strong enough to depolarize Str neurons near to the spike-threshold level, NMDA receptors are activated and become a significant component in the synaptic transmission (Kita 1996). NMDA receptors in the Str may have a variety of roles. The activation of the NMDA receptors is considered to be essential for synaptic plasticity, which is thought to underlie memory acquisition and learning (Calabresi et al. 1996, 1997). In addition, activation of NMDA receptors has been demonstrated to produce nitric oxide, which acts as a neuromodulator and neurotoxic molecule (Ayata et al. 1997; Weiss et al. 1998). Overactivation of NMDA receptors is thought to culminate in excitotoxic neuronal death, which is suggested to be a main causal factor of Huntington's disease (Calabresi et al. 1997).

Methamphetamine is a potent psychostimulant, known to cause a large release of dopamine (DA) from dopaminergic fiber and to deplete DA in the Str. It also causes a decrease in the number of DA transporters (Nakayama et al. 1993). Repeated administration of methamphetamine produces a sensitization and subsequent augmentation of the motor stimulant effects of methamphetamine, referred to as behavioral methamphetamine sensitization, even after long-term withdrawal (Hirabayashi and Alam 1981; Kuczenski and Segal 1989). The precise mechanism underlying behavioral methamphetamine sensitization remains unclear, although evidence suggests that NMDA receptor-mediated corticostriatal synaptic transmission is markedly enhanced after behavioral methamphetamine sensitization. Stimulation-induced DA release in the Str of methamphetamine-sensitized rats is enhanced (Cattaneda et al. 1988; Patrick et al. 1991) and DA potentiates NMDA responses (Blank et al. 1997; Cepeda et al. 1998). Administration of the noncompetitive NMDA receptor antagonist MK-801 suppresses the behavioral amphetamine sensitization (Karler et al. 1989, 1991; Ohmori et al. 1994, 1996). MK-801 also suppresses the increased expression of the Fos protein in the Str that is considered to be associated with behavioral methamphetamine sensitization (Ohno et al. 1994). More recently, we have found a dramatic change in the long-term plasticity of the corticostriatal synaptic transmission in methamphetamine-sensitized rats (Nishioku et al. 1999).

In the present study, we conducted an electrophysiological study to directly examine possible changes in the properties of NMDA receptor channels in Str neurons following methamphetamine sensitization. We provide, for the first time, evidence for enhancement of NMDA receptor-mediated corticostriatal synaptic transmission in Str medium spiny neurons of methamphetamine-sensitized rats. Our observations further suggest that this enhancement may be a potential mechanism underlying behavioral methamphetamine sensitization.

Materials and methods

Animals and treatments

This study was approved by the Animal Research Committee of the Kyushu University and also followed by the guidelines laid down by the NIH (*Principles of laboratory animal care*). Male Wistar rats (250–300 g; *n*=46) were purchased from Kyudo (Kumamoto, Japan). They were kept in a room at 24±2°C with a 12-h light-dark cycle and were given free access to commercial food and tap water. Rats received three types of treatment before behavioral or physiological examinations. An acute treatment of a single injection of saline or methamphetamine (1.0 mg/kg i.p., Dainippon Pharmaceuticals, Japan) was given per day prior to the examinations. Chronic treatment was an injection of saline or methamphetamine (1.0 mg/kg i.p.) once daily for 6 consecutive days. These rats were examined 1 day or 7 days after the chronic treatment. The degree of behavioral methamphetamine sensitization was evaluated by effectiveness of methamphetamine challenge on locomotor activity. Home cages of each rat were moved into the counter box, which was equipped with area sensors (Omuron, F5B, Japan). Locomotor activity was recorded for 90 min after an injection of methamphetamine (0.5 mg/kg i.p.) or saline as described previously (Inoue et al. 1996). For electrophysiological studies, we used rats that received a chronic methamphetamine treatment followed by 7 days' withdrawal without the methamphetamine challenge. These animals were tentatively referred to as methamphetamine-withdrawal (MW) rats in the present study.

Electrophysiology

Detailed methods for preparation of slices have been described elsewhere (Nakanishi et al. 1997; Yamamoto et al. 1999). Saline-treated (naive) and MW rats were decapitated under light ether anesthesia. The brains were rapidly removed and block containing the region of the Str and cortex were obtained. Coronal or horizontal slices (400 μm thick) were cut on a vibratome (Vibroslice 752M; Campden Instruments). A single slice was placed in an interface-type recording chamber and preincubated for at least 2 h in Krebs Ringer solution oxygenated with 95% O_2 and 5% CO_2 at 36±0.1°C.

Whole-cell recordings from Str neurons of adult rats (250–300 g) were made by using the blind slice patch technique (Blanton et al. 1989; Lovinger et al. 1993). Briefly, patch electrodes of 5–8 MΩ were fabricated on a micropitette puller (model P-97; Sutter Instruments, Novato, Calif.) from borosilicate glass (outer diameter 1.5 mm and inner diameter 0.9 mm; Narishige, Tokyo, Japan). The internal solution was composed of: potassium glucuronate 120.0 mM, HEPES 10.0 mM, EGTA 1.0 mM, KCl 20.0 mM, $MgCl_2 \cdot 6H_2O$ 2.0 mM, sodium adenosine triphosphate 2.0 mM, sodium guanosine triphosphate 0.25 mM, and Neurobiotin 3 mM. Positive pressure was applied by using a 10-ml syringe to clear a patch-electrode tip. A patch electrode was then advanced slowly through the slice by using a motor drive (PC-5N; Narishige, Tokyo, Japan) while monitoring the amplitude of voltage responses induced by current-pulse injections. When a cell was encountered, an increase in a patch-electrode tip resistance was evident by a decrease in the amplitude of voltage responses. A light suc-

tion was then applied to the patch electrode until a gigaohm seal was formed between the patch-electrode tip and the cell surface. Whole-cell recordings in current clamp mode were initiated by rupturing the cell membrane with an additional suction. An EPC-9 patch-clamp amplifier (HEKA, Lambrecht, Germany) was interfaced to a Power Macintosh computer for pulse application and data recordings. To evoke cortico-Str postsynaptic responses, a single electrical stimulation or trains of electrical stimulation (intensity 1–20 V, duration 200 μs, frequency 0.6 Hz) were applied to the subcortical white matter through a bipolar electrode.

To examine long-term plasticity, extracellular field potentials to single white matter stimulation were recorded before and after the application of four trains of tetanic stimulation (100 Hz for 3 s, interval of 20 s). The field potentials were recorded with a high-input impedance amplifier (Neurodata IR 183) through 2 M NaCl-filled glass microelectrodes (3–5 MΩ) placed on the surface of the slice preparation. The field responses were stored in a MacLab recording system.

The Krebs solution for superfusion of the slices was composed of: NaCl 126.0 mM, KCl 2.5 mM, NaH_2PO_4 1.2 mM, $MgCl_2$ 1.2 mM, $CaCl_2$ 2.4 mM, glucose 11.0 mM, and $NaHCO_3$ 25.0 mM. The following drugs were used for bath application: 3-(2-carboxypiperzin-4-yl)-propyl-1-phosphonic acid (CPP; Sigma); bicuculline methiodide (BMI; Sigma); 3-dihydroxy-6-nitro-7-sulfamony-benzo[*f*]quinoxaline (NBQX; Research Biochemicals International, Natick, Mass.); phorbor-12, 13-DL-acetate (PDA, Sigma); chelerythrine chloride (Research Biochemicals International); Sp-cAMPS-triethylamine salt (Sp-cAMPS; Research Biochemicals International); Rp-cAMPS-triethylamine salt (Rp-cAMPS; Research Biochemicals International); and Neurobiotin (Vector Laboratories, Burlingame, Calif.). All drugs were pH-balanced (pH 7.3–7.4). Numerical data are represented as mean ± SEM.

Histological identification of the recorded cells

Slices containing Neurobiotin-containing cells were fixed with 4% paraformaldehyde overnight at 4°C, washed with phosphate-buffered saline (PBS), and then immersed in 20% sucrose for 24 h at 4°C. Floating sections (30 μm) were prepared by a cryostat and stained with streptavidin-Alexa 488 (Molecular Probes, Eugene, Ore.) for 2 h at room temperature. After washes with PBS, sections were mounted on slide glasses with antifading medium (Vectashield; Vector Laboratories).

Results

Behavioral sensitization after methamphetamine treatment

The degree of methamphetamine-induced behavioral sensitization was assessed by measuring the effect of a methamphetamine challenge (0.5 mg/kg i.p.) on locomotor activity (activity counts/90 min). A significant increase in locomotor activity after methamphetamine challenge (i.e., behavioral methamphetamine sensitization) was observed in rats who received a chronic methamphetamine treatment followed by 7 days' withdrawal (Table 1). On the other hand, rats receiving an acute treatment or chronic treatment followed by 1-day withdrawal did not increase locomotor activity after the methamphetamine challenge.

Table 1 Effect of methamphetamine challenge on locomotor activity (activity counts/90 min) after treatment with methamphetamine (1 mg/kg i.p.) in various schedules (*Acute* a single treatment of methamphetamine, *1 d* chronic treatment of methamphetamine for 6 days followed by withdrawal for 1 day, *7 d* chronic treatment of methamphetamine for 6 days followed by withdrawal for 7 days). At the end of these schedules, locomotor activity of each animal was measured during 90 min after injection of saline or methamphetamine (0.5 mg/kg i.p.). Values represent mean ± SEM (*MW* methamphetamine withdrawal)

	Saline		MW	
	Mean±SEM	*n*	Mean±SEM	*n*
Acute	480.0±129.9	5	517.0±107.5	5
1 d	490.2±124.6	5	752.4±181.1	5
7 d	458.8±59.5	5	1745.8±227.3**	5

**$P<0.01$ compared with saline (Student's *t*-test)

Methamphetamine withdrawal did not change the resting the membrane potentials, the input resistance, or the spike threshold of Str neurons

The results are based on current clamp recordings from 32 and 34 Str neurons from naive and MW rats, respectively. All the neurons recorded were morphologically identified by intracellular labeling with Neurobiotin as medium spiny neurons, based on their medium-sized somata and extensive dendrites laden densely with spines (data not shown). The neurons lacked spontaneous discharges and exhibited an inward rectification upon injection of negative current pulses. These membrane characteristics were similar to those of the medium spiny neurons described in previous studies.

The Str neurons with stable resting potentials lasting for at least 60 min were included in the analysis. The input resistance of the neuron was determined from the slope of the current-voltage curves crossing at zero current point. As summarized in Table 2, there was no significant difference in the resting membrane potential and the input resistance of Str spiny neurons from naive and MW rats. The latency of excitatory postsynaptic potentials (EPSPs) evoked by subcortical white matter stimulation was constant in spite of differences in intensity, indicating that the nature of the response was monosynaptic (data not shown). The spike threshold of the neurons, assessed by evoking the EPSPs with threshold amplitude, was also unaffected after methamphetamine withdrawal.

Synaptic responses of Str neurons to repetitive timulation of the subcortical white matter

Previous studies suggest that NMDA receptor-mediated corticostriatal synaptic transmission is markedly enhanced after methamphetamine sensitization (Nishioku et al. 1999). To test this possibility, we compared responses to repetitive stimulation (10 stimuli with 5-ms interspike intervals) of the subcortical white matter. The

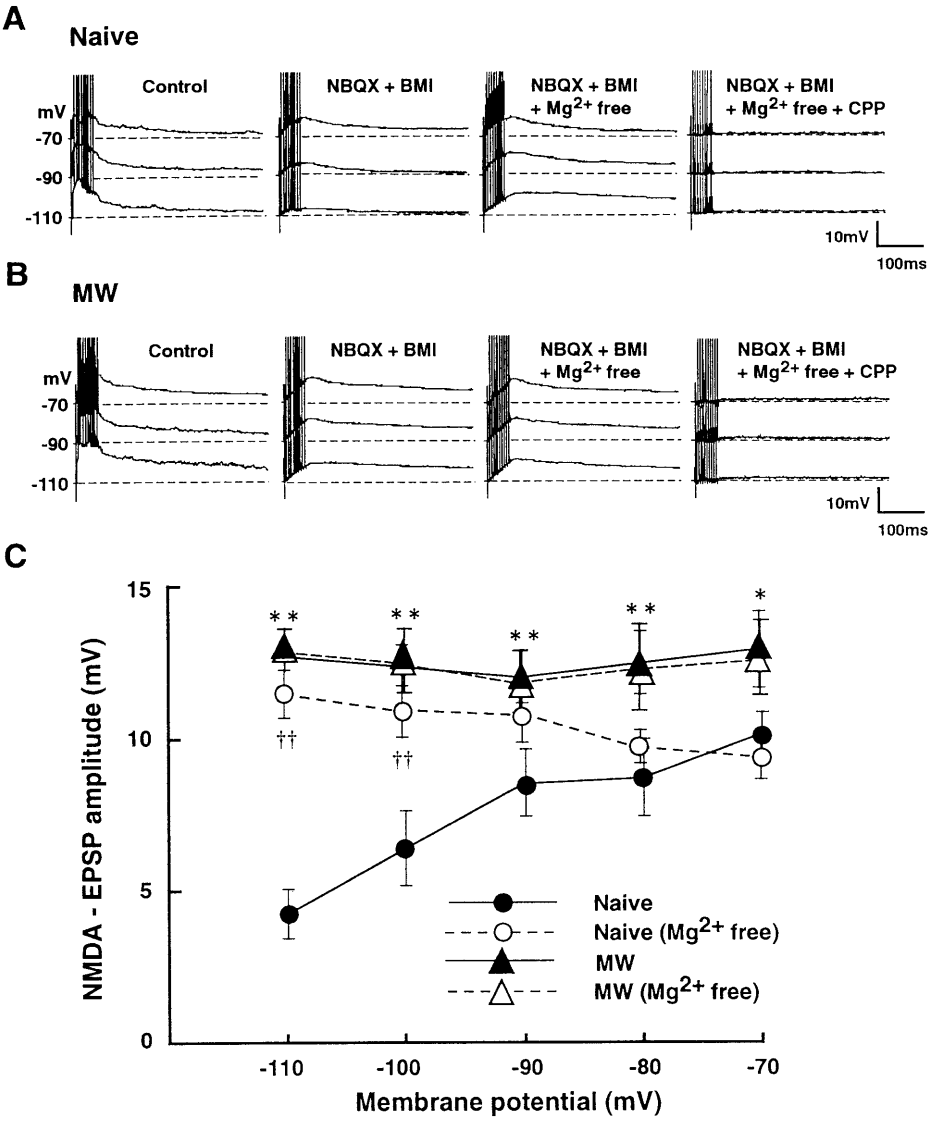

Fig. 1A–C Postsynaptic responses evoked in Str neurons from naive and MW rats by repetitive stimulation of the subcortical white matter. **A, B** Postsynaptic responses in Str neurons from naive (**A**) and MW (**B**) rats. NMDA-EPSPs were isolated by application of NBQX (10 µM) and BMI (100 µM). NMDA-EPSPs were augmented in the Mg^{2+}-free condition. Addition of CPP (5 µM) totally suppressed responses. The intensity of the stimulus was adjusted to evoke subspike EPSPs. Stimulus artifacts in this and Fig. 2 are truncated. **C** The voltage dependence of NMDA-EPSPs in Str neurons of naive and MW rats. The peak amplitude of NMDA-EPSPs in Str neurons of naive and MW rats was plotted as a function of the membrane potential. *Asterisks* indicate significant differences between naive and MW rats (**P*<0.05, ***P*<0.01; Student's *t*-test). *Swords* indicate significant differences between naive and Mg^{2+}-free ($^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01; Student's *t*-test)

Table 2 Passive membrane properties and excitatory postsynaptic potentials (*EPSPs*) of neostriatal neurons in naive and methamphetamine-withdrawal (*MW*) rats. Values represent mean ± SEM

	Naive		MW	
	Mean±SEM	*n*	Mean±SEM	*n*
Resting membrane potential (mV)	–74.9±2.0	32	–73.1±1.6	34
Input resistance (MΩ)	36.8±1.8	7	35.7±1.1	8
Subspike EPSP amplitude (mV)	17.0±1.3	9	16.3±1.0	10
Half-duration EPSPs evoked by repetitive stimulation (ms)	275.4±19.9	5	443.9±16.5**	5

***P*<0.01 compared with naive (Student's *t*-test)

repetitive stimulation induced large EPSPs with a substantial NMDA component in Str neurons. Figure 1 shows examples of EPSPs evoked in Str neurons of the naive and MW rats after repetitive stimulation with subspike intensity. The records clearly show that the decay of the EPSPs in Str neurons of MW rats was much slower than in the control. The half-duration of EPSPs recorded in Str neurons of MW rats was significantly longer than that of naive rats (Table 2). It was also noted that the difference was more prominent at hyperpolarized membrane potentials (e.g., –110 mV).

CPP-sensitive NMDA receptor-mediated excitatory postsynaptic potentials (NMDA-EPSPs) induced by stimulation of the subcortical white matter were isolated by a bath application of the AMPA/KA receptor antagonist NBQX and the $GABA_A$ receptor antagonist BMI (Fig. 1).

When the isolated NMDA-EPSPs were recorded over the range that was practical for examination without large changes of K^+ conductance, there was a striking difference in their voltage dependence between naive and MW rats. In Str neurons of naive rats, the amplitude of NMDA-EPSPs greatly decreased when the neurons were hyperpolarized (Fig. 1A). In Mg^{2+}-free medium, the amplitude of NMDA-EPSPs was increased when the neurons were hyperpolarized, indicating that the decrease in the amplitude of NMDA-EPSPs at hyperpolarized membrane potentials was due to the Mg^{2+} blockade of NMDA channels (Fig. 1A, C). In contrast, the amplitude of NMDA-EPSPs in Str neurons of MW rats was not decreased by hyperpolarizing the neurons (Fig. 1B, C). Furthermore, the perfusion of Mg^{2+}-free medium did not affect either the voltage dependence or the amplitude of NMDA-EPSPs in Str neurons of MW rats (Fig. 1C). Figure 1 also indicates that amplitudes of NMDA-EPSPs recorded from Str neurons of MW rats were significantly larger than those recorded from Str neurons of naive rats (Fig. 1C). These observations suggest that multiple factors are involved in the enhancement of the NMDA-EPSPs after methamphetamine withdrawal, and one important factor is a reduction of the Mg^{2+} blockade of NMDA channels. We also examined the membrane potential dependence of AMPA/KA receptor-mediated EPEPs which were pharmacologically isolated by application of CPP (5 μM) and BMI (100 μM). We found no difference in AMPA/KA receptor-mediated EPSPs recorded from naive and MW rats (data not shown).

To examine the correlation between the augmentation of NMDA-EPSPs with behavioral methamphetamine sensitization, pharmacologically isolated NMDA-EPSPs were also recorded from the Str of rats that received an acute methamphetamine treatment or chronic methamphetamine treatment followed by 1-day withdrawal. In animals that had received these treatments, the methamphetamine challenge did not induce a significant increase in the locomotor activity (Table 1). These results clearly indicated that either an acute methamphetamine treatment or a chronic methamphetamine treatment failed to develop methamphetamine sensitization. The experimental procedure was the same as that used with for MW rats (see Fig. 1). In the control medium, the intensity of repetitive stimulation was adjusted to evoke subspike DPSPs in the neurons at the resting membrane potential. Then NMDA-EPSPs were isolated using NBQX (10 μM) and BMI (100 μM), and their amplitudes recorded at –110 mV were compared (Table 3). The peak amplitudes of NMDA-EPSPs recorded from the Str neurons of rats that received either an acute methamphetamine treatment or a chronic methamphetamine treatment followed by a 1-day withdrawal were not significantly different from that of naive rats. As mentioned already, Str neurons of MW rats had a significantly increased duration of NMDA-EPSPs (Fig. 1 and Table 3). These results suggest that an enhancement of NMDA-EPSPs is closely associated with behavioral methamphetamine sensitization.

Table 3 Peak amplitude of corticostriatal NMDA-EPSPs in slice from rats with various schedules. (*Acute* A single treatment of methamphetamine, *1 d* chronic treatment of methamphetamine for 6 days followed by withdrawal for 1 day, *7 d* chronic treatment of methamphetamine for 6 days followed by withdrawal for 7 days). NBQX (10 mM) and BMI (100 mM) were used to isolate NMDA-EPSPs induced by repetitive cortical white matter stimulation. The peak amplitude of isolated subspike NMDA-EPSPs was measured at the membrane potential of –110 mV. Values represent mean ± SEM

	Control (mV)		MW (mV)	
	Mean±SEM	*n*	Mean±SEM	*n*
Acute	3.98±0.62	5	3.75±0.85	5
1 d	4.18±0.52	5	3.95±0.90	5
7 d	4.20±0.80	5	12.65±0.59**	5

**P<0.01 compared with control (Student's t-test)

Effects of PKC and PKA activators on the NMDA-EPSPs and the long-term synaptic plasticity

There is increasing evidence that the phosphorylation through activation of protein kinase C (PKC) and c-AMP-dependent protein kinase A (PKA) play a pivotal role in regulating the functional activity of NMDA receptors (Gerber et al. 1989; Chen and Huang 1992; Colwell and Levine 1995; Zhang et al. 1996; Blank et al. 1997). For this reason, we examined whether activation of PKC and/or PKA is involved in the enhancement of NMDA-EPSPs after methamphetamine withdrawal. In Str neurons of naive rats after treatment of the specific PKC activator PDA (10 μM), the amplitude of NMDA-EPSPs was greatly increased when the neurons were hyperpolarized (Fig. 2A, C). This voltage dependence of NMDA-EPSPs was very similar to that recorded in Str neurons of naive rats under the Mg^{2+}-free condition (Fig. 1C). When slices were treated with the specific PDA inhibitor chelerythrine (1 μM) 20 min before and during application of PDA, PDA failed to affect NMDA-EPSPs (Fig. 2A). Once PDA changed the voltage dependence of NMDA-EPSPs, chelerythrine could not reverse it (data not shown). On the other hand, the specific PKA activator Sp-cAMPS (10 μM) enhanced the amplitude of NMDA-EPSPs, especially when neurons were current clamped at near-resting membrane potential. Sp-cAMPS did not alter the voltage dependency of NMDA-EPSPs (Fig. 2B,C). Finally, the combined application of PDA and Sp-cAMPS made the amplitude of NMDA-EPSPs relatively voltage independent, very similar to that observed in Str neurons of MW rats (Fig. 2C). However, the voltage dependence or amplitude of NMDA-EPSPs in Str neurons of MW rats were not affected by chelerythrine (1 μM) or Rp-cAMPS (10 μM; data not shown). This apparent discrepancy may be due to the competitive nature of these inhibitors, which could not reverse the phosphorylation state of the proteins.

According to our assumption, activation of PKC and/or PKA should change the long-term synaptic plas-

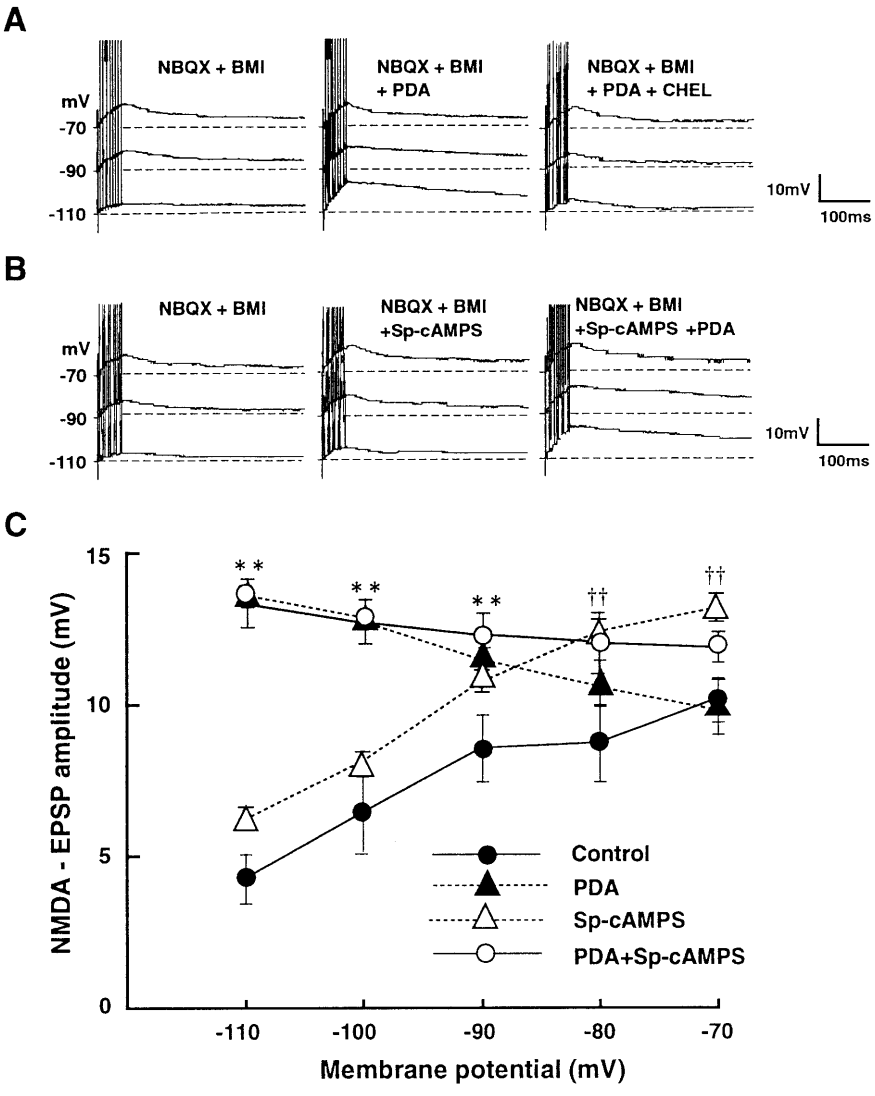

Fig. 2A–C Effects of PKC and PKA activators on the NMDA-EPSPs in Str neurons of naive rats. **A** NMDA-EPSPs were isolated by application of NBQX (10 µM) and BMI (100 µM). PDA (10 µM) greatly enhanced NMDA-EPSPs that were evoked during membrane hyperpolarization. When the specific PKC inhibitor chelerythrine (*CHEL*; 1 µM) was treated 20 min before and during application of PDA, PDA failed to affect NMDA-EPSPs. **B** The PKA activator Sp-cAMPS (10 µM) enhanced NMDA-EPSPs at potentials close to the resting membrane potential without affecting their voltage dependence. **C** A graphic presentation of the voltage dependence of NMDA-EPSPs in Str neurons of naive rats in control, during application of PDA, Sp-cAMPS, and their combination. The peak amplitude of NMDA-EPSPs was plotted as a function of the membrane potential. *Asterisks* indicate significant differences between control and PDA (***P*<0.01; Student's *t*-test). *Swords* indicate significant differences between control and Sp-cAMPS (††*P*<0.01; Student's *t*-test)

ticity of the corticostriatal projections as observed in Str slices of MW rats. To test this, we examined effects of PKC or PKA activator on the long-term synaptic plasticity of field potential amplitude after four high-frequency stimulations (3 s duration, 100 Hz frequency, at 20-s intervals) of the subcortical white matter. In Str slices from naive rats, tetanic stimulation induced long-term depression (LTD) of the field potential, as has been reported previously (Calabresi et al. 1992; Yamamoto et al. 1999; Fig. 3). When PDA (10 µM) or Sp-cAMPS (10 µM) was applied to Str slices, there was no significant change in either latency or amplitude of field potentials (Fig. 3). However, as was seen in the Str of MW rats (Nishioku et al. 1999), tetanic stimulation of PDA or Sp-cAMPS-treated slices increased the field potential amplitude to a stable level, forming long-term potentiation (LTP; Fig. 3).

Discussion

The present study examined alterations of NMDA-EPSPs in the Str spiny projection neurons of rats after methamphetamine treatment and withdrawal. The major finding was that NMDA-EPSPs induced in Str neurons by the subcortical white matter stimulation were significantly augmented after methamphetamine withdrawal. Experiments using rats with different schedules of methamphetamine treatment schedules indicated that the enhancement of NMDA-EPSPs was only induced by the schedule that also resulted in behavioral methamphetamine sensitization. These present findings strongly suggest that the enhancement of NMDA-EPSPs is required for the development of methamphetamine sensitization.

There is increasing evidence that PKC and PKA regulate the activity of NMDA receptor channels through phosphorylation of the NMDA receptor subunits. The PKC activator PDA enhances NMDA currents in various brain areas including the Str (Pisani et al. 1997; Calabresi et al. 2000), trigeminal dorsal horn neurons (Gerber et al.

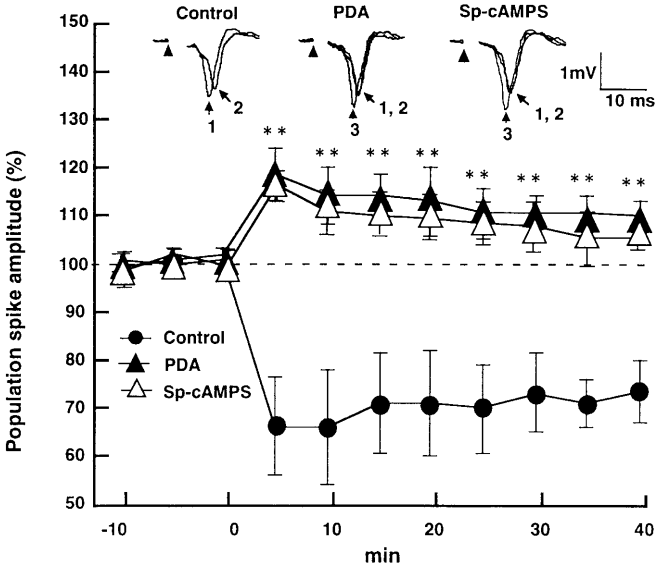

Fig. 3 Effects of PKC and PKA activators on the long-term synaptic plasticity. In Str slices of naive rats, the tetanic stimulation induced LTD of field potentials in the standard solution, whereas LTP was induced after treatment of slices with PDA (10 μM) or Sp-cAMPS (10 μM). The amplitude of negative potentials was measured. *Asterisks* indicate significant differences between before and after application of PDA and Sp-cAMPS (**P<0.01; two-way repeated-measures analysis of variance followed by Dunnett's test). *Inserts* indicate superimposed records of field potentials: *Control*, before (*1*) and 30 min after (*2*) four high-frequency stimulations; *PDA*, *Sp-cAMPS*, superimposed records of field potentials in the standard solution (*1*), during application of PDA or Sp-cAMPS and (*3*) at 30 min after four high-frequency stimulations

1989; Chen and Huang 1992), cortical neurons (Zhang et al. 1996), and *Xenopus* oocytes expressed with recombinant NMDA receptors (Kutsuwada et al. 1992; Meguro et al. 1992). Activation of G protein-coupled receptors, including metabotropic glutamate receptors (Pisani et al. 1997) and muscarinic receptors (Calabresi et al. 1998), also potentiate NMDA currents in Str neurons via activation of PKC. The PKA activator Sp-cAMPS also enhances NMDA-EPSPs (Colwell and Levine 1995) in Str neurons and NMDA currents in *Xenopus* oocytes expressed with recombinant NMDA receptors (Blank et al. 1997), via an indirect action through regulation of phosphatase activity, as well as a direct phosphorylation of the NR1 subunit (Greengard et al. 1999). To address the possible involvement of PKC and PKA activities in the enhancement of NMDA-EPSPs after methamphetamine withdrawal, we examined effects of PDA and Sp-cAMPS on NMDA-EPSPs induced in Str neurons by subcortical white matter stimulation. Our observations here showed that PDA and Sp-cAMPS affected NMDA-EPSPs of Str neurons in a different way. PDA increased the amplitude of NMDA-EPSPs, especially at a membrane potential more hyperpolarized than the resting membrane potential, probably due to the reduction of the Mg^{2+} blockade of NMDA receptors. On the other hand, Sp-cAMPS greatly increased the amplitude of NMDA-EPSPs at near-resting membrane potential. The Sp-cAMPS effect may be due to the involvement of L-type Ca^{2+} channels, because somatodendritic L-type Ca^{2+} channels in Str neurons were enhanced by PKA (Surmeier et al. 1995). Our present observations suggest that both PKC and PKA activities are involved in the enhancement of NMDA-EPSPs after methamphetamine withdrawal, because the enhancement was well mimicked by coapplication of PDA and Sp-cAMPS to the Str neurons of naive rats. There is increasing evidence that phosphorylation states of various substrates for PKC and PKA were increased after application of amphetamine or methamphetamine. Injection of amphetamine increased PKC-mediated phosphorylation of the neural-specific calmodulin-binding protein neuromodulin in rat Str (Gnegy et al. 1993). Similarly, preincubation of Str synaptosomes with a PKC inhibitor blocked the amphetamine-mediated increase in the level of phosphorylated neuromodulin (Iwata et al. 1997). Treatment with methamphetamine or cocaine resulted in increased phosphorylation state of cAMP-regulated phosphoprotein of mol wt. 21 kDa, another substrate for PKA in Str neurons (Caporaso et al. 2000). Furthermore, pretreatment with a PKC inhibitor suppresses amphetamine-stimulated DA release in the nucleus accumbens (Browman et al. 1998). More recent studies have indicated that amphetamine-mediated DA release through the plasmalemmal transporter is regulated by activators of PKC (Kantor and Gnegy 1998).

We have previously observed that only Str slices obtained from MW rats did tetanic stimulation of the corticostriatal pathway induce LTP of the field potentials, while slices obtained from control rats showed LTD of the field potentials and that the LTP was NMDA receptor-dependent (Nishioku et al. 1999). Those observations suggest that the development of the LTP in the Str neurons of MW rats might be due to an augmentation of NMDA responses. This suggestion was further substantiated by the present observations. We observed that only the Str neurons of MW rats but not of naive rats showed enhanced NMDA-EPSPs. We have also observed that application of PDA or Sp-cAMPS to slices obtained from naive rats which augment NMDA responses, induced LTP of the field potentials as was seen in the Str slices obtained from MW rats (Nishioku et al. 1999). In this study, we used field potential recordings to demonstrate LTP of corticostriatal inputs. We conducted a similar experiment using the blind-patch whole-cell recording method. We were unable to observe prominent LTP using the whole-cell recording method. Because the field potential or the sharp intracellular recording methods mainly have been used to demonstrate LTP of corticostriatal inputs in the past, we consider that this failure may be due to the problem of intracellular dialysis.

Conclusions

We demonstrated in the present study that methamphetamine treatment that causes behavioral methamphet-

amine sensitization results in an augmentation of the subcortical white matter stimulation-induced NMDA-EPSPs in the Str, consequently altering the long-term plasticity of the corticostriatal inputs. These changes were mimicked by the simultaneous activation of both PKC and PKA. Although the precise functions of these phosphorylated proteins remain unclear, these observations strongly suggest that changes in PKC and PKA activities are closely associated with multiple aspects of methamphetamine sensitization.

Acknowledgements The authors express thanks to Dr. N. Hori (Department of Environmental Health and Toxicology, School of Public Health, University of Albany) and Dr. T. Shimazoe (Graduate School of Pharmacology, Faculty of Pharmaceutical Sciences, Kyushu University) for their kind help and advice. We also thank to Mr. S. Fujii for the technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (1167184) to H. N. and also by a NINDS grant (NS-36720) to H.K.

References

Alexander E, Crucher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266–271

Ayata C, Ayata C, Hara H, Matthews RT, Beal MF, Ferrante RJ, Endres M, Kim A, Christie RH, Waeber C, Huang PL, Hyman BT, Moskowitz MA (1997) Mechanisms of reduced striatal NMDA excitotoxicity in type I nitric oxide synthase knockout mice. J Neurosci 17:6908–6917

Blank T, Nijholt I, Teichert U, Kügler Behrsing H, Fienberg A, Greengard P, Spiess J (1997) The phosphoprotein DARPP-32 mediates cAMP-dependent potentiation of striatal *N*-methyl-D-aspartate responses. Proc Natl Acad Sci USA 94:14859–14864

Blanton M, Turco JJL, Kriegstein AR (1989) Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. J Neurosci Methods 30:203–210

Browman KE, Kantor L, Richardson S, Badiani A, Robinson TE, Gnegy ME (1998) Injection of the protein kinase C inhibitor Ro31-8220 into the nucleus accumbens attenuates the acute response to amphetamine: tissue and behavioral studies. Brain Res 814:112–119

Calabresi P, Maj R, Pisani A, Mercuri NB, Bernardi G (1992) Long-term synaptic depression in the striatum: physiological and pharmacological characterization. J Neurosci 12:4224–4233

Calabresi P, Pisani A, Mercuri NB, Bernardi G (1996) The corticostriatal projection: from synaptic plasticity to dysfunctions of the basal ganglia. Trends Neurosci 19:19–24

Calabresi P, De Murtas M, Bernardi G (1997) The neostriatum beyond the motor function: experimental and clinical evidence. Neuroscience 78:39–60

Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G (1998) Endogenous ACh enhances striatal NMDA-responses via M1-like muscarinic receptors and PKC activation. Eur J Neurosci 10:2887–2895

Calabresi P, Gubellini P, Centonze D, Picconi B, Bernardi G, Chergui K, Svenningsson P, Fienberg AA, Greengard P (2000) Dopamine and cAMP-regulated phosphoprotein 32 kDa controls both long-term depression and long-term potentiation, opposing forms of synaptic plasticity. J Neurosci 20:8443–8951

Caporaso GL, Bibb JA, Snyder GL, Valle C, Rakhilin S, Fienberg AA, Hemmings HC, Narin AC, Greengard P (2000) Drugs of abuse modulate the phosphorylation of ARPP-21, a cyclic AMP-regulated phosphoprotein enriched in the basal ganglia. Neuropharmacology 39:1637–1644

Cattaneda E, Becker JB, Robinson TE (1988) The long-term effects of repeated amphetamine treatment in vivo on amphetamine, KCl and electrical stimulation evoked striatal dopamine release in vitro. Life Sci 42:2447–2456

Cepeda C, Colwell CS, Itri JN, Chandler SH, Levine MS (1998) Dopaminergic modulation of NMDA induced whole cell currents in neostriatal neurons in slices: contribution of calcium conductances. J Neurophysiol 79:82–94

Chen L, Huang LM (1992) Protein kinase C reduces Mg^{2+} block of NMDA-receptor channel as a mechanism of modulation. Nature 356:521–523

Colwell CS, Levine MS (1995) Excitatory synaptic transmission in neostriatal neurons: regulation by cyclic AMP-dependent mechanisms. J Neurosci 15:1704–1713

Gerber G, Kangrga I, Ryu PD, Larew JS, Randic M (1989) Multiple effects of phorbol esters in the rat spinal dorsal horn. J Neurosci 9:3603–3617

Gnegy ME, Hong P, Ferrell ST (1993) Phosphorylation of neuromodulin in rat striatum after acute and repeated, intermittent amphetamine. Brain Res Mol Brain Res 20:289–298

Götz T, Kraushaar U, Geiger J, Lügke J, Berger T, Jonas P (1997) Functional properties of AMPA and NMDA receptors expressed in identified types of basal ganglia neurons. J Neurosci 17:214–215

Greengard P, Allen PB, Narin AC (1999) Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron 23:435–447

Hirabayashi M, Alam M (1981) Enhancing effect of methamphetamine on ambulatory activity produced by repeated administration in mice. Pharmacol Biochem Behav 15:925–932

Inoue H, Arai I, Shibata S, Watanabe S (1996) NG-nitro-L-arginine methyl ester attenuates the maintenance and expression of methamphetamine-induced behavioral sensitization and enhancement of striatal dopamine release. J Pharmacol Exp Ther 277:1424–1430

Iwata S, Hewlett GH, Gnegy ME (1997) Amphetamine increases the phosphorylation of neuromodulin and synapsin I in rat striatal synaptosomes. Synapse 26:281–291

Kantor L, Gnegy ME (1998) Protein kinase C inhibitors block amphetamine-mediated dopamine release in rat striatal slices. J Pharmacol Exp Ther 284:592–598

Karler R, Calder LD, Chaudhry IA, Turkanis SA (1989) Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. Life Sci 45:599–606

Karler R, Calder LD, Turkanis SA (1991) DNQX blockade of amphetamine behavioral sensitization. Brain Res 552:295–300

Kita H (1996) Glutamatergic and GABAergic postsynaptic responses of striatal spiny neurons to intrastriatal and cortical stimulation recorded in slice preparations. Neuroscience 70:925–940

Kuczenski R, Segal D (1989) Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis. J Neurosci 9:2051–2065

Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, Arakawa M, Mishina M (1992) Molecular diversity of the NMDA receptor channels. Nature 358:36–41

Lovinger DM, Tyler EC, Merritt A (1993) Short- and long-term depression in rat neostriatum. J Neurophysiol 70:1937–1949

Meguro H, Mori H, Araki K, Kushiya E, Kutsuwada T, Yamazaki M, Kumanishi T, Arakawa M, Sakimura K, Mishina M (1992) Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. Nature 357:70–74

Mori A, Takahashi T, Miyashita Y, Kasai H (1994) Two distinct glutamatergic synaptic inputs to striatal medium spiny neurones of neonatal rats and paired-pulse depression. J Physiol (Lond) 476:217–228

Nakanishi H, Tamura A, Kawai K, Yamamoto K (1997) Electrophysiological studies of rat substantia nigra neurons in an in vitro slice preparation after middle cerebral artery occlusion. Neuroscience 77:1021–1028

Nakayama M, Koyama T, Yamashita I (1993) Long-lasting decrease in dopamine uptake sites following repeated administration of methamphetamine in the rat striatum. Brain Res 601:209–212

Nishioku T, Shimazoe T, Yamamoto Y, Nakanishi H, Watanabe S (1999) Expression of long-term potentiation of the striatum in methamphetamine-sensitized rats. Neurosci Lett 268:81–84

Ohmori T, Abekawa T, Muraki A, Koyama T (1994) Competitive and noncompetitive NMDA antagonists block sensitization to methamphetamine. Pharmacol Biochem Behav 48:587–591

Ohmori T, Abekawa T, Koyama T (1996) The role of glutamate in behavioral and neurotoxic effects of methamphetamine. Neurochem Int 29:301–307

Ohno M, Yoshida H, Watanabe S (1994) NMDA-receptor mediated expression of Fos protein in the rat striatum following methamphetamine administration: relation to behavioral sensitization. Brain Res 665:135–140

Patrick SL, Thompson TL, Walker JM, Patrick RL (1991) Concomitant sensitization of amphetamine-induced behavioral stimulation and in vivo dopamine release from rat caudate nucleus. Brain Res 538:343–346

Pisani A, Calabresi P, Centonze D, Bernardi G (1997) Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones. Br J Pharmacol 120:1007–1014

Surmeier DJ, Bargas J, Hemmings HC Jr, Narin AC, Greengard P (1995) Modulation of calcium currents by D_1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. Neuron 14:385–397

Weiss SW, Albers DS, Iadarola MJ, Dawson TM, Dawson VL, Standaert DG (1998) NMDAR1 glutamate receptor subunit isoforms in neostriatal, neocortical, and hippocampal nitric oxide synthase neurons. J Neurosci 18:1725–1734

Yamamoto Y, Nakanishi H, Takai N, Shimazoe T, Watanabe S, Kita H (1999) Expression of *N*-methyl-D-aspartate receptor-dependent long-term potentiation in the neostriatal neurons in an in vitro slice after ethanol withdrawal of the rat. Neuroscience 91:59–68

Zhang L, Baverly A, Rzigalinski BA, Ellis EF, Satin LS (1996) Reduction of voltage-dependent Mg^{2+} blockade of NMDA current in mechanically injured neurons. Science 274:1921–1923