Spermine modulates neuronal excitability and NMDA receptors in juvenile gerbil auditory thalamus

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Abstract

Medial geniculate body (MGB) neurons process synaptic inputs from auditory cortex. The glutamatergic responses vary markedly in time-course and configuration during development. The late component of excitatory postsynaptic potentials (EPSPs) is prolonged during second postnatal week, but becomes significantly shorter until adulthood. The EPSP-prolongation depends on interactions between spermine and an extracellular polyamine-sensitive site on receptors for N-methyl-D-aspartate (NMDA). We examined spermine's effects on membrane properties, EPSPs, and firing modes in MGB neurons during the period of highest polyamine sensitivity (P14). Spermine slowed decay of the late EPSP component. Spermine application increased the frequency of tonic firing evoked with current injection by up to ~150% and induced firing on electrically evoked EPSPs. These effects were concentration-dependent (ED_{50} = 100 μM), reversible, and attributable to NMDA-receptor mechanisms. Spermine lowered action potential threshold. NMDA-receptor antagonist, 2-amino-5-phosphonovalerate (APV), completely blocked the increase in firing rate and reduction in threshold. In contrast to effects on tonic firing, spermine initiated low threshold Ca^{2+}-spike (LTS) firing by a mechanism that was insensitive to blockade of NMDA receptors or persistent Na^{+}-conductance. The LTS enhancement due to spermine was greater at –55 mV than at hyperpolarized potentials. In summary, spermine increased neuronal excitability through NMDA and non-NMDA mechanisms.

Key words: Spermine, auditory thalamic neurons, medial geniculate body, N-methyl-D-aspartate, NR2B

1. Introduction

Neurons of the medial geniculate body (MGB) integrate synaptic information from glutamatergic projections of the auditory cortex and inferior colliculus (Gonzalez-Lima and Scheich, 1984; Morel and Imig, 1987; Hu et al., 1994; Metherate and Cruikshank, 1999). Stimulation of the corticothalamic pathway activates postsynaptic N-methyl-D-aspartate (NMDA) and non-NMDA receptors on MGB neurons (Bartlett and Smith, 1999). The receptors for NMDA, robustly expressed in the MGB (Sucher et al., 1995; Khan et al., 2000), become functional at an early age. By the end of the second postnatal week in the rat, NMDA receptors on thalamocortical neurons mediate the transformation of sound-related synaptic inputs to output firing patterns (Heierli et al., 1987). The NR2B subunit, which MGB neurons express in high levels at this time (Hsieh et al., 2002), confers an NMDA-receptor sensitivity to spermine and other polyamines which enhance the response to glutamatergic stimulation (Williams et al., 1994). In visual cortex, the glutamatergic responses of neurons depend on the subunit composition of the NMDA receptor (Roberts and Ramoa, 1999), including the NR2B subunit.

Abnormal polyamine modulation of glutamatergic systems may occur in pathological states of auditory function (reviewed by McCann and Pegg, 1992). Pharmacological inhibition of polyamine synthesis decreases polyamine concentrations in the cochlea (Schweitzer et al., 1986) and induces a temporary hearing loss in humans and rats (McCann and Pegg, 1992). On the other hand, excessive NMDA-mediated excitation contributes to *absence* epilepsy in an animal model (Koerner et al., 1996). Audiogenic seizure activity, initiated in brainstem nuclei (Faingold et al., 1989), enhances MGB responsiveness to acoustic stimuli (N'Gouemo and Faingold, 1997). Hence, high

concentrations of polyamines may induce excessive NMDA-receptor activation in MGB neurons which can impair auditory information processing.

Despite the potential significance, there is little information about the effects of polyamines on the firing modes as well as other functional aspects of thalamocortical neurons, especially during postnatal stages of development. The present studies on MGB neurons examines, for the first time, the effects of spermine and receptor antagonists on the tonic and burst firing modes, excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs), and the electrical membrane properties. For these experiments, we used gerbils which have a hearing capacity similar to humans (Kraus et al., 1987). At P14, the gerbil's thalamus has abundant NR2B subunits, implying a neuronal sensitivity to polyamines (Hsieh et al., 2002).

2. Methods

The procedures and conditions for thalamic slice preparation were similar to those described previously for rats (Tennigkeit et al., 1996). In brief, young gerbils (10-14 days old) were decapitated while under deep isoflurane anesthesia. For the preparation of slices, the cerebral hemispheres were removed rapidly (?1 min) from the cranial vault and immersed for 1-2 min in ice-cold (0-2 ?C) artificial cerebrospinal fluid (ACSF). The ACSF contained (in mM): NaCl, 124; $NaHCO_3$, 26; glucose, 10; KCl, 4; $CaCl_2$, 2; $MgCl_2$, 2; and KH_2PO_4, 1.25. The ACSF, on saturation with 95% O_2 and 5% CO_2, had a pH of 7.3. The brain tissue was trimmed into a cube (?0.125 cm^3) that contained the cortex and thalamus. We used a Vibroslicer (Campden Instruments, London, England) to cut slices (thickness, 300-500 ?m) in a horizontal orientation. The slices included

portions of the medial geniculate, inferior collicular, and reticularis thalami nuclei. The slices were incubated for 2-3 h in ACSF at room temperature (21-25 ?C), until required for recording, which was carried out at 21-25?C.

Whole-cell patch-clamp techniques were used to record from thalamocortical neurons of a slice situated on a Nylon mesh, and perfused with oxygenated ACSF. (Tennigkeit et al., 1996). The electrode pipettes were pulled from borosilicate glass tubing (WP-Instruments, Sarasota, FL), using a Narishige puller (Narishige Instruments, Tokyo, Japan, Model PP83). The pipette solution contained (in mM): K-gluconate, 140; N-2-hydroxyethylpiperazine-N-2-ethanesulfonate (HEPES), 10; KCl, 5; NaCl, 4; adenosine 5-triphosphate (disodium salt), 3; guanosine 5-triphosphate (trisodium salt), 0.3; ethylene glycol-bis-(?-aminoethyl-ether)-N,N,N?,N?-tetraacetic acid (EGTA), 10; and $CaCl_2$, 1. This combination of EGTA and Ca^{2+} yielded a final [Ca^{2+}] of 10 nM (calculated with Max Chelator software). In experiments with the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N?,N?-tetraacetate (BAPTA), we substituted EGTA with an equal concentration of BAPTA (10 mM), which yielded a final [Ca^{2+}] of 1 nM. The calculated E_{Cl} was –55 mV. The pipette solution had a pH of 7.3. The tip resistances of the pipettes were 5-10 M? and access resistances were below 25 M? .

Guided by differential interference contrast microscopy (100x objective) and using a micromanipulator, we positioned the electrode tip in the ventral partition of the medial geniculate nucleus. The electrical recordings were performed in the current-clamp mode with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). We used pClamp 8 software (Axon Instruments) on a Pentium computer for data acquisition, storage, and analysis. The input resistance (R_i) of the neuron was computed from the voltage

displacements (range, 5-8 mV) to intracellular injection of hyperpolarizing current pulses or from the linear slope of current-voltage (I-V) relationships. The membrane time constant was estimated from a single exponential fit to a voltage response of 5-10 mV to a hyperpolarizing current pulse. The voltage values have been corrected for a measured junction potential of -10 mV.

We evoked tonic firing of action potentials from neurons at DC-held potentials near ~-60 mV by injecting depolarizing current pulses with an amplitude of 1.5 times the amplitude of "just-threshold" pulses, which was estimated from the voltage deflection that resulted in action potentials in 50% of the trials. We evoked EPSPs by electrically stimulating the slice, using a bipolar tungsten electrode (tip diameter ?100 ?m), placed at 0.2-0.3 mm mediodorsal to the MGB and near corticothalamic axons. Stimulation at this position resulted only in EPSPs. The stimuli consisted of single pulses of approximately 30 V in amplitude (range, 10-100 V) and 100-200 ?s in duration. The stimulation rate was 0.5 Hz. We evoked inhibitory postsynaptic potentials (IPSPs) when the electrode was placed in the brachium, midway between the inferior colliculus and MGB, and using the above stimulus parameters. The postsynaptic potentials were averaged and fitted with an alpha function (pClamp 8 software), yielding the rise and decay time constants for the EPSPs.

The drugs were prepared in distilled water as stock solutions. The stock solutions were frozen for storage and thawed just before the experiment. Spermine, tetrodotoxin (TTX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and D-2-amino-5-phosphono-valerate (APV) were diluted ~1000 times with ACSF to the concentrations required for the particular experiment. These substances, ATP, EGTA, GTP, HEPES, and the

inorganic chloride salts were obtained from Sigma (St. Louis, MO). As with the ACSF, the drugs were applied with a roller-type pump at a rate of 2 ml/min through a submersion-type chamber that had a volume of ~0.3 cc.

In most cases, we used 14 days old gerbils; however, some exceptions were made due to the availability of the age group. We incorporated 4 neurons from P10, P12 and P13, including 1) 1 out of 19 neurons from a 13 day old gerbil in the spermine group in studies of tonic firing; 2) 2 out of 8 neurons from a 12 day old gerbil in the APV group; and 3) 1 out of 4 neurons from a 13 day old gerbil in the BAPTA group. All Figures are from neurons of 14 days old gerbils. The data are presented as means ? S.E.M. A Student t-test was used for comparison of responses to drug application between 2 groups. For comparison of more than 2 groups, we used ANOVA. $P < 0.05$ was considered significant.

3. Results

3.1. Spermine application increases tonic firing

Spermine application reversibly increased the number of action potentials in all neurons depolarized from rest by current pulse injection. Spermine (100 ? M) applied for 3 to 6 min induced tonic firing of action potentials on top of subthreshold responses (Fig. 1A). When action potentials were present in the control, spermine application increased the rate of firing. Long recovery times of 35 to 45 min characterized spermine's effects on thalamic firing modes after 6 min applications. In the neuron of Fig. 1A, substantial recovery occurred at ~32 min after discontinuing the spermine application.

The spermine-induced increase in the firing frequency was concentration-dependent over the range between 50 and 500 ?M (n = 19, Fig. 1B). In addition, application of a spermine concentration of 1 ?M did not affect firing (n = 2); however, at 1 mM, there was a marked increase in the firing rate (n = 2), without any apparent recovery (data not shown). Spermine, applied at an ED_{50} of 100 ?M (Fig. 1B), reversibly increased the number of action potentials per pulse by an average of ~80% in 9 neurons (control, 1.8 ? 0.3 action potentials/pulse and spermine application, 3.3 ? 0.4 action potentials/pulse, paired t-test, $P < 0.01$).

The increased firing due to spermine did not likely result from changes in the passive membrane properties which did not greatly change during 3 to 6 min spermine applications (cf. subthreshold responses in Figs. 1A, 3A). The average resting potentials were -67 ? 4 mV during the control period, and -66 ? 5 mV during applications of spermine at 50-500 ?M (n = 19). Spermine application significantly changed the mean membrane time constant (t_m = 64 ? 6 ms, control, and 76 ? 6, spermine, paired t-test) and mean input resistance (R_i = 772 ? 38 M? , control, and 756 ? 61 M? , spermine, paired t-test), computed from the responses to hyperpolarizing current pulse injections in 19 neurons held at –65 mV. Similarly, the spermine-induced effects on the passive properties could not account for the changes in firing threshold.

3.2. Spermine application reduces firing threshold

Spermine (100 ?M) decreased the latency to tonic firing by decreasing the threshold (Fig. 2). Spermine decreased the firing threshold from -51.7 ? 0.8 mV to -57.9 ? 2.2 mV (n = 6, paired t-test, $P < 0.05$). Significant changes in action potential amplitude did not

accompany the decreased threshold. Figure 2B summarizes the effects of spermine on firing threshold for 6 neurons.

The spermine-induced decrease of the action potential threshold depended on extra- and intracellular [Ca^{2+}]. The omission of Ca^{2+} from the ACSF, which normally contained 2 mM Ca^{2+}, decreased the threshold from -53.7 ± 2.9 mV to -60.6 ± 3.1 mV (n = 3, ANOVA, $P < 0.05$). On application of spermine in ACSF that was nominally Ca^{2+} free, we observed no change in the threshold (control, -60.6 ± 3.1 mV, and spermine, -60.2 ± 3.2 mV; n = 3). Recovery of the threshold from the effects of the Ca^{2+}-free solution occurred at 10 min after returning to normal Ca^{2+} perfusion. A subsequent application of spermine for 3 min in normal solution (2 mM Ca^{2+}) reversibly reduced the firing threshold to –60.0 ± 2.6 mV (n = 3, ANOVA, $P < 0.05$). These effects, observed when the pipette solution contained 10 mM EGTA, were largely reversible (recovery, -55.3 ± 2.3 mV). We then examined the effects of the fast Ca^{2+} chelator, BAPTA (10 mM), in the internal pipette on the spermine-induced reduction in action potential threshold. A 3 min application of spermine did not significantly change the threshold in 4 neurons recorded with BAPTA-containing pipettes (control, -49.3 ± 2.1 mV, and spermine, -49.3 ± 2.3 mV). These experiments demonstrated that the effects of spermine on action potential threshold depended on a slow accumulation of intracellular Ca^{2+} and the presence of extracellular Ca^{2+}.

3.3. NMDA-receptors mediate the spermine-induced increase in firing

We examined the possibility that NMDA-receptors mediated the effects on the firing threshold and tonic firing rate by determining the interactions of spermine and the competitive antagonist, APV (40 ?M). As shown in Figure 2B, spermine reduced the

threshold voltage for an action potential evoked with a 500 ms current pulse, by an average of 6 ? 1.1 mV. On recovery from spermine (Fig. 2B), application of APV alone, or in combination with spermine, did not significantly change the firing threshold (during APV, -52.1 ? 1.9 mV and APV + spermine, –53.0 ? 1.7 mV; n = 6) or changes in membrane properties that could account for the blockade of spermine action on firing. This signified a substantial blockade of spermine action at NMDA receptors by APV. In separate experiments (n = 5), we applied APV to neurons that had not previously received a drug application. Here, APV produced an increase in threshold, which remained largely unaltered by a subsequent, combined application with spermine (Fig. 2C). All neurons showed substantial recovery at 15 min after discontinuing the application. These data implicated an NMDA-receptor mechanism in the spermine-induced decrease in the threshold.

NMDA-receptors appeared to mediate the spermine-induced increase in firing rate. In the neuron of Fig. 3A, spermine application (100 ?M, 3 min) increased the number of action potentials during a 500 ms current pulse injection from one action potential in the initial control, to three action potentials. Recovery to one action potential required 15 min. In the neurons that had not previously received a spermine application (Fig. 3B), an increase in the amount of current during action potential blockade by APV produced a return of the action potential. Similarly, the blockade of spermine action by APV was overcome by an increase in the current amplitude. The APV blockades of action potentials and spermine action were not attributable to an APV-associated increase in input conductance and completely reversible. Figure 3C summarizes these data for 6 neurons, which implicated NMDA-receptor mediation.

We assessed the possibility that non-NMDA receptors for glutamate may have contributed to the increased firing during spermine application. In these studies on 5 neurons, we determined the interactions of spermine with an AMPA receptor antagonist, CNQX. Application of CNQX (30 ?M) for 6 min did not result in significant changes in evoked action potential firing, configuration, or membrane electrical properties. A subsequent, combined CNQX and spermine application did not greatly alter the threshold amount of current injection or the ~200% increase in firing rate evoked by current pulses (amplitude ~1.5 x threshold) in all 5 neurons (CNQX, 1.4 ± 0.3 action potentials/pulse, and CNQX + spermine, 4.2 ± 0.4 action potentials/pulse, data not shown). Hence, the increase in tonic firing rate due to spermine application did not likely involve AMPA receptors.

3.4. Effects on membrane rectification

We examined the possibility that the spermine-induced increase in tonic firing involved voltage-dependent membrane properties. Spermine (100 ?M) application for 3 min increased rectification in a range between the resting potential and firing threshold but did not appreciably change rectification at hyperpolarized potentials, down to -100 mV (n = 19; Fig. 4A). Quantification of the increase in rectification was difficult because spermine application shortened the latency to firing on depolarization (cf. arrows in Figs. 2A and 4A). Application of APV (40 ?M, 6 min) completely blocked the rectification in the upper right quadrant of the current-voltage (I-V) relationship. A subsequent co-application with spermine (100 ?M) did not produce a further change in this rectification. The graph of Figure 4A (right) summarizes these findings for 6 neurons.

There was little or no involvement of AMPA receptors in the increased rectification produced by depolarizing current pulses during application of spermine (100 ?M, 3 min). The application of CNQX (30 ?M, 6 min) did not affect spermine's actions on the rectification in response to depolarizing current pulses in 5 neurons. The value for this rectification was 15.9 ± 0.6 mV during co-application of CNQX and spermine which was significantly different from 11.5 ± 0.5 mV in the control or 11.3 ± 0.4 mV during CNQX (ANOVA, $P < 0.05$). Hence, the spermine-induced increase in rectification observed with depolarizing current pulse injection involved NMDA receptors, but not likely AMPA-receptors.

We investigated the possibility that spermine increased the rectification in the upper right quadrant of the I-V relationship by interacting with a persistent Na^+ conductance. The rectification observed on depolarization from ~-70 mV to threshold involves a persistent Na^+ conductance, sensitive to TTX-blockade (Parri and Crunelli, 1998). Blockade of voltage-dependent Na^+-channels with TTX (0.6 ?M, 6 min) decreased the slope in the I-V relationship, producing a lower slope in the depolarizing quadrant than in the hyperpolarizing quadrant. The blockade with TTX nullified the ability of spermine (100 ?M, 3 min) to increase rectification on depolarization (n = 6; Fig. 4B). The results imply that spermine increased rectification in the upper right quadrant of the I-V relationship by increasing a TTX-sensitive, voltage-dependent Na^+-conductance.

The spermine-induced enhancement of rectification on depolarization of the neuron also may depend on extra- or intracellular Ca^{2+}, as demonstrated for neocortical neurons (Crill 1996). Hence, we measured the spermine-induced changes in rectification in the depolarizing responses to current pulse injections during intracellular application of

BAPTA (10 mM) and extracellular perfusion with Ca^{2+}-free ACSF. Figure 5A shows that perfusion of Ca^{2+}-free ACSF did not greatly alter this rectification. For example, a 50 pA current pulse evoked a response of 9.8 ± 0.6 mV in the control and 10.0 ± 0.5 mV in 0 mM [Ca^{2+}] (n = 4); the voltage response increased to 15.2 ± 1 mV during spermine application in control ACSF ($P < 0.01$) and did not significantly change during combined application of spermine and 0 mM [Ca^{2+}] (10.2. ± 0.3 mV).

In contrast, the intracellular application of BAPTA, a more rapid Ca^{2+} chelator than EGTA, eliminated the spermine-induced enhancement of rectification, observed on depolarization. In neurons recorded with BAPTA-containing pipettes (cf. Fig. 5B), spermine application did not alter the rectification in the depolarizing responses from the average values of 12.4 ± 1 mV in the control and 12.4 ± 0.7 during spermine (n = 4). This implicated a Ca^{2+}-conductance in the spermine-induced enhancement of rectification in the subthreshold depolarizing responses.

3.5. Effects of spermine on low threshold Ca^{2+} spike (LTS) firing

Application of Ca^{2+}-free ACSF abolished the transient, low threshold spike (LTS), evoked at the offset of hyperpolarizing current pulse injections or by step depolarization in neurons held at hyperpolarized potentials. This confirmed their Ca^{2+} mediation. Spermine application increased action potential firing on top of a LTS in only 10 out of 19 neurons, in contrast to the increased tonic firing rate on spermine application, observed in all neurons. As shown in Figure 6A, spermine induced an action potential on the rebound depolarizing response at the termination of a hyperpolarizing current pulse injection. When a rebound LTS was present in the control, spermine application

increased the LTS amplitude in 5 out of the 10 neurons, eliciting an action potential. In the remaining 5 neurons, spermine induced one or two action potentials on the rebound response at the termination of current pulses that hyperpolarized the membrane potential beyond –80 mV. These effects were reversible, requiring 20 to 40 min for recovery.

Blockade of voltage-dependent Na^+-channels with TTX did not significantly alter the ability of spermine to enhance the LTS in 6 neurons (cf. Figs. 6A and B). During TTX-blockade, however, the spermine-enhancement of the LTS depended on the holding potential. In these experiments, we evoked the LTS by injecting hyperpolarizing currents of different amplitudes into neurons held at different holding potentials (Fig. 6C). Application of spermine (100 ?M) induced a LTS in neurons at potentials that caused marked inactivation of the control LTS. At potentials where a LTS was present, a spermine application increased its amplitude and rate of rise (dV/dt). There was a greater increase in the dV/dt of the LTS when the neuron was at held -55 mV than at -85 mV (Fig. 6C). After a spermine application, the LTS evoked in a neuron held at -55 mV had an average dV/dt of 3.1 ± 0.2, mV/ms compared to 1.5 ± 0.3 mV/ms of the control. The average rate of decay was -1.7 ± 0.3 mV/ms (n = 6) with fast (26 ± 5 ms, n = 5) and slow (146 ± 12 ms, n = 5) components (trace 2, Fig. 6B). Figure 6C summarizes the effects of spermine on the dV/dt of the LTS, showing a maximal effect at a holding potential (V_h) = –55 mV and a minimal increase at V_h = -85 mV in 5 neurons (paired t-test, $P < 0.01$).

Since the hyperpolarization-activated current influences the rate of rise of the LTS, we examined whether spermine affected the voltage sag, mediated by this current. The voltage sag was not prominent in the majority of neurons. Spermine application (100 ?M) produced no change in the rate of rise or configuration of a voltage sag induced by a

hyperpolarizing current pulse in 3 neurons. Hence, the increase in the rate of rise of the LTS more likely was due to a direct effect of spermine on T-type Ca^{2+} channels than to its interactions with the persistent Na^{+}- or hyperpolarization-activated conductances.

We determined if the spermine-potentiation of the LTS involved NMDA receptors in 8 neurons. In 6 of these neurons, APV application decreased the amplitude of the subthreshold voltage responses to current pulses (Fig. 6D, lower traces). As shown for the neuron of Figure 6D, APV application also decreased a LTS burst to a single action potential, increased the latency to the first action potential on top of the LTS and decreased its rate of rise. Despite APV-antagonism of NMDA receptors, spermine application transformed a subthreshold depolarizing voltage response into a LTS, as well as decreased the latency to an action potential on top of the LTS (cf. APV and spermine traces in Fig. 6D). In 8 out of 8 neurons, APV application (50 ?M, 6 min) reduced the average rate of rise of the LTS from 1.6 ± 0.3 mV/ms in naïve controls to 1.2 ± 0.2 mV/ms in APV, however this was not statistically significant. A subsequent co-application of APV and spermine caused a significant increase in the rate of rise of the LTS to a value of 2.2 ± 0.1 mV/ms (ANOVA, $P < 0.05$).

We determined if the spermine-potentiation of the LTS involved interactions with AMPA receptors. Spermine significantly increased the dV/dt of the LTS (control, 1.8 ± 0.1 mV/ms and spermine, 3.6 ± 0.2 mV/ms; $n = 5$, ANOVA, $P < 0.05$) during blockade of AMPA receptors with CNQX (30 ?M, 6 min). Hence, the effects of spermine on the LTS did not likely involve NMDA or AMPA receptors.

3.6. Effects of spermine on excitatory and inhibitory postsynaptic potentials

Spermine application (100 ?M) to 18 neurons resulted in bursts of action potentials on EPSPs evoked by electrical stimulation of corticothalamic projections (Fig. 7A). Spermine had little or no effects on the rate of rise of the EPSP, but always enhanced the late decay phase amplitude (Table 1). In 5 neurons, the EPSP amplitude increased slightly (3-5 mV) during spermine application, but this was not a consistent finding. The spermine-induced action potentials on the EPSPs were reversible in all 18 neurons. Complete recovery was observed in 13 of 18 neurons at 35 min after terminating the application.

Spermine prolonged the EPSP decay phase (Fig. 7B), thereby promoting the occurrence of action potentials on top of the EPSPs (Fig. 7A). Table 1 summarizes these results for single or cumulative applications of spermine in 18 neurons. Spermine application increased the time to 90-10% of peak amplitude and decay time constant, as estimated with an ?-function fit of the EPSPs. The ED_{50} for the spermine-induced prolongation of EPSPs was ~100 ?M which was approximately the same for the spermine-induced increase in firing (cf. Fig. 1B). Spermine application significantly increased the mean decay time constant from 477 ? 11 ms to 710 ? 23 ms (Table 1). The prolongation of EPSPs was also apparent in the longer time constant of the ?-function fits that was 142 ? 8 ms in control and 217 ? 22 ms during spermine (Bar graph in Fig. 7B). Recovery to the control value occurred after 30 min (148 ? 15 ms). Figure 7B summarizes these results for 6 neurons.

Spermine application (100 ?M) did not significantly affect the amplitude or time course of depolarizing potentials evoked by stimulation of the brachium colliculi inferioris (n = 6; data not shown). These potentials of 100-200 ms duration were likely

GABAergic IPSPs because they reversed at DC-held potentials near E_{Cl} (–55 mV) and were sensitive to blockade by bicuculline (50 ?M, n = 6).

Spermine prolonged the decay of the late EPSP component mediated by a NMDA-type receptor. The application of APV (50 µM), blocked the late component, resulting in shorter rise and decay times of the EPSPs (Table 1). During NMDA-receptor blockade, the decay time constant was 100 ? 14 ms, compared to 143 ? 15 ms in control (Fig. 7C; n = 8, ANOVA, P <0.01). Subsequent co-application of APV and spermine did not significantly affect the amplitude, rate of rise and decay time of the remaining non-NMDA mediated component of the EPSP. The mean decay time constant was 100 ? 14 ms in the control and 103 ? 11 ms during combined spermine and APV application (Fig. 7B). Consistent with these observations, spermine application did not alter the amplitude or duration of the EPSPs in a Ca^{2+}-free solution, presumably mediated by AMPA receptors (n = 3; data not shown).

By applying spermine in combination with CNQX (30 µM, 6 min), we determined if the prolongation of EPSPs resulted from interactions with non-NMDA receptors. We found that spermine prolonged the EPSP decay during AMPA-receptor blockade by CNQX to the same extent as in the absence of AMPA receptor blockade. The 90-10% decay time was 250 ± 7 ms in control, 280 ± 9 ms in CNQX, and 484 ± 13 ms during combined CNQX and spermine application (Table 1). In 3 additional neurons, spermine was applied before the co-application of CNQX and spermine. The co-application resulted in a significant prolongation of the EPSP to the same extent as in the absence of AMPA receptor blockade (Table 1). This confirmed that spermine affected only the NMDA-mediated component. Co-application of CNQX (30 µM) and APV (40 µM)

abolished both the early and late components of the EPSP which remained absent despite a subsequent spermine application (n = 4). From these results, we suggest that spermine increased the duration of the EPSP decay phase by interacting with NMDA-receptors.

We considered the possibility that spermine prolonged the EPSPs by acting on an extracellular polyamine-sensitive site of the NMDA receptor (cf. Benveniste and Mayer, 1993). We studied the interactions of spermine and arcaine, an antagonist that acts at the polyamine-sensitive site on the NMDA receptor (Reynolds 1990). In these studies, we sequentially applied arcaine (40 µM), spermine (100 µM), arcaine and spermine, each for 3 min (n = 3). Arcaine, alone, did not greatly alter the configuration of the EPSP (Fig. 8A) or produce changes in the EPSP amplitude, decay time, and half-width (Table 1). After a 15 min washout from arcaine application, spermine significantly prolonged the EPSP decay time constant from 101 ? 16 ms in the control to 180 ? 32 ms (Fig. 8A). A subsequent co-application of spermine and arcaine abolished the actions of spermine, resulting in a decay constant of 118 ? 16 ms (Fig. 8A). The graph in Fig. 8A summarizes the spermine-induced changes in the EPSP decay times and the arcaine blockade of spermine action.

We also determined if spermine increased the NMDA-mediated component of the EPSP by potentiating the actions of glycine on the NMDA receptor. In the presence of a saturating concentration of the co-agonist glycine (40 µM), spermine still prolonged the EPSP by ~49% (Fig. 8B). In 3 neurons, spermine increased the decay time constant from 255 ? 44 ms to 379 ? 53 ms (ANOVA, $P < 0.05$). In summary, spermine actions on the EPSP likely involved an extracellular polyamine-sensitive site, and not a glycine-sensitive site of the NMDA receptor.

We attempted to assess a contribution of extracellular Mg^{2+} to the spermine-induced enhancement of EPSPs. In 2 neurons, the omission of Mg^{2+} from ACSF perfusion resulted in subthreshold oscillations of the membrane potential and spontaneous firing of action potentials. These observations were consistent with previous studies on thalamocortical neurons (Jacobsen et al., 2001) which prevented critical assessment of an agonist role of Mg^{2+} at the polyamine-sensitive site on the NR2B receptor subunit (Kemp and Kew, 1998).

4. Discussion

These studies demonstrate that extracellular spermine has distinctive actions on thalamocortical neurons, consistent with a neuromodulator role in the medial geniculate body. Spermine actions on NMDA receptors produced a heightened state of excitability which we viewed as prolonged corticothalamic EPSPs, and increased EPSP-bursting and tonic firing of action potentials. To a large extent, these effects resulted from increased membrane rectification on depolarization and reduction in threshold for action potential genesis. Spermine also modulated the burst firing mode by increasing the rate of rise and amplitude of low threshold Ca^{2+} spikes (LTSs). This outstanding effect did not involve interaction with glutamate receptors. The modulation of corticothalamic excitation and LTSs of MGB neurons may be critical in the transformation of auditory signals in gerbil thalamus at the P14 stage of development.

4.1 Enhancement of late component of NMDA-mediated EPSPs

Spermine application increased the decay time constant of corticothalamic EPSPs mediated by NMDA receptors. This finding is consistent with the increased amplitude of NMDA-evoked currents during spermine application to cultured hippocampal and spinal neurons (Lerma, 1992; Benveniste and Mayer, 1993). The action of spermine on MGB neurons was selective. Spermine did not significantly alter the early EPSP component mediated by AMPA receptors, and did not appreciably affect IPSPs mediated by GABA receptors that were sensitive to bicuculline antagonism. Application of APV completely blocked the spermine-induced increase in the EPSP decay time constant. This implicated spermine actions on NMDA-receptors.

The effects of spermine on MGB neurons involved a polyamine-sensitive site on the NR2B subtype of NMDA receptors, as we demonstrated with arcaine and glycine applications. Arcaine, itself, did not have significant effects on the passive and active membrane properties but reversed the spermine-induced decrease of the EPSP decay. Previous studies have shown that arcaine blocks spermine actions by inverse agonism, antagonism, and open-channel blockade of the polyamine-sensitive site on NMDA receptors (Reynolds, 1990; Pritchard et al., 1994). The actions of spermine at the NR2B-receptor subunit decreased the EPSP decay, despite saturating concentrations of glycine. These observations are consistent with the glycine-independent potentiation of NMDA currents by spermine at the NR2B-receptor subunit in cultured hippocampal neurons (Benveniste and Mayer, 1993). In thalamocortical neurons, the persistence during high glycine concentrations and arcaine-reversal imply that spermine acted independently of

the glycine site at a specific polyamine-sensitive site on the NR2B-receptor subunit of the NMDA receptor.

The NR2B subunit may modulate the decay time constant of the NMDA receptor-mediated EPSP during the development in MGB neurons. At the end of the second postnatal week, thalamocortical neurons express an abundance of the NR2B polyamine-sensitive receptor-subtype in the MGB and lateral geniculate body (LGB) of the rat (Chen and Regehr, 2000). The duration of EPSPs mediated by NMDA receptors in LGB neurons of the rat is similar at P14 to that in gerbil MGB neurons. The decay time constant in LGB neurons is longer at P14 than at earlier (P7-P13) or later (P16-P28) stages of development in rats (Chen and Regehr, 2000; cf. also rat MGB at P21-P42, Bartlett and Smith, 1999). Hence, spermine modulation of the NR2B subunit may cause the longer EPSP duration in MGB neurons at the P14 stage.

4.2 Enhancement of membrane rectification on depolarization

Spermine enhanced excitability by increasing inward rectification on depolarization, without greatly affecting the passive properties of MGB neurons. It is not known if the passive and active membrane properties of MGB neurons mature by P14 in gerbil, as in the rat (Tennigkeit et al., 1998). Thalamocortical neurons of the adult guinea pig and P7-P28 rat inwardly rectify because the activation of persistent Na^+-conductance on depolarization results in an amplification of the voltage response (Jahnsen and Llinas, 1984; Tennigkeit et al., 1996; Parri and Crunelli, 1998). In the present studies, blockade of the TTX-sensitive rectification or NMDA receptors eliminated the spermine-induced enhancement of rectification on depolarization. These findings imply that spermine

interactions with NMDA receptors led to activation of a persistent Na^+ conductance in MGB neurons.

An elevation in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) due to NMDA-receptor activation (Jahr, 1992) may have enhanced rectification on depolarization. We did not observe a spermine-induced increase in rectification on depolarization during Ca^{2+}-free perfusion or rapid chelation of Ca^{2+} with intracellular BAPTA. It seems likely that an elevation of $[Ca^{2+}]_i$ initiated by spermine actions at NMDA receptors activated intracellular messengers and increased this rectifying behavior. In neocortical neurons, transmitter activation of dendritic NMDA receptors increases Ca^{2+} entry (Schwindt and Crill, 1995) that may increase channel phosphorylation (Siekevitz, 1991) and a persistent Na^+-conductance (Schwindt et al., 1992). Hence, the spermine-induced enhancement of TTX-sensitive rectification on depolarization may result from NMDA-mediated Ca^{2+} entry in MGB neurons.

The effects of spermine on membrane rectification and firing threshold may involve the recruitment of a Ca^{2+}-dependent second messenger, subsequent to NMDA receptor activation. Activation of NMDA receptors enhances Ca^{2+} entry, resulting in a Ca^{2+} gradient in the dendrites (Connor et al., 1988) and activation of a Ca^{2+}-dependent protein kinase C (PKC) pathway. A rise in intracellular $[Ca^{2+}]$ also may activate calmodulin kinase II which enhances Na^+ currents (Carlier et al., 2000). PKC activation increases membrane excitability by shifting the activation curve for the persistent Na^+ current along the voltage-axis to more hyperpolarized potentials (Astman et al. 1998; Franceschetti et al. 2000). This voltage range is consistent with the range of spermine-enhancement of voltage rectification in our experiments.

The increased rectification on depolarization may have reduced the threshold for an action potential in MGB neurons (cf. neocortical neurons, Stafstrom et al., 1982). Antagonism of NMDA receptors, perfusion with Ca^{2+}-free ACSF or rapid chelation of Ca^{2+} with BAPTA, eliminated the reduction in threshold and increased tonic firing due to spermine application. Hence, the modulation of NMDA receptor-mediated Ca^{2+} entry likely increased membrane rectification on depolarization and reduced firing threshold. This mechanism explains the ability of spermine to increase postsynaptic excitability and tonic firing in MGB neurons.

4.3 Facilitation of low threshold Ca^{2+} spike (LTS) firing

Spermine facilitated LTS firing by a mechanism that did not involve interactions with NMDA receptors. Spermine increased the rate of rise and amplitude of the LTS, despite APV blockade of NMDA receptors. This was evident on depolarization to the action potential threshold where there is a smaller gradient for Ca^{2+} as well as greater inactivation of T-type Ca^{2+}-channels (Hernandez-Cruz and Pape, 1989). Spermine enhanced the LTS during blockade of voltage-dependent Na^{+} channels by TTX. Hence, a change in some parameter of the T-type Ca^{2+}-current, e.g., voltage-dependence of the inactivation-activation relationship, may have increased the LTS.

4.4 Significance

Spermine is widely distributed in rat and human brain (Harman and Shaw, 1981; Morrison et al., 1995) with extracellular concentrations of <1 ?M (Dot et al., 2000). The effects of spermine on NMDA receptors and low threshold Ca^{2+} spikes in juvenile MGB

neurons (ED_{50} = ?100 µM) are consistent with a neuromodulatory role (Williams, 1997). Modulation by spermine is likely important for development, including learning processes (Chida et al., 1992).

The present results are relevant to the normal function of the central auditory system. The NMDA-receptor mediated effects of spermine would enhance the ability of MGB neurons to detect simultaneous inputs, as in coincidence detection. For example, an over-expression of the spermine-sensitive NR2B subunit (Williams et al., 1994) prolongs EPSPs and shortens the time window between two coincident signals in hippocampal neurons (Tang et al., 1999). In thalamic neurons, the generation of synchronous activity may involve coincidence detection (Roy and Alloway, 2001) as well as amplitude selectivity in the MGB neurons (Kuwabara and Suga, 1993).

The effects of spermine on the low threshold Ca^{2+} spikes of MGB neurons may have relevance for conscious or sleep states and disorders of consciousness. The LTS is essential in the generation of bursting and oscillatory activity in the auditory nuclei (Hu, 1995; Tennigkeit et al., 1996). By increasing the rate of rise and amplitude of the LTS and slowing its decay, spermine modulation may increase a MGB neuron's responsiveness to inputs during these states. Modulation by spermine may have importance for bursting behavior during sleep states whereas excessive modulation may occur in absence epilepsy as in audiogenic seizures (Porta et al., 1981), sensitive to blockade by polyamine antagonists (Kotlinska and Liljequist, 1996).

The present study has shown that spermine enhanced the excitability of thalamocortical neurons in specific ways that were consistent with a neuromodulator role in the medial geniculate body at P13-15 stage of development. Spermine acted on a

polyamine site of NMDA receptors, to increase membrane rectification on depolarization, reduce firing threshold and slow the decay of corticothalamic EPSPs in MGB neurons. The heightened excitability increased tonic firing evoked by depolarizing current pulses and EPSP bursts of action potentials. Spermine also increased the rates of rise and amplitudes of low threshold Ca^{2+}-spikes by an unknown mechanism, not mediated by NMDA receptors. By increasing the efficacy of corticothalamic excitation, spermine actions have importance in the transformation of auditory signals to tonic and burst firing during the early stages of development.

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Table 1

Effects of spermine on EPSP variables

	Amplitude (mV)	Rise time (ms)	Decay time (ms)	Half-width (ms)	n
Control	6.3 ? 1.8	37 ??	477 ? 11	238 ? 11	19
Spermine	9.1 ????	20 ????	710 ? 23*	297 ???	19
APV	6.4 ??????????	??.1	147 ????	59 ????	9
APV + Spermine	6.1 ????	12 ? 1.6	153 ????	63 ?2.4	9
CNQX	6.2 ?????	90 ?5	534 ????	247 ????	9
CNQX + Spermine	6.3 ?????	95 ? 4	925 ????**	433 ????**	9
Arcaine	4.5 ??????	36 ? 7	419 ??????	204 ????	5
Arcaine + Spermine	4.6 ????	42 ? 11	394 ????	205 ?38	3
Glycine	6.9 ????	24 ??	190 ?7	245 ? 41	3
Glycine + Spermine	9.0 ????	28 ? 9	400 ????*	437 ?45	3

Values are mean ??SE. The spermine group was analyzed with a paired t-test and all other groups with an ANOVA test. * P < 0.05 , ** P < 0.01

Legends

Fig. 1. Spermine enhanced tonic firing in a concentration-dependent manner in MGB neurons. (A) Spermine application (100 µM, 3 min) enhanced action potential firing evoked by current pulses (25 and 50 pA, 500 ms, 1.5x threshold). Holding potential, –65 mV. Vertical upper bar, 30 mV and lower bar, 60 pA; horizontal bar, 150 ms. (B) Increase in number of action potentials per pulse was concentration dependent. The control firing was 1.8 ± 0.4 action potentials/pulse (n = 7). The ED_{50} was ~100 µM for spermine-enhanced firing which approached saturation at 200 µM.

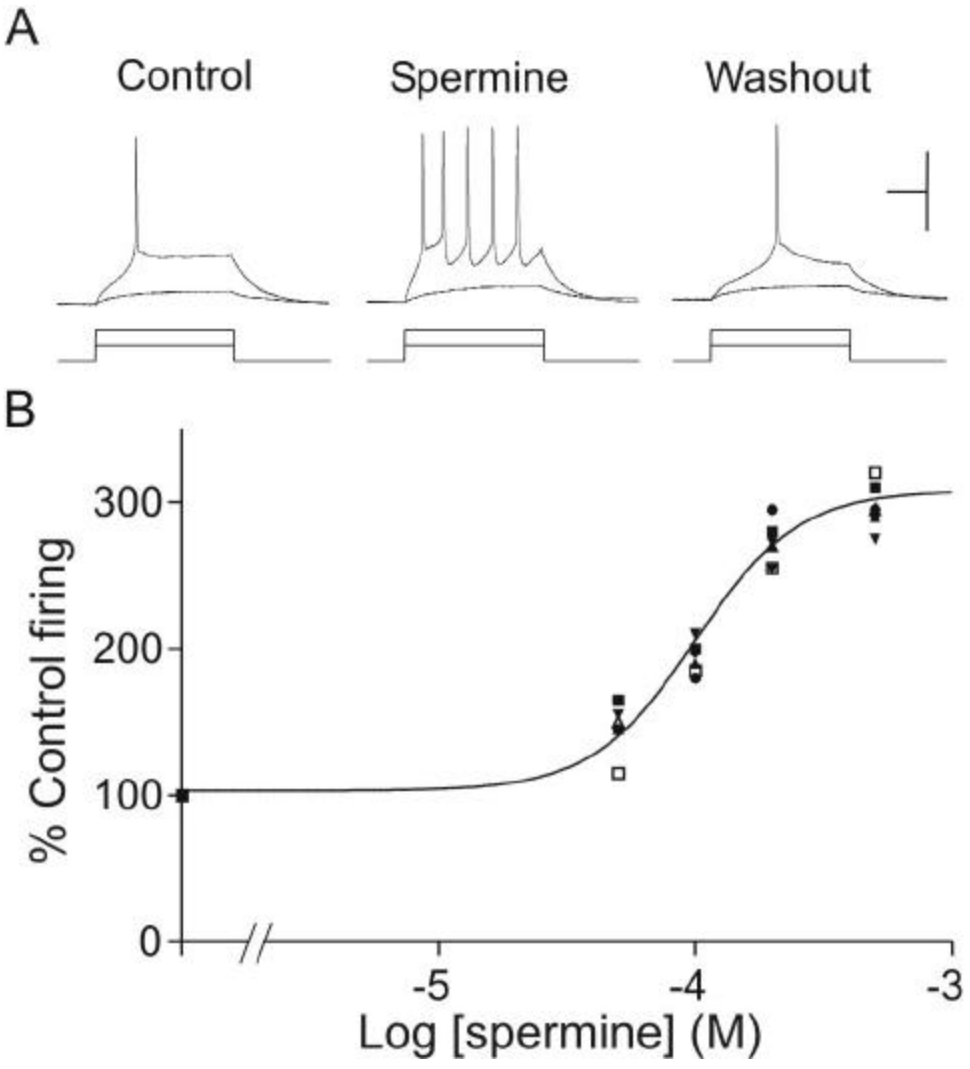


Fig. 2. Spermine (100 µM) decreased the action potential threshold. (A) Spermine application produced a leftward shift in action potential latency (current pulse duration, 500 ms). Arrows point to threshold in control and spermine. (B) NMDA receptors mediated spermine effects on action potential threshold. The control threshold was -50.9 ? 0.6 mV, n = 6 which spermine reduced to -57.1 ? 2.2 mV (n = 6, P <0.05). Partial recovery was observed after 15 min (-52.4 ? 0.6). Blockade of NMDA receptors by APV (50 ?M) reduced the threshold by <1 mV. A reduction in threshold was not observed during co-application of APV and spermine (-0.9 ? 0.6 mV, n = 6). (C) APV (50 ?M) increased firing threshold in 5 neurons from -52.3 ? 0.7 to 48.7 ? 0.5 mV. Subsequent spermine application did not alter the increased threshold (-49.1 ? 0.6 mV). Two-way ANOVA; * in B indicates P < 0.01 and in C, P < 0.05. A: vertical bar, 15 mV; horizontal bar, 50 ms.

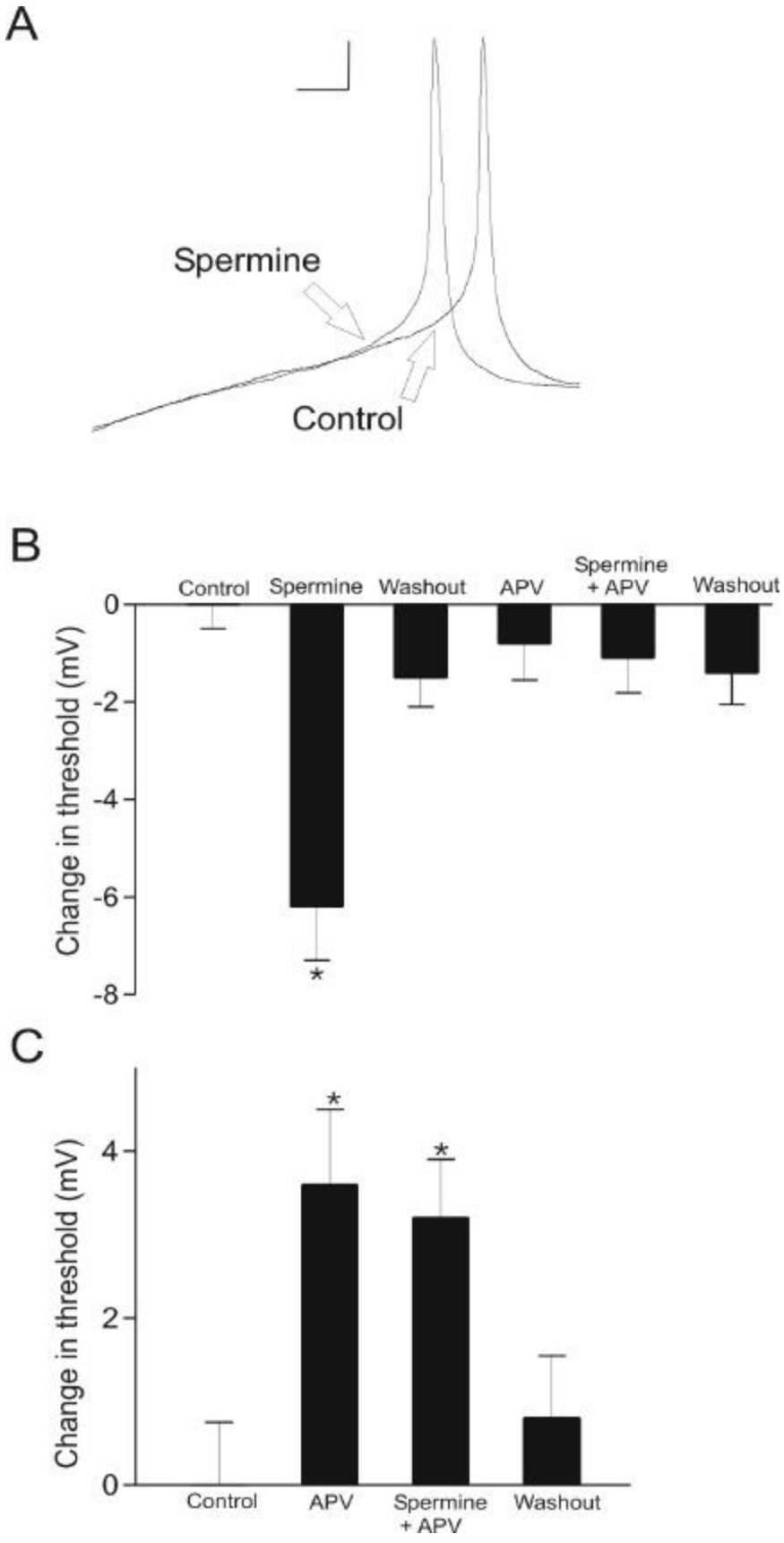

A
Spermine
Control
B
Control
Spermine
Washout
APV
Spermine + APV
Washout
Change in threshold (mV)
0
-2
-4
-6
-8
*
C
Change in threshold (mV)
4
2
0
*
*
Control
APV
Spermine + APV
Washout

Fig. 3. Spermine increased tonic firing by interacting with NMDA receptors. (A) Spermine application (100 µM, 3 min) reversibly induced firing. After a 25 min washout from spermine, APV (50 µM, 6 min), an NMDA-receptor antagonist, blocked the evoked action potential. Firing was not observed during co-application of APV and spermine (3 min). Washout shows recovery at 10 min after discontinuing the co-application. Lower traces show hyperpolarizing tests for input resistance. (B) Application of APV (50 µM, 6 min) abolished firing induced by just-threshold current pulse (40 pA). A subsequent 3 min application of spermine and APV did not alter this suppression (lower superimposed traces in middle panel). A two-fold increase in current amplitude overcame the blockade during APV application, alone, or during co-application with spermine (upper superimposed traces in middle panel). Recovery was observed after 10 min washout. (C) Summary of spermine effects on firing in 6 neurons. ANOVA; * $P < 0.01$, ** $P < 0.05$. Vertical bar, 30 mV; horizontal bar, 200 ms.

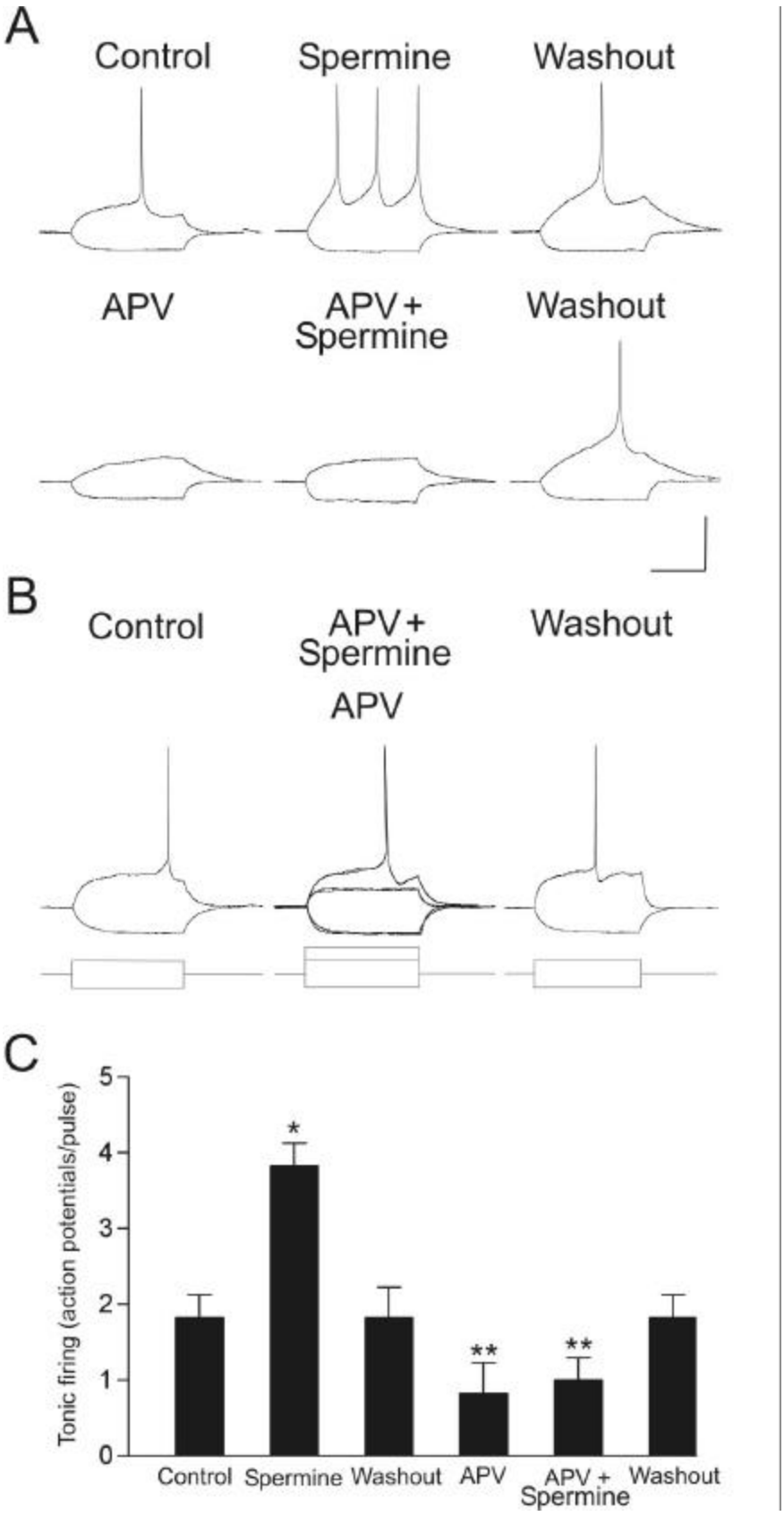

A
Control
Spermine
Washout
APV
APV + Spermine
Washout
B
Control
APV + Spermine
Washout
APV
C
Tonic firing (action potentials/pulse)
0
1
2
3
4
5
*
**
**
Control
Spermine
Washout
APV
APV + Spermine
Washout

Fig. 4. Effects of spermine (100 µM, 3 min) on membrane rectification. (A) Current-voltage (I-V) relationship of neuron shows that spermine increased depolarizing response which was abolished during combined application (3 min) with APV. APV (50 µM, 6 min), alone, reduced rectification in upper right quadrant. Curve after 15 min washout shows substantial recovery. Graph at right summarizes effects of spermine, APV, and their co-application on rectification. The response on depolarization increased from 12.5 ± 0.5 mV (control) to 17.5 ± 0.8 mV during spermine application, and after 15 min washout, recovered to 12.9 ± 0.7 mV. Subsequent APV application reduced the response on depolarization from 12.9 ± 0.7 mV (first washout) to 9.2 ± 0.4 mV (APV). Co-applied APV and spermine did not greatly alter rectification (8.7 ± 0.3 mV). Recovery from APV and spermine occurred after 15 min (12.7 ± 0.5 mV). Holding potential, –70 mV. (B) I-V diagram for neuron shows that TTX application (0.6 ?M, 6 min) decreased rectification on depolarization over a 10-15 mV range. Co-applied with TTX, spermine did not alter rectification in upper right quadrant. A 20 pA pulse was sufficient to observe rectification on depolarization, whereas a -50 pA pulse was required for observation of rectification on hyperpolarization. Graph at right summarizes effects of TTX and spermine on rectification. Rectification on depolarization decreased from 13.1 ± 0.8 mV (control) to 10.2 ± 0.8 mV during TTX application. A subsequent co-application with spermine did not greatly alter depolarizing responses (10.8 ± 0.7 mV). *Inserts* in upper left quadrants of A and B show superimposed responses (± 7 mV) to depolarizing and hyperpolarizing current pulses (duration 500 ms) of matched amplitude (60, -60 pA), during control (C), spermine (S) and at 3 min of co-application of TTX and spermine (TTX+S). Bar graph values are mean ± S.E.M. ANOVA; * $P < 0.05$.

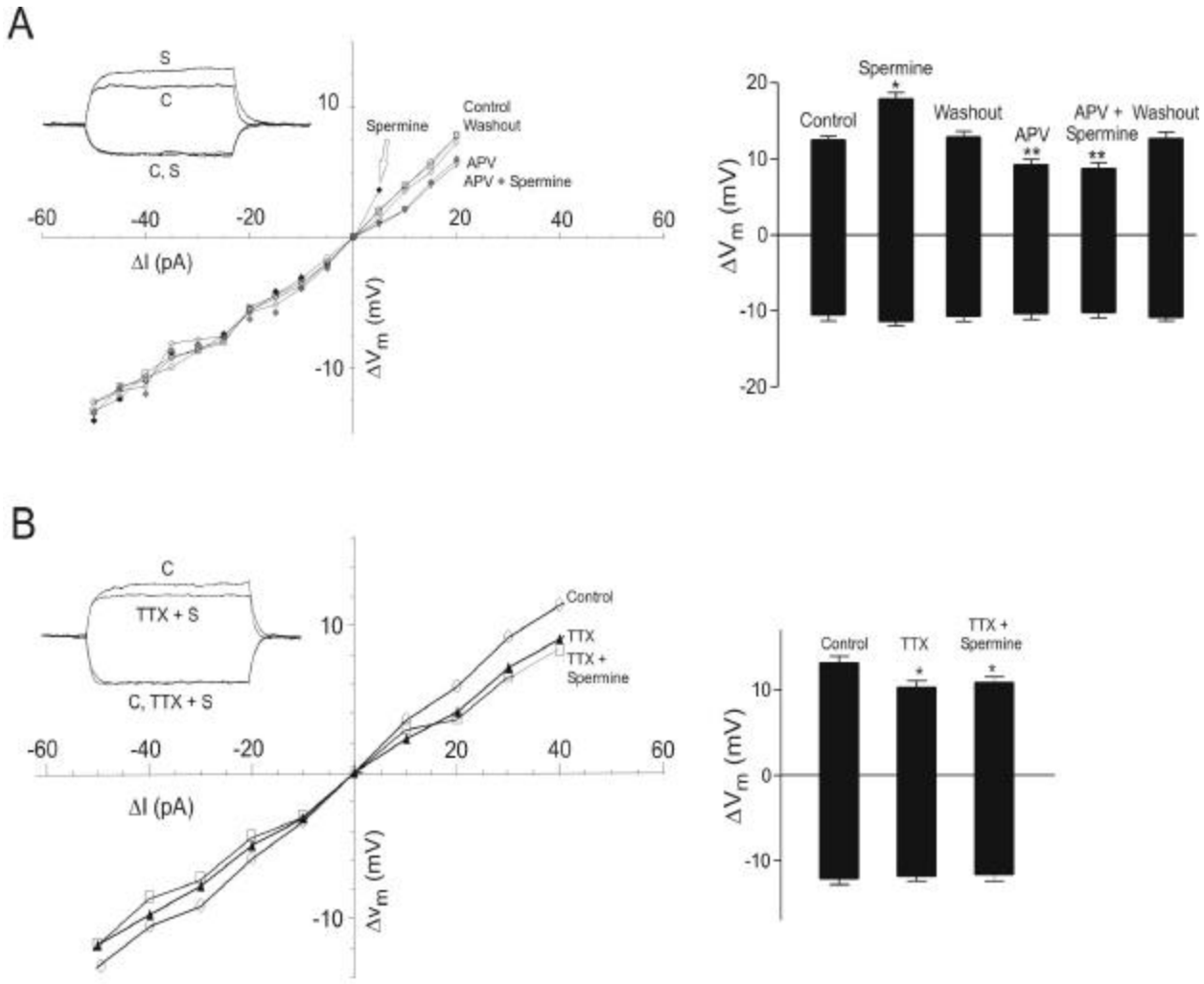

A
S
C
C, S
10
-10
-60
-40
-20
20
40
60
ΔI (pA)
ΔVm (mV)
Spermine
Control
Washout
APV
APV + Spermine
20
10
0
-10
-20
Control
Spermine
Washout
APV
APV + Spermine
Washout
B
C
TTX + S
C, TTX + S
Control
TTX
TTX + Spermine
Control
TTX
TTX + Spermine

Fig. 5. Spermine effects on voltage rectification depend on extra- and intracellular [Ca^{2+}]. (A) Spermine-induced increase in voltage rectification depended on extracellular [Ca^{2+}]. *Upper traces*: Voltage responses to depolarizing current pulses (80 pA, 500 ms). After sampling the control, a Ca^{2+}- free medium was applied extracellularly for 6 min and did not change the amplitude or shape of the voltage response. A subsequent application of spermine for 3 min (100 ?M) with 0 [Ca^{2+}] did not alter the voltage response to injected current. Recovery was observed after a 10 min washout. A subsequent spermine application (100 ?M, 3 min) in normal medium (2 mM Ca^{2+}) shows an increase in the voltage response to depolarization by current injection. *Bottom*: The I-V diagram of the same neuron shows that spermine does not change the voltage rectification in response to depolarizing pulses in 0 Ca^{2+}. When spermine was applied in normal Ca^{2+}, it increased voltage rectification for depolarizing pulses > 25 pA. (B) Spermine-induced increase in voltage rectification depended on intracellular Ca^{2+}. Neurons contained intracellular BAPTA. *Upper traces*: Voltage responses to depolarizing current pulses (80 pA, 500 ms). Spermine application for 3 min did not cause any change in the voltage response induced by depolarizing current pulses. *Bottom*: The I-V diagram of the same neuron shows that spermine does not change the voltage rectification in response to depolarizing pulses. Vertical bar, 10 mV; horizontal bar, 100 ms.

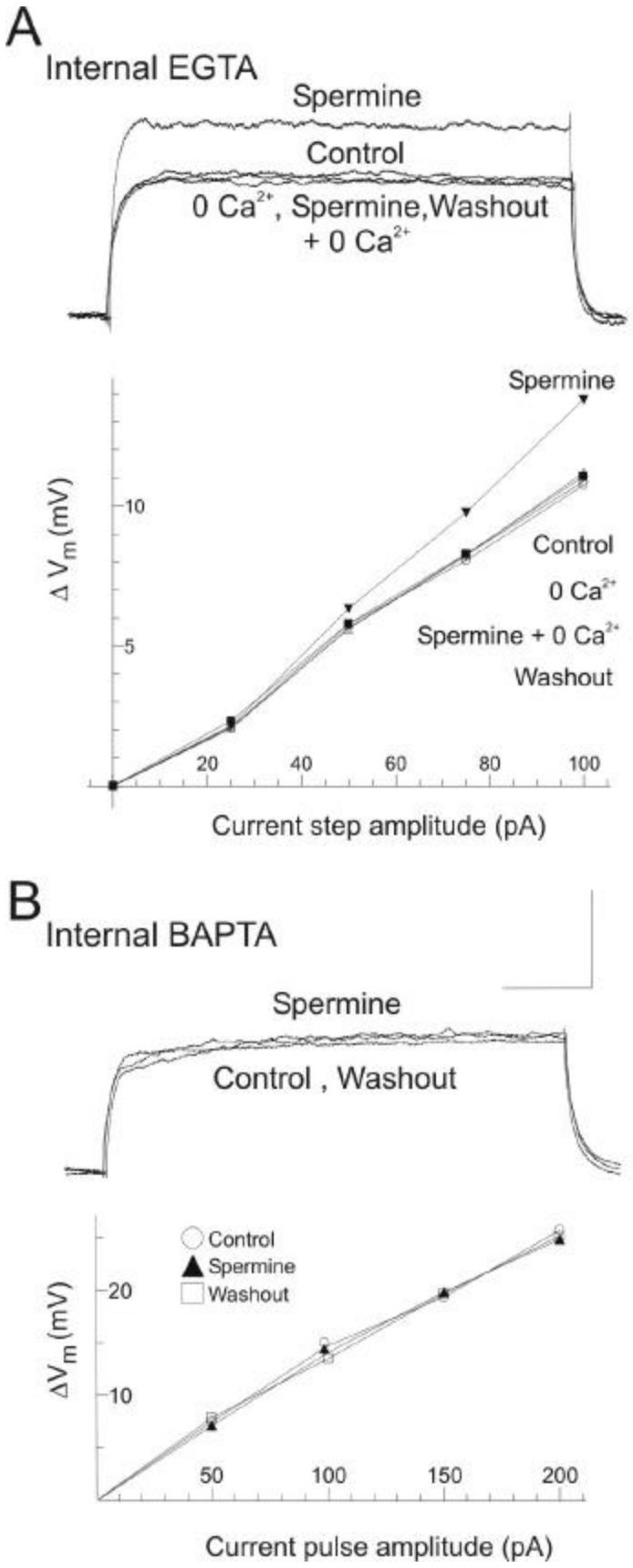

A
Internal EGTA
Spermine
Control
0 Ca2+, Spermine,Washout
+ 0 Ca2+
Spermine
Control
0 Ca2+
Spermine + 0 Ca2+
Washout
Δ Vm (mV)
10
5
20
40
60
80
100
Current step amplitude (pA)
B
Internal BAPTA
Spermine
Control , Washout
Control
Spermine
Washout
ΔVm (mV)
20
10
50
100
150
200
Current pulse amplitude (pA)

Fig. 6. Effects of spermine (100 ? M, 3 min) on the low threshold Ca^{2+}-spike (LTS) firing. (A) With spermine application, a LTS occurred on termination of the hyperpolarizing current pulse (- 40 pA, 500 ms). Superimposed voltage responses in control, spermine, and recovery. (B) Effects of spermine on the rate of rise (dV/dt) of the LTS. Spermine increased the dV/dt and the amplitude of the LTS. Voltage responses show the LTS at the end of a hyperpolarizing current pulse (-80 pA) just before (1), during (2), and after (3) spermine application. Holding potential, -55 mV. (C) Bar graph summarizes spermine effects on dV/dt of the LTS. Neurons were kept at 4 holding potentials and injected with hyperpolarizing current pulses. Spermine increased dV/dt in neurons held at -85, -75, -65, and, maximally, at -55 mV (n = 6, paired t-test, * P <0.01, ** P< 0.005). (D) APV did not block the effects of spermine on the LTS. Sub- and suprathreshold voltage responses to current pulses during sequential applications of APV, alone, and co-applied with spermine. Application of APV reduced the subthreshold voltage response in response to depolarizing current pulses, increased the rate of rise of the LTS and the latency to the first action potential on the LTS, and transformed the burst to a single action potential. A subsequent co-application of APV and spermine (3 min) resulted in a transformation of a subthreshold voltage response to an LTS, an increase in the rate of rise of the LTS, and a shortening of the latency to the action potential on the LTS. Recovery (in APV) was observed after a 10 min washout in voltage responses that were subthreshold in the absence of spermine (Control, APV and Washout (in APV)). The current pulse amplitude that evoked a subthreshold voltage response was + 60 pA and suprathreshold responses +120 pA. Vertical bar, 15 mV in A and B; 30 mV in C. Horizontal bar, 150 ms. *Insert in B (right)*: Vertical bar, 3 mV; horizontal bar, 30 ms.

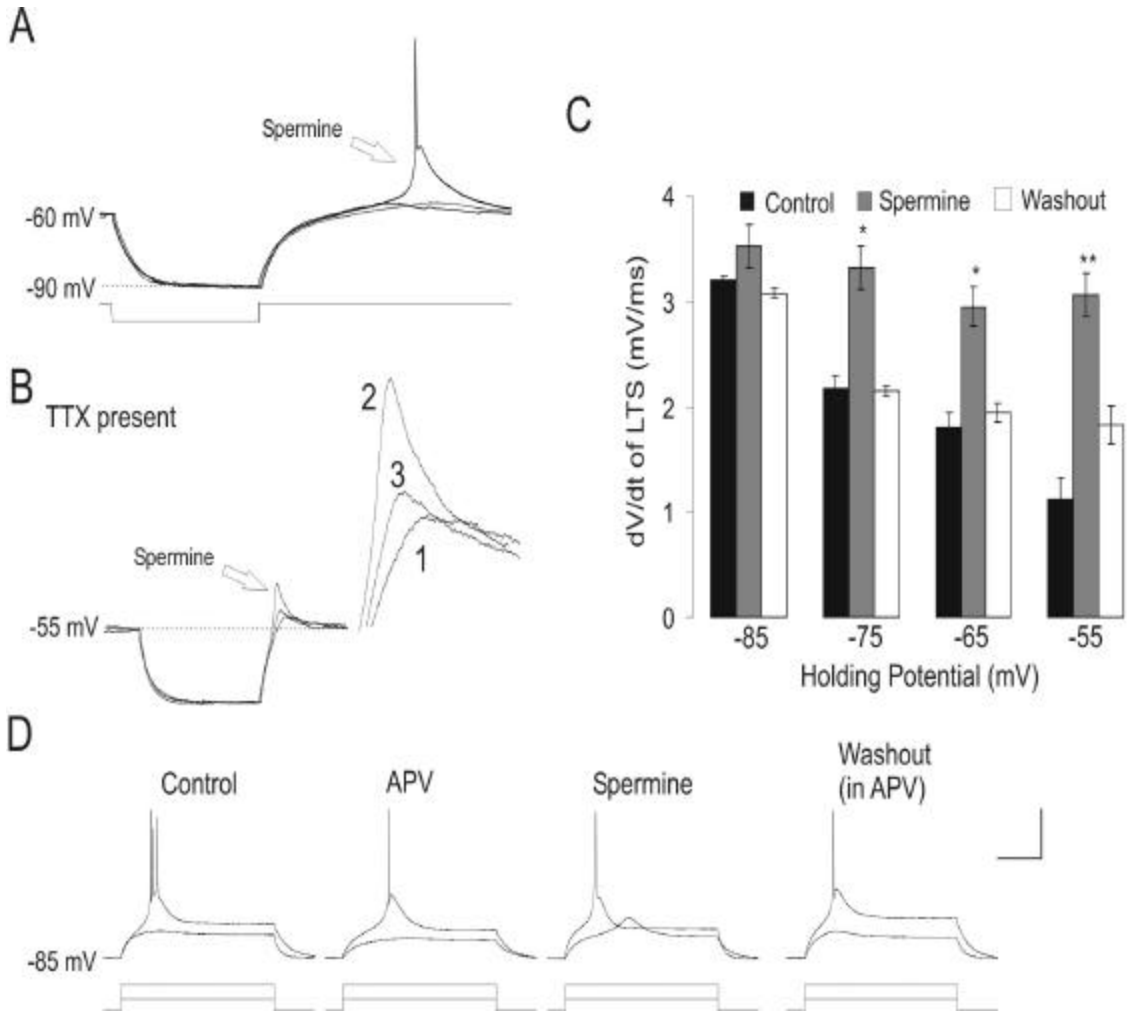

A
Spermine
-60 mV
-90 mV
B
TTX present
2
3
1
Spermine
-55 mV
C
Control
Spermine
Washout
dV/dt of LTS (mV/ms)
0
1
2
3
4
*
*
**
-85
-75
-65
-55
Holding Potential (mV)
D
Control
APV
Spermine
Washout
(in APV)
-85 mV

Fig. 7. Spermine (100 ?M, 3 min) prolonged the NMDA receptor-mediated component of EPSPs. EPSPs evoked by extracellular electrical stimulation. (A) Spermine application boosted the subthreshold EPSP evoked by electrical stimulation resulting in 3 action potentials. Spermine prolonged the decay phase of NMDA-mediated EPSPs in an MGB neuron. (B) Spermine had little or no effect on the early component but delayed the late component (2) of the EPSPs. The records were taken during control (1) spermine (2) and after a 30 min recovery period (3). The bar graph summarizes the spermine-induced changes in EPSP decay time constant expressed as % of the control. Control decay time constant was 142 ± 8.5 ms (n = 15, paired t-test, * $P < 0.01$). (C) Spermine affected the EPSPs mediated by NMDA-receptors. Spermine did not significantly change the EPSP components that were not mediated by NMDA-receptors. After a control period (1), a 6 min application of an NMDA receptor antagonist, APV, abolished the late component of the EPSP (2). A subsequent co-application of spermine (3 min) had little or no effect on the amplitude or duration of the remaining EPSP (3). Bar graph summarizes the changes in EPSP decay time constant expressed as % of the control. Control decay time constant was 143.6 ± 14 ms (n = 8, ANOVA, * $P < 0.01$). Holding potential, –60 mV in A,C and –65 mV in B. Vertical bar, 5 mV; horizontal bar, 200 ms.

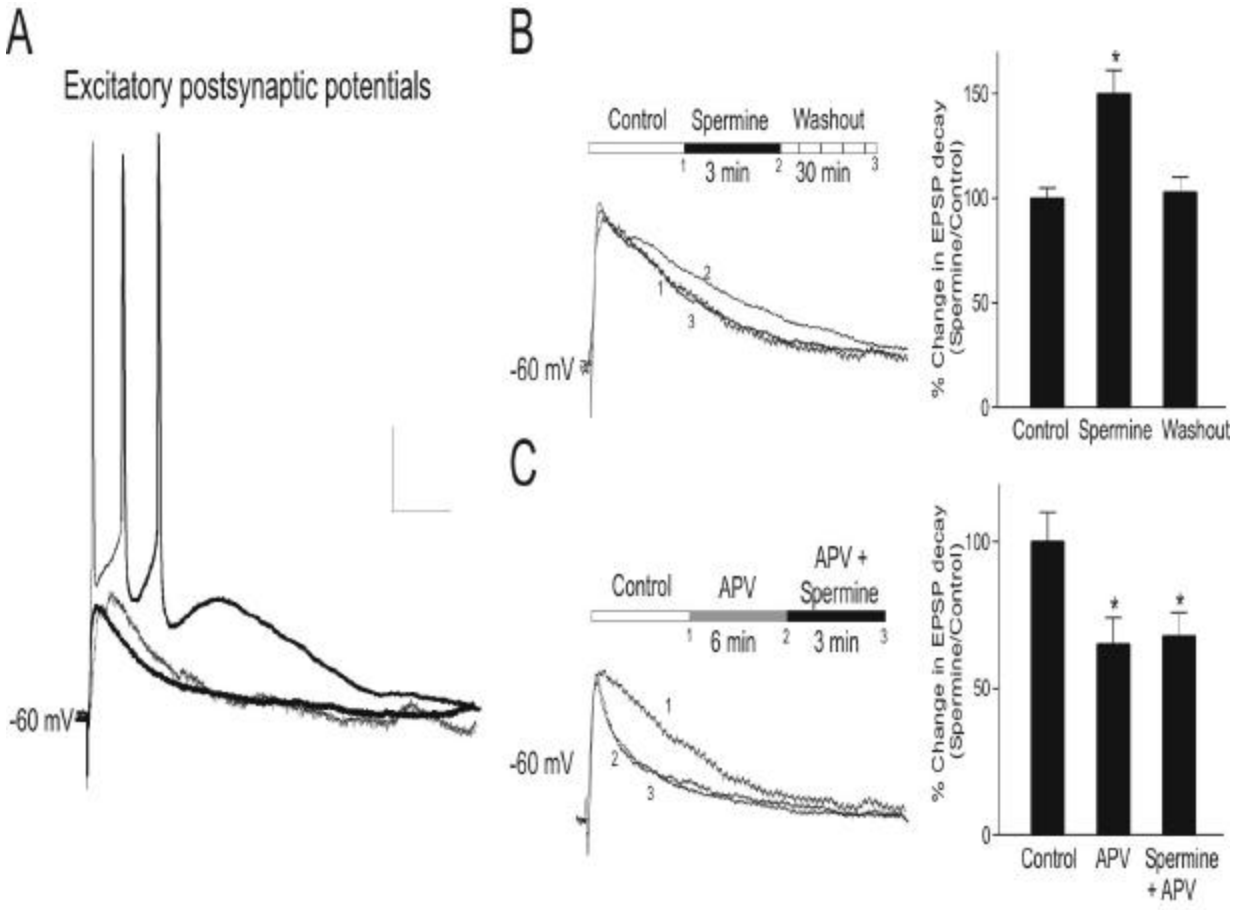
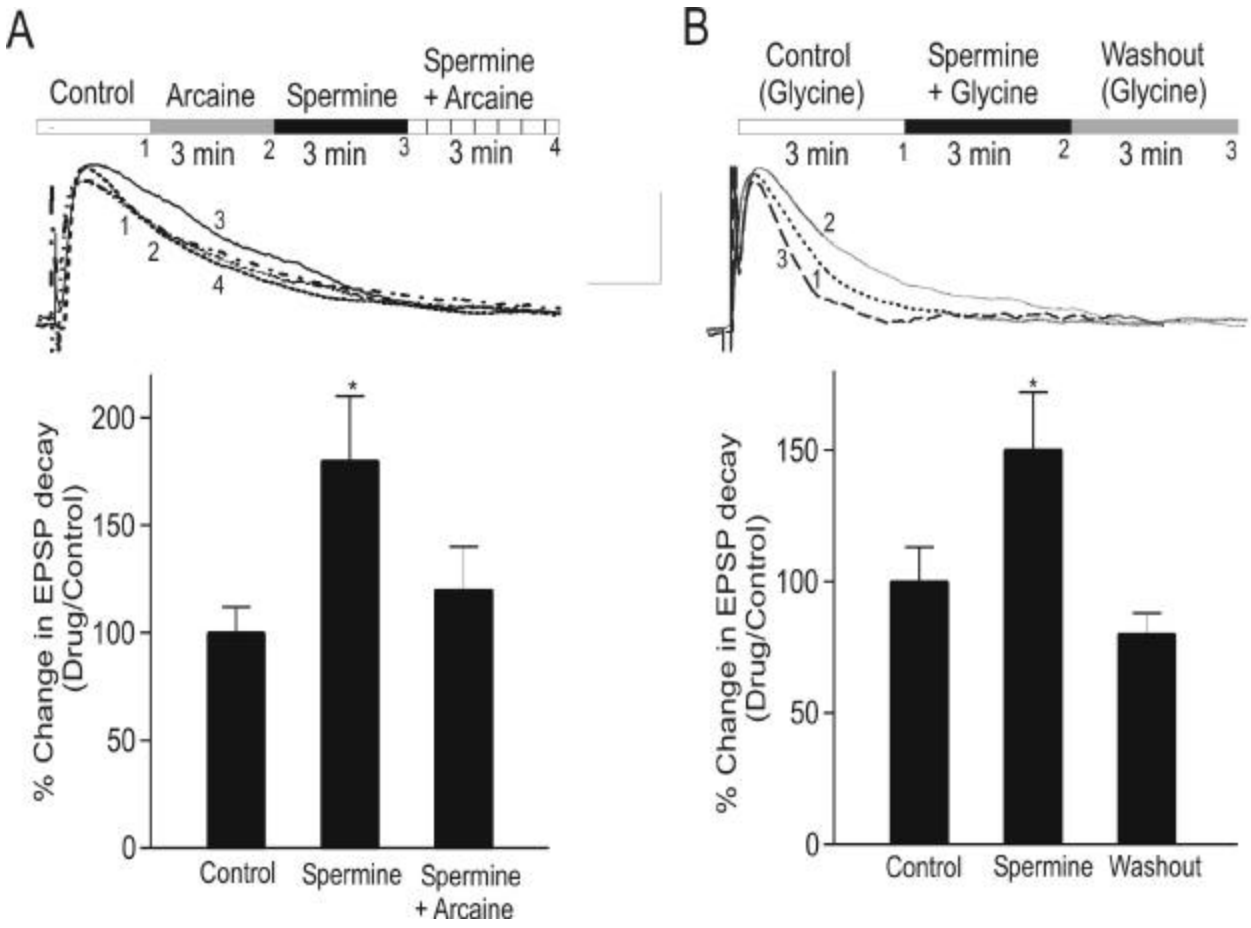

A
Excitatory postsynaptic potentials
-60 mV
B
Control
Spermine
Washout
3 min
30 min
-60 mV
% Change in EPSP decay (Spermine/Control)
Control
Spermine
Washout
C
Control
APV
APV + Spermine
6 min
3 min
-60 mV
% Change in EPSP decay (Spermine/Control)
Control
APV
Spermine + APV

Fig. 8. Spermine prolonged the EPSPs by interacting with the polyamine sensitive site on NMDA receptor. (A) Arcaine (100 μM), an antagonist at the polyamine sensitive site on NMDA receptor, greatly alleviated the slow decay of the EPSP induced by spermine (100 μM). The superimposed EPSPs were taken during a control period just prior to application of arcaine (1), and at the end of 3 min applications of arcaine (2), spermine alone (3), and spermine plus arcaine (4). Control time decay was 100.4 ± 16 ms (n = 3, ANOVA, * $P < 0.05$). Compared to the control (1), arcaine alone (2) had little or no effect on the amplitude or duration of the EPSP. Arcaine (4) almost completely blocked the spermine-induced slow decay of the EPSP (3). (B) Spermine (100 μM) slowed the decay of the EPSP during co-application with glycine (10 μM). The superimposed EPSPs were taken at the end of 3 min sequential applications of glycine (1), glycine + spermine (2), and recovery in glycine (3). Control decay time = 255 ± 44 ms (n = 3, paired t-test, * $P < 0.05$). Neurons in A and B were held at – 60 mV. Bar graphs in A and B summarize the changes in EPSP decay time constants (ratio of drug/control). Decay values were taken from the alpha-fits of the EPSPs, as in the previous Figure. Calibration: Vertical bar, 5 mV; horizontal, 100 ms.


A
Control
Arcaine
Spermine
Spermine + Arcaine
1
3 min
2
3 min
3
3 min
4
1
2
3
4
% Change in EPSP decay (Drug/Control)
200
150
100
50
0
*
Control
Spermine
Spermine + Arcaine
B
Control (Glycine)
Spermine + Glycine
Washout (Glycine)
3 min
1
3 min
2
3 min
3
2
3
1
% Change in EPSP decay (Drug/Control)
150
100
50
0
*
Control
Spermine
Washout