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Xenon Attenuates Excitatory Synaptic Transmission in the Rodent Prefrontal Cortex and Spinal Cord Dorsal Horn

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Background: **The molecular mechanisms of the inhalational anesthetic xenon are not yet fully understood. Recently, the authors showed that xenon reduces both *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor–mediated synaptic transmission in a brain slice preparation of the amygdala. In the current study, the authors examined the effects of xenon on synaptic transmission in the prefrontal cortex and the spinal cord dorsal horn (substantia gelatinosa).**

Methods: **In rodent brain or spinal cord slice preparations, the authors used patch clamp technique to investigate the impact of xenon on NMDA and AMPA receptor–mediated excitatory postsynaptic currents, as well as on γ-aminobutyric acid type A receptor–mediated inhibitory postsynaptic currents. The currents were either evoked upon electrical stimulation (NMDA-eEPSCs and AMPA-eEPSCs) or upon photolysis of caged L-glutamate (p-NMDA-Cs and p-AMPA-Cs). In addition, the authors investigated the effects of xenon on AMPA receptor–mediated miniature excitatory postsynaptic currents.**

Results: **In both central nervous system regions, xenon had virtually no effect on inhibitory postsynaptic currents. In the prefrontal cortex (spinal cord), xenon reversibly reduced NMDA-eEPSCs to approximately 58% (72%) and AMPA-eEPSCs to approximately 67% (65%) of control. There was no difference in the xenon-induced reduction of NMDA-eEPSCs and p-NMDA-Cs, or AMPA-eEPSCs and p-AMPA-Cs. Xenon did not affect the frequency of miniature excitatory postsynaptic currents but reduced their amplitude.**

Conclusions: **In the current study, the authors found that xenon depresses NMDA and AMPA receptor–mediated synaptic transmission in the prefrontal cortex and the substantia gelatinosa without affecting γ-aminobutyric acid type A receptor–mediated synaptic transmission. These results provide evidence that the effects of xenon are primarily due to postsynaptic mechanisms.**

THE inhalational anesthetic xenon combines profound anesthetic and analgesic properties with a low side effect profile. A number of clinical trials during the past years have evaluated the safety and efficacy of xenon anesthesia in human patients.[1,2] However, the detailed mechanisms by which xenon exerts its anesthetic and analgesic properties are still a matter of debate.[3]

Anesthesia, when simplistically described as a status with reduced neuronal activity, might emerge from an enhancement of inhibitory or reduction of excitatory central nervous system (CNS) signaling or a combination of both.

Current knowledge speaks in favor of the excitatory rather than the inhibitory transmitter system to transduce the molecular mechanisms of xenon. Studies using heterologously expressed *N*-methyl-D-aspartate (NMDA) receptors[4] or neurons in culture[5,6] brought evidence that the NMDA receptor might be a major target for xenon action. However, meanwhile, several studies provide evidence for non-NMDA receptors to play an important role in the xenon anesthetic state. Current flow through heterologously expressed non-NMDA receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate type are reduced in the presence of xenon,[7,8] as are non-NMDA receptor–mediated currents recorded from cultured cortical neurons.[8] In addition, the two-pore domain–related potassium channel type 1 has been shown to be activated by xenon,[9] possibly contributing to the xenon anesthetic state. Consistent with these *in vitro* findings, *in vivo* studies using the model organism *Caenorhabditis elegans*[10] or human volunteers[11,12] provide evidence that mechanisms beyond an exclusive NMDA receptor antagonism play a role in mediating the xenon anesthetic state.

In a recently published study,[13] we demonstrated that xenon depresses both NMDA and AMPA receptor–mediated excitatory while not affecting γ-aminobutyric acid type A ($GABA_A$) receptor–mediated inhibitory synaptic transmission in an acute murine brain slice preparation of a subcortical brain structure, the basolateral amygdala. Because the basolateral amygdala is critically involved in anesthetic-induced amnesia,[14,15] addictive behavior, and the formation of negative emotions such as fear (review: Sah *et al.*[16]), these results may explain the amnestic and memory-ablating properties of xenon. However, detailed investigations on the neuronal mechanisms of xenon action in other CNS areas are missing.

In the current study, we therefore determined the impact of xenon on $GABA_A$, NMDA, and AMPA receptor–mediated synaptic transmission in two distinct CNS areas that might be critically involved in producing the hypnotic and analgesic effects of xenon. The prefrontal cortex (PFC) was chosen as a cortical brain area being critically involved in higher brain functions such as consciousness (review: Bodovitz[17]), whereas the substantia

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gelatinosa (SG) of the spinal cord dorsal horn, as the first site of synaptic integration of nociceptive inputs,[18,19] is involved in mechanisms of pain processing.

Materials and Methods

The experimental protocols were approved by the Ethical Committee on Animal Care and Use of the Government of Bavaria, Germany. For the experiments in the PFC, 28- to 42-day-old male C57Bl6 mice were killed by cervical dislocation, and the brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing 125 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 25 mM D-glucose, and 1.25 mM NaH_2PO_4 (all from RBI/Sigma, Deisenhofen, Germany). Saturation with a mixture of 95% O_2–5% CO_2 (carbogen gas) led to a pH of 7.4. Sagittal slices (350 μm thick) were prepared using a microtome (HM 650 V; Microm International, Walldorf, Germany). For the experiments in the SG of the spinal cord dorsal horn, 14- to 28-day-old male Wistar rats were killed by cervical dislocation, and coronary slices of lumbar spinal cord were obtained as described previously.[20] All slices were allowed to recover in a storage chamber (34°C) for at least 1 h before being transferred to the recording chamber.

A platinum ring with nylon filaments was used to fix the slices on the bottom of the recording chamber, which was continuously perfused (2 ml/min) with ACSF. We used infrared videomicroscopy and the gradient contrast system (Zeiss, Oberkochen, Germany; for details, see Dodt *et al.*[21]) to visualize the neurons within layer V of the PFC or within the SG. The SG was clearly identifiable as a translucent band across the superficial dorsal horn.

The patch pipettes were pulled from thin-walled borosilicate glass tubes with inner filament (OD 1.5 mm, ID 1.17 mm, GC150TF-10; Clark Electromedical Instruments, Pangbourne Reading, United Kingdom) and heat polished using a two-step horizontal puller (DMZ-Universal Puller; Zeitz-Instruments, Munich, Germany). Pipettes had an open tip resistance of 4–6 MΩ when filled with a solution containing 130 mM K-D-gluconate, 5 mM KCl, 0.5 mM EGTA, 2 mM $MgCl_2$, 10 mM HEPES, 5 mM D-glucose, and 20 mM Na_2-phosphocreatine (all from RBI/Sigma). Currents were recorded with a switched voltage clamp amplifier (SEC 10 l; NPI electronic, Tamm, Germany) with switching frequencies of 60–80 kHz (25% duty cycle). Series resistance was monitored continuously and compensated in bridge mode. All patch clamp experiments were performed at room temperature (22°–25°C) to ameliorate oxygenation of the neurons.

Electrically evoked postsynaptic currents (ePSCs) were elicited by square pulse stimuli (6–100 V, 0.05–0.5 ms, interstimulus interval 15 s) delivered *via* a bipolar tungsten electrode, which was placed either in layer V near the apical dendrite of the recorded neuron (5-μm electrode tip diameter) or in the dorsal root entry zone of the spinal cord slices (50-μm electrode tip diameter). Preceding each tissue stimulation, neuronal input resistance was determined by injecting a current pulse hyperpolarizing by 10 mV for 200 ms. In the PFC, in a subset of experiments, paired-pulse stimulation was performed by delivering the same stimulus at 50-ms interpulse intervals. The paired-pulse ratio was determined by dividing the amplitude of the second current response by the amplitude of the first one.

Photolytically evoked excitatory currents were induced upon focal photolysis of caged L-glutamate.[21] For this purpose, the beam of an ultraviolet laser (355 nm wavelength, frequency-tripled Nd:YVO4, 100-kHz pulse repetition rate; DPSS Lasers, San Jose, CA) was focused by the objective (×60, 0.9 numerical aperture; Olympus, Tokyo, Japan) on a small spot (5 μm in diameter) positioned on a dendrite approximately 10–20 μm from the soma. Laser stimulation was delivered alternating with electrical stimulation in intervals of 15 s. When a stable whole cell recording had been obtained, γ-α-carboxy-2-nitrobenzyl caged glutamate at a final concentration of 0.25 mM was added to the recirculating perfusate. Caged glutamate had no discernible effect on neurons *per se*.[21] Glutamate was released by Q-switching brief laser pulses (3–5 ms; intensity 1–2 mW), applied at regular intervals of 30 s throughout the experiment.

To isolate specific currents, we used d(−)-2-amino-5-phosphonopentanoic acid (AP5; 50 μM), 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX; 5 μM), 3-amino-propyl(diethoxymethyl)phosphinic acid (CGP35348; 200 μM), and bicuculline methiodide (20 μM). Of these four receptor antagonists, an appropriate cocktail was used, with NBQX omitted for AMPA, AP5 omitted for NMDA, and bicuculline methiodide omitted for $GABA_A$ receptor–mediated responses. For the experiments in the spinal cord, strychnine at a final concentration of 2 μM was added to the ACSF, and for recordings of NMDA receptor–mediated currents in the spinal cord, $MgCl_2$ was reduced to 0.5 mM. For AMPA receptor–mediated currents, the holding potential was set to −70 mV, and for NMDA and $GABA_A$ receptor–mediated currents, the holding potential was set to −30 and −50 mV, respectively. Miniature excitatory postsynaptic currents (mEPSCs) were continuously recorded (10 min) at −70 mV in the presence of 1 μM tetrodotoxin, 50 μM AP5, 200 μM CGP35348, and 20 μM bicuculline methiodide. mEPSCs were completely blocked in the presence of 5 μM NBQX (data not shown).

The whole cell responses were amplified, low-pass filtered (3 kHz), and then digitized (ITC-16 Computer Interface; Instrutech Corp., Port Washington, NY) with a sampling frequency of 9 kHz and stored to a hard drive (Power Macintosh G3 computer, data acquisition soft-

ware Pulse version 8.5; Heka electronic GmbH, Lambrecht, Germany). The obtained data were analyzed with IGOR Pro software (WaveMetrics, Portland, OR). For detection of mEPSCs, the amplitude threshold was defined as the threefold amplitude of the baseline variance (noise level), and the events identified were subsequently verified visually. We then quantified the frequency and peak amplitudes of the detected events.

Under control conditions, the superfusing ACSF was exposed to a mixture of 65% N_2–30% O_2–5% CO_2; for xenon application, the ASCF was gassed with a mixture of 65% xenon–30% O_2–5% CO_2. In a subset of experiments, xenon was applied at a lower concentration using a gas mixture of 30% xenon–35% N_2–30% O_2 and 5% CO_2. Replacement of N_2 by xenon did not change the pH of the gassed ACSF. We have shown repeatedly[13,22] that it is feasible to obtain stable synaptic responses from brain slice preparations for at least 40 min under these conditions with relatively low oxygen concentration. All gas mixtures were purchased from Linde AG (Unterschleissheim, Germany) and applied at a flow rate of 0.3–0.5 l/min to the ACSF reservoir. Oxygen and carbon dioxide concentrations were verified with a calibrated gas monitor (Datex Capnomac Ultima, Duisburg, Germany). Tubing was made of polytetrafluoroethylene (Teflon; VWR International, Darmstadt, Germany) to minimize loss of xenon.

Concentration measurements of dissolved xenon were accomplished using headspace gas chromatography (RCC Ltd., Itingen, Switzerland). When gassing the ACSF with the 65% (30%) xenon gas mixture, the concentration of xenon in ACSF was 1.9 ± 0.2 mM (1.1 ± 0.1 mM) (mean ± SD; n = 4). For comparison, other studies describe xenon saturation of 3.9 mM [23] or 2.0 mM [4] when gassing with pure xenon, 3.4 mM when gassing with 80 vol% xenon,[6,7] or 3.54 mM when gassing with 84 vol%,[8] which would result in a calculated mean of approximately 2.3 mM at an assumed 65 vol% xenon saturation. The difference from our obtained concentration of 1.9 mM might be explained by an increased evaporation due to our open-chamber system.

Tetrodotoxin, AP5, NBQX, bicuculline methiodide, strychnine, γ-α-carboxy-2-nitrobenzyl caged glutamate (all from RBI/Sigma), and CGP35348 (Novartis Laboratories, Basel, Switzerland) were bath applied at known concentrations *via* the superfusion system.

Statistical Analysis

For all statistical evaluations, SPSS Statistics version 16 (SPSS GmbH Software, Munich, Germany) was used. The 40-min recording time of ePSCs, electrically evoked inhibitory postsynaptic currents (eIPSCs), electrically evoked excitatory postsynaptic currents (eEPSCs), and photolytically evoked currents was first partitioned in equidistant subintervals of 5 min, and then the averaged relative amplitudes in each of them were determined. A two-factorial multivariate analysis of variance (MANOVA) with repeated-measures design was applied on the averaged relative amplitudes with interval as a within-subjects factor and kind of receptor as a between-subjects factor. Differences between the various intervals were tested by tests with contrasts, and differences between the various receptors and stimulation methods were tested by *post hoc* tests. MANOVA was also performed for testing differences in the amplitudes and frequency of mEPSCs between the control and xenon recordings. As the nominal level of significance, we accepted $\alpha = 0.05$. It was corrected (according to Bonferroni procedure) for all *a posteriori* tests (tests with contrasts and *post hoc* tests). Numerical data are presented as mean ± SEM with the number of experiments (= neurons) indicated, if not stated otherwise. In graphs where error bars are not shown, they are smaller than the size of the symbol.

Results

In a first set of experiments, the effect of 1.9 mM xenon on compound synaptic transmission in the PFC or SG was examined. Compound postsynaptic currents upon electrical stimulation (ePSCs) were recorded from neurons in the PFC (layer V) or the SG. Ten to 15 min after xenon application, the ePSCs recorded from the PFC and SG were reduced to 70.1 ± 10.3% and 74.3 ± 4.2%, respectively (figs. 1A and B). The averaged relative amplitudes in this interval were significantly lower than in the two control intervals (tests with contrasts in MANOVA, PFC: $P = 0.022$, SG: $P = 0.003$), with no significant difference between PFC and SG (Wilks multivariate test, $P = 0.661$). Upon termination of xenon application, the ePSCs nearly reversed to control level. Application of xenon for a longer period (up to 25 min) did not result in an additional reduction of current responses (data not shown). Neither resting membrane potential (PFC: control −71.3 ± 0.9 mV, xenon −71.1 ± 0.2 mV; SG: control −50.6 ± 2.2 mV, xenon −52.8 ± 4.7 mV) nor input resistance (PFC: control 272.2 ± 26.7 MΩ, xenon 294.3 ± 29.9 MΩ; SG: control 823.7 ± 117.8 MΩ, xenon 789.9 ± 117.0 MΩ) of the neurons were changed in the presence of 1.9 mM xenon.

From neurons in the PFC and SG, electrically evoked $GABA_A$ receptor–mediated eIPSCs ($GABA_A$-eIPSCs) were recorded in the presence of 50 μM AP5, 5 μm NBQX, and 200 μM CGP35348 at a holding potential of −50 mV. Xenon, 1.9 mM, did not affect the amplitudes of $GABA_A$-eIPSCs (figs. 1C and D) in the PFC or in the SG. The decay of the $GABA_A$-eIPSCs was fitted biexponentially, with two time constants, τ_{decay} fast and τ_{decay} slow, and total charge transfer was calculated by integrating the area under the $GABA_A$-eIPSCs. In both CNS regions, we did not detect an apparent influence of xenon on either of these parameters (table 1).

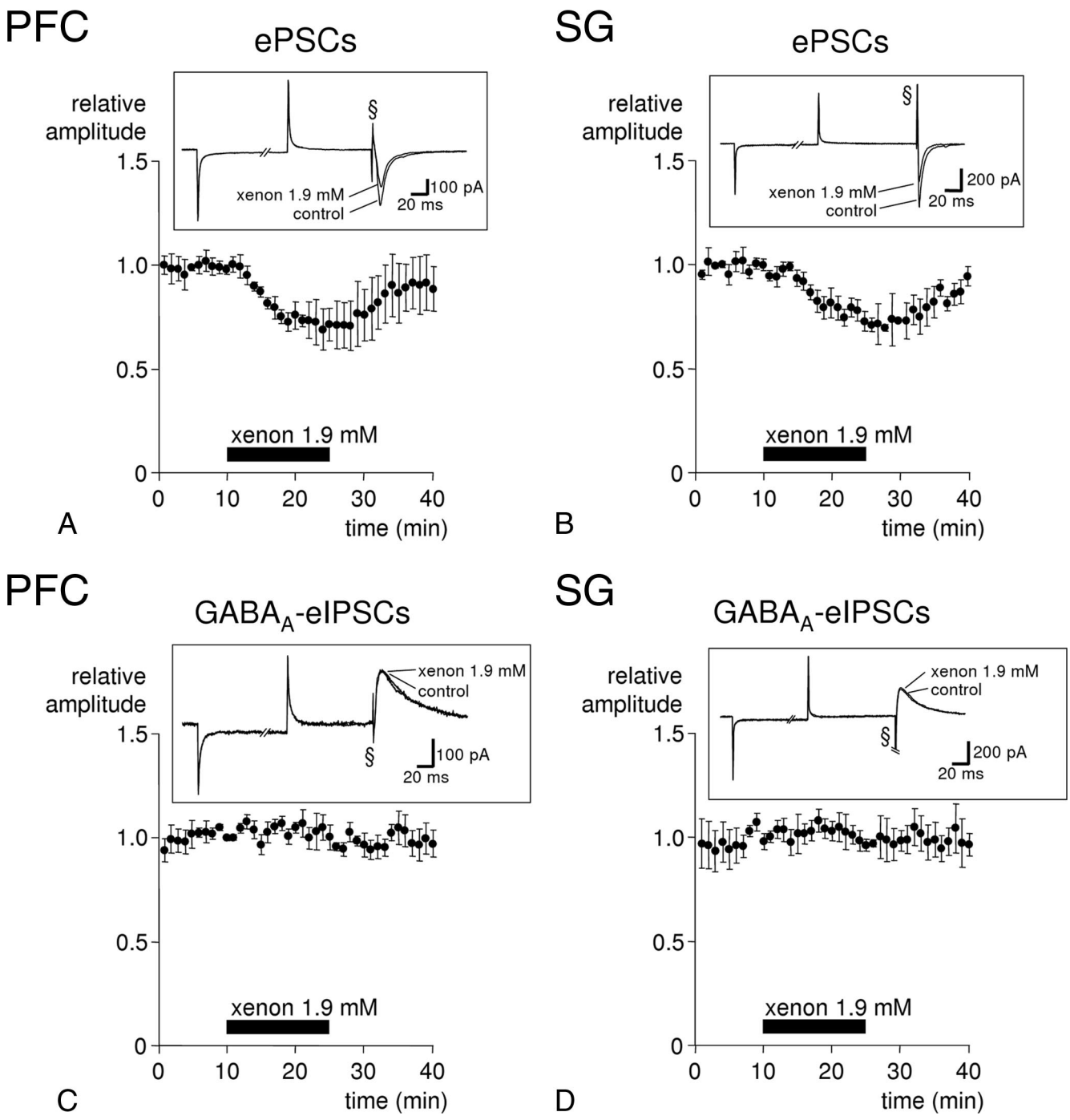

Fig. 1. In acute slice preparations of the prefrontal cortex (PFC) and substantia gelatinosa (SG), xenon reversibly reduces compound synaptic transmission but has no effect on γ-aminobutyric acid type A ($GABA_A$) receptor–mediated inhibitory synaptic transmission. (*A* and *B*) Compound postsynaptic currents, evoked upon electrical stimulation (ePSCs), were recorded from 5 (6) neurons in the PFC (SG). Application of 1.9 mM xenon diminished the ePSC amplitudes to 70.1 ± 10.3% (74.3 ± 4.2%) of control responses. Upon termination of xenon application, the ePSCs nearly recovered to control level. (*C* and *D*) $GABA_A$ receptor–mediated inhibitory postsynaptic currents evoked upon electrical stimulation ($GABA_A$-eIPSCs) were isolated using specific receptor antagonists and recorded at a holding potential of −50 mV. Xenon, 1.9 mM, did not affect amplitudes of $GABA_A$-eIPSCs in the PFC (*C*; n = 5) or in the SG (*D*; n = 4). Each *data point* represents either mean ePSC or $GABA_A$-eIPSC amplitude ± SEM normalized to the respective control values (5 min before start of xenon application, which is indicated by the *black bars*). *Insets* show representative current traces. § Stimulation artifact.

In further sets of experiments, the impact of xenon on NMDA and AMPA receptor–mediated excitatory synaptic transmission in the PFC (fig. 2) and SG (fig. 3) was investigated.

N-methyl-D-aspartate receptor–mediated current responses were evoked using either electrical stimulation (NMDA-eEPSCs) or photolytic uncaging of glutamate (p-NMDA-Cs). Xenon, 1.9 mM, reversibly diminished NMDA-

Table 1. Time Constants of Current Decay and Charge Transfer of $GABA_A$-eIPSCs

	Mean ± SEM			Mean Difference (95% CI)	
	Control	Xe	Wash	Control − Xe	Xe − Wash
PFC					
τ_{decay} fast, ms	25.5 ± 1.6	26.7 ± 1.7	32.6 ± 1.9	−2.4 (−7.8 to 2.9)	−5.5 (−12.9 to 1.6)
τ_{decay} slow, ms	75.7 ± 5.4	71.0 ± 2.2	68.6 ± 4.7	4.6 (−12.2 to 21.5)	1.6 (−14.2 to 17.4)
Charge transfer, pC	7.8 ± 2.1	8.8 ± 2.0	7.6 ± 0.7	0.98 (−2.7 to 0.8)	0.14 (−2.2 to 1.9)
SG					
τ_{decay} fast, ms	21.0 ± 4.4	19.8 ± 4.1	21.5 ± 0.5	1.2 (−4.3 to 6.8)	0.5 (−18.6 to 19.6)
τ_{decay} slow, ms	29.8 ± 4.7	34.5 ± 8.2	29.5 ± 5.5	−4.8 (−15.8 to 6.6)	−1.5 (−7.9 to 4.9)
Charge transfer, pC	1.7 ± 0.9	1.5 ± 0.7	1.2 ± 0.4	0.21 (−0.85 to 1.28)	0.35 (−1.0 to 1.69)

Time constants of current decay (τ_{decay} fast and τ_{decay} slow) and charge transfer of electrically evoked γ-aminobutyric acid type A receptor–mediated inhibitory postsynaptic currents ($GABA_A$-eIPSCs) tend not to be changed in the presence of 1.9 mM xenon (Xe) in both prefrontal cortex (PFC; n = 5) and substantia gelatinosa (SG; n = 4).

CI = confidence interval.

eEPSCs recorded from neurons in the PFC (SG) to 57.7 ± 4.4% (71.9 ± 3.4%) of control (PFC: n = 6, SG: n = 5; tests with contrasts in MANOVA, PFC: $P = 0.008$, SG: $P = 0.004$; figs. 2A and 3A). Photolytically evoked p-NMDA-Cs were reduced by xenon to 58.6 ± 3.6% (PFC; n = 6) and 71.5 ± 5.6% (SG; n = 5) of control (tests with contrasts in MANOVA, PFC: $P = 0.001$, SG: $P = 0.002$; figs. 2A and 3A). There was no significant difference in the degree of xenon-induced reduction when comparing NMDA-eEPSCs with p-NMDA-Cs in the PFC or in the SG (Bonferroni *post hoc* tests in MANOVA, PFC: $P = 0.951$, SG: $P = 0.937$). Moreover, when comparing the impact of xenon on NMDA receptor–mediated currents in the PFC with that in the SG, we did not see a significant difference (Wilks multivariate test, NMDA-eEPSC: $P = 0.166$, p-NMDA-Cs: $P = 0.104$). The current decay of NMDA-eEPSCs was fitted biexponentially, with two time constants, τ_{decay} fast and τ_{decay} slow. In both CNS

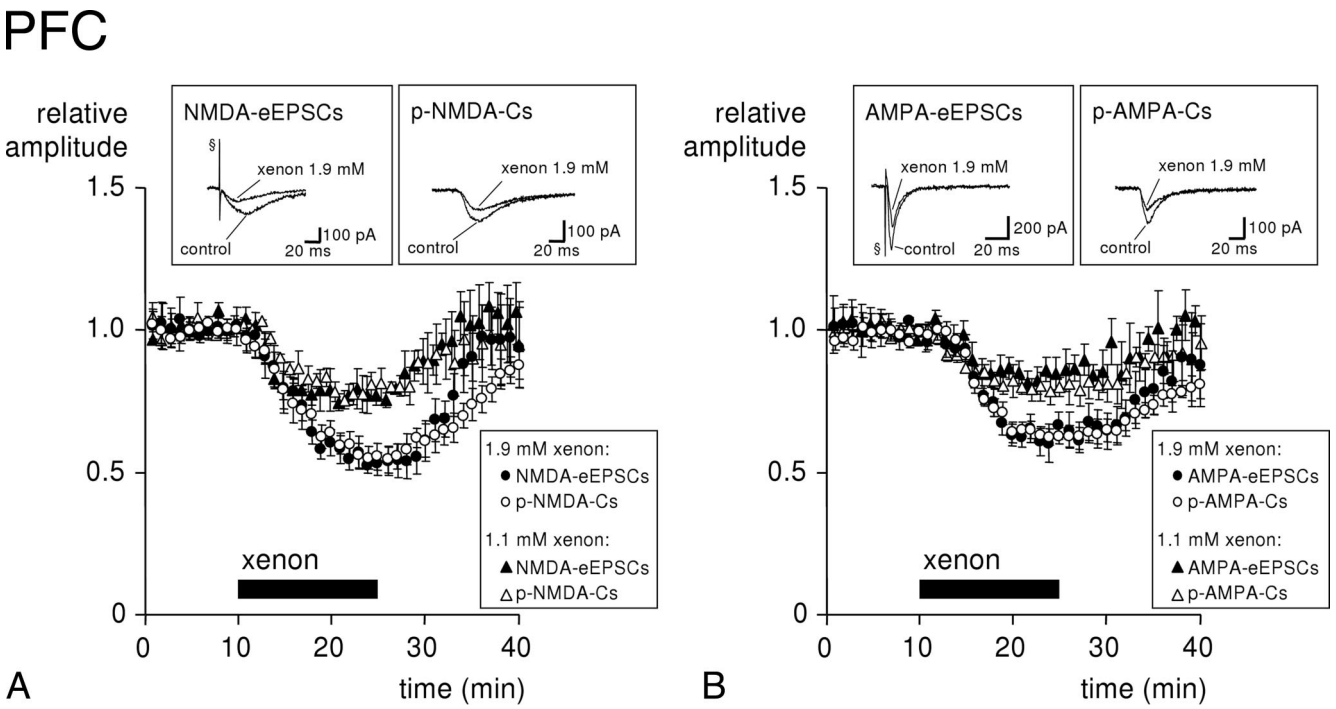

Fig. 2. In the prefrontal cortex (PFC), xenon diminishes *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor–mediated synaptic transmission. Pharmacologically isolated NMDA and AMPA receptor–mediated current responses, evoked either upon electrical stimulation (NMDA-eEPSCs and AMPA-eEPSCs; *closed symbols*) or upon focal photolysis of caged L-glutamate (p-NMDA-Cs and p-AMPA-Cs; *open symbols*), were recorded from neurons in layer V of the PFC. Each *data point* represents mean current amplitude ± SEM from six (*circles*) or four (*triangles*) neurons normalized to the 5-min period before start of xenon application. (*A*) Xenon, 1.9 mM (1.1 mM), reversibly reduced NMDA-eEPSC to 57.7 ± 4.4% (76.5 ± 3.7%) and p-NMDA-C amplitudes to 58.6 ± 3.6% (78.0 ± 4.1%). Under both xenon concentrations, the extent of reduction of NMDA-eEPSC and p-NMDA-C amplitudes did not differ (1.9 mM xenon: $P = 0.951$, 1.1 mM xenon: $P = 0.435$). (*B*) Likewise, xenon-induced reduction of AMPA-eEPSCs and p-AMPA-Cs did not differ significantly at 1.9 mM xenon (66.9 ± 4.1% *vs.* 63.7 ± 2.9%; $P = 0.826$) or at 1.1 mM xenon (83.3 ± 3.4% *vs.* 79.9 ± 4.2%; $P = 0.430$). *Insets* show representative current traces. § Stimulation artifact.

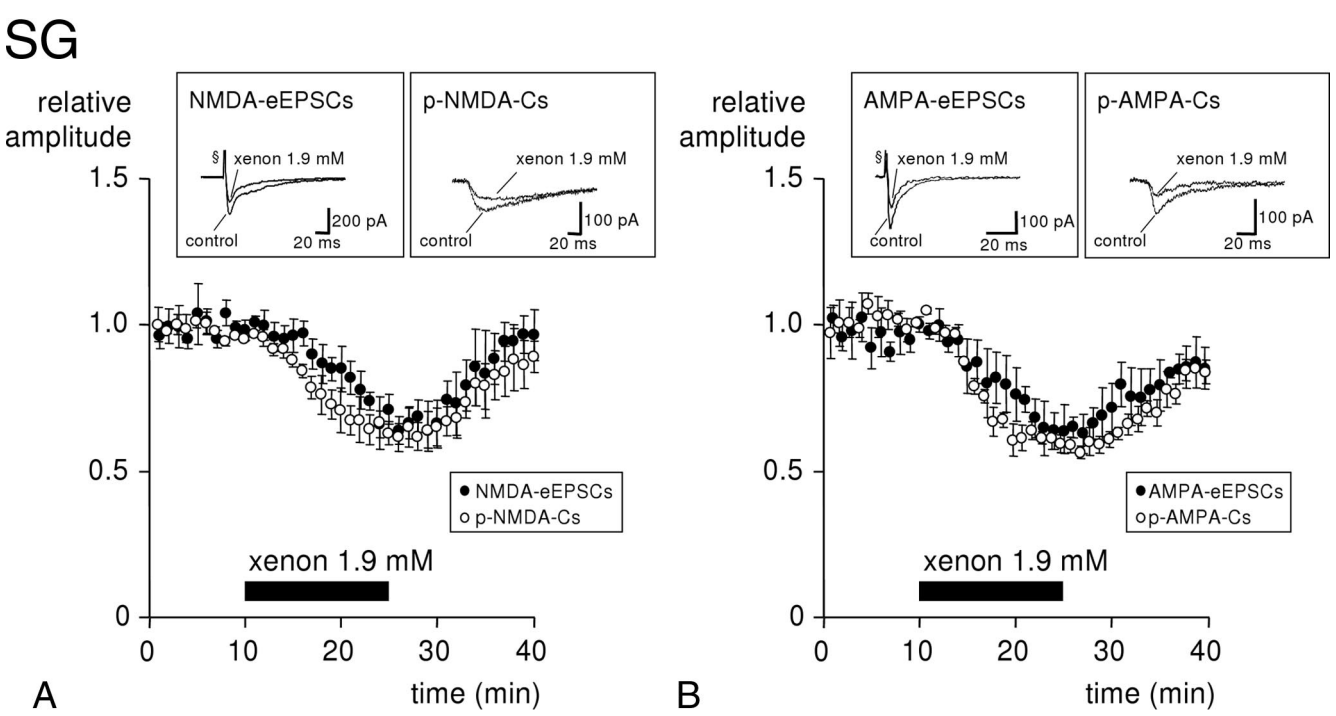

Fig. 3. In the substantia gelatinosa (SG), xenon diminishes *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor–mediated synaptic transmission. Pharmacologically isolated NMDA and AMPA receptor–mediated current responses were recorded from neurons in the SG of rat lumbar spinal cord slices. The currents were evoked either upon electrical stimulation of the dorsal root entry zone (NMDA-eEPSCs and AMPA-eEPSCs; *closed circles*) or upon focal photolysis of caged L-glutamate (p-NMDA-Cs and p-AMPA-Cs; *open circles*). Similar to the xenon action in the prefrontal cortex, neither the xenon-induced reduction of NMDA-eEPSCs (71.9 ± 3.4%, n = 5) and p-NMDA-Cs (71.5 ± 5.6%, n = 5) (*A*) nor the xenon-induced reduction of AMPA-eEPCSs (64.5 ± 6.5%, n = 5) and p-AMPA-Cs (58.2 ± 3.1%, n = 5) (*B*) was significantly different. Because photolytically evoked current responses are generated beyond the influence of the presynaptic terminal, the lacking difference between the reduction of NMDA-eEPSCs and p-NMDA-Cs on the one hand and AMPA-eEPSCs and p-AMPA-Cs on the other hand suggests a postsynaptic mechanism of xenon action for both receptors. Each *symbol* represents mean current amplitude ± SEM normalized to the 5-min period before start of xenon application. *Insets* show representative current traces. § Stimulation artifact.

regions, a clear influence of xenon on deactivation kinetics of NMDA-eEPSCs was not detectable (table 2).

Electrically (AMPA-eEPSCs) and photolytically (p-AMPA-Cs) evoked AMPA receptor–mediated currents were recorded in the PFC and SG. Xenon reduced the amplitudes of AMPA-eEPSCs to 66.9 ± 4.1% (PFC; n = 6; fig. 2B) and 64.5 ± 6.5% (SG; n = 5; fig. 3B). p-AMPA-Cs were reduced in the presence of xenon to 63.7 ± 2.9 (PFC; n = 5; fig. 2B)

Table 2. Time Constants of Current Decay of NMDA-eEPSCs and AMPA-eEPSCs

	Mean ± SEM			Mean Difference (95% CI)	
	Control	Xe	Wash	Control − Xe	Xe − Wash
PFC					
NMDA-eEPSC					
τ_{decay} fast, ms	31.6 ± 2.4	31.0 ± 2.1	33.8 ± 1.5	0.7 (−10.8 to 12.2)	−2.8 (−11.3 to 5.7)
τ_{decay} slow, ms	121.3 ± 21.2	100.6 ± 17.3	112.0 ± 13.4	20.8 (−8.5 to 50.0)	−11.4 (−40.7 to 17.9)
AMPA-eEPSC					
τ_{decay}, ms	9.8 ± 1.0	10.2 ± 1.4	10.9 ± 1.1	−0.4 (−2.7 to 1.9)	−0.8 (−1.9 to 0.4)
SG					
NMDA-eEPSC					
τ_{decay} fast, ms	8.7 ± 1.9	9.3 ± 1.3	8.3 ± 1.9	−0.7 (−6.9 to 5.6)	1.0 (−1.5 to 3.5)
τ_{decay} slow, ms	62.0 ± 1.5	69.3 ± 6.9	56.7 ± 11.1	−7.3 (−46.0 to 28.4)	12.7 (−40.9 to 66.2)
AMPA-eEPSC					
τ_{decay}, ms	5.8 ± 1.1	5.6 ± 0.9	5.2 ± 0.6	0.3 (−1.9 to 2.5)	−0.5 (−1.3 to 0.3)

Xenon, 1.9 mM (Xe), showed no definite effect on time constants τ_{decay} fast or τ_{decay} slow of electrically evoked *N*-methyl-D-aspartate receptor–mediated excitatory postsynaptic currents (NMDA-eEPSCs) or time constant of current decay of electrically evoked α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor–mediated excitatory postsynaptic currents (AMPA-eEPSCs) recorded from neurons in the prefrontal cortex (PFC, n = 6) or substantia gelatinosa (SG, n = 5).

CI = confidence interval.

and 58.2 ± 3.1% (SG; n = 5; fig. 3B) of control. Reduction of the amplitudes of AMPA-eEPSCs and p-AMPA-Cs was not significantly different in the PFC or in the SG (Bonferroni *post hoc* tests in MANOVA, PFC: $P = 0.826$, SG: $P = 0.209$). Comparison of the xenon effect on AMPA receptor–mediated current responses in the PFC with that in the SG revealed no statistical significant difference (Wilks multivariate test, AMPA-eEPSC: $P = 0.452$, p-AMPA-Cs: $P = 0.640$). We did not see a conspicuous influence of 1.9 mM xenon on current decay kinetics of AMPA-eEPSCs (table 2).

In a subset of experiments, we tested whether a lower concentration of xenon (1.1 mM), which most likely corresponds to minimum alveolar concentration $(MAC)_{awake}$, affected NMDA and AMPA receptor–mediated current responses in the PFC. Xenon, 1.1 mM, reversibly diminished NMDA-eEPSCs to 76.5 ± 3.7% of control (n = 4; tests with contrasts in MANOVA, $P < 0.05$) with no significant difference from the reduction of p-NMDA-Cs caused by 1.1. mM xenon (78.0 ± 4.1%; n = 4; fig. 2A, triangles). Likewise, 1.1 mM xenon reduced the amplitudes of AMPA-eEPSCs (83.3 ± 3.4%; n = 4) and p-AMPA-Cs (79.9 ± 4.2%; n = 4) to a similar extent (fig. 2B).

Miniature excitatory postsynaptic currents were recorded in the presence of 1 μM tetrodotoxin (fig. 4). mEPSCs recorded from neurons in the PFC (SG) occurred at a frequency of 9.0 ± 0.7 (4.9 ± 2.1) Hz and had a mean amplitude of 4.2 ± 0.3 (5.7 ± 0.4) pA. Application of 1.9 mM xenon reduced the mEPSC amplitudes in the PFC (fig. 4A) and SG (fig. 4E). Figures 4B and F show the effect of xenon on cumulative distributions of mEPSC amplitudes and interevent intervals. Xenon, 1.9 mM, increased the proportion of mEPSCs having smaller amplitudes but had no effect on the distribution of interevent intervals. Figures 4C and G show pooled data from five experiments performed in the PFC and five experiments performed in the SG. Xenon reduced the mean mEPSC amplitude to 3.7 ± 0.1 (5.1 ± 0.2) pA, whereas the mean frequency remained unchanged.

In an additional set of experiments, AMPA-eEPSCs upon paired-pulse stimulation were recorded from PFC neurons. The paired-pulse ratio did not change when 1.9 mM xenon was applied ($P = 0.111$, n = 7; fig. 4D).

Discussion

Recently, we demonstrated that the inhalational anesthetic xenon depresses excitatory while not affecting inhibitory synaptic transmission in the murine basolateral amygdala.[13] Because the effects of an anesthetic on the cellular level may vary depending on the local network under consideration,[24,25] we now investigated the effect of xenon in two additional CNS regions. In the current study, we show that in acute rodent slice preparations of the cortex and the spinal cord, both NMDA and AMPA receptor–mediated synaptic transmission are depressed by xenon, whereas $GABA_A$ receptor–mediated inhibitory synaptic transmission remains unchanged.

Based on published MAC values of xenon for humans (63–71 vol%)[26,27] and a solubility coefficient of 0.0887 for xenon at 37°C,[28] the calculation of MAC equivalents of dissolved xenon reveals a value of 2.2–2.5 mM. Therefore, with a measured xenon concentration of 1.9 ± 0.3 mM dissolved in ACSF at room temperature, the xenon concentration applied during most of our experiments lies close to $MAC_{immobility}$, whereas the lower concentration of 1.1 mM, which was applied in a subset of experiments, corresponds closely to MAC_{awake}.[29]

The results provided in the current study further support the hypothesis that xenon does not act *via* an enhancement of γ-aminobutyric acid (GABA)–mediated synaptic transmission. For intravenous[30,31] and volatile anesthetics,[32] as well as for nitrous oxide,[23,33] a substantial potentiating effect on GABA-mediated currents has been reported, and for intravenous and volatile anesthetics, there is growing evidence that they mediate their anesthetic properties, at least in part, *via* an enhancement of inhibitory synaptic transmission (reviews: Campagna *et al.*,[34] Rudolph and Antkowiak[35]). In the current study, we observed no effect of xenon on the amplitudes of $GABA_A$-eIPSCs recorded from native neurons in the PFC or in the SG. A slight effect of xenon on $GABA_A$-eIPSC deactivation time constants, and total charge transfer, which determines the strength of inhibition,[36,37] cannot be completely excluded by our data. However, we certainly can exclude a pronounced effect, as it has been described, *e.g.*, for volatile anesthetics, prolonging IPSC time constants to 260–500%.[32,38] Our results are in accord with studies[5,6] showing that xenon exerts no effects on $GABA_A$-IPSCs evoked from cultured hippocampal neurons, but contrasts with two studies describing a xenon-induced increase of Cl^- currents through $GABA_A$ receptors, which were heterologously expressed in *Xenopus* oocytes[4] or human embryonic kidney 293 cells.[23] In the latter studies, the xenon-induced potentiation occurred only at nonsaturating GABA concentrations, which are below the range that occurs synaptically,[36] thus possibly explaining the discrepancy with our data. However, the possibility, that extrasynaptic $GABA_A$ receptors, which are activated at subsaturating GABA concentrations (reviews: *e.g.*, Walker and Semyanov,[39] Hemmings *et al.*[40]) and mediate a tonic neuronal inhibition, are affected by xenon, cannot be ruled out by our data.

In both PFC and SG, compound synaptic transmission was reduced under xenon. For a further detailed investigation of the underlying mechanisms, we used pharmacologic isolation to separately investigate the effect of

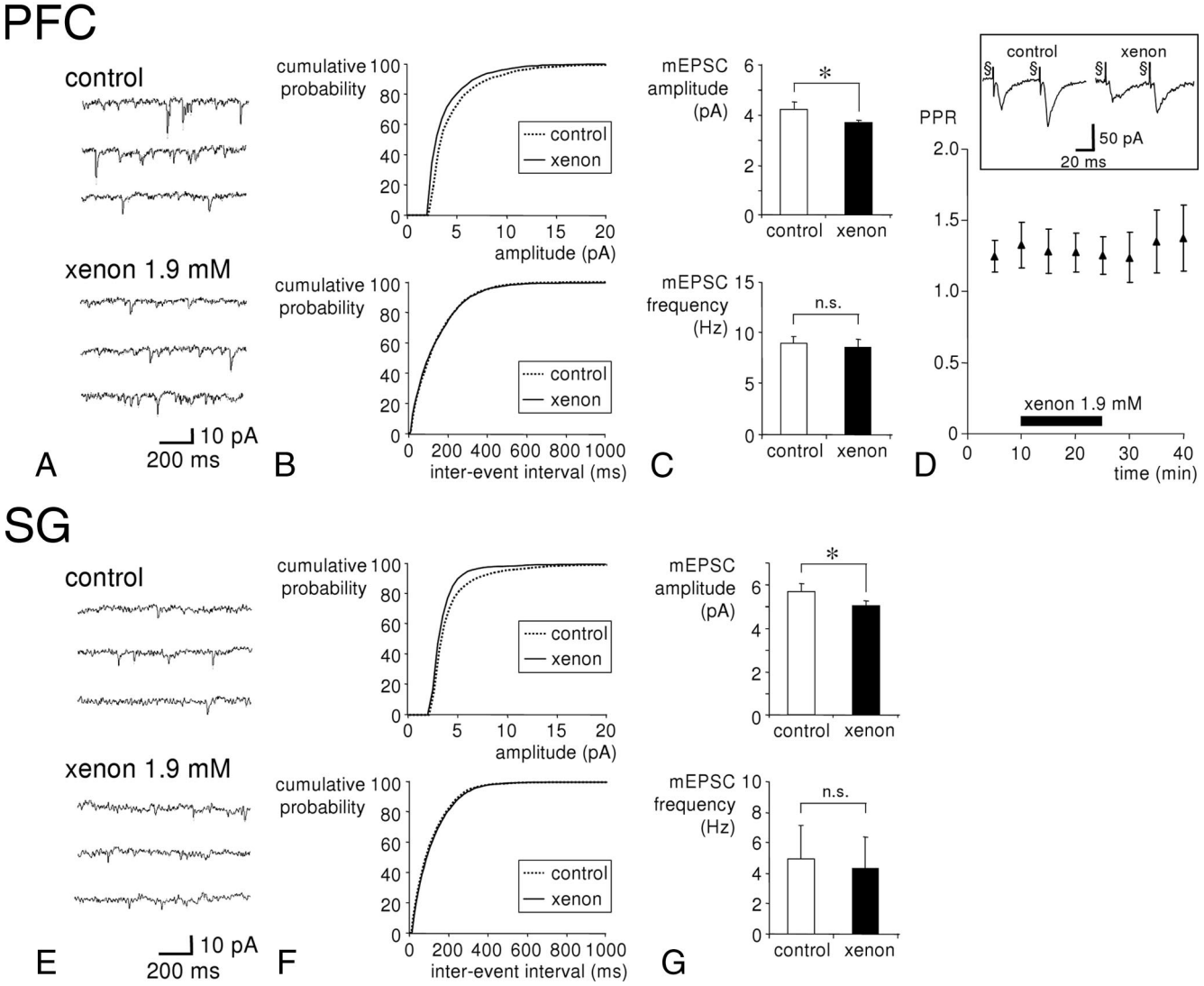

Fig. 4. Xenon has no effect on the frequency of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor–mediated miniature excitatory postsynaptic currents (mEPSCs) and on the paired-pulse ratio of AMPA receptor–mediated electrically evoked excitatory postsynaptic currents. From layer V pyramidal neurons of the prefrontal cortex (PFC; *A–C*) and from neurons in the substantia gelatinosa (SG; *E–G*) of the spinal cord dorsal horn, mEPSCs were recorded in the presence of 1 μM tetrodotoxin, 50 μM AP5, 200 μM CGP35348, and 20 μM bicuculline methiodide at a holding potential of −70 mV. Example traces in the absence and presence of 1.9 mM xenon are shown in (*A*) and (*E*). (*B* and *F*) Cumulative distributions of amplitudes and interevent intervals from one representative experiment. In both PFC (*B*) and SG (*F*) neurons, xenon shifted the distribution of mEPSC amplitudes to smaller amplitudes but had no discernible effect on the distribution of interevent intervals. (*C* and *G*) Pooled data from five experiments in the PFC and five experiments in the SG. (*D*) In a subset of experiments in the PFC, AMPA-eEPSCs were recorded upon paired-pulse stimulation (50-ms interpulse interval), and the paired-pulse ratio was determined by dividing the amplitude of the second AMPA-eEPSC by the amplitude of the first one. Xenon did not change the paired-pulse ratio ($P = 0.111$, n = 7). *Insets* show representative current traces. § Stimulation artifact. *Error bars* are SEMs. PFC: * $P = 0.039$; SG: * $P = 0.035$; n.s. = not significant (PFC: $P = 0.304$; SG: $P = 0.173$).

xenon on NMDA and AMPA receptor–mediated synaptic transmission.

In both CNS regions, we observed a pronounced xenon-induced reduction of NMDA receptor–mediated currents without apparent change of current decay kinetics. An NMDA receptor–depressing effect has been described for, *e.g.*, propofol, nitrous oxide, ketamine, and also some volatile anesthetics (review: Rudolph and Antkowiak[35]), and a depressant effect on the NMDA receptor has been suggested as the main mechanism for the xenon anesthetic state.[5] NMDA receptor–mediated currents recorded from amygdalar neurons in a brain slice preparation,[13] from neurons in culture,[5,6] and from heterologously expressed NMDA receptors[4] were reported to be reduced by xenon. In the current study, in the PFC as well as in the SG, photolytically evoked NMDA receptor currents, which are generated beyond the influence of the presynaptic terminal, were depressed by xenon to the same amount as were electrically evoked NMDA receptor–mediated currents. This finding provides strong evidence for a mainly postsynaptic mechanism of xenon on NMDA receptors in the PFC and SG.

Similar to our recently published results in the basolateral amygdala, also AMPA receptor–mediated currents in the PFC and SG were reduced by xenon. These results further support the hypothesis that the xenon anesthetic state is not only due to an NMDA receptor depression, as has been suggested initially.[5] In fact, evidence for a participation of the AMPA receptor in mediating the xenon anesthetic state derives not only from *in vitro* electrophysiologic[7,8] but also from *in vivo* studies using the model organism *C. elegans* [10] or human volunteers.[11,41]

Because AMPA receptor currents upon electrical stimulation and upon photolytic uncaging of L-glutamate were reduced to the same amount, we propose a postsynaptic mechanism of xenon action, both in the PFC and in the SG. The analysis of the effect of xenon on AMPA receptor–mediated mEPSCs in the PFC and SG, as well the analysis of the xenon effect on the paired-pulse ratio of AMPA-eEPSCs in the PFC, further support this hypothesis. Xenon reduced mEPSC amplitudes while not affecting mEPSC frequency, which speaks, according to the classic interpretation of mEPSC analysis,[42] in favor of a postsynaptic mechanism of xenon action. Changes in paired-pulse ratio and neurotransmitter release are inversely correlated.[43,44] Therefore, our findings of decreased AMPA-eEPSCs with unchanged paired-pulse ratio suggest that xenon inhibits excitatory synaptic transmission without changing the probability of presynaptic neurotransmitter release.

The analysis of current kinetics of $GABA_A$, NMDA, and AMPA receptor–mediated currents revealed considerably faster current responses recorded from SG neurons compared with neurons in the PFC. Possible explanations for these discrepancies might be species differences and/or the approximately threefold higher input resistance of the SG neurons, which reflects a smaller cell size and thus a less pronounced current distortion typically produced by space clamp deficits.

When comparing the amount of reduction of NMDA and AMPA receptor–mediated currents in the PFC and the SG, we did not see a difference between the two CNS regions. Furthermore, when comparing the results of the current study with the recently published results of the xenon effect in the basolateral amygdala,[13] no significant difference occurred in terms of the impact of xenon on NMDA or AMPA receptor–mediated currents recorded in the PFC, SG, or amygdala (Wilks multivariate test, $P > 0.05$). We therefore hypothesize an equipotent xenon action on the cortical, subcortical, and spinal levels.

The PFC is essentially involved in higher cognitive functions such as working memory,[45–47] attention regulation,[48] level of wakefulness,[49–51] and consciousness.[17,52,53] Therefore, the observed xenon action in PFC neurons might account for the hypnotic properties of xenon, in fact, at concentrations close to $MAC_{immobility}$ and close to MAC_{awake}. Moreover, because layer V pyramidal neurons in the PFC represent the major output neurons of the PFC,[54] a xenon-induced disruption of excitatory synaptic transmission to these cells might also affect further important subcortical processes regulated by the PFC.

Current knowledge regarding the action of xenon on the spinal cord level is limited to few studies. It has been shown that xenon depresses spinal cord dorsal horn neuronal activity *in vivo*,[55,56] slows ventral root potentials and spinal monosynaptic reflexes *in vitro*,[57] and attenuates long-term potentiation of C-fiber evoked potentials.[58] However, precise insights into the mechanisms of xenon action on dorsal horn level are missing. In our study, we showed that xenon depresses both NMDA and AMPA receptor–mediated synaptic transmission to SG neurons, presumably *via* postsynaptic mechanisms. Nociceptive signals transmitted by thin (Aδ) and unmyelinated (C) primary afferent fibers are conveyed to neurons in the superficial laminae (SG) of the spinal cord dorsal horn as the first site of their synaptic integration,[18,19] and inhibition of SG neuron activity might be a key mechanism of antinociception.[59] Therefore, the observed xenon effect on NMDA and AMPA receptor–mediated synaptic transmission might crucially account for the profound analgesic[60,61] properties of xenon. In addition, another endpoint of general anesthesia, immobility, is largely produced by anesthetic action on the spinal cord level.[62–65] It is still a matter of debate whether depression of ventral or dorsal horn neuronal activity contributes more to immobility.[66,67] Although accumulating evidence suggests the ventral horn as the primary site,[67,68] the involvement of dorsal horn depression to produce immobility cannot be ruled out.[66,69] Hence, the depression of synaptic transmission to SG neurons observed in our study might also account for the immobilizing properties of xenon.

In summary, we have shown that a clinical relevant concentration of xenon depresses NMDA and AMPA receptor–mediated synaptic transmission in the PFC and the SG, while not affecting GABA-mediated synaptic transmission. Our data provide evidence that the depression might be mainly due to postsynaptic mechanisms. According to the primary functions of the investigated CNS areas, the observed depression of excitatory synaptic transmission might account for the hypnotic and analgesic properties of the general anesthetic xenon.

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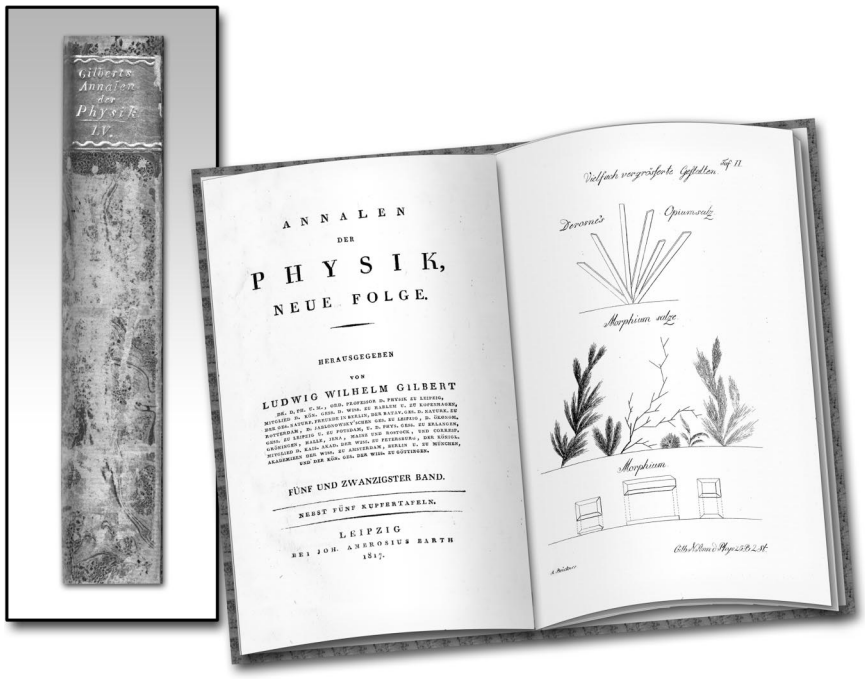
ANESTHESIOLOGY REFLECTIONS

Sertürner Crystallizes Morphine



Initially practicing pharmacy near his birthplace, outside Paderborn, Germany, Friedrich Wilhelm Adam Sertürner (1783–1841) first isolated morphine from a poppy's raw opium in 1803. After he and three of his friends had each ingested an astonishing total of 90 mg of morphine (in three divided doses) over 45 minutes, Sertürner had to induce vomiting for the ensuing abdominal pains and sleepiness. Sadly, fellow scientists ignored this "sleeping agent" reported by Sertürner in a letter published by a pharmacy journal in 1805. Sertürner then moved to Einbeck to practice at a second pharmacy and persevere with his groundbreaking alkaloidal research. In 1817 in Gilbert's *Annalen der Physik* (*pictured above, courtesy of the Wood Library-Museum*), Sertürner presented the world with the "crystallizable" isolate of opium that he had aptly named "morphine," after Morpheus, the ancient Greek god of dreams. (Copyright © the American Society of Anesthesiologists, Inc. This image appears in color in the *Anesthesiology Reflections* online collection available at www.anesthesiology.org.)

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