1. Title Page

Depression of synaptic NMDA responses by xenon and nitrous oxide

Naoki Kotani, Il-Sung Jang, Michiko Nakamura, Kiku Nonaka,
Hideaki Nagami, and Norio Akaike

Kitamoto Hospital, Saitama, Japan (N.K., N.A.); Kyungpook National University, Daegu, Republic of Korea (I.S.J., M.N.); Kumamoto Health Science University, Kumamoto, Japan (K.N.) and Kumamoto Kinoh Hospital, Kumamoto, Japan (H.N., N.A.)
2. Running Title Page

a) Running Title: (46/60 characters)

Synaptic NMDA response and gaseous anesthetics

b) Correspondence to:

Norio Akaike, Research Division for Clinical Pharmacology, Medical Corporation, Juryo Group, Kumamoto Kinoh Hospital, 6-8-1 Yamamuro, Kita-ku, Kumamoto 860-8518, Japan.
Tel: +81 96 345 8111, Fax: +81 96 345 8188, E-mail address: akaike.sachin715@juryo.or.jp

c) The number of Text pages: 48,

The number of Tables: 3

Figures: 8

References: 58

The number of words in the Abstract: 216,
d) Abbreviations:

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CA, cornu ammonis; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione disodium; D-APV, D-2-Amino-5-phosphonovaleric acid; EGTA, ethylene glycol tetraacetic acid; GABA, γ-aminobutylic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; I_{AMPA}, I_{Glu}, I_{KA}, I_{NMDA}, AMPA, glutamate, KA, and NMDA receptor-mediated whole-cell currents; KA kainic acid; NMDA, N-methyl-D-aspartate; PPR, paired-pulse rate; Rf, failure rate; s(e)EPSCs, spontaneous and (action potential-evoked) excitatory inward postsynaptic currents; s(e)EPSC_{AMPA/KA}, s(e)EPSC_{NMDA}, AMP/KA (non-NMDA) and NMDA receptor-mediated spontaneous and (action potential-evoked) excitatory postsynaptic currents; SEM, mean ± standard error of the mean; S/N; signal-to-noise ratio, τ_{Decay}, 1/e decay time constant; V_{H}, holding potential.
e) A recommended section assignment to guide the listing in the table of contents.

Section options are: Neuropharmacology
In "synapse bouton preparation" of rat hippocampal CA3 neurons, we examined how Xe and N₂O modulate N-methyl-D-aspartate (NMDA) receptor-mediated spontaneous and evoked excitatory post-synaptic currents (sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA}). This preparation is a mechanically isolated single neuron attached with nerve endings (boutons) preserving normal physiological function and promoting the exact evaluation of sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA} responses without influence of extrasynaptic, glial and other neuronal tonic currents. These sEPSC and eEPSC are elicited by spontaneous glutamate (Glu) release from many homologous glutamatergic boutons and by focal paired-pulse electric stimulation of a single bouton, respectively. The s/eEPSC\textsubscript{AMPA/KA} and s/eEPSC\textsubscript{NMDA} were isolated pharmacologically by their specific antagonists. Thus, independent contributions of pre- and postsynaptic responses be could also quantified. All kinetic properties of s/eEPSC\textsubscript{AMPA/KA} and s/eEPSC\textsubscript{NMDA} were detected clearly. The s/eEPSC\textsubscript{NMDA} showed smaller amplitude, slower rise and $1/e$ decay time constant ($\tau_{\text{Decay}}$) than s/eEPSC\textsubscript{AMPA/KA}. Xe (70%) and N₂O (70%) significantly decreased the frequency and amplitude without altering the $\tau_{\text{Decay}}$ of sEPSC\textsubscript{NMDA}. They also decreased the amplitude but increased the Rf and PPR without altering the $\tau_{\text{Decay}}$ of the eEPSC\textsubscript{NMDA}. These data show clearly that "synapse bouton preparation" can be an accurate model for
evaluating s/eEPSC$_{NMDA}$. Such inhibitory effects of gas anesthetics are primarily due to presynaptic mechanisms. Present results may explain partially the powerful analgesic effects of Xe and N$_2$O.
4. Significance Statement (62 word)

We could record pharmacologically isolated sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA} and clearly detect all kinetic parameters of sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA} at synaptic levels by using "synapse bouton preparation" of rat hippocampal CA3 neurons. We found that Xe and N\textsubscript{2}O clearly suppressed both sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA}. Different from previous studies, present results suggest that Xe and N\textsubscript{2}O predominantly inhibit the NMDA responses by presynaptic mechanisms.

5. Visual Abstract: None
6. INTRODUCTION (524/750 words)

Xe and N₂O induce anesthesia primarily via interactions with glutamate receptors, in which the NMDA receptor has been proposed as a major target. In fact, Xe (Dinse et al., 2005; Ogata et al., 2006; Haseneder et al., 2008; Weigt et al., 2009; Yamamoto et al., 2012) and N₂O (Jevtovic-Todorovic et al., 1998; Mennerick et al., 1998; Yamakura and Harris, 2000; Ranft et al., 2007; Georgiev et al., 2008) depressed NMDA receptor-mediated responses in cultured cells and slice preparations. However, the previous results using slice preparation are controversial. Haseneder et al. (2008; 2009b) and Georgiev et al. (2010) found that Xe significantly decreased eEPSC\textsubscript{NMDA}. Conversely, Yamamoto et al. (2012) demonstrated that Xe did not affect eEPSC\textsubscript{NMDA} at all in the spinal cord.

The most plausible explanation for these differences might be that electric recording purely isolated sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA} is very challenging; this may be because of the slow time course and small amplitude of s/eEPSC\textsubscript{NMDA} as well as the low s S/N ratio induced by NMDA receptor-mediated tonic currents from surrounding glial neurons and extrasynaptic responses (Dalby and Mody, 2003; Myme et al., 2003; Vargas-Caballero and Robinson, 2003; Hardingham and Bading, 2010; Petralia et al., 2010; De-Miguel and Fuxe, 2012). In fact, the determination of sEPSC\textsubscript{NMDA} is extremely difficult;
eEPSC\textsubscript{NMDA} is the usual choice for characterizing the NMDA receptor-mediated electric responses in slice preparations (Dalby and Mody, 2003).

The “synapse bouton preparation” of single hippocampal CA3 neurons dissociated mechanically is preserving functional many synaptic terminals (boutons). This preparation thus facilitates the evaluation of sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA} at pure synaptic level (Akaike and Moorhouse, 2003). As shown in schematic illustration (Fig. 1A-C), by exposing test solution with drug to a whole cell, many homologous sEPSCs could be detected (Fig 1B). Furthermore, focal single- or paired-pulse stimulation of a single nerve ending (a bouton) attached to the neuronal cell bodies elicits single synaptic eEPSCs (Nonaka et al., 2019). (Fig 1C).

Focal paired-pulse electric stimulation of a single bouton enables us to accurately quantify how test drugs modulate pre- and postsynaptic transmission. The frequency of sEPSCs, and the PPR and Rf of eEPSCs reflect in presynaptic events while the $\tau_{\text{Decay}}$ is specific for postsynaptic one. The amplitude of both sEPSCs and eEPSC could be influenced by pre- and postsynaptic elements (Stocca and Vicini, 1998; Mohrmann et al., 2000; Tovar et al., 2000; Townsend et al., 2003).

We recently reported in “synapse bouton preparation” of rat hippocampal and spinal neurons that Xe (Nonaka et al., 2019; Kubota et al., 2020) and N\textsubscript{2}O (Wakita et al., 2015)
inhibited \( I_{\text{AMPA/KA}} \), \( \text{sEPSC}_{\text{AMPA/KA}} \), and \( \text{eEPSC}_{\text{AMPA/KA}} \). The primary effects of Xe and \( \text{N}_2\text{O} \) on both \( \text{sEPSC}_{\text{AMPA/KA}} \) and \( \text{eEPSC}_{\text{AMPA/KA}} \) were on the presynaptic side. These findings are completely different from those of previous studies.

Since all kinetic properties of \( \text{sEPSC}_{\text{NMDA}} \) and \( \text{eEPSC}_{\text{NMDA}} \) could now be recorded, we precisely investigated the actions of Xe and \( \text{N}_2\text{O} \) on both \( \text{sEPSC}_{\text{NMDA}} \) and \( \text{eEPSC}_{\text{NMDA}} \) at the pre- and postsynaptic levels. Furthermore, the clinical significance in NMDA receptor-mediated responses in the presence of Xe and \( \text{N}_2\text{O} \) were discussed by comparison with our previously reported AMPA/KA receptor-mediated responses (Wakita et al., 2015; Nonaka et al., 2019; Kubota et al., 2020).
7. MATERIALS AND METHODS

Ethical Approval

All experiments were performed in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan and were approved by the Ethics Committee of Kumamoto Health Science University (approval number, 20-07) and Kumamoto Kinoh Hospital. All efforts were made to minimize animal suffering and to reduce the number of animals used in this study.

Animals

Wistar rats of either sex (11–17 days old, SLC, Shizuoka, Japan) were decapitated after deep pentobarbital anesthesia (50 mg/kg, intraperitoneally) (Nonaka et al., 2019). The brain was dissected and transversely sliced at a thickness of 350 µm using a microslicer (7000smz; Campden Instruments LTD, Leicester, England). Slices containing the hippocampal CA3 region were kept in an incubation medium (124 mM NaCl, 5 KCl, 1.5 KH2PO4, 24 mM NaHCO3, 2 CaCl2, 1.3 MgSO4, and 10 mM glucose) saturated well with 95% O2 and 5% CO2 at room temperature (22-24ºC) for at least 1 h before mechanical dissociation. For mechanical dissociation without using any enzymes, slices
were transferred into a 35-mm culture dish (Primaria 3801; Becton Dickinson, Rutherford, NJ, USA) containing a standard extracellular solution (150 mM NaCl, 5 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM glucose, and 10 mM HEPES, pH 7.4). The hippocampal CA3 region was identified using a binocular microscope (SMZ-1; Nikon, Tokyo, Japan). Details of the mechanical dissociation of CA3 neurons can be found in previous studies (Akaike and Moorhouse, 2003; Wakita et al., 2016). Mechanically dissociated CA3 neurons were allowed to settle and left to adhere to the bottom of the dish for 15 mins.

**Electrophysiology**

Detail of electrophysiological measurements were reported in previous papers (Nonaka et al., 2019). Neurons were voltage-clamped at a holding potential ($V_H$) of $-60$ mV which is close Cl$^-$ equilibrium potential ($E_{Cl}$). The resistance of the recording pipettes filled with internal solution (140 mM CsF, 10 mM CsCl, 2 mM EGTA, 2 mM ATP-Na$_2$, and 10 mM HEPES, pH adjusted to 7.2 with Tris-base) was 4–6 MΩ. During recordings, 10-mV hyperpolarizing step pulses (30 ms in duration) were periodically applied to monitor the access resistance (routinely 15–25 MΩ), and recordings were discontinued if
access resistance changed by more than 10%. All experiments were performed at room temperature (22–24 °C).

sEPSC_{AMP/A} and eEPSC_{AMP/A} were recorded in the standard extracellular solution. On the other hand, sEPSC_{NMDA} and eEPSC_{NMDA} were recorded in the Mg^{2+}-free mixture solution containing 3 μM glycine, 3 μM strychnine (a glycine receptor antagonist), and 30 μM CNQX (an AMPA/KA receptor antagonist). For the Mg^{2+}-free mixture solution, 1 mM MgCl₂ was omitted from the standard extracellular solution. These extracellular solutions also routinely contained 10 µM bicuculline (a GABAₐ receptor antagonist) to inhibit GABAergic synaptic currents.

Focal single- or paired-pulse electrical stimulation was performed to record eEPSC_{AMP/A} and eEPSC_{NMDA} (Akaike et al., 2002; Akaike and Moorhouse, 2003). A stimulating theta electrode was placed near the surface of the distal part of the apical dendrite, which was the nearest to the hippocampal CA3 cell body (distance of 100-150 μm on the soma) (Fig. 1C). Brief single pulses (200 μs duration, 80–120 μA) were applied through the theta electrode at a stimulation frequency of 0.2 Hz using a stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan) equipped with an isolator unit (SS-701J, Nihon Kohden).
Statistical Analysis

sEPSC_{AMPA/KA} and sEPSC_{NMDA} were detected automatically using template and threshold search methods, respectively, with an amplitude threshold of 10 pA (pCLAMP 10.7). The average values of the frequency, amplitude, rise time course, and $\tau_{\text{Decay}}$ of all sEPSCs during the control (5–10 mins) or drug (Xe/N₂O; 3 min) periods were calculated for each neuron. The number of events during each control or drug period ranged several tens to hundreds. The inter-event intervals (frequency) and amplitudes of a large number of synaptic events obtained from the same neuron were examined by constructing cumulative probability distributions, which were then compared using the Kolmogorov-Smirnov test with Stat View software (SAS Institute, Inc., Cary, NC, USA). Numerical values are presented as the mean ± SEM using values normalized to the control. The sample size shown in the Results section represents the number of neurons.

Significant differences in the mean amplitude, frequency, rise time, and $\tau_{\text{Decay}}$ were tested using the Student’s paired two-tailed $t$-test, except where indicated, with absolute values rather than normalized ones. Statistical significance was set at $p < 0.05$. The amplitudes of the eEPSCs were calculated by subtracting the baseline from the respective peak amplitudes (pCLAMP 10.7; Molecular Devices). When electric responses were not evoked by electrical stimulation, these sweeps were regarded as having failed the test and
were not included in the averaged value of the eEPSC amplitude.

Drugs

The reagents used for the test solutions were obtained from Wako Pure Chemicals (Osaka, Japan). Other reagents, such as bicuculline, CNQX, D-APV, strychnine, and glycine, were obtained from Sigma-Aldrich (St. Louis, MO, USA). All test solutions were applied using the ‘Y–tube system’ for rapid solution exchange within 20 ms (Murase et al., 1990).
8. RESULTS

**sEPSC\textsubscript{AMPA/KA} and sEPSC\textsubscript{NMDA} in Hippocampal CA3 Neurons**

Dissociated CA3 pyramidal neurons were held at a $V_H$ of $-60$ mV, and sEPSCs were recorded in normal external solution containing 10 µM bicuculline, a GABA\textsubscript{A} receptor antagonist. The inwardly directed and rapidly decaying sEPSCs were completely blocked by the addition of 30 µM CNQX, an AMPA/KA receptor blocker, consistent with sEPSC\textsubscript{AMPA/KA} ($n = 4$, Fig. 2Aa’, b’). Thus, sEPSCs were referred to as sEPSC\textsubscript{AMPA/KA}. In the continuous presence of CNQX, the inwardly directed but slowly decaying sEPSCs appeared due to the cumulative application of Mg\textsuperscript{2+}-free mixture solution containing 3 µM glycine (Hershkowitz and Rogawski, 1989; Allen et al., 1995) and 3 µM strychnine. The slow synaptic currents were completely and reversibly blocked by the addition of 30 µM D-APV ($n = 4$), a selective NMDA receptor antagonist (Fig. 2Ac’, d’). These slow sEPSCs are referred to as sEPSC\textsubscript{NMDA}. Figure 2B shows the cumulative distributions of the frequency of sEPSC\textsubscript{AMPA/KA} (black) and sEPSC\textsubscript{NMDA} (red), respectively. The mean frequency was $0.73 \pm 0.10$ Hz, $n = 5$ for sEPSC\textsubscript{AMPA/KA} and $0.46 \pm 0.10$ Hz, $n = 5$ for sEPSC\textsubscript{NMDA}. Figure 2C shows the cumulative distributions of the amplitude, the values were $42.2 \pm 4.7$ pA, $n = 5$ for sEPSC\textsubscript{AMPA/KA} and $24.3 \pm 2.4$ pA, $n = 5$ for sEPSC\textsubscript{NMDA}. 

This article has not been copyedited and formatted. The final version may differ from this version. 

JPET Fast Forward. Published on October 22, 2022 as DOI: 10.1124/jpet.122.001346 
This article has not been copyedited and formatted. The final version may differ from this version.
These values were lower at $p < 0.01$ in $s\text{EPSC}_{\text{NMDA}}$ than in $s\text{EPSC}_{\text{AMPA/KA}}$ (Fig. 2B, C, Table 1A).

Figure 3A shows single normalized and superimposed $s\text{EPSC}_{\text{AMPA/KA}}$ (black) and $s\text{EPSC}_{\text{NMDA}}$ (red) with an expanded time scale. The rise time (time to peak) was $1.26 \pm 0.09 \text{ ms}$, $n = 5$ for $s\text{EPSC}_{\text{AMPA/KA}}$ and $9.28 \pm 0.33 \text{ ms}$, $n = 5$ at $p < 0.001$ for $s\text{EPSC}_{\text{NMDA}}$.

Figure 3B summarizes the rise time values for $s\text{EPSC}_{\text{AMPA/KA}}$ and $s\text{EPSC}_{\text{NMDA}}$. The $\tau_{\text{Decay}}$ values of $s\text{EPSC}_{\text{AMPA/KA}}$ and $s\text{EPSC}_{\text{NMDA}}$ are shown in Fig. 3C, where their decay time coursed were well fitted in a single exponential manner. The $\tau_{\text{Decay}}$ values were $6.21 \pm 0.69 \text{ ms}$, $n = 5$ for $s\text{EPSC}_{\text{AMPA/KA}}$ (white) and $70.8 \pm 7.4 \text{ ms}$, $n = 5$ at $p < 0.001$ for $s\text{EPSC}_{\text{NMDA}}$ (red) (Fig. 3D, Table 1A).

**eEPSC\text{AMPA/KA} and eEPSC\text{NMDA}**

When hippocampal CA3 pyramidal neurons were held at a $V_H$ of $–60 \text{ mV}$, the focal single-pulse electrical stimulation of a bouton elicited eEPSCs in normal external solution. The eEPSCs were completely blocked by adding $30 \mu\text{M} \text{CNQX}$ ($n = 4$, Fig. 4Aa’, b’). The eEPSCs were thus referred to as $e\text{EPSC}_{\text{AMPA/KA}}$. In the continued presence of $30 \mu\text{M} \text{CNQX}$, the inwardly directed but slowly decaying synaptic currents appeared after the cumulative application of the $\text{Mg}^{2+}$-free mixture solution. The evoked synaptic
currents were reversibly blocked by the addition of 30 μM D-APV (n = 4) (Fig. 4Ac’, d’). The eEPSCs were referred to as eEPSC\textsubscript{NMDA}. Figure 4B shows the normalized and superimposed eEPSC\textsubscript{AMPA/KA} (black) and eEPSC\textsubscript{NMDA} (red) with an expanded time scale. The mean amplitude in eEPSC\textsubscript{NMDA} was smaller than that in eEPSC\textsubscript{AMPA/KA} (483.7 ± 56.3 pA, n = 8 for eEPSC\textsubscript{AMPA/KA} and 184.2 ± 20.0 pA, n = 8, p < 0.001 for eEPSC\textsubscript{NMDA}) (Fig. 4Ca, Table 1B). The rise time values were 1.57 ± 0.08 ms, n = 8 for eEPSC\textsubscript{AMPA/KA} and 10.1 ± 0.5 ms, n = 8 for eEPSC\textsubscript{NMDA} (Fig. 4Cb). There was highly significant difference between the two. The $\tau_{\text{Decay}}$ values of both eEPSC\textsubscript{AMPA/KA} and eEPSC\textsubscript{NMDA} were also well fitted in a single exponential function; the resultant $\tau_{\text{Decay}}$ values were 6.35 ± 0.23 ms, n = 8 for eEPSC\textsubscript{AMPA/KA} and 80.7 ± 8.3 ms, n = 8 for eEPSC\textsubscript{NMDA} (Fig. 4Cc). The difference between two values was p < 0.001.

**Effects of Xe on sEPSC\textsubscript{NMDA}**

The effect of 70% Xe was examined on sEPSC\textsubscript{NMDA} recorded in a Mg\textsuperscript{2+}-free mixture solution containing 30 μM CNQX. As shown in Fig. 5Aa’, b’, Xe decreased both the frequency and amplitude of sEPSC\textsubscript{NMDA} at p < 0.01. Figure 5B shows the cumulative distributions of the frequency (a) and amplitude (b) of sEPSC\textsubscript{NMDA} with and without Xe. Xe shifted the cumulative probability of the frequency and amplitude of sEPSC\textsubscript{NMDA} to
the right and left, respectively, indicating that Xe decreased the mean frequency and amplitude of sEPSC\textsubscript{NMDA} at p < 0.01 (Fig. 5C) while Xe did not alter the $\tau$\textsubscript{Decay} of sEPSC\textsubscript{NMDA} (Fig. 5D). The inset in Fig. 5D (left panel) shows normalized and superimposed sEPSC\textsubscript{NMDA} with (red) and without (black) Xe with an expanded time scale.

Effects of 70% Xe on sEPSC\textsubscript{NMDA} were summarized in Table 2A.

**Effects of Xe on eEPSC\textsubscript{NMDA}**

In Mg\textsuperscript{2+}-free mixture solution with CNQX, we examined the effects of 70% Xe on eEPSC\textsubscript{NMDA}. When the focal paired-pulse stimuli were delivered with an interval of 200 ms to the surface of the apical dendrites of CA3 pyramidal neurons (see Fig. 1C) using a theta glass electrode, the electrical stimuli evoked two eEPSCs (eEPSC\textsubscript{1} and eEPSC\textsubscript{2}) as shown in Fig. 6A(a). The amplitude of eEPSC\textsubscript{1}, R\textsubscript{f} (failure rate) of eEPSC\textsubscript{1}, and PPR (paired-pulse ratio) of eEPSC\textsubscript{2}/eEPSC\textsubscript{1} were analyzed and calculated. Figure 6A shows the typical time course of the amplitude (a) and PPR (b) of NMDA receptor-mediated response obtained before, during, and after adding 70% Xe. Xe decreased the mean eEPSC\textsubscript{1} amplitude at p < 0.01, but Xe increased the R\textsubscript{f} and PPR at p < 0.01 (Fig. 6B, Table 2A). However, Xe did not affect the $\tau$\textsubscript{Decay} of eEPSC\textsubscript{NMDA} at all (Fig. 6Cb).
Normalized and superimposed eEPSC\textsubscript{NMDA} with (red) and without (black) Xe are shown at an expanded time scale in Fig. 6Ca.

**Effects of N\textsubscript{2}O on sEPSC\textsubscript{NMDA}**

We also examined the effect of 70\% N\textsubscript{2}O on sEPSC\textsubscript{NMDA}. The application of N\textsubscript{2}O test solution also depressed the frequency and amplitude of sEPSC\textsubscript{NMDA} (Fig. 7A a’, b’). Figure 7B shows the cumulative distributions for the frequency (a) and amplitude (b) of sEPSC\textsubscript{NMDA} with (red) and without (black) N\textsubscript{2}O. N\textsubscript{2}O shifted the cumulative probability of the frequency and amplitude of sEPSC\textsubscript{NMDA} to the right and left, indicating that N\textsubscript{2}O decreased the mean frequency and amplitude of sEPSC\textsubscript{NMDA} at $p < 0.01$ and $p < 0.05$, respectively (Fig. 7C). However, N\textsubscript{2}O did not affect the $\tau_{\text{decay}}$ of sEPSC\textsubscript{NMDA} (Fig. 7D). Normalized and superimposed sEPSC\textsubscript{NMDA} with (red) and without (black) N\textsubscript{2}O at an expanded time scale are shown in Fig. 7D (left panel). All data were summarized in Table 3A.

**Effects of N\textsubscript{2}O on eEPSC\textsubscript{NMDA}**

We investigated the effects of 70\% N\textsubscript{2}O on eEPSC\textsubscript{NMDA}. Figure 8A shows a typical time course of the amplitude (a) and PPR (b) of evoked NMDA receptor-mediated

This article has not been copyedited and formatted. The final version may differ from this version.
response obtained before, during, and after the application of N₂O. N₂O also decreased the mean eEPSC₁ amplitude and increased the Rᵣ and PPR (Fig. 8B). But N₂O did not affect the τᵣDecay of eEPSCₑNMDA (Fig. 8 Ca, b). All data were summarized in Table 3B.
9. DISCUSSION (1438/1500 words)

Kinetic parameters of sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA} were detected in the “synapse bouton preparation”.

Detail current parameters (frequency, amplitude, rise and decay time) of sEPSC\textsubscript{NMDA} recorded from “synapse bouton preparation” are shown in Figs. 2, 3, 5 and 7, and summarized in Table 1-3. The frequency of sEPSC\textsubscript{NMDA} was much lower than that of sEPSC\textsubscript{AMPA/KA}. The sEPSC\textsubscript{NMDA} also showed smaller amplitude, slower rise time and much prolonged $\tau$\textsubscript{Decay} than sEPSC\textsubscript{AMPA/KA}. Such clear current parameters might be resulted from high S/N ratio (Fig. 2A, Fig. 5A, and Fig. 7A) in current recording using “synapse bouton preparation” not like “slice preparation” of low S/N ratio having large dendrites and poor space clamp (Hestrin, 1992; Stocca and Vicini, 1998).

Figure 4 shows eEPSC\textsubscript{NMDA} that have the same wave pattern as in sEPSCs; the amplitude is smaller while the rise time and $\tau$\textsubscript{Decay} are more prolonged in eEPSC\textsubscript{NMDA} than in eEPSC\textsubscript{AMPA/KA}. The $\tau$\textsubscript{Decay} of sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA} recorded in CA3 neurons were well-fitted to a single exponential function. However, according to Lindlbauer et al (1998), the $\tau$\textsubscript{Decay} of eEPSC\textsubscript{NMDA} was initially single exponential in newborn rats but changed to double exponential in relation to animal growth. Such age-dependent charge of eEPSC\textsubscript{NMDA} $\tau$\textsubscript{Decay} were also noted (Hestrin et al., 1990; Khazipov et al., 1995; Kirson
et al., 1998; Lindlbauer et al., 1998). We consider that age-dependent meshed-dendrites made it more difficult to have enough space-clamp for patch clamp measurement.

In this study, we used the “synapse bouton preparation” of the hippocampal CA3 neurons from 11-17-days-old rats. This preparation provides a better space-clamp because of several short dendrites without complex meshed dendritic processes, as compared with culture or slice preparations. Thus our “synapse bouton preparation” is unlikely to interfere with the measurement of prolonged and small $s\text{EPSC}_{\text{NMDA}}$ and $e\text{EPSC}_{\text{NMDA}}$. In addition, present focal electrical stimuli were applied 70-120 μm from the cell body and could be transmitted very close distance to the recording patch pipette.

Even a single axon from one neuron in slice studies diverges into many branches. The input of nerve branches from many neurons to a target neuron have several hundred synapses (Katsurabayashi et al., 2016; Kawano et al., 2017). Each synapse shows different responses (Rosenmund et al., 2002). Therefore, the synaptic $e\text{EPSCs}$ from slice preparation were the sum of many synaptic currents (Pouzat and Marty, 1998; Hou et al., 2016). In contrast to previous studies of Xe and N$_2$O, analyses of kinetic parameters (amplitude, $R_f$, PPR, rise time and $\tau_{\text{Decay}}$) of $e\text{EPSC}_{\text{NMDA}}$ at a pure single synaptic level was performed in this study. Present findings confirmed that the “synapse
bouton preparation” is a more accurate and promising method for investigating NMDA receptor-mediated small synaptic responses.

Xe and N₂O seriously depress sEPSC_{NMDA}

In the present study, we found that 70% Xe or N₂O decreased the frequency and amplitude of sEPSC_{NMDA} but did not change the $\tau_{\text{Decay}}$ (Figs. 5, 7). The results on CNQX-sensitive sEPSC_{AMP\/KA} were similar to those of our previous studies (Wakita et al., 2015; Nonaka et al., 2019; Kubota et al., 2020), which showed that 70% Xe and N₂O inhibited sEPSC_{AMP\/KA}. However, decreases in the frequency and amplitude of sEPSC_{NMDA} without altering $\tau_{\text{Decay}}$ differed from the results of previous studies using slice preparations. Xe (Haseneder et al., 2008; Haseneder et al., 2009a) and N₂O (Georgiev et al., 2008) decreased the amplitude of sEPSC_{NMDA} but did not change the frequency. This discrepancy may be due to the preparation used in these studies. Because the S/N ratio was low in slice preparation (Roberts et al., 1992; Sumie et al., 2016) it was critically challenging to accurately detect sEPSC_{NMDA} (Dalby and Mody, 2003). Moreover, a significant portion of the small amplitude of sEPSC_{NMDA} might be missed in slice preparation. Spontaneous currents recorded on slice preparation may also have been affected by glutamate exocytosis from astrocytes (Fiacco and McCarthy, 2006; Jourdain
et al., 2007; Perea and Araque, 2007; McCullumsmith et al., 2016); the ambient glutamate released from astrocytes activates NMDA receptors and induces a tonic current (Le Meur et al., 2007).

**Xe and N₂O seriously depress eEPSC\textsubscript{NMDA}**

The eEPSC\textsubscript{NMDA} is the usual choice for characterizing NMDA receptor-mediated responses (Dalby and Mody, 2003; Myme et al., 2003). In the present study of hippocampal CA3 neurons, Xe (Fig. 6) and N₂O (Fig. 8) significantly decreased the amplitude of eEPSC\textsubscript{NMDA} at a single synapse level. Our results were comparable to those of previous studies using slice preparations, which reported that Xe decreased the amplitude but did not affect the $\tau_{\text{Decay}}$ of glutamatergic eEPSCs in the prefrontal cortex and substantia gelatinosa neurons (Haseneder et al., 2009b). According to Georgiev et al. (Georgiev et al., 2010), Xe decreased the amplitude of both eEPSC\textsubscript{AMPA/KA} and eEPSC\textsubscript{NMDA} without altering $\tau_{\text{Decay}}$. They also reported that N₂O directly inhibited eEPSC\textsubscript{NMDA} in spinal cord neurons (Georgiev et al., 2008).

We previously reported that Xe (Nonaka et al., 2019; Kubota et al., 2020) and N₂O (Wakita et al., 2015) decreased $I_{\text{Glu}}$, $I_{\text{AMPA}}$, $I_{\text{KA}}$, and $I_{\text{NMDA}}$ as well as the sEPSC\textsubscript{AMPA/KA} and eEPSC\textsubscript{AMPA/KA} using the “synapse bouton preparation” of brain and...
spinal neurons. Together with the decreases in $s\text{EPSC}_{\text{NMDA}}$ and $e\text{EPSC}_{\text{NMDA}}$ by gas anesthetics in this study, a series of our studies suggest that Xe and $\text{N}_2\text{O}$ critically suppress all three subtypes of glutamate receptors.

**Xe and $\text{N}_2\text{O}$ presynaptically depress NMDA receptor-mediated synaptic transmission**

Our study is the first report of all kinetic parameters of $s\text{EPSC}_{\text{NMDA}}$ and $e\text{EPSC}_{\text{NMDA}}$. The frequency and amplitude of $s\text{EPSC}_{\text{NMDA}}$ were decreased by Xe and $\text{N}_2\text{O}$, while $\tau_{\text{Decay}}$ was not changed. Furthermore, the amplitude of $e\text{EPSC}_{\text{NMDA}}$ also decreased with increasing the Rf and PPR of eEPSCs without changing the $\tau_{\text{Decay}}$.

The frequency of sEPSCs and the PPR, and Rf of eEPSCs are well-established indicators of presynaptic mechanisms. On the other hand, the $\tau_{\text{Decay}}$ is specific for postsynaptic contributions. Therefore, Xe and $\text{N}_2\text{O}$ affected the kinetic properties of established presynaptic mechanisms, including the frequency of sEPSCs, PPR, and Rf of eEPSCs. In contrast, changes in $\tau_{\text{Decay}}$, which suggests a postsynaptic contribution, were absent. These findings clearly support that both Xe and $\text{N}_2\text{O}$ act exclusively on presynaptic sites. Although Xe and $\text{N}_2\text{O}$ had no effect on the $\tau_{\text{Decay}}$ of postsynaptic NMDA receptors, our previous studies have clearly shown that Xe and $\text{N}_2\text{O}$ decrease
the amplitude of the $I_{\text{NMDA}}$ elicited by exogenous application of NMDA (Wakita et al., 2015; Nonaka et al., 2019; Kubota et al., 2020). Therefore, it can be concluded that Xe and N$_2$O decrease synaptic NMDA responses by the direct inhibition of postsynaptic NMDA receptors as well as decrease of synaptic glutamate release.

In contrast, all previous studies concluded that the inhibitory effects of Xe and N$_2$O on glutamatergic transmission are exclusively on the postsynaptic side (Haseneder et al., 2008; Haseneder et al., 2009b; Yamamoto et al., 2012). However, their interpretation may be problematic. Although there was a lack of alterations in $\tau_{\text{Decay}}$ they concluded that the inhibitory effects of Xe and N$_2$O are primarily due to postsynaptic mechanisms. Also, they evaluated a very limited number of parameters for $s\text{EPSC}_{\text{NMDA}}$ and $e\text{EPSC}_{\text{NMDA}}$. Finally, studies using these slice preparations cannot evaluate sufficiently the independent presynaptic contributions. Therefore, the conclusion of anesthetic-induced postsynaptic dominant NMDA receptor-mediated responses have no theoretical basis. However, “synapse bouton preparation” offered a unique and independent evaluation of the effects on both pre- and postsynaptic transmission at the synaptic levels.

Comparison with AMPA/KA receptor-mediated responses and its clinical implication
N\textsubscript{2}O is a very weak anesthetic and is clinically used together with more powerful general anesthetics and analgesics. In contrast, Xe has profound anesthetic and analgesic potency and can be used alone. The inhibitory effects of Xe on sEPSC\textsubscript{AMPA/KA} and eEPSC\textsubscript{AMPA/KA} were much greater than those of N\textsubscript{2}O (Nonaka et al., 2019). However, differences in sEPSC\textsubscript{NMDA} and eEPSC\textsubscript{NMDA} between Xe and N\textsubscript{2}O were not significant in this study.

The spinal cord is the site of the first sensory synapse within the central nervous system and is easier to stop the influx of acute nociceptive stimuli without affecting higher brain function (Zhuo, 2003; Basbaum et al., 2009). In fact, NMDA-receptor mediated responses in the spinal cord critically modulate acute nociceptive pathways. It is well known that sensory dorsal horn neurons to noxious stimuli are enhanced by intrathecal application of NMDA (Willis, 2002). To the contrary, intrathecally administrated NMDA receptor antagonists significantly reduce acute pain sensation (Zahn et al., 1998; Bennett et al., 2000; Pogatzki et al., 2000; Hama et al., 2003).

In contrast, the NMDA receptors in the brain rather play an important role in chronic pain such as hyperalgesia and allodynia than acute nociceptive pathway by sustainably increasing glutamate sensory transmission (Zhuo, 2016; Zhuo, 2017).
Detailed studies are mandatory to elucidate how Xe and N\textsubscript{2}O influence the NMDA receptors in the spinal cord.
10. ACKNOWLEDGMENTS

The authors thank Dr. Mami Noda (Kyushu University) for her kind comments. We would also like to thank Editage (www.editage.jp) for English language editing.

11. AUTHOR CONTRIBUTIONS

Participated in research design: Kotani, Jang, and Akaike.

Conducted experiments: Jang, Nakamura, and Nonaka.

Contributed new reagents or analytic tools: Usually ones.

Performed data analysis: Jang, Nakamura, Nonaka and Nagami.

Wrote or contributed to the writing of the manuscript: Kotani, Jang, Nakamura, Nonaka, Nagami, Akaike
12. References


Bennett AD, Everhart AW and Hulsebosch CE (2000) Intrathecal administration of an NMDA or a non-NMDA receptor antagonist reduces mechanical but not thermal allodynia in a rodent model of chronic central pain after spinal cord injury. *Brain Res* **859**:72-82.


synaptic transmission in the rodent prefrontal cortex and spinal cord dorsal horn. *Anesthesiology* **111**:1297-1307.


McCullumsmith RE, O'Donovan SM, Drummond JB, Benesh FS, Simmons M, Roberts R, Lauriat T,


Yamakura T and Harris RA (2000) Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated
ion channels. Comparison with isoflurane and ethanol. *Anesthesiology* **93**:1095-1101.


13. GRANTS

This work was supported by a Grant-in-Aid from Kumamoto Health Science University [18-02, 20-07], JSPS KAKENHI grant to [JP21K08984], Grant-in-Aid from Kitamoto Hospital (to N.K. and N.A.), and Grant-in-Aid from Kumamoto Kinoh Hospital (to H.N. and N.A.)

14. FOOTNOTES

No another has an actual or perceived conflict of interest with the contents of this article.
15. LEGENDS for FIGURES

Figure 1. Schematic illustration of three types of electrical recordings in whole-cell mode.

(A) Conventional whole-cell recording in “synapse bouton preparation” of rat hippocampal CA3 neurons isolated mechanically without using any enzymes. The test solution was exogenously applied using the Y-tube method. Extrasynaptic receptors on postsynaptic cell membrane (red circle). Single nerve ending (bouton) (blue half triangle). The lower panel shows the glutamate-induced current ($I_{\text{Glu}}$) by exogenous application of $10^{-5}$ M glutamate. In this case, extrasynaptic and synaptic glutamate receptors are activated.

(B) CNQX-sensitive sEPSC$_{\text{AMPA/KA}}$ induced by many glutamatergic homogenous boutons. The lower panel shows the sEPSC$_{\text{AMPA/KA}}$ recorded in normal external solution containing Mg$^{2+}$.

(C) Focal electrical stimulation of a single nerve ending (bouton) adhered to an isolated single CA3 neuron. In this case, eEPSC$_{\text{AMPA/KA}}$ was elicited by focal single electrical stimulation of a single bouton. The lower panel is action potential-triggered eEPSC$_{\text{AMPA/KA}}$. 
Figure 2. Isolation of sEPSC\textsubscript{NMDA} from sEPSC\textsubscript{AMPA/KA} in hippocampal CA3 neurons.

(A) A typical trace of glutamatergic sEPSCs observed before, during, and after the cumulative application of 30 µM CNQX, Mg\textsuperscript{2+}-free mixture solution and 30 µM D-APV. Insets (a’ to d’) represent the current traces with an expanded time scale of the upper panel (a’ to d’). The Mg\textsuperscript{2+}-free mixture external solution contained 3 µM glycine and 3 µM strychnine.

(B-C) Cumulative probability distribution for inter-event interval (Ba) and current amplitude (Ca) of CNQX-sensitive sEPSC\textsubscript{AMPA/KA} (Aa’) as well as D-APV-sensitive sEPSC\textsubscript{NMDA} (Ac’). The plot includes 97 events for sEPSC\textsubscript{AMPA/KA} (black) and 70 events for sEPSC\textsubscript{NMDA} (red) obtained from a single same neuron. The mean frequency (Bb) and amplitude (Cb) of sEPSC\textsubscript{AMPA/KA} (white) and sEPSC\textsubscript{NMDA} (red). Each column represents the mean ± SEM of five neurons. **; p < 0.01.

Figure 3. Kinetics of sEPSC\textsubscript{AMPA/KA} and sEPSC\textsubscript{NMDA}.

(A) Rise time course of sEPSC\textsubscript{AMPA/KA} (black) and sEPSC\textsubscript{NMDA} (red). The current amplitudes were normalized and superimposed with an expanded time scale.
(B) Rise time values of both sEPSC\textsubscript{AMPA/KA} (white) and sEPSC\textsubscript{NMDA} (red). Each column represents the mean ± SEM of five neurons. ***, p < 0.001.

(C) Typical current traces of single sEPSC\textsubscript{AMPA/KA} (left, gray) and sEPSC\textsubscript{NMDA} (middle, red) with an expanded time scale. $\tau_{\text{Decay}}$ of sEPSC\textsubscript{AMPA/KA} and sEPSC\textsubscript{NMDA} were well-fitted to a single exponential function (left and middle panels, thin solid lines). Normalized and superimposed single sEPSCs (fast sEPSC\textsubscript{AMPA/KA}, black; slow sEPSC\textsubscript{NMDA}, red) with an expanded time scale (left panel).

(D) $\tau_{\text{Decay}}$ of sEPSC\textsubscript{AMPA/KA} (black) and sEPSC\textsubscript{NMDA} (red) are summarized. Each column represents the mean ± SEM of five neurons. ***, p < 0.001.

Figure 4. Kinetics of eEPSC\textsubscript{AMPA/KA} and eEPSC\textsubscript{NMDA}.

(A) A typical time course of the amplitude of eEPSC\textsubscript{AMPA/KA} and eEPSC\textsubscript{NMDA} observed before, during, and after the cumulative application of 30 µM CNQX, Mg$^{2+}$-free mixture solution and 30 µM D-APV. The upper insets (a'–d') represent current trace of eEPSC\textsubscript{AMPA/KA} (a') and eEPSC\textsubscript{NMDA} (c') with or without their antagonists CNQX.
(b’) and D-APV (d’) respectively. The currents were elicited by focal single-pulse
electric stimuli.

**Figure 5. Effect of Xe on sEPSC_{NMDA}**

- **(A)** Typical trace of sEPSC_{NMDA} observed before, during, and after application of 70% Xe in the Mg^{2+}-free mixture solution with 30 µM CNQX. Lower insets (a’ and b’) represent current traces with an expanded time scale.

- **(B)** Cumulative probability distribution for the inter-event interval (frequency) (a) and amplitude (b) of sEPSC_{NMDA} with (red) and without (black) 70% Xe. The plot includes 121 events for the control and 54 events for the Xe. Data were obtained from a single same neuron.
(C) Columns (frequency and amplitude) represent the mean ± SEM of six neurons. The horizontal dotted line represents the relative control of the basal frequency and amplitude of sEPSC\textsubscript{NMDA}. **; p < 0.01.

(D) Xe-induced change in the $\tau_{\text{Decay}}$ of sEPSC\textsubscript{NMDA}. The column represents the mean ± SEM of the eight experiments. n.s.; not significant. The inset (left) represents the normalized and superimposed current traces with an expanded time scale of single sEPSC\textsubscript{NMDA} obtained in the control (black) and Xe (red).

**Figure 6. Effect of Xe on eEPSC\textsubscript{NMDA}**

(A) Typical time course of eEPSC\textsubscript{1} amplitude (a) and the PPR (eEPSC\textsubscript{2}/eEPSC\textsubscript{1}; b) before, during, and after application of 70% Xe in the Mg\textsuperscript{2+}-free mixture solution with 30 µM CNQX. Insets (left and middle) represent typical raw current traces in regions indicated by arrows (a’ and b’). The scaled and superimposed traces (right upper panel) show the PPR in the control (black) and Xe (red). (a) Time courses of eEPSC\textsubscript{NMDA} (eEPSC\textsubscript{1}) amplitude with and without Xe. (b) Time courses of paired-pulse ratio (PPR) with and without Xe.
(B) Xe-induced changes in eEPSC$_1$ amplitude, R$_f$, and PPR. The eEPSC$_1$ amplitude, R$_f$, and PPR obtained during the application of Xe were normalized to those obtained under the respective control (dotted line). Each column and error bar represents the mean ± SEM of eight neurons. **; p < 0.01.

(C) (a) Normalized and superimposed current traces of single eEPSC$_{NMDA}$ obtained in the control (black) and Xe (red) with an expanded time scale. (b) Xe-induced change in $\tau_{\text{Decay}}$ of eEPSC$_{NMDA}$. Each column represents the mean ± SEM of the eight experiments. n.s; not significant.

Figure 7. Effect of N$_2$O on sEPSC$_{NMDA}$

(A) A typical trace of sEPSC$_{NMDA}$ observed before, during, and after application of 70% N$_2$O in the Mg$^{2+}$-free mixture containing 30 µM CNQX. Insets (a’ and b’) in lower panel represent traces with an expanded time scale.

(B) Cumulative probability distribution for the inter-event interval (a) and current amplitude (b) of sEPSC$_{NMDA}$ with (red) or without (black) N$_2$O. The plot includes 156 events for the control and 45 events for the N$_2$O. Data were obtained from a single same neuron.
(C) Inset columns represent the mean ± SEM of six neurons. Dotted line represents the relative control of the basal frequency and amplitude of sEPSC$_{\text{NMDA}}$. *; p < 0.05, **; p < 0.01.

(D) N$_2$O-induced change in τ$_{\text{decay}}$ of sEPSC$_{\text{NMDA}}$. The column represents the mean ± SEM of the six neurons; n.s.; not significant. The inset (left) represents the normalized and superimposed traces of single sEPSC$_{\text{NMDA}}$ obtained in the control (black) and N$_2$O (red) with an expanded time scale. n.s; not significant.

Figure 8. Effect of N$_2$O on eEPSC$_{\text{NMDA}}$

(A) Typical time course of eEPSC$_1$ amplitude (a) and PPR (eEPSC$_2$/eEPSC$_1$; b) before, during, and after application of 70% N$_2$O in the Mg$^{2+}$-free mixture solution with 30 µM CNQX. Insets (left and middle) represent typical raw traces in regions indicated by arrows (a’ and b’). The scaled and superimposed traces (right panel) show the PPR in the control (black) and N$_2$O (red). (a) Time courses of eEPSC$_{\text{NMDA}}$ (eEPSC$_1$) amplitude with and without N$_2$O. (b) Time courses of PPR with and without N$_2$O.

(B) N$_2$O-induced changes in eEPSC$_1$ amplitude, R$_f$, and PPR. The eEPSC$_1$ amplitude, R$_f$, and PPR obtained during the application of N$_2$O were normalized to those obtained
under the respective control (dotted line). Columns and error bars represent the mean ± SEM of the six neurons. **; p < 0.01.

(C) (a) Normalized and superimposed traces of single eEPSC$_{\text{NMDA}}$ obtained in the control (black) and N$_2$O (red) with an expanded time scale. (b) N$_2$O-induced change in $\tau_{\text{Decay}}$ of eEPSC$_{\text{NMDA}}$. Each column represents the mean ± SEM of the six neurons. n.s; not significant.
16. TABLES

TABLE 1

Kinetic properties of sEPSCs and eEPSCs elicited by synaptic AMPA/KA (non-NMDA) (referred as AMPA/KA)- and NMDA-receptor channels in the rat hippocampal CA3 neurons.

<table>
<thead>
<tr>
<th></th>
<th>sEPSC&lt;sub&gt;AMPA/KA&lt;/sub&gt;</th>
<th>sEPSC&lt;sub&gt;NMDA&lt;/sub&gt;</th>
<th>n</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Hz)</td>
<td>0.73 ± 0.10</td>
<td>0.46 ± 0.10</td>
<td>5</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Amplitude (pA)</td>
<td>42.2 ± 4.7</td>
<td>24.3 ± 7.4</td>
<td>5</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Time to peak (ms)</td>
<td>1.26 ± 0.09</td>
<td>9.28 ± 0.33</td>
<td>5</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>τ&lt;sub&gt;Decay&lt;/sub&gt; (ms)</td>
<td>6.21 ± 0.69</td>
<td>70.8 ± 7.4</td>
<td>5</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>eEPSC&lt;sub&gt;AMPA/KA&lt;/sub&gt;</th>
<th>eEPSC&lt;sub&gt;NMDA&lt;/sub&gt;</th>
<th>n</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (pA)</td>
<td>483.7 ± 56.3</td>
<td>184.2 ± 20.0</td>
<td>8</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Time to peak (ms)</td>
<td>1.57 ± 0.08</td>
<td>10.1 ± 0.5</td>
<td>5</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>τ&lt;sub&gt;Decay&lt;/sub&gt; (ms)</td>
<td>6.35 ± 0.22</td>
<td>80.7 ± 8.3</td>
<td>8</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>
**TABLE 2**

Effect of xenon (Xe) on sEPSC\textsubscript{NMDA} (A) and eEPSC\textsubscript{NMDA} (B)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>sEPSC\textsubscript{NMDA}</th>
<th>n</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency (Hz)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.39 ± 0.07</td>
<td></td>
<td>8</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Xe</td>
<td>0.23 ± 0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amplitude (pA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>41.9 ± 3.5</td>
<td></td>
<td>8</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Xe</td>
<td>35.6 ± 3.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(\tau\text{Decay} (ms))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>76.4 ± 3.0</td>
<td></td>
<td>8</td>
<td>p = 0.382</td>
</tr>
<tr>
<td>Xe</td>
<td>74.3 ± 3.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>eEPSC\textsubscript{NMDA}</th>
<th>n</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amplitude (pA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>68.5 ± 9.8</td>
<td></td>
<td>8</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Xe</td>
<td>39.2 ± 6.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Failure rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.29 ± 0.05</td>
<td></td>
<td>8</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Xe</td>
<td>0.49 ± 0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Paired-pulse ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.94 ± 0.07</td>
<td></td>
<td>8</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Xe</td>
<td>1.37 ± 0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(\tau\text{Decay} (ms))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>80.7 ± 8.3</td>
<td></td>
<td>8</td>
<td>p = 0.875</td>
</tr>
<tr>
<td>Xe</td>
<td>80.4 ± 8.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 3

Effect of nitrous oxide (N$_2$O) on sEPSC$_{NMDA}$ (A) and eEPSC$_{NMDA}$ (B)

<table>
<thead>
<tr>
<th></th>
<th>sEPSC$_{NMDA}$</th>
<th>n</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.49 ± 0.06</td>
<td>6</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Xe</td>
<td>0.32 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (pA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>53.9 ± 5.6</td>
<td>6</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>Xe</td>
<td>41.8 ± 3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\tau_{\text{Decay}}$ (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>87.9 ± 4.5</td>
<td>6</td>
<td>$p = 0.780$</td>
</tr>
<tr>
<td>Xe</td>
<td>89.9 ± 7.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>eEPSC$_{NMDA}$</th>
<th>n</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (pA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>72.9 ± 8.9</td>
<td>6</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Xe</td>
<td>49.4 ± 6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failure rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.50 ± 0.07</td>
<td>6</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Xe</td>
<td>0.66 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paired-pulse ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.84 ± 0.07</td>
<td>6</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Xe</td>
<td>1.10 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\tau_{\text{Decay}}$ (ms)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>78.2 ± 9.1</td>
<td>6</td>
<td>$p = 0.416$</td>
</tr>
<tr>
<td>Xe</td>
<td>73.9 ± 8.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1

A. Whole-cell Rec. 
B. Many homogenous synaptic Rec. 
C. Single synaptic Rec.

Glu $10^{-5}$ M

Extrasynaptic receptor

Rec.

Bouton

50 pA

15 s

sEPSC_{AMPA/KA}

50 pA

1 min

Stim.

Synaptic receptor

Rec.

eEPSC_{AMPA/KA}

100 pA

5 ms
Figure 3
Figure 5

A

sEPSC_{NMDA}

70% Xe  
CNQX + Mg^{2+}-free mixture

1 min

50 pA

a'

b'

100 pA

B

a

Control  
Xe

Cumulative Prob.

Interval (s)

0  
1  
20  
30

b

Cumulative Prob.

Amplitude (pA)

0  
200

C

Xe-induced change

**

**

Frequency  
Amplitude

D

Control  
Xe

Xenon-induced change

n.s

\tau_{Decay}

100 ms
**Figure 6**

**A**

- **a**
  - Graph showing eEPSC$_{\text{NMDA}}$ Amp. (pA) with time (min) on the x-axis and eEPSC$_{1}$ and eEPSC$_{2}$ with control (a') and Xe (b') conditions. CNQX + Mg$^{2+}$-free mixture.
  - Time points 0 to 15 min.
- **b**
  - Graph showing PPR (eEPSC$_{2}$/eEPSC$_{1}$) with time (min) on the x-axis and a' and b' conditions.

**B**

- Graph showing Xe-induced change in eEPSC$_{1}$ Amp., R$_{f}$, and PPR with significance indicated.

**C**

- **a**
  - Graph showing control and Xe conditions with 100 ms time scale.
- **b**
  - Bar graph showing $\tau_{\text{decay}}$ (ms) for control and Xe with n.s. (not significant).
Figure 8

**eEPSC\textsubscript{NMDA}**

**A**
- Control (a’)
- N\textsubscript{2}O (b’)
- Scaled
- CNQX + Mg\textsuperscript{2+}-free mixture

- eEPSC\textsubscript{1} and eEPSC\textsubscript{2} Amplitude (pA)

**B**
- N\textsubscript{2}O-induced change
- eEPSC\textsubscript{1} Amp.
- R\textsubscript{f}
- PPR

**C**
- Control
- N\textsubscript{2}O

**Figure 8**

N\textsubscript{2}O-induced change in eEPSC\textsubscript{1} Amp., R\textsubscript{f}, and PPR. 

**C**
- Control
- N\textsubscript{2}O

**Figure 8**

N\textsubscript{2}O-induced change in eEPSC\textsubscript{1} Amp., R\textsubscript{f}, and PPR. 

**C**
- Control
- N\textsubscript{2}O

**Figure 8**

N\textsubscript{2}O-induced change in eEPSC\textsubscript{1} Amp., R\textsubscript{f}, and PPR. 

**C**
- Control
- N\textsubscript{2}O

**Figure 8**

N\textsubscript{2}O-induced change in eEPSC\textsubscript{1} Amp., R\textsubscript{f}, and PPR. 

**C**
- Control
- N\textsubscript{2}O