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Nitrous Oxide at Dental-Used Low Concentration Adequately Depresses NMDA Receptor-Mediated Spontaneous and Evoked Responses in Rat Hippocampal CA3 Neurons

Kiku Nonaka[1], Il-Sung Jang[2], Mami Noda[3, 4], Hideaki Nagami[5] and Norio Akaike[5, 6, *]

[1]Research Division for Life Science, Kumamoto Health Science University, Kumamoto, Japan

[2]Department of Pharmacology, School of Dentistry, Kyungpook National University, 2177 Dalgubeol-daero, Jung-gu, Daegu, Republic of Korea

[3]Laboratory of Pathophysiology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

[4]RUDN University, Moscow, Russian Federation

[5]Research Division for Clinical Pharmacology, Medical Corporation, Juryo Group, Kumamoto Kinoh Hospital, Kumamoto, Japan

[6]Research Division of Neurophysiology, Kitamoto Hospital, Saitama, Japan

[*]**Corresponding Author:** 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
E-mail: akaike.sachin715@juryo.or.jp

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Abstract

Nitrous oxide (N_2O) induces anesthesia via interaction with N-methyl-D-aspartate (NMDA) and low concentration (30%) of N_2O is frequently used in dental procedure. Present study was examined how 30% N_2O modulates spontaneous (s) and evoked (e) NMDA receptor-induced excitatory postsynaptic currents (s/eEPSC$_{NMDA}$) by using "synapse bouton preparation" of rat hippocampal CA3 neurons to evaluate. N_2O significantly decreased the frequency and amplitude without altering the *1/e* decay time constant (τ_{Decay}) of sEPSC$_{NMDA}$, while it decreased the amplitude but increased the failure rate (Rf) and paired-pulse ratio (PPR) without altering the current decay time course (τ_{Decay}) of eEPSC$_{NMDA}$. The results suggest that N_2O acts not only postsynaptic but also presynaptic sides. In addition, the inhibitory effect of 30% N_2O on s/eEPSC$_{NMDA}$ was almost the same as compared with a high concentration of 70% N_2O. Weak anesthetic action of 30% N_2O is combined with other intravenous or volatile anesthetics in the pediatric fields. In the previous studies show that N_2O works on hippocampal CA3 neurons as well as in spinal dorsal horn and amygdala. These results suggest that N_2O may also take away negative influences such as pain and terror-induced memories induced by clinical examination, helping to understand why 30% N_2O is used in pediatric dentistry and surgery.

Keywords: N_2O; AMPA/KA response; NMDA response; spontaneous EPSC$_{NMDA}$; evoked EPSC$_{NMDA}$; hippocampal CA3 neuron; synaptic transmission

Introduction

Different from the most of intravenous and volatile anesthetics, the gas anesthetics such as nitrous oxide (N_2O) and xenon (Xe) induce anesthesia mainly via interaction with three kinds of ionotropic glutamate receptors: i.e. N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainic acid (KA). AMPA/KA receptors (non-NMDA receptors) effect short-term changes in synaptic strength, whereas NMDA receptors regulate genes that are required for the long-term maintenance of these changes [1]. As for their clinical relevance, dysfunction of AMPA/KA receptors play important role in the development of neurological or psychiatric disorders such as anxiety, schizophrenia, epilepsy, neuropathic pain, and migraine [2], while NMDA receptors are reported to contribute to the amnestic and analgesic components of anesthesia [3]. Inhibition of excitatory NMDA or AMPA/KA receptor-gated excitatory transmission is the main anesthetic action of N_2O [4-8], while the NMDA receptors had been considered as major targets for general anesthetics [3]. We had succeeded to detect pure NMDA receptor-mediated s/eEPSC$_{NMDA}$ using the "synapse bouton preparation" of acutely and mechanically isolated single neuron [9-11] and reported previously that N_2O inhibited the activation of both AMPA/KA and NMDA receptors [12]. A few slice studies also demonstrated that 50-65% N_2O depressed both s/eEPSC$_{NMDA}$ in spinal dorsal horn neurons and amygdala [4, 13].

High concentration of N_2O (50-70%) is commonly inhaled in major surgery. At the dental procedure, however, low concentration of N_2O (30%) is sufficient for sedative effect together with the anxiolytic, analgesic, anesthetic properties and the rapid onset and recovery with minimal side effects [14]. However, it is still unclear about the effects of 30% N_2O on other region of the brain, for example, on hippocampal neurons responsible for short-term memory. Therefore, we investigated the effect of 30% N_2O on s/eEPSC$_{NMDA}$ using "synapse bouton preparation" of the rat hippocampal CA3 neurons. Functional role of 30% and 70% N_2O were discussed based on our present and previous results [9].

Methods

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 in Kumamoto Health Science University (approved number 20-07) and Kumamoto Kinoh Hospital. All efforts were made to minimise animal suffering and reduce the number of animals used in this study.

Acute Isolation

Wistar rats of either sex (aged 11-17 days, SLC, Shizuoka, Japan) were decapitated after deep pentobarbital anaesthesia (50 mg·kg^{-1} i.p.) [15]. The brain was dissected and transversely sliced at a thickness of 350 μm using a micro-slicer (7000 smz; Campden Instruments LTD, Leicester, England). Slices containing the hippocampal CA3 neurons were kept in an incubation medium at room temperature (22°C–24°C) for at least 1 h before acute mechanical dissociation, as reported in previous study [9]. The mechanically dissociated CA3 neurons were allowed to settle and left to adhere to the bottom of the dish for 15 min.

Electrophysiological Recordings

Electrophysiological recordings were performed using a conventional whole-cell patch-clamp technique. Neurons were clamped at a holding potential (V_H) of −60 mV. The resistance of 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. The liquid junction potential, series resistance (40%–70%), and pipette capacitance were compensated. The membrane currents were filtered at 1 kHz (Multiclamp 700 B), digitized at 4 kHz (Digidata 1440; Molecular Devices), and stored on a computer equipped

with pCLAMP 10.7 (Molecular Devices). During recording, 10-mV hyperpolarizing step pulses (30 ms in duration) were periodically delivered to monitor access resistance. Experiments were performed at room temperature (22ºC–24ºC) [9].

The s/eEPSC$_{NMDA}$ were recorded in Mg^{2+}-free extracellular mixture (Mg^{2+}-free mixture) solution containing 3 μM glycine, 3 μM strychnine (a strychnine-sensitive glycine receptor-operated Cl^- channel antagonist), and 30 μM CNQX (an AMPA/KA receptor antagonist). The extracellular test solutions routinely contained 10 μM bicuculline (a GABA$_A$ receptor blocker) to inhibit GABAergic currents [9].

Focal single and/or paired-pulse electrical stimulation was applied to elicit eEPSCs [10, 11]. A stimulating theta electrode was placed to the nearest surface of the apical dendrites of CA3 neurons, and the focal paired-pulse stimulation with an interval of 200 ms was delivered to evoke two eEPSCs (eEPSC$_1$ and eEPSC$_2$).

Synapse Bouton Preparation

This preparation is mechanically dissociated single neuron attached with many nerve endings (boutons) preserving normal physiological function. We can exactly evaluate s/eEPSC$_{NMDA}$ without either glial and neuronal tonic currents or extrasynaptic substances. The sEPSC$_{NMDA}$ and eEPSC$_{NMDA}$ are recorded from many homologous glutamatergic boutons and a single glutamatergic bouton evoked by focal electrical stimuli using conventional whole-cell patch clamp technique, respectively. The frequency of sEPSC$_{NMDA}$ and the Rf (failure rate) and PPR (paired-pulse ratio) of eEPSC$_{NMDA}$ are realized as presynaptic events, while the τ_{Decay} s/eEPSC$_{NMDA}$ and the I_{NMDA} induced by exogenous NMDA application are identified as postsynaptic events [16, 17]. Schematic illustration of synapse bouton preparation in rat hippocampal CA3 neuron was presented in our previous paper [9].

Drugs and Application of N_2O

The reagents 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). The 30% N_2O gas mixtures (N_2O 30%, O_2 28.5%, CO_2 1.5% N_2 40%) were purchased from a gas supplier (Kumamoto Sanso Co., Kumamoto, Japan). To manufacture N_2O solutions, N_2O gas mixtures were bubbled into the external test solution in a 5-mL glass test tube for a minimum of 3-5 min, and then the test tube was tightly sealed using a parafilm. N_2O test solution was applied using the 'Y-tube system' for rapid solution exchange within 20 ms [18] with a perfusion rate of 0.7–0.8 mL/min. The tip of the Y-tube (0.2-mm inner diameter) was positioned 1 mm away from the patched neuron.

Data Analysis

All data of s/eEPSCNMDA were analyzed using Origin Pro 7.5 software (Origin-Lab Corporation, Northampton, MA, USA). Data are reported as the means ± S.E.M. Data were tested by Student t-test. Two-tailed *P* values of less than 0.05 were considered statistically significant [9].

Results

CNQX-Sensitive sEPSC$_{AMPA/KA}$ and D-APV-Sensitive sEPSC$_{NMDA}$ in Rat Hippocampal CA3 Neurons

When the isolated CA3 neurons were held at a V_H of –60 mV, sEPSCs were recorded in normal external solution containing Mg^{2+} (NMDA receptor channel blocker) and 10 μM bicuculline. The inwardly directed and rapidly decaying synaptic currents was completely blocked by adding 30 μM CNQX (Figure 1Aa', b'). Thus, the fast sEPSCs were referred as sEPSC$_{AMPA/KA}$ (Figure 1 Aa', Ba and C). In a continuous presence of CNQX, the inwardly directed and slowly decaying spontaneous synaptic currents appeared by the cumulative application of external Mg^{2+}-free mixture solution containing 3 μM strychnine and 3 μM glycine (a

strychnine-insensitive NMDA receptor agonist) [19, 20]. Since the slow synaptic currents were completely and reversibly blocked by adding 30 μM D-APV (a selective NMDA receptor antagonist) (Figure 1Ac', d', e'), the slow sEPSCs were identified as $sEPSC_{NMDA}$. The *1/e* current decay time course (τ_{Decay}) of both $sEPSC_{AMPA/KA}$ and $sEPSC_{NMDA}$ was well fitted to a single exponential function (Figure 1 Bb). As reported previously [9], the mean current amplitude was smaller in $sEPSC_{NMDA}$ than in $sEPSC_{AMPA/KA}$ (Figure 1 Aa', c', e'). The τ_{Decay} values were 4.8 ± 0.7 ms for $sEPSC_{AMPA/KA}$ [15] and 83.4 ± 5.5 ms for $sEPSC_{NMDA}$. But the τ_{Decay} values were much greater in $sEPSC_{NMDA}$ than in $sEPSC_{AMPA/KA}$ [9] (Figure 1C).

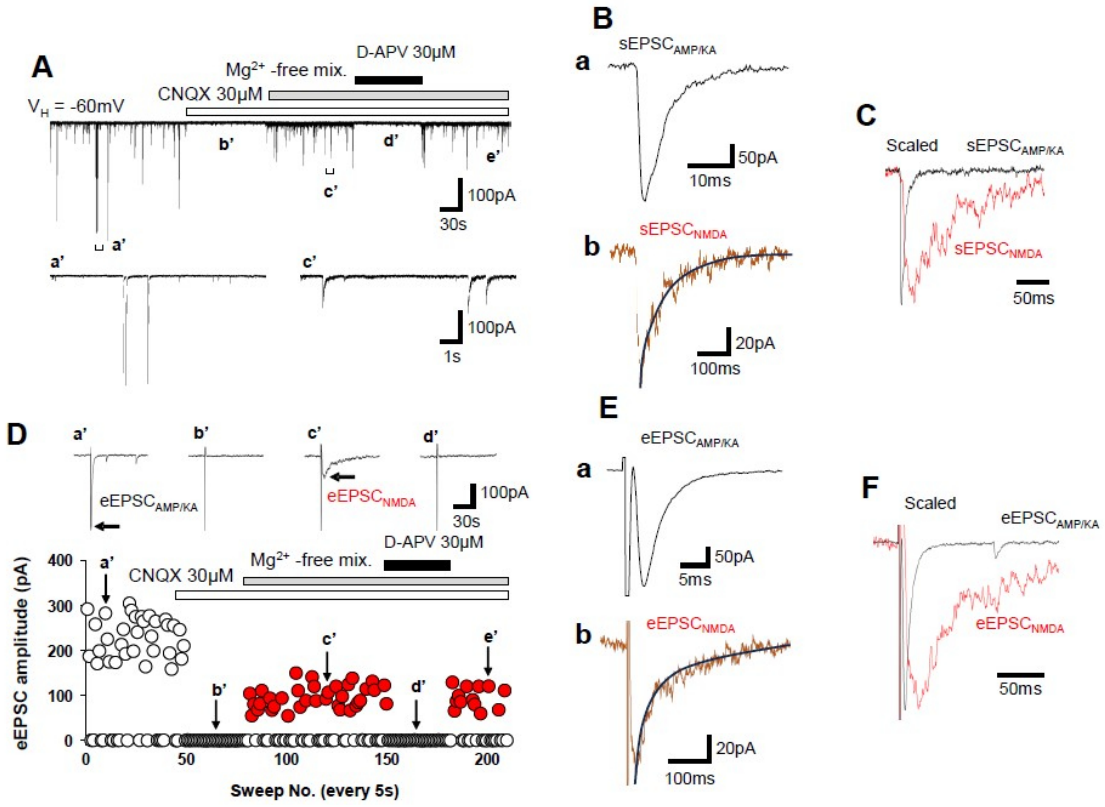

Figure 1: Separation of $s/eEPSC_{NMDA}$ from $s/eEPSC_{AMPA/KA}$ in rat hippocampal CA3 neurons

(A) Recording of fast $sEPSC_{AMPA/KA}$ (a') and slow $sEPSC_{NMDA}$ (c', e') induced by multiple homogenous glutamatergic synaptic boutons at a V_H = -60mV with and without the application of CNQX, Mg^{2+}-free mixture solution containing glycine and strychnine, or D-APV.

(B) Typical current trace of a single $sEPSC_{AMPA/KA}$ (a) and a single $sEPSC_{NMDA}$ (b) with different expanded time scales. The current decay time courses of $sEPSC_{NMDA}$ (b) was well fitted in a single exponential manner (black line), as well as $sEPSC_{AMPA/KA}$ [15].

(C) Normalized and superimposed single sEPSCs (fast $sEPSC_{AMPA/KA}$, black; slow $sEPSC_{NMDA}$, red) with same expanded time scale.

(D) (Upper) Typical current traces of $eEPSC_{AMPA/KA}$ (a') and $eEPSC_{NMDA}$ (c') at the time indicated in the lower panel. The data were obtained using focal single-pulse electrical stimuli.

(Lower) The time course of peak amplitude during control (a'), in the presence of 30 μM CNQX (b'), CNQX + Mg^{2+}-free mix (c' and e'), and CNQX + Mg^{2+}-free mix + 30μM D-APV (d'), respectively. Data were obtained from a single neuron.

(E) Typical current traces of $eEPSC_{AMPA/KA}$ (a) and $eEPSC_{NMDA}$ (b) that were fitted by single exponential decay.

(F) Normalized and superimposed $eEPSC_{AMPA/KA}$ (black) and $eEPSC_{NMDA}$ (red) with the same expanded time scale. The current data were quoted from Da', c'.

Separation of $eEPSC_{NMDA}$ from $eEPSC_{AMPA/KA}$

The focal single-pulse stimulation elicited the inwardly directed and rapidly decaying currents in normal external solution which were completely blocked by adding 30 μM CNQX (Figure 1Da', b'). Thus, the fast eEPSCs were identified as $eEPSC_{AMPA/KA}$. In a continuous presence of 30 μM CNQX, the inwardly directed and slowly decaying currents appeared by the cumulative application of Mg^{2+}-free mixture solution containing 3 μM glycine and 3 μM strychnine. The slow currents were completely and reversibly blocked by 30 μM D-APV (Figure 1Dc', d'), indicating that the slow eEPSCs are $eEPSC_{NMDA}$. The τ_{Decay} of both $eEPSC_{AMPA/KA}$ and $eEPSC_{NMDA}$ was also well fitted to a single exponential function (Figure 1Eb). The mean amplitude of $eEPSC_{NMDA}$ was significantly smaller than that of $eEPSC_{AMPA/KA}$ (Figure 1Da', c'). Also, τ_{Decay} was greater in $eEPSC_{NMDA}$ than in $eEPSC_{AMPA/KA}$ (Figure 1Da', c', Ea, b, F): τ_{Decay} for $eEPSC_{NMDA}$ is 79.4 ± 6.2 ms (Table 1B), while τ_{Decay} for $eEPSC_{AMPA/KA}$ is 5.4 ± 0.73 ms [9]. There were no differences in kinetic parameters between $sEPSC_{NMDA}$ and $eEPSC_{NMDA}$ (Table 1A, B), as well as between $sEPSC_{AMPA/KA}$ and $eEPSC_{AMPA/KA}$ [9].

Effects of 30% N_2O on $sEPSC_{NMDA}$

The application of 30% N_2O significantly decreased both the frequency and amplitude of $sEPSC_{NMDA}$ (Figure 2Aa', b'). Figure 2B shows the cumulative distributions of the frequency (a) and amplitude (b) of $sEPSC_{NMDA}$ in the control (black) and 30% N_2O (red) conditions. N_2O shifted the cumulative probability of the interval (frequency) to the right and the amplitude to the left, indicating that N_2O decreased the mean frequency and amplitude of $sEPSC_{NMDA}$. However, the τ_{Decay} of $sEPSC_{NMDA}$ was not affected by 30% N_2O (Figure 2C, Table 1A).

Table 1: Effects of 30% N_2O on s/$eEPSC_{NMDA}$

30% N_2O effect				
A	$sEPSC_{NMDA}$		n	t test
Frequency (Hz)	Control	0.40 ± 0.06	6	$p < 0.05$
	N_2O	0.21 ± 0.04		
Amplitude (pA)	Control	40.8 ± 3.6	6	$p < 0.01$
	N_2O	32.6 ± 3.4		
τ_{Decay} (ms)	Control	83.4 ± 5.5	6	$p = 0.681$
	N_2O	83.0 ± 5.6		

30% N_2O effect				
B	$eEPSC_{NMDA}$		n	t test
Amplitude (pA)	Control	76.4 ± 10.7	6	$p < 0.05$
	N_2O	57.3 ± 7.2		
Failure rate	Control	0.50 ± 0.06	5	$p < 0.05$
	N_2O	0.65 ± 0.06		
Paired-pulse ratio	Control	0.85 ± 0.03	5	$p < 0.05$
	N_2O	1.19 ± 0.10		
τ_{Decay} (ms)	Control	79.4 ± 6.2	6	$p = 0.199$
	N_2O	83.9 ± 6.1		

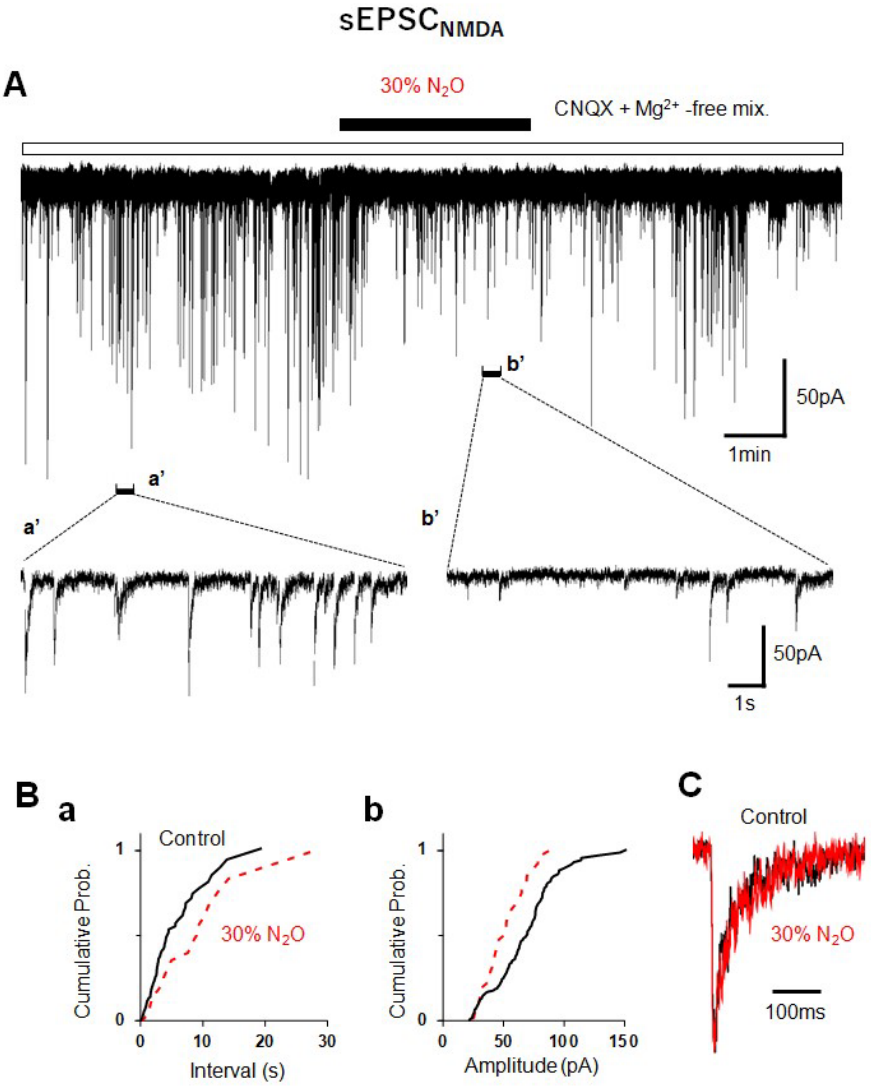

Figure 2: Effects of 30% N_2O on sEPSC$_{NMDA}$

(A) A typical current trace of sEPSC$_{NMDA}$ observed before, during, and after application of 30% N_2O in the Mg^{2+}-free mixture solution with 30 μM CNQX. The lower panel of sEPSC$_{NMDA}$ shows an expanded time scale at a' and b' that were shown by horizontal bar in the upper current traces.

(B) Cumulative probability distributions for the inter-event interval (frequency) (a) and amplitude (b) of sEPSC$_{NMDA}$ with (red) and without (black) 30% N_2O. The plot includes 155 events for the control and 44 events for the 30% N_2O conditions, respectively. Data were obtained from a single neuron.

(C) Effect of 30% N_2O on the τ_{Decay} of sEPSC$_{NMDA}$. The current traces represent the normalized and superimposed current traces with an expanded time scale of single sEPSC$_{NMDA}$ obtained in control (black) and 30% N_2O (red) conditions.

Effects of 30% N_2O on eEPSC$_{NMDA}$

Figure 3A shows typical time course of eEPSC$_{NMDA}$ including the amplitude of NMDA receptor-mediated eEPSC$_1$ (a) and paired-pulse ratio (PPR) (b) obtained before, during, and after the application of 30% N_2O. N_2O decreased the mean amplitude of eEPSC$_1$ and increased the R_f and PPR as compared to the control (Figure 3Aa, b). The N_2O-induced increase in either the Rf or PPR suggests that 30% N_2O acts presynaptically to decrease the probability of evoked glutamate release. In contrast, N_2O did

not affect the τ_{Decay} of $eEPSC_{NMDA}$ (Figure 3B and Table 1B).

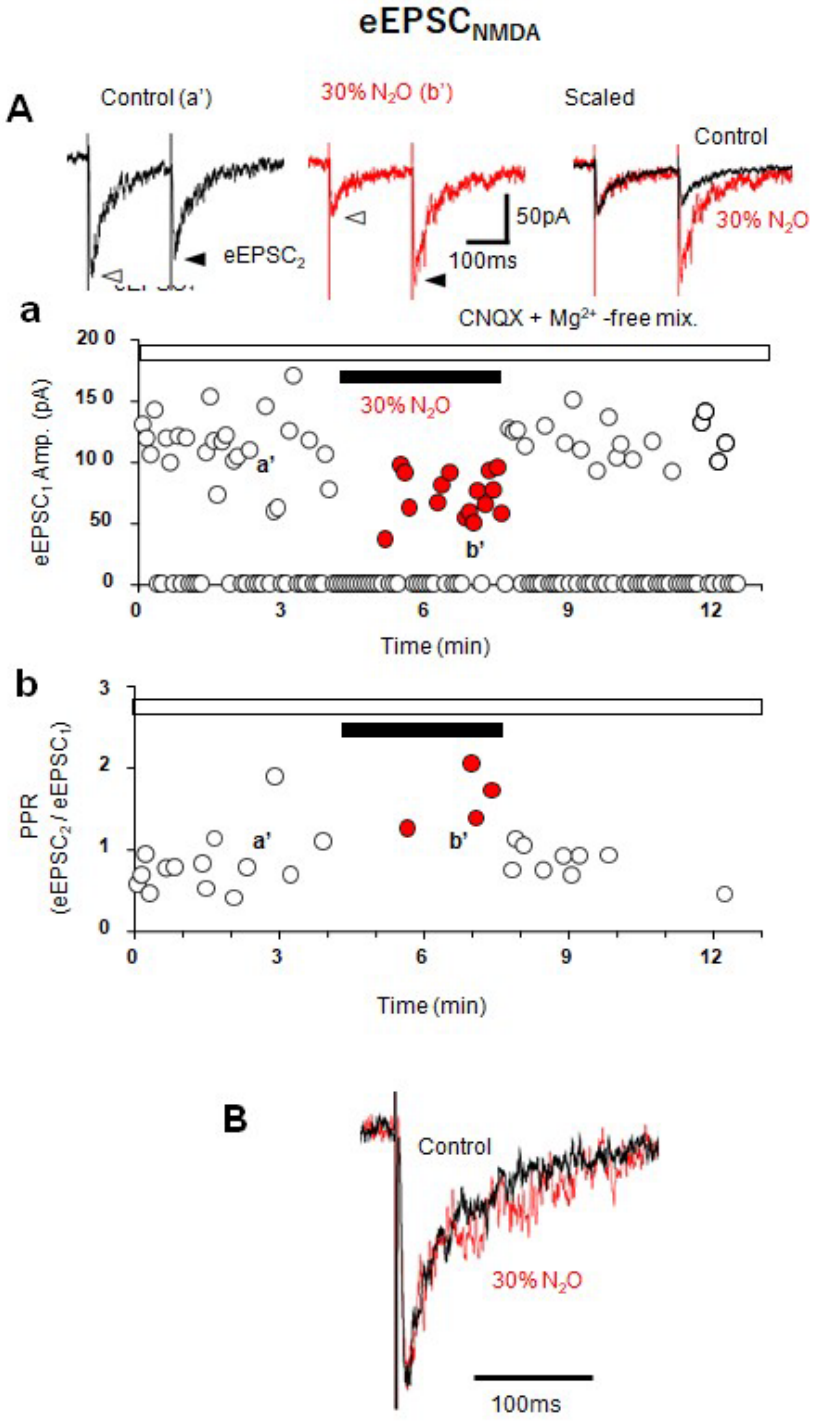

Figure 3: Effects of 30% N_2O on $eEPSC_{NMDA}$.

(A) (Upper panel) The typical raw current traces ($eEPSC_1$ and $eEPSC_2$) induced by the focal paired pulse stimulation in control (obtained at a' point in the lower panel) and during application of 30% N_2O (at b' point in the lower panel). The right traces show the superimposed traces of the paired-pulse stimulation in the control (black) and N_2O (red) conditions. (a) Typical time course of $eEPSC_1$ amplitude before, during, and after application of 30% N_2O in the Mg^{2+}-free mixture solution with 30 μM CNQX. (b) Time course of the paired-pulse ratio ($eEPSC_2/eEPSC_1$, PPR).

(B) Normalized and superimposed current traces of single $eEPSC_{NMDA}$ obtained in the control (black) and 30% N_2O (red) conditions with an expanded time scale.

Discussion

Kinetic properties of $sEPSC_{NMDA}$ and $eEPSC_{NMDA}$

The $sEPSC_{NMDA}$ clearly showed smaller amplitude, slower rise, and prolonged τ_{Decay} than those of $sEPSC_{AMPA/KA}$, as shown in present and previous studies [9]. In slice preparation, $sEPSC_{NMDA}$ are affected by relatively limited spillover of transmitter to synaptic and extrasynaptic receptors or neighboring postsynaptic sites. When analyzing synaptic responses in the slice preparation, one cannot avoid interference by extrasynaptic NMDA receptor-mediated and glial tonic currents in Mg^{2+}-free external solution [21-23]. In the "synapse bouton preparation", the tonic current is absent. Thus, the recorded sEPSCs have high signal--to-noise (S/N) ratio.

Even in a single axon from one neuron diverges into many branches. These axons have the inputs of several hundred synapses on a single neuron [24, 25], and each synapse shows different responses [26]. Therefore, the polysynaptic $eEPSC_{NMDA}$ measured in slice preparations are the sum of many different synaptic currents [27, 28]. In contrast, we could accurately analyze PPR, Rf, amplitude and τ_{Decay} of $eEPSC_{NMDA}$ at pure single synapse level. The measured D-APV-sensitive $eEPSC_{NMDA}$ had the same kinetic parameters as seen in $sEPSC_{NMDA}$ (Table 1). The amplitude of $s/eEPSC_{NMDA}$ was smaller than $e/eEPSC_{AMPA/KA}$ respectively, and the rise and τ_{Decay} of $s/eEPSC_{NMDA}$ were more prolonged than $s/eEPSC_{AMPA/KA}$ [9].

NMDA and AMPA/KA Receptor-Mediated Responses and Anesthetic Potency

In our previous papers, N_2O of 30 and 70% significantly decreased $s/eEPSC_{AMPA/KA}$ in a concentration-dependent manner [12, 29]. These findings confirm that inhibitions of AMPA/KA receptors-mediated responses may correspond to anesthetic potency of N_2O as well as the those of NMDA receptor-mediated ones. Our findings are further supported by growing evidences, showing the clinical role of AMPA/KA receptors in acute surgical and inflammatory pain [30-34]. Instead, NMDA receptor-dependent responses have more important role for chronic hyperalgesia and allodynia [35-37]. The NMDA receptor-mediated long-term potentiation (LTP), in which intense tetanic stimulation of the dorsal root corresponding to strong noxious stimuli induces long-lasting enhancement of synaptic responses, critically result in chronic intractable pain [38-40]. As a NMDA receptor antagonist, N_2O acts as a potent antihyperalgesic property [41], and improves antidepressant responses in treatment-resistant depression [42]. As described in our previous paper, both N_2O and Xe significantly decreased $eEPSC_{AMPA/KA}$ in a concentration-dependent manner [12, 15, 29]. In this study, however, 30% and 70% N_2O equally inhibited $s/eEPSC_{NMDA}$ (Figure 4). The finding can explain the established concept that glutamate (Glu) first activates AMPA/KA receptors by depolarizing postsynaptic membrane. The depolarization leads to remove Mg^{2+} ions that block NMDA receptor-channels, resulting in further activation of NMDA receptors. Thus, it is concluded that the concentration-dependent blockage of AMPA/KA receptors by N_2O may contribute more effectively to the N_2O anesthesia in clinical use.

The $s/eEPSC_{Glu}$ consists of three types of Glu subtype receptors (AMPA, KA, and NMDA receptors)-induced currents. The results may suggest that 30 and 70% N_2O inhibited the currents of AMPA and KA subtypes concentration-dependently but equally for NMDA-induced current. In other words, the inhibitory effect of N_2O on $eEPSC_{NMDA}$ might be saturated at low concentration of 30%. Therefore, the low concentration of N_2O in dental field may be reasonable to prevent enough the negative influence such as pain and terror *etc*. followed by examination in pediatric fields [43, 44].

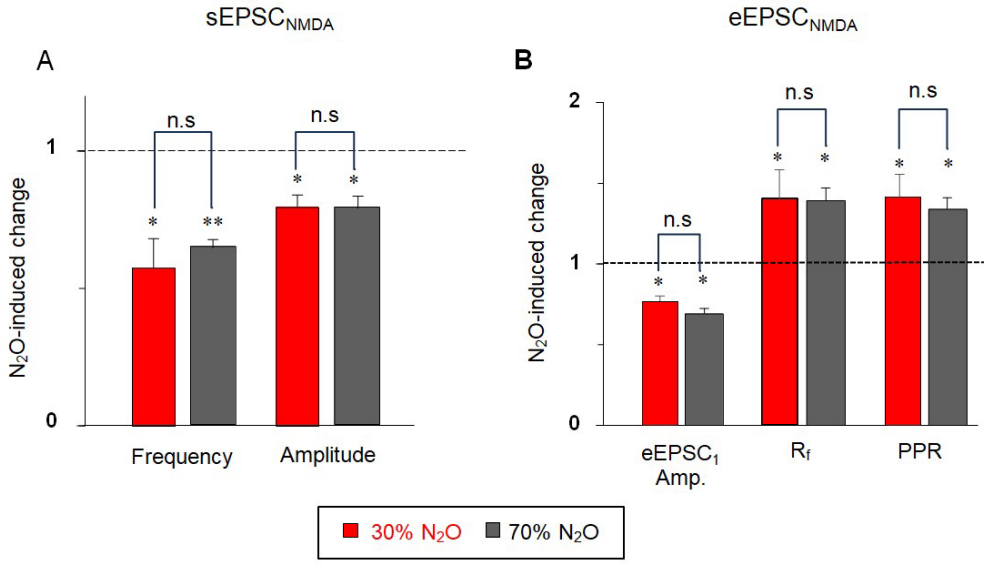

Figure 4: Effects of 30% and 70% N_2O on $sEPSC_{NMDA}$ and $eEPSC_{NMDA}$

(A) The relative N_2O-induced changes of frequency and amplitude of $sEPSC_{NMDA}$. The columns represent the mean ± SEM of six neurons. The horizontal dotted line represents the relative control values of $sEPSC_{NMDA}$. *$p < 0.05$, **$p < 0.01$, n.s; not significant. There were no differences between relative inhibitory effects on $sEPSC_{NMDA}$ by 30% N_2O (red, $n = 6$) and 70% N_2O (black, $n = 6$). Data of 70% N_2O were quoted by our previous paper [24].

(B) The relative amplitude of $eEPSC_1$, the failure rate (R_f), and the PPR of $eEPSC_{NMDA}$ obtained during the application of 30% N_2O (red) and 70% N_2O (black) were normalized to those obtained under the respective control conditions (dotted line). The columns represent the mean ± SEM of five to six neurons. *; $p < 0.05$, n.s; not significant. Data of 70% N_2O were quoted from our previous study [9].

Comparison of Gas Anesthetics

In two kinds of gas anesthetics such as N_2O an Xe, Xe suppressed Glu-, AMPA-, KA- and NNDA-induced postsynaptic currents (I_{Glu}, I_{AMPA}, $I_{KA,}$ I_{NMDA}) in rat isolated CA3 neurons in a non-competitive manner [15]. Similarly, N_2O inhibited the I_{Glu} in concentration-dependent manner. The inhibition of I_{Glu} by 70% Xe was 1.6 and 2.7 times greater than those by 30% and 70% N_2O, respectively [12, 15]. These results indicate both Xe and N_2O act directly on postsynaptic Glu receptor, but the inhibition by N_2O is less than Xe. Both Xe and N_2O decreased the frequency of sEPSC in a concentration-dependent manner, suggesting that Xe and N_2O also act on the presynaptic site [12, 15]. Moreover, 70% Xe and 70% N_2O inhibited the amplitude but increased the Rf and PPR of $eEPSC_{Glu}$ without affecting τ_{Decay}. The inhibition was also much greater in Xe than N_2O [9, 12, 15]. All these results indicate clearly that the two kinds of gas anesthetics directly act on both pre- and post-synaptic level.

Clinical Relevance

Low concentration of N_2O (30%) is sufficient for sedative effect together with the anxiolytic, analgesic, anesthetic properties and the rapid onset and recovery with minimal side effects in dentistry [14]. We found that 30% N_2O works on hippocampal neurons, as well as in spinal dorsal horn and amygdala with 70% N_2O. These results suggest that N_2O inhibits not only pain and terror but also related negative memories. Though the results are from limited parts of the animal brain, our study may help to understand why 30% N_2O is used in pediatric dentistry and surgery.

Conclusion

Low (30%) and high (70%) concentration of N_2O inhibited equally NMDA receptor-operated spontaneous and evoked synaptic currents in the rat hippocampal CA3 neurons. Low N_2O combined with other kinds of anesthetics may take away negative influences such as pain and terror-induced memories in clinical examination of pediatric dentistry and surgery.

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