Suppressive effect of nitrous oxide on motor evoked potentials can be reversed by train stimulation in rabbits under ketamine/fentanyl anaesthesia, but not with additional propofol

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The effect of nitrous oxide on myogenic motor evoked potentials (MEPs) after multipulse stimulation is controversial. We investigated the effects of propofol in this paradigm. MEPs were elicited electrically by a single pulse and by trains of three and five pulses in rabbits anaesthetized with ketamine and fentanyl. Nitrous oxide 30–70% was given and MEPs were recorded. After washout of nitrous oxide, propofol was given as a bolus of 10 mg kg⁻¹ followed by 0.8 (n=9) or 1.6 mg kg⁻¹ min⁻¹ (n=8) as a continuous infusion. Nitrous oxide was then re-administered and MEPs were recorded. Without propofol, nitrous oxide significantly reduced the amplitude of MEPs dose-dependently, but this effect was reversed by multipulse stimulation. Administration of low-dose propofol enhanced nitrous oxide-induced suppression, and this effect was reversed by five-pulse stimulation. However, high-dose propofol produced a greater increase in suppression, such that even five-pulse stimulation did not overcome the suppression. The results suggest that the degree of reversal of nitrous oxide-induced MEP suppression produced by multipulse stimulation is affected by the administration of propofol.


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Intraoperative monitoring of myogenic motor evoked potentials (MEPs) in response to transcranial stimulation of the motor cortex provides a method for monitoring the functional integrity of descending motor pathways during invasive manipulation of the spine or thoracoabdominal aortic replacement surgery. However, the clinical and experimental use of these techniques with a single pulse as the stimulus has shown that the potentials elicited are very sensitive to suppression by most anaesthetic agents.

Recently, to overcome anaesthetic-induced depression of myogenic MEPs, multiple-stimulus setups with paired or a train of pulses for stimulation of the motor cortex have been proposed. The advent of multipulse stimulation for intraoperative monitoring of myogenic MEPs may allow a wider choice and dose range of anaesthetic agents.

Nitrous oxide has been used commonly during MEP monitoring as a supplementary anaesthetic, although a number of investigators have shown that it suppresses MEPs elicited by single-pulse stimulation. However, the effect of nitrous oxide on myogenic MEPs in response to stimulation with paired pulses or a train of pulses is still controversial.

One report demonstrated that 50% nitrous oxide did not affect the amplitude of MEPs under fentanyl/low-dose propofol anaesthesia when paired-pulse stimulation was applied. By contrast, other reports have shown that, even with a train of six pulses, increasing the concentration of nitrous oxide to 60% resulted in reduced MEP amplitude. These controversial results suggest that the reversing effect of multipulse stimulation on nitrous oxide-induced suppression of MEPs may be overcome by adding more anaesthetic in the form of propofol. However, there has been no report confirming this hypothesis, probably because many permutations of nitrous oxide concentration, propofol infusion rate and stimulus train size would make this difficult to study in surgical patients.

The present study was conducted to investigate the effects of nitrous oxide on myogenic MEPs when multipulse stimulation was applied in rabbits under ketamine/fentanyl anaesthesia, which produces minimal depression of MEP. Furthermore, we infused propofol at two rates (low- and high-dose regimens) with nitrous oxide to elucidate the above controversy about the reversing effect of multipulse stimulation on nitrous oxide-induced MEP suppression.
Materials and methods

The study was approved by the Animal Experiment Committee of Nara Medical University. Seventeen male New Zealand White rabbits weighing 2.0–2.5 kg (mean 2.3 kg) were used. They were housed and maintained on a 12 h light–dark cycle with free access to food and water. Each rabbit was given ketamine 50 mg kg⁻¹ i.m. and a 24-gauge catheter was placed in the right marginal ear vein. Thereafter, continuous infusion of ketamine 25 mg kg⁻¹ h⁻¹ and fentanyl 30 μg kg⁻¹ h⁻¹ in lactated Ringer solution was initiated at the rate of 4 ml kg⁻¹ h⁻¹. Another 24-gauge catheter was inserted in the left ear vein for the administration of propofol. The trachea was intubated via a tracheostomy and the lungs were ventilated mechanically to maintain end-tidal carbon dioxide at 30–35 mm Hg. End-tidal concentrations of carbon dioxide and nitrous oxide were monitored continuously with a gas analyser (Hewlett Packard, Andover, MA, USA). Oesophageal temperature was monitored continuously with a thermometer (Mon-a-Therm, Mallinckrodt, St Louis, MO, USA) and maintained at 40°C with a warm blanket.

The animals were placed prone and the head was fixed in a stereotactic frame. The scalp was infiltrated with 1% lidocaine and reflected laterally to expose the calvarium. Two small craniotomies were performed with an air drill. A point 0.5 mm lateral to the sagittal suture and 14.5 mm rostral to the lamboid suture on the left hemisphere was chosen as the anodal stimulating site. A point 0.5 mm to the right of the sagittal suture at the level of the lamboid suture was used for the cathode. Silver ball electrodes (1 mm in diameter) were placed epidurally via holes, in which mineral oil was placed. Two standard recording needle electrodes were inserted in the left soleus muscle. A ground electrode was placed at the tail. Constant-voltage anodal stimulation was delivered through an electrical stimulator (SEN-3301; Nihon Kohden, Tokyo, Japan). The strength of the electrical stimulus was increased gradually until MEP amplitude no longer increased. The recording device (Neuropack Sigma, Nihon Kohden) was triggered by the stimulating device. Low- and high-cutoff filters were set at 30 Hz and 3 kHz respectively. Peak-to-peak amplitude was determined from the average of three to five individual responses. After the MEPs in response to a single-pulse stimulation had been recorded, a train of three or five pulses was applied. The duration of each pulse was 200 μs. The interstimulus interval of each pulse was set at 2 ms. The interval between each stimulation was set at 30 s.

After control MEPs had been recorded, nitrous oxide was administered at a concentration of 30, 50 or 70%. The order of concentrations was randomized to eliminate time-course bias. At least 10 min was allowed to elapse between each targeted concentration, and the end-tidal concentration of nitrous oxide was confirmed with a gas analyser. At each concentration of nitrous oxide, MEPs in response to a single pulse and a train of three or five pulses were recorded. After completion of all recordings at the three concentrations of nitrous oxide, administration of nitrous oxide was discontinued. MEPs were again recorded when the end-tidal

| Table 1 Physiological variables. Data are mean (SEM). *P<0.01 vs before propofol |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Nitrous oxide (%)** | 0 | 30 | 50 | 70 | Re-zeroed |
| Mean arterial pressure (mm Hg) | | | | | |
| Propofol 0.8 (n=9) Before | 84 (4) | 83 (4) | 87 (4) | 90 (4) | 83 (4) |
| During | 86 (4) | 87 (5) | 89 (3) | 90 (3) | 82 (3) |
| Propofol 1.6 (n=8) Before | 80 (4) | 80 (5) | 82 (5) | 88 (5) | 83 (3) |
| During | 90 (5) | 87 (3) | 89 (4) | 88 (3) | 92 (2) |
| Heart rate (beats min⁻¹) | | | | | |
| Propofol 0.8 (n=9) Before | 250 (11) | 250 (7) | 273 (9) | 272 (12) | 255 (9) |
| During | 234 (7) | 250 (11) | 254 (12) | 254 (15) | 251 (9) |
| Propofol 1.6 (n=8) Before | 249 (12) | 237 (11) | 257 (12) | 258 (15) | 247 (10) |
| During | 215 (8)* | 208 (19)* | 231 (11)* | 246 (12) | 221 (4)* |
| Oesophageal temperature (°C) | | | | | |
| Propofol 0.8 (n=9) Before | 40.3 (0.2) | 40.4 (0.2) | 40.3 (0.1) | 40.3 (0.2) | 40.3 (0.2) |
| During | 40.3 (0.2) | 40.1 (0.2) | 40.1 (0.2) | 40.1 (0.2) | 40.1 (0.2) |
| Propofol 1.6 (n=8) Before | 39.9 (0.1) | 39.9 (0.1) | 39.8 (0.1) | 39.7 (0.1) | 39.8 (0.2) |
| During | 39.8 (0.2) | 39.7 (0.2) | 39.7 (0.3) | 39.8 (0.2) | 39.8 (0.2) |
The concentration of nitrous oxide was 0%, as assessed by the gas analyser. After the complete elimination of nitrous oxide, all animals were allocated randomly to one of two groups. In one group (Propofol 0.8, \( n = 9 \)), a bolus of propofol 10 mg kg\(^{-1}\) was administered, followed by a continuous infusion of propofol at 0.8 mg kg\(^{-1}\) min\(^{-1}\). In the other group (Propofol 1.6, \( n = 8 \)), a bolus of propofol 10 mg kg\(^{-1}\) was followed by a continuous infusion of propofol at 1.6 mg kg\(^{-1}\) min\(^{-1}\). Thirty minutes after administration of the bolus of propofol, nitrous oxide was administered at 30, 50 or 70% and MEPs were recorded in the same fashion as described above. During administration of propofol, a continuous infusion of phenylephrine was given if arterial blood pressure decreased by more than 10% of control. At the end of the study, the animals were killed with an injection of potassium chloride, which caused cardiac arrest.

### Statistical analysis

All values are expressed as mean (SEM). Parametric methods were used for the analysis of all variables, as a normal distribution was confirmed with the Kolmogorov–Smirnov test. For comparisons of physiological variables and MEP amplitudes between the two experimental groups, we used two-way analysis of variance with repeated measures to test for multiple comparisons. To compare values within groups, we used multiple analysis of variance with repeated measures followed by Fisher’s protected least significant difference test for multiple comparisons. Differences were considered significant when \( P < 0.05 \).

### Results

Physiological variables are shown in Tables 1 and 2. There were no significant differences between the two experimental groups in mean arterial pressure (MAP), heart rate, oesophageal temperature, pH, \( P_{\text{aCO}_2} \), and \( P_{\text{aO}_2} \) before the administration of propofol. With the exception of heart rate, all variables were similar in the two groups during the administration of propofol. Heart rate was significantly reduced during the administration of propofol 1.6 mg kg\(^{-1}\) min\(^{-1}\) (\( P < 0.01 \)). MAP, heart rate and oesophageal temperature did not change significantly before, during or after the administration of nitrous oxide. Phenylephrine was used in all animals in both groups during propofol infusions; the doses were approximately 0.05–0.7 (mean 0.33) mg h\(^{-1}\) for the Propofol 0.8 group and 0.1–1.5 (mean 0.6) mg h\(^{-1}\) for the Propofol 1.6 group.

#### The effect of propofol on MEPs

Distinct MEPs could be recorded in all animals before the administration of propofol, and there were no significant differences between the two groups in the amplitudes of MEPs in response to stimulation with a single pulse or a train of three or five pulses. The changes in the amplitudes of MEPs induced by electrical stimulation with a single pulse or a train of three or five pulses in the two groups are shown in Fig. 1.

During the administration of propofol 0.8 mg kg\(^{-1}\) min\(^{-1}\), MEPs could be recorded in all animals, and the amplitude of MEPs in response to single-pulse stimulation was significantly reduced (\( P < 0.05 \)). MEP amplitudes in response to stimulation with a train of three or five pulses were not significantly different from baseline during administration of propofol 0.8 mg kg\(^{-1}\) min\(^{-1}\).

During the administration of propofol 1.6 mg kg\(^{-1}\) min\(^{-1}\), MEPs could be recorded in only three of eight animals after single-pulse stimulation. By contrast, MEPs could be recorded in all animals after stimulation with a train of three or five pulses, and MEP amplitudes were significantly higher than after single-pulse stimulation (\( P < 0.01 \)). The amplitudes of MEPs after stimulation with a single pulse or a train of three or five pulses were significantly reduced during propofol administration (\( P < 0.01 \)).

For later analysis of the effects of nitrous oxide and number of pulses, data on MEPs before the administration of propofol in both groups were pooled as group Propofol(−).

#### Effects of nitrous oxide on MEPs

**Propofol(−)**

Without administration of propofol, the amplitudes of MEPs in response to stimulation with a single pulse or a train of three pulses were significantly reduced after the administration of 50% (\( P < 0.01 \)) or 70% (\( P < 0.01 \)) nitrous oxide and after 70% (\( P < 0.05 \)) nitrous oxide, respectively (Fig. 2).
contrast, the amplitudes of MEPs induced by a stimulation with a train of five pulses did not change significantly during the administration of nitrous oxide (Fig. 3). The amplitudes of MEPs in response to a stimulation with a train of three pulses during 70% nitrous oxide and a train of five pulses during administration of 30, 50 or 70% nitrous oxide were significantly higher than those after single-pulse stimulation.

**Propofol 0.8**

During the administration of propofol 0.8 mg kg\(^{-1}\) min\(^{-1}\), MEPs in response to single-pulse stimulation could be recorded in six, four and one of nine animals during the administration of 30, 50 and 70% nitrous oxide respectively. During the administration of nitrous oxide, the amplitudes of MEPs in response to single-pulse stimulation were significantly reduced in a dose-dependent manner \((P<0.01)\) (Fig. 2). However, amplitudes returned to the baseline after

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**Fig 1** Changes in amplitude of myogenic MEPs in response to electrical stimulation with a single pulse or a train of three or five pulses before and during the administration of propofol in rabbits under ketamine/fentanyl anaesthesia. Propofol was administered as a bolus of 10 mg kg\(^{-1}\) followed by continuous infusion at 0.8 mg kg\(^{-1}\) min\(^{-1}\) (A) or 1.6 mg kg\(^{-1}\) min\(^{-1}\) (B). Data are expressed as mean (SEM). *\(P<0.05\) vs before propofol; **\(P<0.01\) vs before propofol.

**Fig 2** Effects of nitrous oxide on amplitude of myogenic MEPs without \((A)\) and with \((B, C)\) propofol administration in rabbits under ketamine/fentanyl anaesthesia. Electrical stimulation was performed with a single pulse or a train of three or five pulses. After a control recording, the concentration of nitrous oxide (N\(_2\)O) was changed to 30, 50 or 70% and then re-zeroed. Propofol was administered as a bolus of 10 mg kg\(^{-1}\) followed by continuous infusion at 0.8 mg kg\(^{-1}\) min\(^{-1}\) (B) or 1.6 mg kg\(^{-1}\) min\(^{-1}\) (C). Data are expressed as mean (SEM). *\(P<0.05\) vs one pulse; **\(P<0.01\) vs one pulse.
Nitrous oxide was discontinued. MEPs in response to stimulation with a train of three or five pulses could be recorded in all animals during the administration of nitrous oxide, but not in two animals after stimulation with a train of three pulses during administration of 70% nitrous oxide. The amplitudes of MEPs after stimulation with a train of three pulses were significantly reduced during the administration of 50% ($P < 0.05$) or 70% ($P < 0.01$) nitrous oxide. The amplitudes of MEPs in response to stimulation with a train of five pulses did not change significantly during propofol administration. The amplitudes of MEPs in response to stimulation with a train of three pulses during 30 or 50% nitrous oxide or with a train of five pulses during 30, 50 or 70% nitrous oxide were significantly higher than those after single-pulse stimulation.

During the administration of propofol 1.6 mg kg$^{-1}$ min$^{-1}$, MEPs in response to a single pulse could be recorded only in the absence of nitrous oxide. MEPs in response to stimulation with a train of three pulses could be recorded in eight, five and four of eight animals during the administration of 30, 50 and 70% nitrous oxide respectively. MEPs could be recorded in all animals in response to stimulation with a train of five pulses. During the administration of nitrous oxide, the amplitudes of MEPs in response to stimulation with a train of three or five pulses were significantly reduced in a dose-dependent manner (Figs 2 and 4). The amplitudes of MEPs in response to stimulation with a train of three pulses during 30 or 50% nitrous oxide or with a train of five pulses during 30, 50 or 70% nitrous oxide were significantly higher than those after single-pulse stimulation.
five pulses during the administration of nitrous oxide were significantly higher than those after single-pulse stimulation.

Discussion

Results obtained in the present study show that nitrous oxide dose-dependently reduced the amplitudes of MEPs in response to single-pulse stimulation in rabbits anaesthetized with ketamine and fentanyl, and that this effect was reversed by the application of a train of pulses. After the administration of propofol 0.8 mg kg$^{-1}$ min$^{-1}$, significant nitrous oxide-induced suppression was observed in the amplitudes of MEPs in response to stimulation with a single pulse or a train of three pulses, but not with a train of five pulses. However, after the administration of propofol 1.6 mg kg$^{-1}$ min$^{-1}$, significant nitrous oxide-induced suppression was noted even after a train of five pulses. These results suggest that the reversing effect of multipulse stimulation on nitrous oxide-induced suppression of MEPs was overcome by high-dose administration of propofol.

In previous work that investigated the effects of nitrous oxide on myogenic MEPs, $^{15-18}$ the dose-dependence of the suppressive effect of nitrous oxide on MEPs in response to single-pulse stimulation has often been demonstrated, $^{15-18}$ regardless of differences in combined anaesthetic regimens. These reports are compatible with the present results, which were obtained with ketamine/fentanyl anaesthesia. However, there have been few reports on the effect of nitrous oxide on myogenic MEPs when paired pulses or a train of pulses were used for stimulation, and the results are controversial. $^{19-21}$ Van Dongen et al. $^{19}$ compared MEPs in response to a six-pulse train of transcranial electrical stimuli when 20, 40 or 60% nitrous oxide was given in 10 patients anaesthetized with fentanyl and low-dose propofol. Compared with 20% nitrous oxide, 40 or 60% nitrous oxide significantly reduced MEP amplitude. Pechstein et al. $^{20}$ also reported that 60% nitrous oxide significantly reduced the amplitude of MEPs induced by transcranial stimulation with a train of five pulses in four patients anaesthetized with alfentanil and propofol. By contrast, in another report by van Dongen et al., $^{21}$ it was noted that 50% nitrous oxide did not affect the amplitude of MEPs induced by transcranial electrical stimulation with paired pulses during fentanyl and low-dose propofol anaesthesia in 10 patients. The reasons for the different results are unknown. However, it is possible that co-administration of propofol with nitrous oxide affected the results.

In the present study, the degree of reversal of the effect of nitrous oxide-induced suppression of multipulse stimulation was affected by propofol. In the absence of propofol, nitrous oxide-induced suppression could be reversed after the application of a train of five pulses. A similar tendency was also observed during the administration of propofol at 0.8 mg kg$^{-1}$ min$^{-1}$ (low dose). However, when the propofol infusion rate was increased to 1.6 mg kg$^{-1}$ min$^{-1}$ (high dose), even a train of five pulses was unable to reverse the nitrous oxide-induced suppression of MEPs. These findings may be important for the determination of the optimal combination of anaesthetics during intraoperative MEP monitoring. Our data in a rabbit model suggest that, when nitrous oxide is used as a supplement, high-dose propofol is probably better avoided. In contrast, when multipulse stimulation is used the effect of nitrous oxide on MEPs may be minimal during low-dose administration of propofol in the rabbit MEP model.

For ethical reasons, a baseline anaesthetic was necessary. We selected ketamine and fentanyl because both drugs have minimal effects on MEPs, $^{4}$ $^{13}$ $^{14}$ $^{23}$ $^{24}$ although Thees et al. $^{25}$ have recently reported a dose-dependent suppressive effect of fentanyl. However, the dosage of fentanyl used in the present experiment was much lower than that used in their study. Moreover, there was evidence that, with this regimen, multipulse stimulus resulted in maximal MEP amplitudes, suggesting near-maximal recruitment of cortical and spinal motor neurones.

Our propofol regimen comprised a bolus of 10 mg kg$^{-1}$ followed by continuous infusion at 0.8 or 1.6 mg kg$^{-1}$ min$^{-1}$. These doses were selected on the basis of the results of a preliminary study that we made (unpublished), in which the basic procedure was identical to that used in the present study. Infusion rates after a bolus of propofol 10 mg kg$^{-1}$ were doubled from 0.1 to 3.2 mg kg$^{-1}$ min$^{-1}$ in a step-wise manner. Amplitudes of MEPs in response to single-pulse stimulation were recorded 30 min after each step. On the basis of the results obtained, in the present study we chose 0.8 mg kg$^{-1}$ min$^{-1}$ as the low-dose regimen, which resulted in a mild reduction in MEP amplitude, and 1.6 mg kg$^{-1}$ min$^{-1}$ as the high-dose regimen, which produced a severe reduction in MEP amplitude. These doses were similar to those used in a previous study in which a mean propofol infusion rate of 0.876 mg kg$^{-1}$ min$^{-1}$ produced a light plane of anaesthesia in which the palpebral reflex, the reaction to ear pinching, and the front and hind limb withdrawal reflexes were abolished in rabbits. $^{26}$

Ma et al. $^{27}$ measured the blood concentration of propofol when propofol was infused at 0.8 mg kg$^{-1}$ min$^{-1}$ in rabbits. They demonstrated that the blood concentration remained constant from 15 min after starting the infusion until the withdrawal of propofol at 105 min. In our study, MEP recording began 30 min after the propofol infusion and was completed within 120 min. Therefore, we believe that blood concentration was constant during the administration of propofol 0.8 mg kg$^{-1}$ min$^{-1}$. By contrast, regarding propofol 1.6 mg kg$^{-1}$ min$^{-1}$, we cannot exclude the possibility that the variation in blood propofol concentration observed during the administration of propofol 1.6 mg kg$^{-1}$ min$^{-1}$ affected the results obtained in the present study. In order to avoid time-course bias, the order of administration of the different nitrous oxide concentrations was randomized. Moreover, we measured MEP amplitudes again after nitrous oxide had been discontinued, and did not find any differences between...
the values obtained before and after the administration of nitrous oxide. Therefore, we believe that the influence of variation in propofol concentration on MEPs was minimal.

In summary, we have investigated the influence of stimulation paradigm and the administration of propofol on the nitrous oxide-induced suppression of myogenic MEPs in rabbits anaesthetized with ketamine and fentanyl. We have demonstrated that the application of a train of five pulses reverses nitrous oxide-induced suppression of MEPs in the absence of propofol infusion and during the administration of low- but not high-dose propofol. These results suggest that nitrous oxide-induced suppression of MEPs can be modified by the use of multipulse stimulation and the administration of propofol. Further clinical investigations are required to determine optimal anaesthetic regimens for intraoperative MEP monitoring.

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