Corresponding minimum alveolar concentrations of isoflurane and isoflurane/nitrous oxide have divergent effects on thalamic nociceptive signalling

C. Vahle-Hinz1*, O. Detsch2, C. Hackner2 and E. Kochs2

1Institut für Neurophysiologie und Pathophysiologie, Zentrum für Experimentelle Medizin, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany. 2Klinik für Anaesthesiologie, Technische Universität München, Klinikum rechts der Isar, Munich, Germany

*Corresponding author: Institut für Neurophysiologie und Pathophysiologie, Universitätsklinikum Hamburg-Eppendorf, 20246 Hamburg, Germany. E-mail: vahle-hinz@uke.uni-hamburg.de

Background. Suppression of nociceptive signalling in the thalamus is considered to contribute significantly to the anaesthetic state. Assuming additivity of anaesthetic mixtures, our study assessed the effects of corresponding minimum alveolar concentrations (MACs) of isoflurane and isoflurane/nitrous oxide on thalamic nociceptive signalling.

Methods. Nociceptive response activity (elicited by controlled radiant heat stimuli applied to cutaneous receptive fields) of single thalamic neurons was compared in rats anaesthetized at 1.1 and 1.4 MAC isoflurane with that at 1.1 and 1.4 MAC isoflurane/nitrous oxide.

Results. Under baseline anaesthesia (~0.9 MAC isoflurane), noxious stimulation elicited excitatory responses in all neurons (n = 19). These responses were uniformly suppressed at ~1.1 and ~1.4 MAC isoflurane. In contrast, at ~1.1 and ~1.4 MAC isoflurane/nitrous oxide, excitatory responses no different to baseline were still present in 64 and 37% of the neurons, respectively.

Conclusions. These data demonstrate a pronounced nitrous oxide-induced response variability. It appears that, with respect to thalamic transfer of nociceptive information, the interaction of isoflurane and nitrous oxide may not be compatible with the concept of additivity and that the antinociceptive potency of nitrous oxide is considerably less than previously reported.


Keywords: anaesthetics, isoflurane; anaesthetics, nitrous oxide; brain, thalamus, nociception; potency, anaesthetic, MAC

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Nitrous oxide has been used in clinical anaesthesia for more than 150 years. Its analgesic action1 led to its widespread use as a supplement to volatile anaesthetics. As part of the concept of minimum alveolar concentration (MAC), a generally accepted tenet holds that inhalational anaesthetics such as isoflurane and nitrous oxide act in an additive manner.2 3 Despite not being proved definitively and indications for a non-linearity of additive interaction, it is still considered the best working hypothesis,4 notwithstanding the distinct mechanisms and sites of action within the central nervous system of both inhaled anaesthetics.4 5

Information about noxious events affecting the body is conveyed via the spinal dorsal horn and trigeminal nucleus to the ventrobasal and posterior complexes of the thalamus and onward to the somatosensory cortex (Fig. 1A). This pathway involving lateral thalamic and cortical areas is associated with processing of the sensory-discriminative aspects of pain.6 Suppression of nociceptive information transfer from the thalamus to the cortex is considered a key mechanism contributing to the state of general anaesthesia;7 8 a hypothesis which has recently gained strong support from functional imaging studies both in animals9 and humans.4 10 Therefore, in the present study, we focused our attention on the underlying nociceptive activity from single thalamic neurons and—assuming additivity of anaesthetic mixtures—compared the effects of isoflurane with those of isoflurane/nitrous oxide at corresponding MAC concentrations in rats in vivo.
Methods

Surgical preparation

With Institutional Animal Care and Use Committee (IACUC) approval, adult Wistar rats (400 g body weight) were prepared for acute electrophysiological recordings using methods previously described.11–13 Anaesthesia was induced with a single dose of ketamine (100 mg kg\(^{-1}\) i.p.) in five rats and by inhalation of isoflurane in two rats to allow cannulation of the trachea. After tracheotomy, anaesthesia was maintained with isoflurane (1.5–2.0% in oxygen) during surgical preparation. The rat’s lungs were mechanically ventilated, and end-tidal \(P_{CO_2}\) was maintained at 35–40 mm Hg. End-tidal isoflurane and nitrous oxide concentrations were monitored continuously. Body temperature was maintained at 37.0–37.5°C by means of a feedback-controlled heating pad coupled to a rectal probe. The femoral artery was cannulated for measurement of mean arterial blood pressure (MAP) and heart rate was monitored via the ECG. MAP was >70 mm Hg in all rats throughout the study period without the use of vasopressors. The femoral vein was cannulated for administration of vecuronium bromide (4 mg kg\(^{-1}\) h\(^{-1}\)) during the recording session. The animal’s head was mounted with blunt ear bars in a stereotaxic holder. A lateral 3 × 3 mm square craniotomy was performed and the dura mater was removed. At the end of the experiment the rats were killed with pentobarbitone.

Neuronal recordings and stimulation

For extracellular recordings of single neurons, a tungsten electrode (2 MΩ impedance at 1 kHz) was inserted into the medial posterior complex (POm) of the lateral thalamus initially guided by stereotaxic coordinates. After amplification, neuronal activity was filtered and displayed on an oscilloscope as well as stored on a digital tape recorder. Off-line analysis was performed using a window discriminator, an A/D-converter, and Spike2 software (Cambridge Electronic Design, UK). Single neurons were identified electrophysiologically as deriving from neuronal somata by criteria such as spike form (initial segment/soma inflection), spike duration, and the occurrence of high frequency injury discharges after advancing the microelectrode. The recording sites were marked by small electrolytic lesions for histological verification (Fig. 1B).

Fig 1 (A) Recording site in the pathway of the nociceptive system of the rat. DH, dorsal horn; POm, medial posterior complex; SI, primary somatosensory cortex; SpV, spinal trigeminal nucleus. (B) Camera lucida drawing of the frontal histological section holding the lesion made at the recording site of a nociceptive neuron. VP, ventroposterior nucleus; scale bar: 1 mm. (C) The neuron’s response to a radiant heat stimulus applied to the receptive field on the nose (middle trace) is shown as original spike record (upper trace) and peristimulus time histogram (lower trace). Segments taken for measurements of ongoing and response activities are marked.
Radiant heat stimuli applied to small areas on the nose, lips, ears, and paws. Only neurons that responded at least twice to a given noxious stimulus were classified as nociceptive. For quantitative analyses, a feedback-controlled radiant heat source was used to apply stimuli of known temperature as measured on the surface of the skin (10–15 s duration, 30°C baseline, 1.5 s rise time per 50°C). At least 5 min were allowed to elapse between consecutive noxious stimuli and care was taken to use the lowest effective intensity, in order to avoid changes in skin tissue by repeated stimulus application. Under baseline anaesthesia, neurons displayed ongoing activity, which is defined as the discharge activity present when no experimental stimulus (i.e. here: heat stimulation of the peripheral receptive field) is presented (Fig. 1c). After constructing peristimulus time histograms (PSTH, bin width 1 s), ongoing activity was determined as discharge rate (spikes s⁻¹) from a 10-s window immediately before stimulus onset. The response activity (spikes s⁻¹) per stimulus was determined in a window covering the last 5 s of the stimulus and the first 5 s after stimulus end and was corrected for the preceding ongoing activity (Fig. 1c). This 10-s period was chosen because of the characteristic pattern of heat responses of thalamic neurons with an onset after a latency of about 5 s and a pronounced after-discharge for several tens of seconds (Fig. 1c). The stimulus-induced neuronal activity was considered a ‘response’ when the increment of the discharge rate was greater than 30% of the ongoing activity.

**Experimental protocol**

Neuronal discharge activity was recorded at: (i) baseline, ~0.9 MAC isoflurane; (ii) addition of 70% nitrous oxide with the isoflurane concentration kept constant, yielding a total MAC of ~1.4; (iii) ~1.4 MAC isoflurane (without nitrous oxide); (iv) recovery, ~0.9 MAC isoflurane; (v) addition of 70% nitrous oxide with a decreased isoflurane concentration to yield a total MAC of ~1.1; (vi) ~1.1 MAC isoflurane (without nitrous oxide); and (vii) recovery, ~0.9 MAC isoflurane. After each change in anaesthetic mixture, an equilibration period of at least 20 min was allowed before data acquisition. Assumed equipotencies of isoflurane and isoflurane/nitrous oxide mixtures were based on previous MAC determinations in Wistar rats for isoflurane (1.3% = ~1 MAC)¹⁴–¹⁸ and in various rat strains for nitrous oxide (70% = ~0.5 MAC).¹⁹–²³

**Data analysis**

Since nitrous oxide apparently exerted divergent effects on thalamic neuronal activity, the nociceptive responses of each neuron were retrospectively classified as either suppressed or maintained, the criterion for a maintained response being a discharge rate >30% above ongoing activity. For statistical comparisons, multiple Wilcoxon tests and Mann–Whitney U-tests were used (where appropriate) with P<0.05 considered significant. Data are reported as mean (SEM).

**Results**

**Effects of isoflurane vs isoflurane/nitrous oxide on nociceptive responses**

At baseline (~0.9 MAC isoflurane), noxious stimulation elicited excitatory responses [19.5 (4.3) spikes s⁻¹] in 19 thalamic nociceptive neurons. As shown in Figure 2a, isoflurane and isoflurane/nitrous oxide exerted divergent effects at ~1.4 MAC: the nociceptive response of one neuron was suppressed by nitrous oxide (b) while the response of the other was maintained and even increased in discharge rate (b'); in contrast, isoflurane alone caused a uniform response suppression in both neurons (c, c'). Figure 2n summarizes the population data (n=19). While administration of ~1.4 MAC isoflurane resulted in a complete suppression of nociceptive responses in all neurons (19/19, 100%), administration of ~1.4 MAC isoflurane/nitrous oxide caused a response suppression in only 12/19 (63%) neurons; 7/19 (37%) neurons still showed an excitatory response [18.2 (5.6) spikes s⁻¹] not different from that during baseline anaesthesia (~0.9 MAC). Following return to baseline anaesthesia the nociceptive responses recovered [18.6 (4.3) spikes s⁻¹] demonstrating the stability of the preparation over time. Baseline response and ongoing activity [14.9 (2.6) and 5.5 (3.3) spikes s⁻¹, respectively] of neurons showing nitrous oxide induced response suppression were not different in neurons with maintained responses [27.3 (10.7) and 3.5 (0.8) spikes s⁻¹], suggesting that the divergent effects of nitrous oxide were neither caused by differences in sensitivities to nociceptive input nor in basal neuronal excitability. In a larger population (n=32) studied solely for the effects of addition of nitrous oxide to baseline isoflurane anaesthesia, the same divergent results were found: 10/32 (31%) neurons still showed excitatory nociceptive responses while 22/32 (69%) neurons were suppressed.

Baseline responses [19.0 (5.2) spikes s⁻¹] of the 14 neurons studied for the ~1.1 MAC sequence were not different from the baseline of the preceding sequence. Figure 3a demonstrates the variable effects of isoflurane vs isoflurane/nitrous oxide at ~1.1 MAC. Administration of ~1.1 MAC isoflurane/nitrous oxide caused a response suppression in 5/14 neurons (36%) while 9/14 (64%) neurons still showed an excitatory response [25.5 (6.5) spikes s⁻¹] not different from that under baseline anaesthesia (Fig. 3b). In some cases even a marked increase in discharge rate was found (Fig. 3ab'). In contrast, ~1.1 MAC isoflurane produced a suppression of the nociceptive responses of all neurons (100%). The initial excitatory responses recovered in all cases after return to baseline anaesthesia [14.8 (4.2) spikes s⁻¹]. When the effects of isoflurane/nitrous oxide at ~1.4 and ~1.1 MAC were compared for each neuron tested,
response suppression and maintenance, respectively, under both concentrations were found in four neurons each, suppression under \(C_241.4\) MAC and maintenance under \(C_241.1\) MAC in five neurons (see Neuron 1 in Figs 2A and 3A), and vice versa in one neuron.

**Effects of isoflurane vs isoflurane/nitrous oxide on ongoing activity**

The addition of nitrous oxide to baseline anaesthesia (yielding a total MAC of \(\sim1.4\)) did not change ongoing activity \([2.8 (0.1) \text{ spikes s}^{-1}]\) as compared with baseline.
[4.8 (2.1) spikes s\(^{-1}\)]; isoflurane alone at \(\sim 1.4\) MAC, however, caused a reduction to 0.3 (0.1) spikes s\(^{-1}\) \((P<0.001)\). Under \(\sim 1.1\) MAC isoflurane/nitrous oxide, ongoing activity significantly increased to 16.1 (5.3) spikes s\(^{-1}\) \((P<0.05)\). Under isoflurane alone (\(\sim 1.1\) MAC), again, ongoing activity was significantly reduced to 0.7 (0.2) spikes s\(^{-1}\) \((P<0.05\) vs baseline and vs isoflurane/ nitrous oxide). Ongoing activity recovered at the end of the experimental sequences to baseline values [3.3 (0.8) spikes s\(^{-1}\) and 2.9 (0.8) spikes s\(^{-1}\)].
Effects of isoflurane vs isoflurane/nitrous oxide on haemodynamics

MAP and heart rate were recorded during data acquisition at the different experimental steps. The ranges for mean MAP and heart rate were 79 (2) to 102 (5) mm Hg and 345 (3) to 361 (6) beats min$^{-1}$, respectively. These haemodynamic parameters in general showed no significant changes compared with baseline (98 (4) mm Hg and 352 (9) beats min$^{-1}$). The only exception was a significant decrease in MAP at ~1.4 MAC isoflurane/nitrous oxide [79 (2) mm Hg; $P<0.05$ vs baseline], while heart rate remained constant [351 (7) beats min$^{-1}$].

Discussion

The major finding of the present study is that thalamic nociceptive responses were uniformly suppressed by ~1.1 MAC and ~1.4 MAC isoflurane, while during co-administration of isoflurane with nitrous oxide at equivalent MACs, the responses were unaffected in about two-thirds and one-third of the neurons, respectively. In other words, isoflurane in concentrations above 1 MAC consistently exerted antinociceptive effects, in contrast to the combination of isoflurane with nitrous oxide, which in a significant number of cases had no antinociceptive effects (and in some cases even facilitated nociceptive activity, see Figs 2Ab and 3Ab). Thus, during ~1.1 MAC and ~1.4 MAC isoflurane/nitrous oxide anaesthesia, the cerebral cortex still receives some information about the presence, duration, and magnitude of noxious stimuli, whereas at MAC-equivalent levels, isoflurane alone reliably effects a functional cortical deafferentation.

Our finding of partly maintained thalamocortical transmission of nociceptive information under an isoflurane/nitrous oxide anaesthetic as high as ~1.4 MAC is surprising, since nitrous oxide has long been considered a weak anaesthetic but potent analgesic.24 One might speculate that the variable effects on nociceptive signalling observed in our study may be responsible, at least partly, for the low potency of nitrous oxide. The estimation of equivalent MAC levels of isoflurane and isoflurane in nitrous oxide was based on the concept of additivity,1–3 which has received support, for example from an EEG study in humans25 as well as a recent study in rats.26 However, it also was hypothesized that the potency of nitrous oxide may be less than anticipated from MAC studies, a view also emerging from a MAC study in rats.27 Thus, one explanation for our results is that the interaction of isoflurane and nitrous oxide may not be additive. The concept of a linearly additive interaction has been disputed before28 29 and an agonist–antagonist relationship for isoflurane and nitrous oxide has been suggested.23 In the light of our data, the latter view appears appealing. It has to be kept in mind, however, that our results are derived from lateral thalamic neurons that are associated with processing of the sensory-discriminative aspects of pain.5 Other pathways (including the medial thalamus) associated with the motivational- affective dimension of pain may well be affected differently. One should also remember that the MAC of inhalational agents reflects their actions mostly at the spinal cord level,5 30 and thus, differences in potency may occur if measures of drug effect other than movement responses are considered. Whether additive or not, our data show that there is no indication for a general anaesthetic sparing effect of nitrous oxide and no predictable reduction of the anaesthetic requirement of isoflurane. The results, therefore, might reflect the distinct mechanisms and sites of action of isoflurane and nitrous oxide within the central nervous system, which may preclude a prediction of their interactive effects on the nociceptive system.4 5

Several studies investigated the effects of isoflurane and nitrous oxide on transmission of nociceptive information in the somatosensory system. Most showed for isoflurane that responses of nociceptive neurons of the ascending pathway (including thalamic neurons31–33) are dose-dependently suppressed.34 In contrast, nitrous oxide has been shown to exert highly variable effects; in the spinal dorsal horn nitrous oxide had suppressive and facilitatory effects on ongoing activity35 and on nociceptive responses.36–38 Under isoflurane/nitrous oxide, these divergent effects were also described for nociceptive responses in the midbrain reticular formation and the medial thalamus.38 Our study now demonstrates similar divergent effects on lateral thalamic neurons. The results may reflect direct or indirect anaesthetic actions on the ascending pathway, modulatory systems, or both. However, our study was not designed to address this question. Isoflurane acts primarily by potentiating neuronal inhibition via direct action at GABA$\alpha_2$ receptors, most prominently affecting the transmission of sensory signals at the thalamocortical projection.4 11–13 This may underlie the robust suppression of nociceptive thalamic responses as well as their ongoing activity under 1.1 and 1.4 MAC isoflurane seen in the present study. In contrast, nitrous oxide appears to affect mainly spinal excitatory pathways primarily via non-competitive antagonism at NMDA and nicotinic acetylcholine receptors.39 Its analgesic effect is at least partly ascribed to activation of the descending noradrenergic inhibitory pathway by release of endogenous opioid peptides in the periaqueductal grey.40 It is conceivable that these mechanisms may be highly susceptible to modulatory influences, hence resulting in the observed variable effects of nitrous oxide.

Several potential limitations must be acknowledged for the present study. We did not determine the MAC of isoflurane for the individual rat concerned, instead we chose 1.3% isoflurane as 1 MAC, a value representing the average of recent MAC determinations in adult Wistar rats.14–18 Differences in susceptibility between rat strains should be taken into account for isoflurane, however, not for nitrous oxide.22 We had to rely on previous MAC
determinations for nitrous oxide, since it can only reliably be determined under hyperbaric conditions due to its value being greater than one atmosphere absolute. Thus, we chose 70% nitrous oxide to represent ~0.5 MAC, a value reflecting the average of most MAC studies in rats.19–23

MAP was well above 70 mm Hg throughout our experiments and the overall changes in heart rate and MAP were minor. We consider a MAP >70 mm Hg in rats to be above critical levels, which may affect cerebral autoregulation41 and systemic perfusion. Potential effects of blood pressure changes on neuronal activity had to be considered, since thalamic neurons receive inputs from nerves innervating the cranial vasculature.42 43 However, we have previously shown that the response characteristics of thalamic low-threshold neurons were unaltered by decreases in MAP to ~70 mm Hg.11 Also, neither extreme MAP levels nor extreme changes in physiological parameters such as body temperature or end-tidal P CO2, which have been demonstrated to influence anaesthetic requirements (i.e., MAC),2 44 occurred in the present study. Thus, we consider it unlikely that the observed changes in thalamic neuronal activity were effected by the different anaesthetic mixtures via changes in haemodynamic or other physiological variables.

We used ketamine for induction of anaesthesia in five animals, which might have suppressed nociceptive responses. However, this is unlikely, because ketamine is a short-acting agent and neuronal recordings commenced not earlier than 3 h after the induction of anaesthesia and the recovery recordings for each experimental sequence were assessed ~2 h after baseline recordings; thus, any residual ketamine effects would have been noted, which, however, was not the case (Figs 2 and 3). Likewise, this holds for our studies on thalamic low-threshold neurons11 13 and for data sampled even earlier after ketamine administration (30 min) in a study on nociceptive trigeminal sensory neurons in cats.45 Furthermore, no differences were found for the occurrence of response classes, that is suppression vs maintenance under isoflurane/nitrous oxide, with respect to absence of ketamine (two rats) and the time after ketamine administration (up to 12 h) or when the experimental sequence was repeated a second or a third time. This shows that neither a residual antinociceptive effect of ketamine varying between early and late phases of the experiments was confounding the measurements nor a development of tolerance to nitrous oxide. The latter has been demonstrated for non-pain and pain-related measures in humans46 and animals,47 however, in rats this may depend on the strain.48

In conclusion, comparing the effects of corresponding MAC concentrations of isoflurane/nitrous oxide with those of isoflurane alone on nociceptive signalling in the thalamus of the rat, we found that isoflurane caused profound response suppression, that is blocked information about noxious stimuli from gaining access to cortical areas involved in pain perception. In contrast, isoflurane plus nitrous oxide had only modest effects on nociceptive responses, that is allowed unimpaired nociceptive information transfer to a great extent.

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