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Ketamine pharmacology revisited

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Chapter 5

Esketamine counters opioid-induced respiratory depression

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5.1 Introduction

The observation that opioids produce life-threatening respiratory depression is not new. The first reported death from intravenous morphine dates from the 1850's when Englishman Alexander Wood injected his wife with morphine just after the introduction of the hollow needle.¹ Public awareness of the potentially life-threatening adverse effects of opioids is new, however, and is related to the recent escalation of prescribed opioid consumption and prescribed opioid deaths in the US and other Western countries.²⁻⁴ Especially the combination of opioid misuse and cardiorespiratory depression is potentially lethal. While it is well established that the rise in deaths occurs in patients that consume opioids in the community (*i.e.* opioids prescribed for treatment of chronic pain), opioid-induced respiratory depression (OIRD) is an equally relevant problem for patients treated with potent opioids in the acute and/or hospital setting.⁵⁻⁷

In recent years various pharmacological interventions have been proposed to offset OIRD, most of which are respiratory stimulants that do not interact with the opioid receptor system, so that opioid analgesia is not compromised.⁸ While some of these drugs are registered respiratory stimulants (*e.g.* doxapram), others are experimental drugs that require further research (ampakines, 5HT-agonists, methylxanthines, drugs acting at background potassium channels of type 1 carotid body cells).⁸⁻¹¹ In the current study we assess whether the commonly used anesthetic esketamine is able to reverse, at subanesthetic-dose, (part of) the respiratory depression induced by a potent opioid. Recent animal and human data suggest that ketamine is a respiratory stimulant and consequently may possibly offset OIRD.¹²⁻¹⁵ Ketamine is different from other respiratory stimulants in that it has inherent analgesic properties. Consequently, if ketamine is able to reverse OIRD, its use in the acute (*e.g.* postoperative) setting may be further advantageous as ketamine treatment may then coincide with reduced opioid consumption.¹⁶

We performed two studies. The first was a double-blind, randomized, placebo-controlled, crossover trial designed as a proof-of-concept study to investigate the effect of dose-escalating infusions of esketamine (four steps with a cumulative dose of 40 mg per 70 kg given in 1 hour) on opioid-induced respiratory depression under isohypercapnic conditions. We measured esketamine plasma concentrations and minute ventilation and performed a population pharmacokinetic (PK) - pharmacodynamic (PD) analysis. We hypothesise that (low-dose) esketamine will effectively reduce remifentanyl-induced respiratory depression. To further understand the esketamine effect on ventilation, we next examined, in an observational study, whether esketamine is a respiratory stimulant when respiration is not depressed by an opioid.

5.2 Materials and Methods

ETHICS AND SUBJECTS

This single-center, double blind, placebo-controlled, crossover study protocol was performed from November 2016 to July 2017 at the Anesthesia & Pain Research Unit of the Department of Anesthesiology at the Leiden University Medical Center. The local Institution Review Board (Commissie Medische Ethiek, Leiden, the Netherlands) and the Central Committee on Research involving Human Subjects (CCMO, The Hague, the Netherlands) approved the study protocol. From all participants written informed consent was obtained prior to enrollment. All study procedures were conducted according to good clinical practice guidelines and adhered to the tenets of the Declaration of Helsinki. Participants were recruited by flyers posted on the campus of the university. The study was registered in the Dutch trial register (identifier 6248).

Healthy volunteers, aged 18 to 40 years, with a body mass index $<30 \text{ kg m}^{-2}$ and able to read and understand the subject information form were recruited. Exclusion criteria were: a medical history of medical or psychiatric disease, any allergy to food or medication, alcohol abuse (i.e. > 21 units per week), smoking, pregnancy or lactation, participation in an investigational drug trial in the three months prior to the current study, illicit drug use in the 30 days prior to the current study, or a positive urine dipstick on the screening or study days. The dipstick (Alere Toxicology Plc, Oxfordshire, UK) tests for cocaine, amphetamine, cannabinoids, phencyclidine, methadone, benzodiazepine, tricyclic antidepressants and barbiturates. Subjects were asked not to eat and drink for the 8 hours before dosing, not to take caffeinated drinks, chocolate drinks or alcohol for the 24 hours before dosing and to refrain from grapefruit (juice) for the seven days prior to the first study visit and thereafter for the duration of the study.

STUDY DESIGN

Subjects visited the research unit on three separate occasions, at least one week apart. On visits one and two the effect of esketamine (Ketanest-S, Pfizer, the Netherlands) on opioid-induced respiratory depression was tested using a double blind, placebo-controlled, crossover design. Subjects were randomized to receive either esketamine or placebo (normal saline) on top of remifentanyl (GlaxoSmithKline BV, the Netherlands) induced respiratory depression. On the third occasion, the effect of just esketamine on ventilation was studied (i.e. without remifentanyl). Subjects received two intravenous access lines (one for esketamine or placebo and the other for remifentanyl infusion) and a 22 G cannula in the left or right radial artery for blood sampling. During the study day, subjects were monitored by ECG, oxygen saturation via a finger probe and blood pressure through the arterial line.

Drug administration (Supplementary Fig. S1). Remifentanyl was administered intravenously by target controlled infusion on visits one and two. The remifentanyl target controlled infusion system makes use of the Minto and colleagues¹⁷ pharmacokinetic data set. The target concentration was started at 0.5 ng ml^{-1} and step-wise increased to a specific end-point (i.e. a decrease in ventilation by 40 to 50% of baseline value). Titration to effect was performed with steps in target remifentanyl concentration of $0.1\text{-}0.5 \text{ ng ml}^{-1}$. After ventilation had reached a steady state for at least 10 min, the esketamine/placebo infusion began. Esketamine or placebo were administered by intravenous dose-escalating infusions over 60 min: 0-15 min 4 mg (step 1), 15-30 min 8 mg (step 2), 30-45 min 12 mg (step 3) and 45-60 min 16 mg (step 4); all doses are per 70 kg. After the 1-hour esketamine infusion, the remifentanyl infusion continued for another 15 min (see also Fig. 1). In case ventilation reached baseline values during steps 1, 2 or 3, a next step increase in ketamine was not performed and the esketamine infusion was ended at the end of the 15 min infusion of that particular step.

Ventilation measurements On all three occasions, ventilation was measured on a breath-to-breath basis using the Dynamic End-Tidal Forcing technique.¹⁸ Subjects breathed through a facemask connected to a pneumotachograph (#4813; Hans Rudolph Inc., USA). The inhaled gas mixture came from three mass flow controllers (for O_2 , N_2 and CO_2 ; Bronkhorst High-Tech BV, the Netherlands) that were controlled using custom-made software (RESREG/ACQ). On visits one and two the inspired CO_2 concentration was manipulated to elevate and clamp the end-tidal PCO_2 to a level that caused an increase in mean [standard deviation (SD)] ventilation to 20 (2) litre min^{-1} , while end-tidal PO_2 was strictly maintained at a normoxic value (14.5 kPa). When ventilation reached a steady state under these isohypercapnic and iso-oxic conditions the remifentanyl infusion was started followed by the esketamine or placebo infusion. After the remifentanyl infusion had ended, ventilation measurements at isohypercapnic and iso-oxic conditions continued for another 15 min.

On the third visit, ventilation was measured under poikilocapnic conditions (i.e. without CO_2 clamping). The esketamine administration was similar to the infusion scheme of visit one or two, however, without administration of remifentanyl. Minute ventilation and end-tidal PCO_2 averages were calculated and used in the data analysis.

Sedation and drug high At the end of drug infusion, sedation and drug high were measured on an 11-point verbal rating scale (VRS) ranging from 0 (no effect) to 10 (maximum effect).

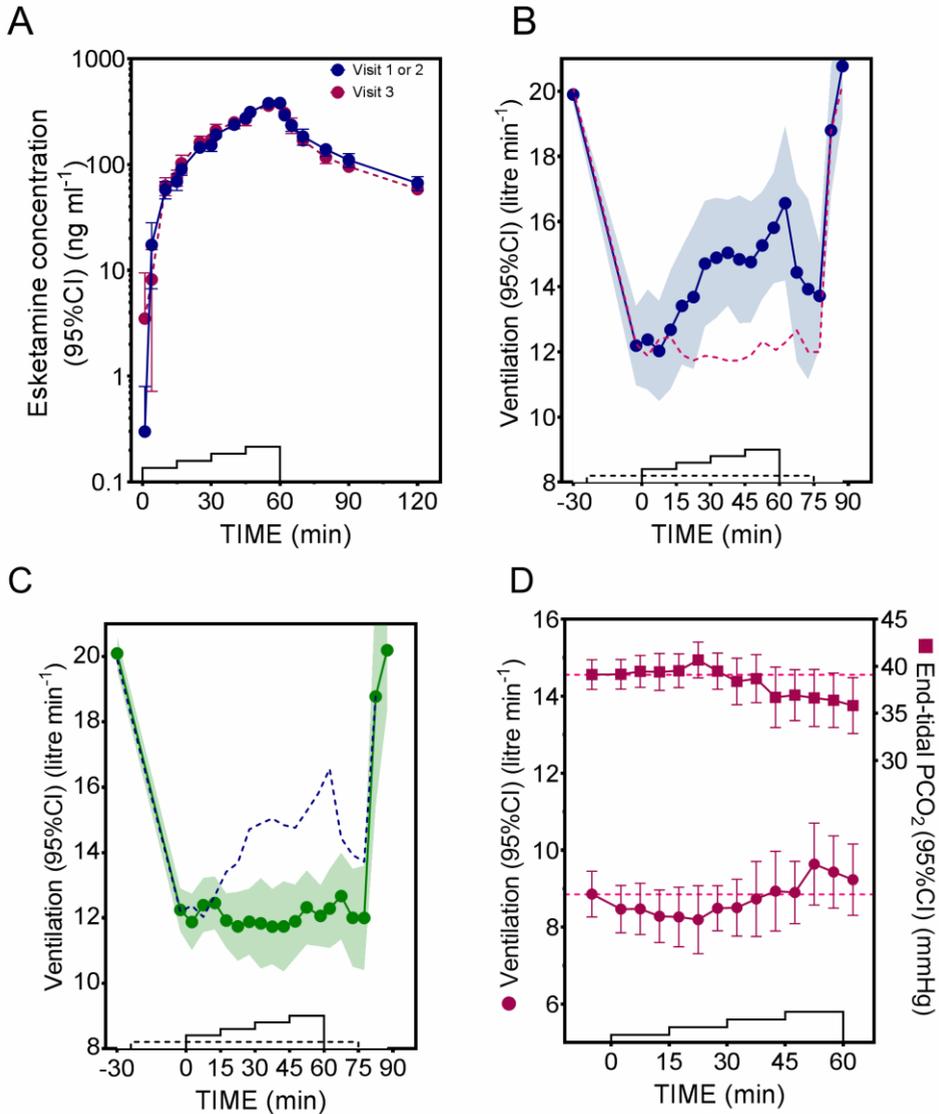


Figure 1. **A.** Population averages of the plasma esketamine concentration of visits 1 or 2 (blue symbols) and visit 3 (pink symbols). **B.** The effect of esketamine on remifentanyl-induced respiratory depression. To guide the eye, the placebo data are plotted on top of the esketamine data (pink broken line). **C.** The effect of placebo on remifentanyl-induced respiratory depression. To guide the eye, the remifentanyl data are plotted on top of the placebo data (blue broken line). **D.** The results of the observational trial of visit 3. All data are mean \pm 95% confidence interval. The black lines are the esketamine infusion scheme, the broken black lines the remifentanyl infusions. The dotted pink lines in panel **D** reflect the baseline values of ventilation and end-tidal PCO_2 .

Blood sampling To quantify the esketamine concentrations, arterial blood samples were collected in 6-ml heparin tubes. Blood samples were taken at baseline (before any esketamine infusion) and $t = 1, 4, 10, 15, 17, 25, 30, 32, 40, 45, 47, 55, 60, 62, 65, 70, 80, 90$ and 120 min after the start of esketamine infusion (Supplementary Figure S1). Within 30 min after collection, blood samples were centrifuged at 1,500 rpm for 15 min at 4°C; 2-3 ml plasma was separated and stored at -80 °C until analysis. Esketamine measurements were performed at the University of Groningen on a TSQ Quantum™ Access MAX Triple Quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) using a Vanquish autosampler (Thermo Fisher Scientific) and injections of 5 µl. The linear range of the assay was 2.5-2,000 ng ml⁻¹; the lower limit of quantitation was 0.5 ng ml⁻¹ for esketamine. The data were analysed using Xcalibur software (Thermo Fisher Scientific).

Randomisation and allocation Randomisation was performed using a computer-generated randomisation list by an individual not involved in the study after all relevant parties (institutional and national review boards, pharmacy, departmental research board) approved the study protocol. The randomisation list was used by the pharmacy to prepare the study medication. The researchers notified the pharmacy of the subject number on the day before the study. Esketamine or saline were delivered to the laboratory on the morning of the study in syringes labelled with subject and visit numbers (1 or 2) only. The research team prepared all other medication. The team remained blinded to treatment until the data acquisition was completed.

DATA ANALYSIS

A population PK- PD model was constructed in which the esketamine's PK was linked to the remifentanyl potency parameter (i.e. C_{50} or the concentration remifentanyl that reduced ventilation by 50%). The population analyses were performed in NONMEM version 7.4.1 (software for nonlinear mixed-effects modelling; Icon plc, Gaithersburg, MD, USA). Model selection was based on the minimum objective function value (χ^2 test), standard error of estimates (SEE) and goodness of fit plots. For both PK and PD analysis, the model parameters were assumed to be log-normally distributed across the population. Residual error was assumed to have both an additive and a relative error for concentrations and only an additive error for ventilation. All values in the PK-PD analysis are median (SEE); P -values < 0.01 were considered significant.

The analyses were performed simultaneously on remifentanyl/esketamine and remifentanyl/placebo data in multiple steps (with estimation of inter-occasion variability for the remifentanyl model parameters). (i) First the esketamine PK data were analysed using a three-compartment model. Initially, the structural parameters of Sigtermans and colleagues¹⁹ were implemented, after which we searched for systematic deviations from

that model. (ii) For remifentanil, it was assumed that the target controlled infusion values correctly reflect the plasma concentrations. (iii) For both esketamine (E) and remifentanil (R), a possible hysteresis between plasma concentration and effect was modelled by assuming effect compartments with blood-effect-site equilibration half-times, $t_{1/2E}$ and $t_{1/2R}$, respectively. (iv) Next, the population PD model parameters were determined with fixed empirical Bayesian individual drug PK model parameters.

Inspired minute ventilation (\dot{V}_E) was modeled as²⁰:

$$\dot{V}_E = \dot{V}_{BLN} + S \times (P_TCO_2 - P_{TB}CO_2) \quad (1)$$

where \dot{V}_{BLN} is baseline ventilation obtained without any inspired CO_2 , S the ventilatory carbon dioxide sensitivity, P_TCO_2 the CO_2 concentration at the site of chemoreception (and instantaneously related to \dot{V}_E) and $P_{TB}CO_2$ the baseline value of P_TCO_2 . In our isohypercapnic experiments P_TCO_2 was assumed to be constant over time, hence $(P_TCO_2 - P_{TB}CO_2)$ is a constant. We next assume that remifentanil depresses ventilation by an effect on \dot{V}_{BLN} , S , or both:

$$Y1 = \dot{V}_{BLN}/[1 + (C_{REM}/C_{501})^{G1}] \quad (2)$$

$$Y2 = S/[1 + (C_{REM}/C_{502})^{G2}] \quad (3)$$

and

$$\dot{V}_E = (1 - \lambda) \times Y1 + \lambda \times Y2 \quad (4)$$

where C_{REM} is the remifentanil effect-site concentration and C_{501} the concentration remifentanil that \dot{V}_{BLN} by 50%, C_{502} the concentration remifentanil that reduces S by 50%, $G1$ and $G2$ shape factors and λ a constant. Since the effect of remifentanil on S and \dot{V}_{BLN} may effectively occur at separate sites in the brainstem, we postulated two distinct equilibration half-times, $t_{1/2R1}$ and $t_{1/2R2}$.

We assume that esketamine may increase C_{501} or C_{502} as follows:

$$C_{501} = C_{501}(0) \times [1 + (C_{KET}/C_D^K1)^Q] \quad (5)$$

$$C_{502} = C_{502}(0) \times [1 + (C_{KET}/C_D^K2)^Q] \quad (6)$$

where $C_{501}(0)$ and $C_{502}(0)$ are the respective remifentanil C_{501} and C_{502} values when the esketamine effect-site concentration is zero, C_{KET} the esketamine effect-site concentration, C_D^K1 and C_D^K2 the esketamine effect-site concentrations causing a doubling of C_{501} and C_{502} , respectively, and Q a shape factor.

The effect of placebo on remifentanil-induced respiratory depression was modeled as follows:

$$C_{502} = C_{502}(0) \times [1 + (C_p / C_D^K)^Q] \quad (7)$$

where C_p is the assumed esketamine concentration during placebo treatment.

A mixture analysis was performed on C_D^K with four possible response subgroups. An individual can be a ketamine responder or non-responder, and a placebo responder or non-responder, giving four possibilities. The mixture analysis was done in NONMEM, by specifying: (i) the probability of being a ketamine responder and the probability of being a placebo responder; and (ii) the C_D^K values of the four subgroups: an estimable parameter for ketamine, an estimable parameter for placebo, and a fixed potency of zero (corresponding to a C_D^K of infinity) for the (ketamine and placebo) non-responders. The probability parameters are estimated by NONMEM by minimising the objective function as usual. In addition, NONMEM estimates to which of the four subgroups the individuals are the most likely to belong, on the basis of which the ketamine/placebo responder/non-responder status of the subjects were assessed and counted. The choice of C_D^K for mixture analysis was based on the objective functions of the various models that were tested. These models included: (i) a model in which remifentanil had an effect at a single component Y versus an effect at Y1 and Y2; (ii) a model with an esketamine effect on C_D^K1 and C_D^K2 with $C_D^K1 = C_D^K2$ versus an effect at just C_D^K2 ; (iii) a model with an esketamine effect on C_D^K1 and C_D^K2 with $C_D^K1 \neq C_D^K2$ versus an effect at just C_D^K2 ; (iv) a model in which Q was not fixed versus Q fixed to 1; (v) a model in which esketamine was compared to placebo without any mixture analysis versus the mixture analysis as described above; and (vi) a model in which placebo and esketamine data were grouped with a mixture analysis with two possible groups (responders and non-responders) versus a model with mixture analysis with four groups as described above.

We calculated Normalized Prediction Discrepancies (NPD; by NONMEM) as a visual predictive check of the final esketamine model.^{21,22} In brief, three hundred Monte Carlo simulations (of the final model output based on the fixed and distributions of the random effects) were performed and the number of times an observation is greater than the model prediction is counted. The NPD are the counts divided by 300, transformed via the inverse normal distribution. Under the null hypothesis that the model is correct, the NPD should have a normal distribution. It was checked by visual inspection that the NPD vs time showed no trends, heteroscedasticity or both.

5.3 Results

Sixteen subjects of either sex were enrolled in the study. Two subjects ended their participation during screening because of facemask discomfort, two others because of esketamine-induced psychomimetic side effects. Their data were discarded. The 12 participating subjects (six men, six women) had a mean age of 24 (range 20-31) years, weight of 68 (52-102) kg and body mass index of 22 (19-30) kg m⁻². All 12 subjects completed the study without major side effects.

SIDE EFFECTS

Side effects included nausea (occurred on seven occasions), headache (two occasions) and anxiety (one occasion). Mean drug high VRS scores (SD) were: remifentanil/esketamine 7.2 (1.9), remifentanil/placebo 1.9 (2.3; $P < 0.001$ vs remifentanil/esketamine) and esketamine (visit 3) 6.9 (2.5; $P = 0.75$ vs remifentanil/esketamine). Sedation VRS scores were remifentanil/esketamine 7.0 (2.5), remifentanil/placebo 3.7 (2.4; $P < 0.001$ vs remifentanil/esketamine) and esketamine (visit 3) 6.3 (2.6; $P = 0.18$ vs remifentanil/esketamine).

EFFECT OF ESKETAMINE VERSUS PLACEBO ON REMIFENTANIL-INDUCED RESPIRATORY

DEPRESSION

The average esketamine concentrations are given in Figure 1A; mean (SD) peak plasma esketamine concentration was 381 (65) ng ml⁻¹. The end-tidal PCO₂ values were similar between treatments: mean end-tidal PCO₂ esketamine 6.6 (0.4) kPa vs placebo 6.5 (0.5) kPa ($P = 0.20$). The target remifentanil concentration was somewhat higher in the esketamine arm [1.0 (0.4) ng ml⁻¹] compared to the placebo arm [0.90 (0.3) ng ml⁻¹, $P = 0.01$].

Figures 1B and C demonstrate that esketamine but not placebo antagonized remifentanil-induced respiratory depression. Remifentanil had similar effects in the two arms of the study with a reduction from 19.9 (0.4) to 12.2 (2.3) litre min⁻¹ in the esketamine arm and from 20.1 (0.9) to 12.2 (1.3) litre min⁻¹ in the placebo arm of the study. Adding placebo had no effect on remifentanil-induced respiratory depression [change in ventilation from 12.2 (1.3) to 12.3 (2.2) litre min⁻¹]. In contrast, esketamine increased ventilation from 12.2 (2.3) to 16.6 (4.1) litre min⁻¹ (an increase of 35%; paired t-test: $P < 0.01$ vs placebo).

In both treatment arms, remifentanil affected both ventilatory frequency and tidal volume (Supplementary Fig. S2). In the remifentanil/esketamine and remifentanil/placebo arms, remifentanil reduced ventilatory frequency from 17.1 (3.7) to 14.7 (3.1) breaths per min (bpm) ($P < 0.01$) and 17.5 (2.9) to 14.9 (2.5) bpm ($P < 0.01$), respectively, and tidal volume from 1,220 (283) to 853 (955) ml ($P < 0.01$) and 1,150 (222) to 812 (113) ml ($P < 0.01$),

respectively. Esketamine had a selective effect on ventilatory frequency with an increase from 14.7 (3.1) to 18.6 (3.9) ($P < 0.01$). Tidal volume showed a small albeit insignificant increase 853 (156) to 955 (396) ml by esketamine. Placebo had no effect on either ventilatory frequency or tidal volume.

PK-PD ANALYSIS

The PK parameters estimates are given in Supplementary Table S1. The parameters estimates of the final three-compartment model were similar to that of Sigtermans and colleagues,¹⁹ with the exception of the clearances that were 83 (2)% of the earlier estimates. Goodness of fit plots are given in Figures 2A-C, showing measured vs individually predicted esketamine concentrations (Fig. 2A), conditional weighted residuals with η - ϵ interaction (CWRESI) vs time (Fig. 2B) and the NPD (Fig. 2C). All indicate that the model adequately described the data.

The best PD model (Table 1 and Supplementary Table S2) is a mixture model with remifentanil effect on Y1 and Y2, an esketamine effect on C_D^K2 (i.e. Y2) with mixture analysis and Q fixed to 1. The PD parameter estimates of the best model are given in Table 1. Examples of data fits are given in Figure 3 for an esketamine responder (Fig. 3A, median fit with $R^2 = 0.719$), an esketamine non-responder (Fig. 3B), a placebo responder (Fig. 3C) and a placebo non-responder (Fig. 3D, best fit with $R^2 = 0.952$). Goodness of fit plots are given in Figure 2D-F. Inspection of the individual fits and the goodness of fit plots indicate that the model adequately described the data.

Remifentanil effect The model has two ventilation components [Y1 and Y2, equations (2) and (3)] at which remifentanil acted. The effect of remifentanil was for 39% at Y2 and for 61% at Y1 [compare parameter λ of equation (4) and Table 1]. Remifentanil acted on the two ventilation components with different potencies and dynamics. Y1 is affected more slowly [$t_{1/2R1} = 12.2$ (2.6) min] with relatively low potency [$C_{501} = 1.24$ (0.15) ng ml⁻¹] and high G1 [8.44 (0.64)] than the remifentanil effect at Y2 [$t_{1/2R2} = 2.15$ (0.49) min, $C_{502} = 0.46$ (0.06) ng ml⁻¹ and $G2 = 4.44$ (0.64)]. The existence of the two components is well illustrated in Figure 3D, a placebo experiment, where an initial rapid decrease in ventilation is followed by a slow further decline. The absence of an Y1 component in some subjects is explained by the fact that the slow component was only present when target remifentanil levels were relatively high and occurred with a steep concentration-response relationship. This reflected by the relatively high values of parameter estimates C_{501} and G1.

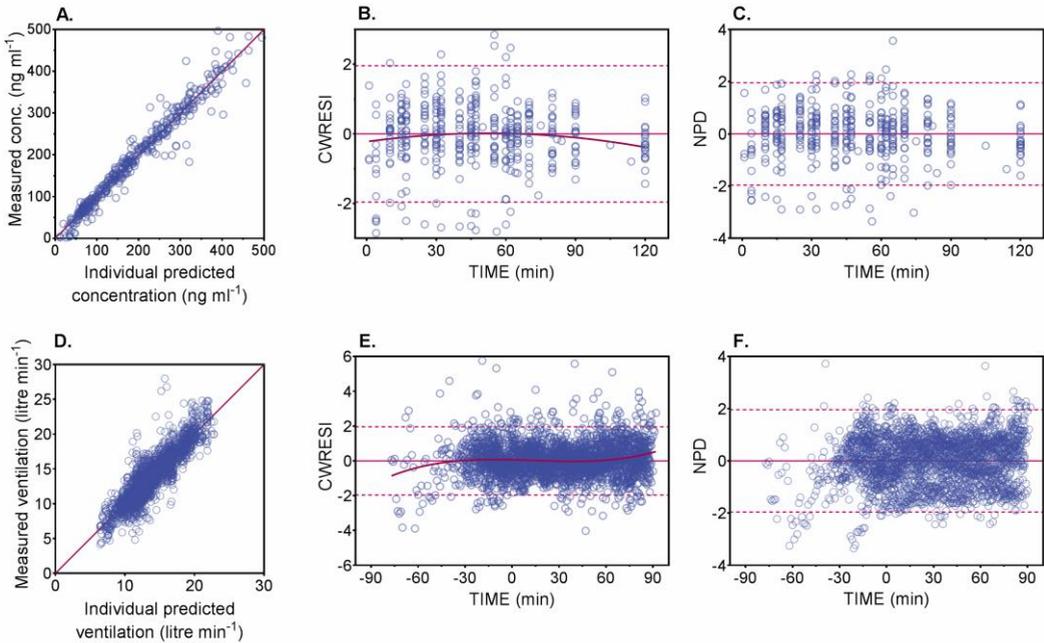


Figure 2. Goodness of fit plots of the pharmacokinetic (A-C) and the pharmacodynamic models (D-F). Panels (A,D) are the measured vs the individual predicted esketamine concentration and ventilation, respectively; (B,E) are the conditional weighted residuals with η - ϵ interaction (CWRESI) vs time for esketamine concentration and ventilation, respectively; and (C,F) the normalised prediction discrepancy for esketamine concentration and ventilation, respectively. In (B,E) the pink line is a smoothing curve; in (B,C,E,F) the pink lines are the median (solid line) with 95% confidence intervals (broken lines). NPD, normalised prediction discrepancies

Esketamine and placebo effects Esketamine had an exclusive effect on component Y2 and doubled C_{502} at a concentration of 127 (34) ng ml⁻¹ (C_D^K2); an effect on C_{501} was not identifiable. Esketamine blood-effect-site equilibration half-time ($t_{1/2E}$) was not significantly different from 0, indicative that the esketamine effect was driven by plasma PK. In some subjects a response to placebo treatment was observed (see Fig. 3C for an example). To quantify the placebo effect, it was assumed that esketamine was present and had an effect of C_{502} . This led to a placebo potency parameter $C_D^K2(\text{placebo})$ with value 363 (37.5) ng ml⁻¹, a factor of 3 smaller than the esketamine C_D^K2 . Probability of being a responder to esketamine or placebo was 83 (12)% and 23 (15)%, respectively, which corresponds to ten responders to esketamine and three to placebo.

Table 1. Pharmacodynamic parameter estimates

	Estimate	SEE	ω^2	SEE	v^2	SEE
Remifentanil effect on ventilation						
\dot{V}_{BLN} (litre min ⁻¹)	20.1	0.26	-	-	0.006	0.002
λ	0.39	0.01	-	-		
C_{501} (ng ml ⁻¹)	1.24	0.15	0.14	0.07		
C_{502} (ng ml ⁻¹)	0.46	0.06	0.14	0.08		
$t_{1/2R1}$ (min)	12.2	2.6	0.26	0.12		
$t_{1/2R2}$ (min)	2.15	0.49	0.49	0.37		
G1	8.44	0.64	0.24	0.08		
G2	4.37	0.64	0.24	0.08		
Esketamine effect on remifentanil-induced respiratory depression						
C_D^K1 (ng ml ⁻¹)	-	-	-	-		
C_D^K2 (ng ml ⁻¹)	127	33.9	0.37	0.17		
Q	1 (FIX)	-	0.72	0.36		
$P_{\text{(Responder to esketamine)}}$	0.83	0.12				
$P_{\text{(Responder to placebo)}}$	0.23	0.15				
σ^2a	2.39	0.54				

\dot{V}_{BLN} is baseline ventilation, C_{501} is the remifentanil concentration causing a 50% decline of V_{BLN} , C_{502} is the remifentanil concentration causing a 50% decline the ventilatory CO₂ sensitivity, $t_{1/2R1}$ and $t_{1/2R2}$ are the blood-effect-site equilibration half-times linked to components Y1 and Y2, respectively [equations (1) and (2)], G1 and G2 are shape factors of components Y1 and Y2, respectively [equations (1) and (2)], λ is a constant, C_D^K1 and C_D^K2 are the esketamine concentration causing a doubling of remifentanil potency parameters C_{501} and C_{502} , respectively, Q a shape parameter [equations (3) and (4)], P the responder rate and σ^2a the additive residual error. SEE, standard error of estimate

Dose-response relationship To get an indication of the steady-state dose-response relationships, the two inputs to the PD model are plotted against ventilation in Figure 4. Figure 4A gives the target remifentanil concentration-ventilation relationship at several esketamine concentrations (ranging from 0 to 400 ng ml⁻¹); Figure 4B gives the esketamine concentration-ventilation relationship at a number of target remifentanil concentrations (ranging from 0 to 2 ng ml⁻¹). In both Figure 4A and B, the maximum observed concentrations are depicted by the grey broken lines. All values beyond these lines are extrapolations. Figure 4C combines the data from Figure 4A and B and gives the response surface of esketamine concentration (y-axis), target remifentanil concentration (x-axis) against ventilation (z-axis).

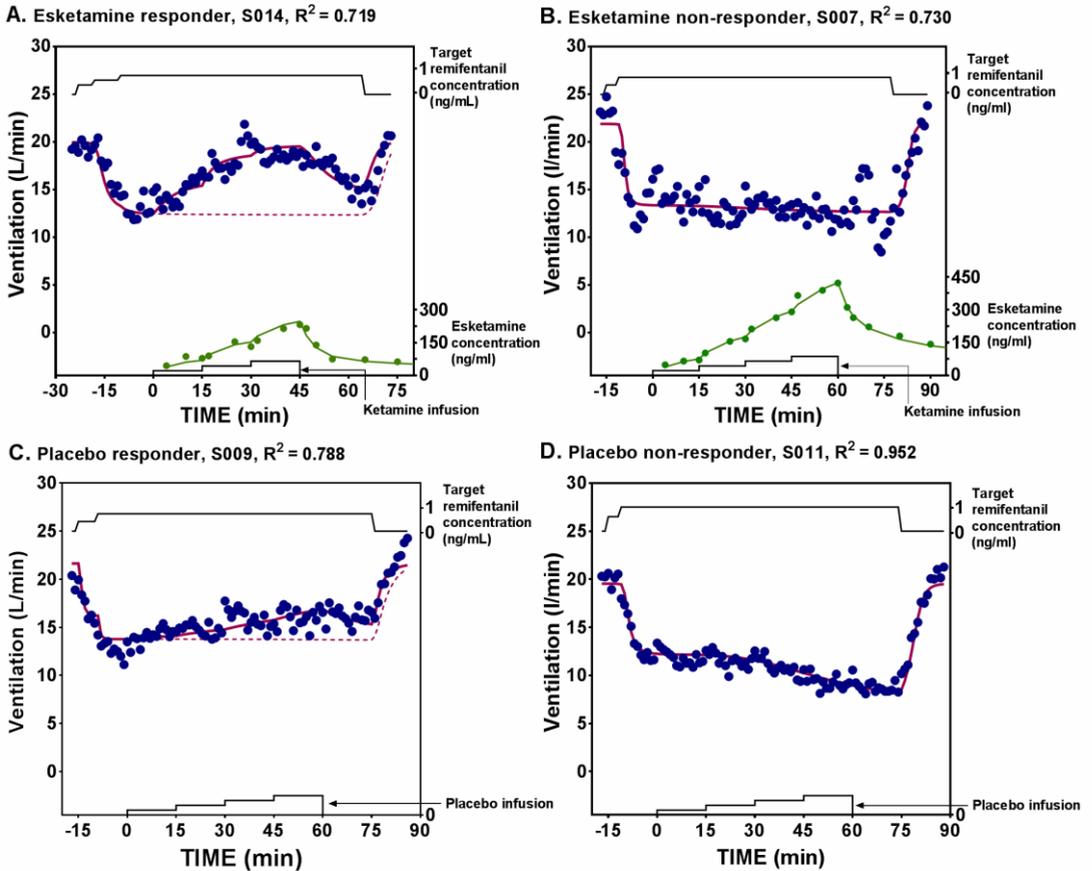


Figure 3. Pharmacokinetic and pharmacodynamic data fits of an esketamine responder (A; median fit), an esketamine non-responder (B), a placebo responder (C) and a placebo non-responder (D; best fit). The data show the esketamine concentration (green dots), pharmacokinetic data fits (green lines), the measured ventilation (blue dots), the pharmacodynamic data fits (continuous pink lines), the effect of remifentanyl *without* the presence of ketamine or placebo (A,C, pink broken lines) and the ketamine or placebo infusion schemes (black lines) and the target remifentanyl concentration (black lines).

EFFECT OF ESKETAMINE ON POIKILOCAPNIC VENTILATION

Esketamine plasma concentrations of the observational study were similar to those observed in the randomised trial (Fig. 1A), with peak plasma concentrations of 378 (24) ng ml⁻¹. Esketamine induced a small decrease in end-tidal PCO₂ of 0.4 kPa from 5.2 (0.3) (baseline) to 4.8 (0.4) kPa (last minute of esketamine infusion; paired-t-test: $P < 0.01$) but had no effect on ventilation (\dot{V}_{BLN} changed from 8.9 (0.6) litre min⁻¹ (baseline) to 9.4 (1.6) litre min⁻¹ (last minute of esketamine infusion), $P = 0.12$). Changes in ventilation and end-tidal PCO₂ were restricted to the final minute of the highest esketamine infusion (Fig. 1D).

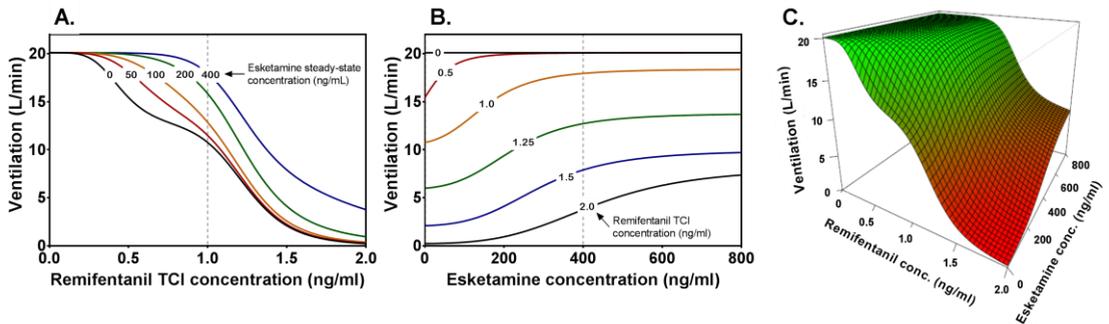


Figure 4. (A) Steady-state relationship of target remifentanil concentration vs ventilation at increasing esketamine steady-state concentrations (0-400 ng ml⁻¹). The grey broken line reflects the mean target remifentanil concentration applied in the study. The effect of target remifentanil concentrations > 1 ng ml⁻¹ on ventilation are extrapolations. (B) Steady-state relationship of esketamine concentration vs ventilation at increasing target remifentanil concentrations (0-2 ng ml⁻¹). The grey broken line reflects the peak esketamine concentration observed in the study. The effect of esketamine concentrations >400 ng ml⁻¹ on ventilation are extrapolations. (C) Response surface of the interactive effect of steady-state esketamine concentration (y-axis), target remifentanil concentration (y-axis) vs ventilation (z-axis).

5.4 Discussion

The main findings from our study are that esketamine dose-dependently reverses remifentanil-induced respiratory depression, while it has a little or no effect when ventilation is not depressed by the opioid.

PD MODEL

Remifentanil effect We constructed a PD model in which both remifentanil and esketamine could affect breathing *via* actions at component Y1, component Y2 [equations (2-4)], or both. We observed that remifentanil reduced ventilation via an effect at both parts, at Y1 with relatively low potency and slow dynamics, and at Y2 with relatively high potency and faster dynamics (Table 1). The initial rapid decrease in ventilation following the start of remifentanil infusion is due to its effect on Y2, while the slow decrease in ventilation that was observed in some subjects was due to an effect at Y1. In mammals, ventilatory control has CO₂-sensitive and CO₂-insensitive components as described in equation (1), where \dot{V}_{BLN} is the CO₂-insensitive and S the CO₂-sensitive component.^{18,23,24} In our analysis, we link a drug effect at Y1 to an action at CO₂-insensitive ventilation, while an effect at Y2 is linked to the CO₂-sensitive operator of ventilation. These associations seem hypothetical but are plausible when we take the results of visit 3 into account where esketamine had little effect on resting ventilation (i.e. Y1 or \dot{V}_{BLN}); see also the following section (**Esketamine effect**).

We did not directly estimate the value of S in our analysis. However, parameter λ is an indirect estimate of S . Since $\lambda \times Y2 = S \times (P_{T}CO_2 - P_{TB}CO_2) \approx 8 \text{ litre min}^{-1}$, the value of S is estimated to be approximately $8 \text{ litre min}^{-1} \text{ kPa}^{-1}$ (assuming that $P_{T}CO_2 - P_{TB}CO_2 \approx 1 \text{ kPa}$),²⁴ which is within the range of ventilatory CO_2 sensitivities observed in healthy young volunteers.²⁴ The reason for a difference in remifentanyl potency and dynamics at components Y1 and Y2 remains unknown but could be related to the opioid effect on central neuronal dynamics, causing a slow reduction of CO_2 -insensitive ventilation.²⁵

Previously we tested the effect of remifentanyl on ventilation in open loop conditions.²⁶ The remifentanyl effect in that study was modelled with just one component with values for C_{50} (1.6 ng ml^{-1}) and $t_{1/2R}$ (0.53 min) in the same range as in the current study for Y2. We relate the inability to detect a slow component (Y1) to the short infusion times ($0.5\text{-}6 \text{ min}$) and open loop (i.e. poikilocapnic) conditions in that study. With respect to the latter condition, any slow reduction in ventilation might have been counteracted by the slow increase in arterial CO_2 . Apparently, the open-loop PD model was unable to detect such a slow effect, if at all it occurred in these short infusion experiments.

Esketamine effect Esketamine (at a cumulative dose of 40 mg) exclusively impacted on Y2. We argue that (low-dose) esketamine interacts with the ventilatory control system under conditions that the central respiratory network is affected by an opioid (i.e. under conditions of hypoventilation). We come to this conclusion as the same esketamine dose had only a limited effect under baseline conditions (i.e. without the presence of an opioid; visit 3). Consequently, as remifentanyl and other opioids reduce ventilatory CO_2 sensitivity,^{20,27-29} our data suggest that esketamine increased CO_2 chemosensitivity (i.e. parameter S) after its reduction by remifentanyl. Also, other respiratory stimulants acting within the respiratory network, such as the ampakines, stimulate breathing exclusively under conditions of hypoventilation by increasing the (reduced) ventilatory CO_2 sensitivity.^{29,30} Still, we cannot exclude some albeit small esketamine effect at \dot{V}_{BLN} as the data from visit 3 still indicate some effect on ventilation, which might have been underestimated as result of the open loop conditions of visit 3.

ESKETAMINE- AND RACEMIC KETAMINE-INDUCED STIMULATION VS DEPRESSION OF BREATHING

While some experimental work in both animals and humans shows racemic ketamine-induced respiratory stimulation in agreement with our observations,¹²⁻¹⁵ others show an inhibitory effect of ketamine on ventilation.³¹⁻³⁵ For example, Bourke and colleagues³¹ studied the interaction of racemic ketamine and morphine in six male volunteers. Using an experimental design very similar to this study, they showed a dose-dependent reduction of isohypercapnic (end-tidal PCO_2 6.6 kPa) ventilation by ketamine (cumulative dose increased from 0.39 to 3.0 mg kg^{-1}) and morphine (0.2 and 0.4 mg kg^{-1}); the combination

of ketamine and morphine had an additive negative effect on breathing.³¹ Using CO₂-rebreathing, Hamza and colleagues³⁵ showed a 40% reduction of the slope of the \dot{V}_E -CO₂ response by an anesthetic dose of racemic ketamine (2 mg kg⁻¹ followed by 0.04 mg kg⁻¹ min⁻¹) in a paediatric population (age 6-10 years). We previously observed in mice that esketamine produced a dose-dependent reduction of the \dot{V}_E -CO₂ response slope in a dose range of 10-200 mg kg⁻¹, with frequent inspiratory pauses in the higher dose range.³² Differences in species, dose, ketamine formulation or experimental set-up can only explain part of the differences in study outcomes. For example our cumulative esketamine dose of 0.57 mg kg⁻¹ esketamine falls well within the dose range studied by Bourke and colleagues,³¹ even when taking into account a possible potency difference between racemic ketamine and the S(+)-isomer.³⁶ Possibly, ketamine's metabolites are involved in the respiratory effects of the parent drug (see below). If so, then differences in PK among species and between isomers may explain some of the observed differences. It is important to realise that we and others observed a ketamine-induced stimulatory effects on breathing at subanesthetic ketamine doses.^{13 15} In rats, Eikermann and colleagues¹⁴ showed that respiratory stimulation persists even at anesthetic doses, while Hamza and colleagues³⁵ found the reverse in children. Further human studies are needed to fully understand the complex behavior of the low- and high-dose ketamine isomers on breathing.

An interesting observation is that, in contrast to remifentanil, which reduced both tidal volume and ventilatory frequency, esketamine selectively increased ventilatory frequency (Supplementary Figure S2). It has recently been proposed that tidal volume is regulated by metabolic stimuli, while respiratory frequency is driven by fast non-metabolic factors.^{37,38} Our findings then suggest that remifentanil has a metabolic effect, while the esketamine effect was related to non-metabolic stimuli (e.g. stress, behavioural stimuli, or both). This seems in disagree with the findings of this study that esketamine increased ventilatory CO₂ sensitivity, which is a component of metabolic control.³⁸ Additionally, the proposed mechanisms of esketamine-induced respiratory stimulation all seem metabolic in nature (see below). Possibly, behavioural effects of isohypercapnia may have affected the study outcome. However, all subjects who completed the study were highly comfortable during all three visits. Further studies are needed to address the mechanistic pathway of esketamine within the ventilatory control system.

Extrapolation of our results to higher remifentanil concentrations suggests that even in case of the complete cessation of breathing (i.e. opioid-induced apnoea), low-dose ketamine may restore breathing activity (Figure 4). Further studies are needed to verify the validity of our model at deep levels of respiratory depression (e.g. at remifentanil plasma concentrations >1.25 ng ml⁻¹). If corroborated, ketamine behaves similarly to

CX717, an ampakine that is able to restore respiratory activity following fentanyl-induced apnea,¹⁰ but contrarily to drugs that act at the carotid bodies and that show reduced efficacy at deeper levels of opioid-induced respiratory depression (i.e. ceiling behavior).³⁹

MECHANISM OF ESKETAMINE-INDUCED RESPIRATORY STIMULATION

Although our study was not specifically designed to unearth the mechanism of the stimulatory effect of esketamine on breathing, it is relevant to discuss possible mechanisms. (i) Esketamine produces a strong increase in sympathetic outflow and reduces the reuptake of neuronal norepinephrine.¹⁹ Monoaminergic neurotransmitters play an important role in ventilatory control.⁴⁰ Increased synaptic concentrations of nor-adrenaline stimulate breathing activity and increase ventilatory CO₂ reactivity.^{41,42} (ii) Esketamine may stimulate breathing though *N*-methyl-D-aspartate receptor blockade. Some indirect evidence comes from an animal model of the Rett syndrome.⁴³ Patients with Rett syndrome have mental retardation and experience severe breathing irregularities because of mutations in the *MECP2* gene. In mice model of Rett syndrome, ketamine reduced the number of apnoeic events by actively stimulation of breathing activity. (iii) Finally, it may well be that the esketamine metabolite hydroxynorketamine contributed to the respiratory effects of the parent drug. Hydroxynorketamine is an agonist at the AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor.⁴⁴ AMPA receptors are expressed in the brainstem respiratory network, for example within the pre-Bötzinger complex, an area of the brain involved in respiratory rhythmogenesis, and play an important role in the maintenance of respiratory drive.^{45,46} Of interest is the observation that so called ampakines, drugs that act selectively at the AMPA receptors, increase the respiratory drive in both animal and human studies, but, as stated earlier, only under conditions of hypoventilation.^{29,30} For example, the ampakine CX717 counters alfentanil-induced respiratory depression to a 50% depression of \dot{V}_E -CO₂ sensitivity.²⁹ An effect of esketamine via hydroxynorketamine at AMPA receptors may explain the absence of response in some subjects who are poor metabolisers. This remains speculative at present, as we did not measure hydroxynorketamine plasma concentrations in our subjects. Future studies will need to resolve whether hydroxynorketamine plays an important role in the respiratory effects of ketamine. If so, this would mirror the observation in rodents that hydroxynorketamine rather than ketamine plays a major role in the generation of its antidepressant effects.⁴⁴

5.5 Conclusion

To summarise, we observed that remifentanyl depressed ventilation in healthy volunteers by reducing ventilatory CO₂ sensitivity, an effect that was partially countered by esketamine. These findings could indicate that the use of low-dose esketamine in postoperative patients may not only reduce opioid consumption but will also stabilize

breathing and consequently reduce the probability of fatal events. The esketamine dose that is needed for such an effect is between 12 and 24 mg hour⁻¹ (for a 70 kg patient). However, the observed advantageous effects of esketamine should be balanced against its side effect profile, most importantly the psychedelic symptoms that may be perceived by some patients as extremely frightful. We believe that additional studies are still needed and we plan to construct esketamine utility functions to determine the optimal esketamine dose that produces respiratory stimulation at minimal psychedelic symptoms.^{47,48}

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Appendix A. Supplementary data

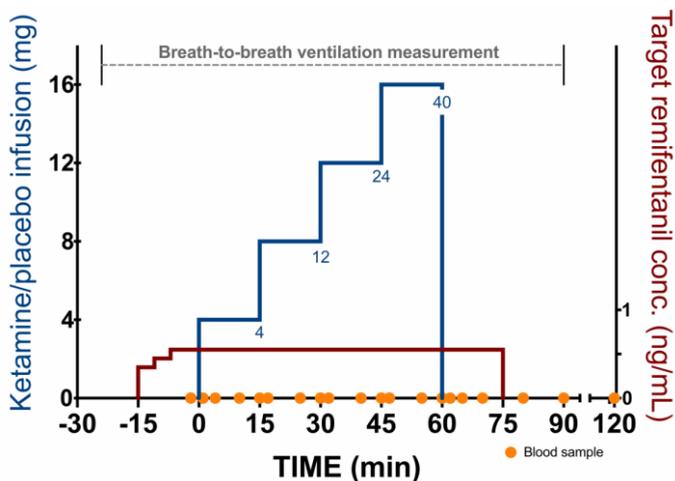


Figure S1 Diagram of the design of the randomised controlled study of visits 1 and 2. The red line depicts the plasma target concentration of the remifentanyl target controlled infusion, the blue line the esketamine or placebo infusion (the numbers near the graph are the cumulative ketamine doses). The orange dots indicate blood samples.

Table S1 Pharmacokinetic parameter estimates

	Estimate	SEE	v^2	SEE
V_1 (litre)	15.8	-	0.129	0.031
V_2 (litre)	24.8	-	0.157	0.088
V_3 (litre)	119	-	-	
CL_1 (litre min^{-1})	1.50	-	0.039	0.013
CL_2 (litre min^{-1})	2.33	-		
CL_3 (litre min^{-1})	1.29	-		
σ^2_r	0.091	0.014		
σ^2_a	12.1	1.94		

SEE, standard error of the estimate; V , volume; CL , clearance; σ^2_r , relative residual error; σ^2_a , additive residual error; v^2 , interoccasion variability.

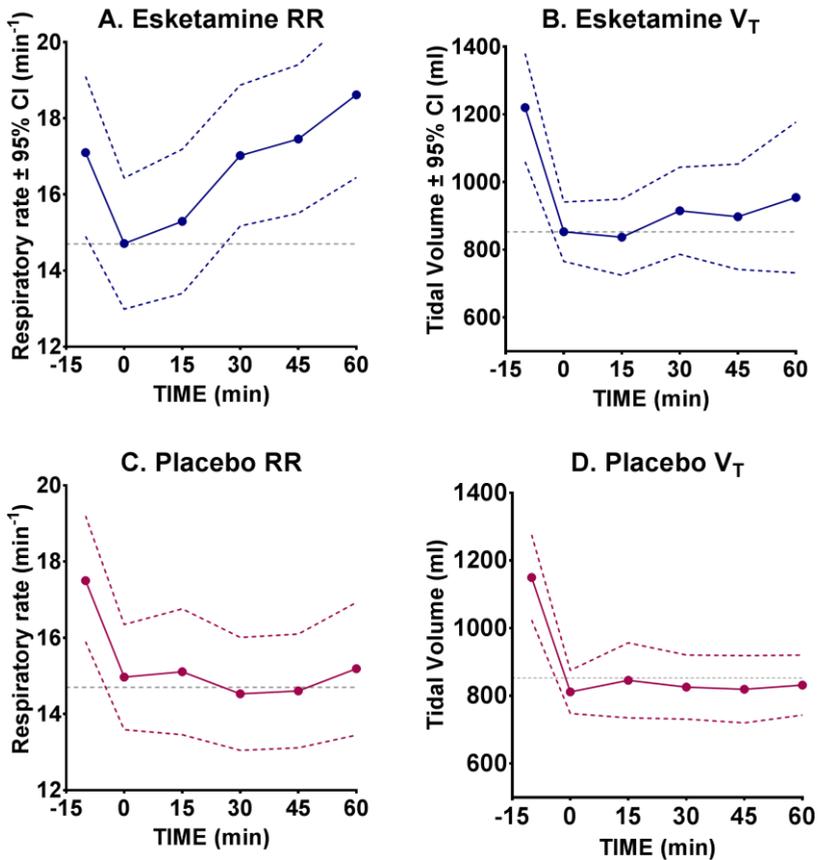


Figure S2 Effect of remifentanyl and esketamine on respiratory rate (RR) and tidal volume (V_T). Each data point is a 5-min average of either RR (A,C) or V_T (B,D). T = -15 min is predrug baseline; T = 0 min is remifentanyl; T = 15 min is the first esketamine infusion step (4 mg); T = 30 min is the second esketamine step (12 mg); T = 45 min is the third ketamine step (24 mg); T = 60 min is the fourth and last ketamine step (40 mg). See also Supplementary Figure S1.

Table S2. Objective functions of the tested pharmacodynamic models

Model	Objective function	p-value vs. best model
Final model	5478.970	
1. Remifentanil on Y	6028.338	p < 0.001
2. Esketamine effect on C_D^K1 and C_D^K2 with $C_D^K1 = C_D^K2$	5559.636	p < 0.001
3. Esketamine effect on C_D^K1 and C_D^K2 with $C_D^K1 \neq C_D^K2$	not identifiable	-
4. Q free	5478.970	p < 0.001
5. No mixture analysis	5555.146	P < 0.001
6. Esketamine and placebo data grouped with mixture analysis	5523.386	p < 0.001

