Safety-efficacy balance of S-ketamine and S-norketamine in acute and chronic pain

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Chapter 6

Negative contribution of norketamine to ketamine-induced acute pain relief but not neurocognitive impairment in healthy volunteers

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Introduction

The *N*-methyl-D-aspartate receptor (NMDAR) antagonist ketamine is used as anesthetic and at low-dose for the treatment of (acute and chronic) pain or combined with opioids in the treatment of perioperative and cancer pain.[1-3] Ketamine is rapidly metabolized into the NMDAR antagonist norketamine.[4] Animal data indicate that norketamine passes the blood-brain barrier, has about 20-30% the potency of ketamine and is thought to contribute significantly to ketamine (side) effects.[5-8] No human data are available on norketamine's contribution to ketamine effect. We previously showed that pretreating humans with rifampicin (an antibiotic that induces multiple hepatic cytochrome P450 enzymes, including CYP 2B6 and 3A4, involved in the ketamine *N*-demethylation into norketamine) caused a small (10%) reduction in S-ketamine concentration, but a large (50%) reduction in S-norketamine concentrations during and following a 2-h S-ketamine infusion (Figure 1).[9] Simulation studies were performed (as no pharmacodynamic measures were obtained), and by using data on norketamine's contribution to effect derived from animal studies, we predicted a 10-20 % contribution of norketamine to ketamine effect.

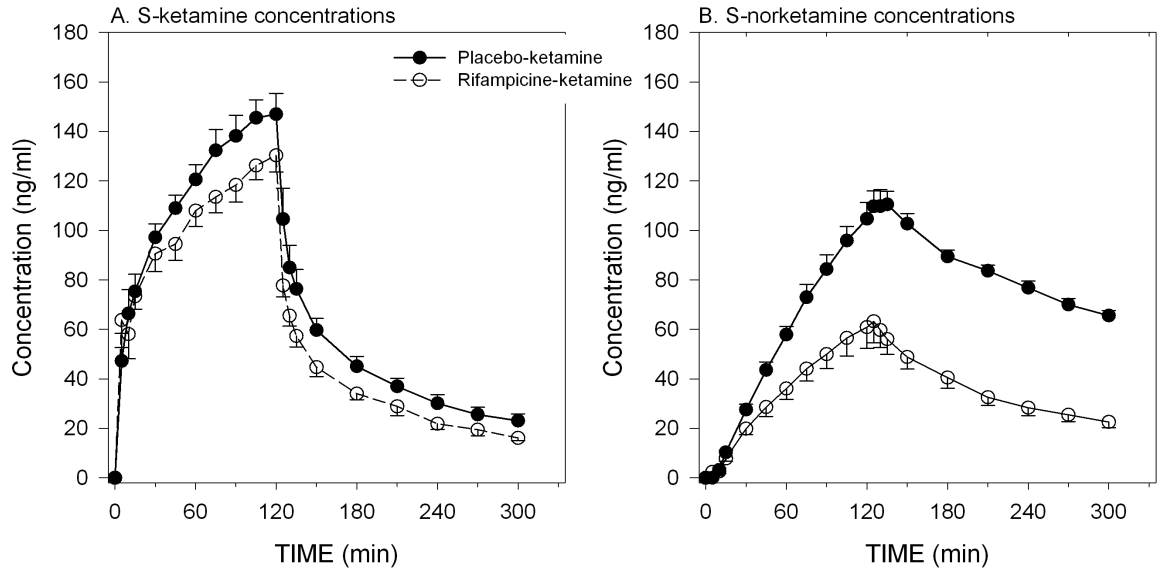

Figure 1 Effect of placebo (closed symbols) and rifampicin (open symbols; pretreatment 600 mg po per day for five days) on **A** S-ketamine and **B** S-norketamine concentrations, during and following a 2-h S-ketamine infusion (from t = 0 to 120 min; dose = 20 mg/h). Values are mean ± SEM. Data are from Noppers et al.[9]

In the current study we measured the effect of rifampicin pretreatment on pain responses and cognitive impairment during and following a 2-h ketamine infusion using a placebo controlled randomized cross-over and single blind

design. This design and the application of an additive ketamine-norketamine pharmacokinetic-pharmacodynamic (PK-PD) model allows the estimation of the norketamine versus ketamine contribution to changes in effect observed after infusion of just ketamine.

The main aims of this study were: (i) to assess the effect of low-dose ketamine on pain responses and cognition during and following a 2-h infusion; and (ii) to get an estimate of the contribution of norketamine to ketamine effect. We hypothesize that, in agreement with our previous simulation study, norketamine contributes 10-20 % to ketamine-induced effect. In order to assess the contribution of norketamine, we performed a population PK-PD analysis using the PK data from our previous study.[9]

Methods

After the protocol was approved by the local Human Ethics Committee and the Central Committee on Research involving Human Subjects, participants were recruited and informed consent was obtained according to the Declaration of Helsinki. The study was registered (www.trialregister.nl) under number NTR1328.

Participants

Twelve healthy male volunteers aged 18-37 were enrolled in the study. Participants were excluded from participation in the presence of one or more of the following criteria: body mass index > 30 kg/m^2; presence or history of major heart, lung, liver, kidney, neurological or psychiatric disease; history of chronic alcohol or illicit drug use; medication use, allergy to study medication; use of contact lenses during the study (to prevent damage by rifampicin) and color-blindness. All participants were subjected to a medical history and physical examination before participation. Participants had to refrain from food and drinks 8 hours prior to the start the study day. Alcohol, coffee and chocolate were not allowed for 24 hours and grapefruit or grapefruit juice was not allowed for 6 days prior to the study day.

Study design

This study had a randomized single-blind, placebo-controlled, crossover design. Participants were studied on three occasions, with at least three weeks between sessions (Figure 2). In the five days before study occasion 1, six subjects took rifampicin 600 mg tablets (Sandoz BV, Almere, The Netherlands) (1 tablet/day taken just before going to sleep), six others took placebo tablets (cellulose tablets

produced by the local pharmacy). On the study day all 12 subjects received a 2-h treatment with normal saline (NaCl 0.9%) (treatment R/PP). In the five days before study occasion 2, all 12 subjects took rifampicin 600 mg tablets (1 tablet/day, taken before going to sleep). On the study day all subjects received a 2-h treatment with S-ketamine (Ketanest S, Pfizer BV, Capelle aan de IJssel, The Netherlands) (treatment RK). Finally, in the five days before study occasion 3, all 12 subjects took placebo tablets (1 tablet/day, taken before going to sleep). On the study day all subjects received a 2-h treatment with S-ketamine (treatment PK). The S-ketamine intravenous infusion dose was 0.29 mg/kg/h (= 20 mg/h for a volunteer of 70 kg). The order of the three occasions was random. Randomization was performed upon inclusion of the subject by the local pharmacy that provided the blinded study material (rifampicin/placebo tablets and S-ketamine/saline infusion).

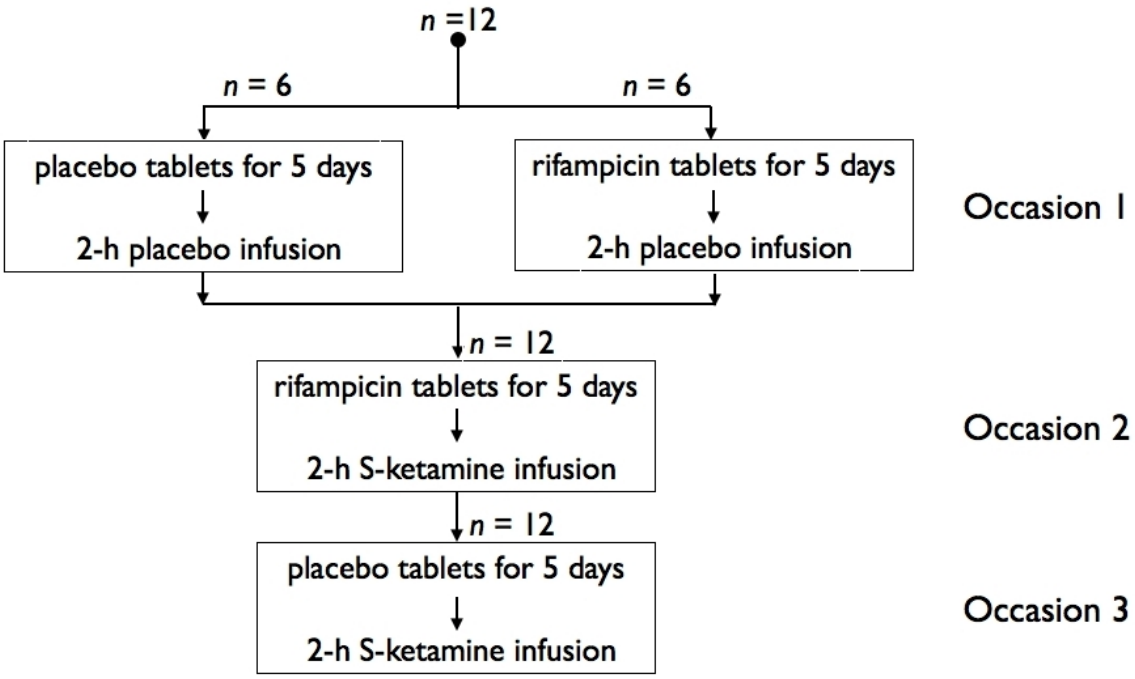

Figure 2 Flow chart of the study. The occasion sequence was random.

Prior to the first study occasion all subjects participated in two training sessions to get accustomed to the cognitive function tests. On the study day, baseline parameters were obtained (pain tests, cognitive function tests, and side effect score: drug high) before treatment. Next, during the 2-h treatment and 3 h following infusion, all tests and scores were performed at regular intervals, except for drug high which was determined at the end of the infusion.

Heat pain

The response to a noxious heat pain stimulus (scores for pain intensity ('strength' of the pain stimulus) and pain appreciation ('unpleasantness' of the pain stimulus)), was obtained. Heat pain was induced with the TSA-II NeuroSensory Analyzer (Medoc Ltd, Ramat Yishai, Israel). A 3 × 3 cm thermode was placed on the skin of the volar side of the forearm. The temperature was increased from 32 °C by 0.5 °C/s to 'peak temperature', after which the temperature was rapidly returned to 32 °C. After each stimulus the Visual Analogue Score (VAS) for pain intensity and pain appreciation was obtained using a 10 cm scale ranging from 0 (= no pain) to 10 (= most severe pain). 'Peak temperature' was determined for each subject individually during a test phase. 'Peak temperature' was varied from 46 to 52 °C at 1°C intervals. The lowest temperature that caused a VAS of 6 or greater was used in the study. Pain tests were performed at t = 0 (baseline), 5, 10, 15 min following the start of drug infusion and subsequently at 30-min intervals. In order to prevent sensitization of the skin, the thermode was repositioned after each stimulus.[10]

Cognition

Cognition was measured with a neurocognitive test battery (CNS Vital Signs LLC, Morrisville, NC, USA) and performed on a laptop computer.[11] The battery consisted of seven tests: 1 symbol digit coding; 2 Stroop test; 3 shifting attention test; 4 finger tapping; 5 continuous performance test; 6 verbal and visual memory test; 7 verbal and visual memory delay test. See for an explanation of the tests Appendix 1. All tests were in the Dutch language. The full battery (i.e., all 7 tests) was performed prior to drug infusion (baseline) and at t = 120 and 300 min following the start of infusion (the duration of the battery was approximately 30 min). At t = 30, 60, 90, 150, 180, 210, 240 and 270 min a short battery was performed that included symbol digit coding, Stroop test and shifting attention test. The full battery generates scores on 5 separate domains: memory, psychomotor speed, reaction time, complex attention and cognitive flexibility (see Appendix 1). The short battery generates scores on the domains: reaction time and cognitive flexibility. Data analysis was performed on the domain scores.

Domain scores are reported as standard scores (*z*-scores standardized to a mean of 100 and a standard deviation of 15).[11] The average of the *z*-scores for the five domains generates a summary score, the NeuroCognition Index (NCI), which is reported as a standard score as well. The NCI is similar to an IQ score, and is generated by averaging the *z*-scores of different subtests, (an NCI score of 100 is at the 50th percentile; 80% of the population scores between 80 and 120, 90% between 75 and 125). The NCI score gives an indication of the impact of treatment on the cognitive functions altogether.

Side effects

Drug high was scored at the end of the infusion on a 10-point numerical rating scale from 0 = no effect to 10 = maximal effect. Only integers were allowed as scores.

Statistical analysis

Descriptive analysis

Prior to the group comparisons the placebo-placebo and rifampicin-placebo data were compared. Since no significant differences were present, these two groups were combined (R/PP) in the remainder of the analysis. The area-under-the-curve divided by the 300 min duration of the study ($AUC_{0 \rightarrow 300}$) of pain intensity and appreciation were calculated. These AUCs of the three treatments were compared with an analysis of variance (and post-hoc Bonferroni's test) or Kruskal-Wallis test (and post-hoc Dunnett's test). The NCI and the five cognition domains were analyzed with a repeated measures analysis of variance (factors: time and treatment) with post-hoc Bonferroni's test. Drug high scores at the end of infusion were compared with an analysis of variance (and post-hoc Bonferroni's test). Data analysis was performed with SPSS 16.0. P-values < 0.05 were considered significant. Data are presented as mean ± standard error of the mean (SEM) unless otherwise stated.

Pharmacokinetic-pharmacodynamic analysis

Since blood sampling has stimulatory effects that may interfere with the measurement of pain, cognition, and side effects, we decided to perform this study without blood sampling. To be able to perform a PK-PD analysis, we assumed that S-ketamine and S-norketamine concentrations under these conditions are well described by earlier established pharmacokinetic models. The pharmacokinetic model that we used has three compartments for S-ketamine and two for S-norketamine linked by three metabolism compartments.[4,9]

To eliminate a possible hysteresis between plasma concentration and effect, an effect compartment was postulated that equilibrates with the plasma compartment with a half-life $t_{½}k_{e0}$ (i.e., the blood-effect-site equilibration half-life). A similar value of $t_{½}k_{e0}$ was assumed for S-ketamine and S-norketamine.

To estimate the contribution of S-norketamine on S-ketamine-induced changes in pain responses, cognition (reaction time and cognitive flexibility) and side effects (drug high) the following linear model was fitted to the data:

$$Y_E(t) = Y_0 + F_K \cdot C_{E,K}(t) + F_N \cdot C_{E,N}(t)$$

where $Y_E(t)$ = the effect at time t, Y_0 = predrug baseline effect, F_K the ketamine contribution to effect, $C_{E,K}(t)$ = the ketamine effect-site concentration at time t, F_N = the norketamine contribution to effect and $C_{E,N}(t)$ = the norketamine effect-site concentration at time t. F_N is parameterized as fraction of F_K, as follows: $F_N = F_{N^*} \cdot F_K$. For example, when F_K = 0.2 and $C_{E,K}$ = 100, the ketamine contribution to effect = 20%. When F_{N^*} = 1 the value of F_N = 1 × 0.2 = 0.2 indicating that norketamine contributes as much to the effect as ketamine (both cause a 20% change in effect).

The pharmacokinetic-pharmacodynamic data were analyzed with the statistical package NONMEM VII (ICON Development Solutions, Ellicott City, MD, USA).[12] Model parameters were assumed to be log-normally distributed. Residual error was assumed to be additive with variance σ^2. P-values less than 0.01 were considered significant.

Results

All subjects completed the protocol without major or unexpected side effects. The subjects' age, weight, height and body mass index averaged to 23 ± 5 years, 183 ± 6 cm, 75 ± 12 kg and 22 ± 3 kg/m^2, respectively (values are mean ± SD).

Descriptive analysis - comparison to placebo

The population averages are given in Figure 3. Based on the AUCs (Table 1), S-ketamine produced antinociception to a greater extent than placebo (R/PP). No differences in antinociception were observed between the PK and RK treatments. As determined from the measurement at the end of infusion, drug high was reduced in the subjects pretreated with rifampicin (RK) compared to those treated with placebo (PK; Table 1). S-ketamine produced cognitive impairment greater than placebo (R/PP) for all measures at t = 120 min (difference ranging between 17 and 24%, except for reaction time where the differences ranged from 5 to 12%) with no difference between treatment groups PK and RK. Most domains showed a decline over time, possibly caused by fatigue. An exception is psychomotor speed which showed an increase over time, which may be related to a learning effect. The results of the full battery are given in Table 2, the results of the short battery in Figure 3. These latter data were used in the PK-PD analysis.

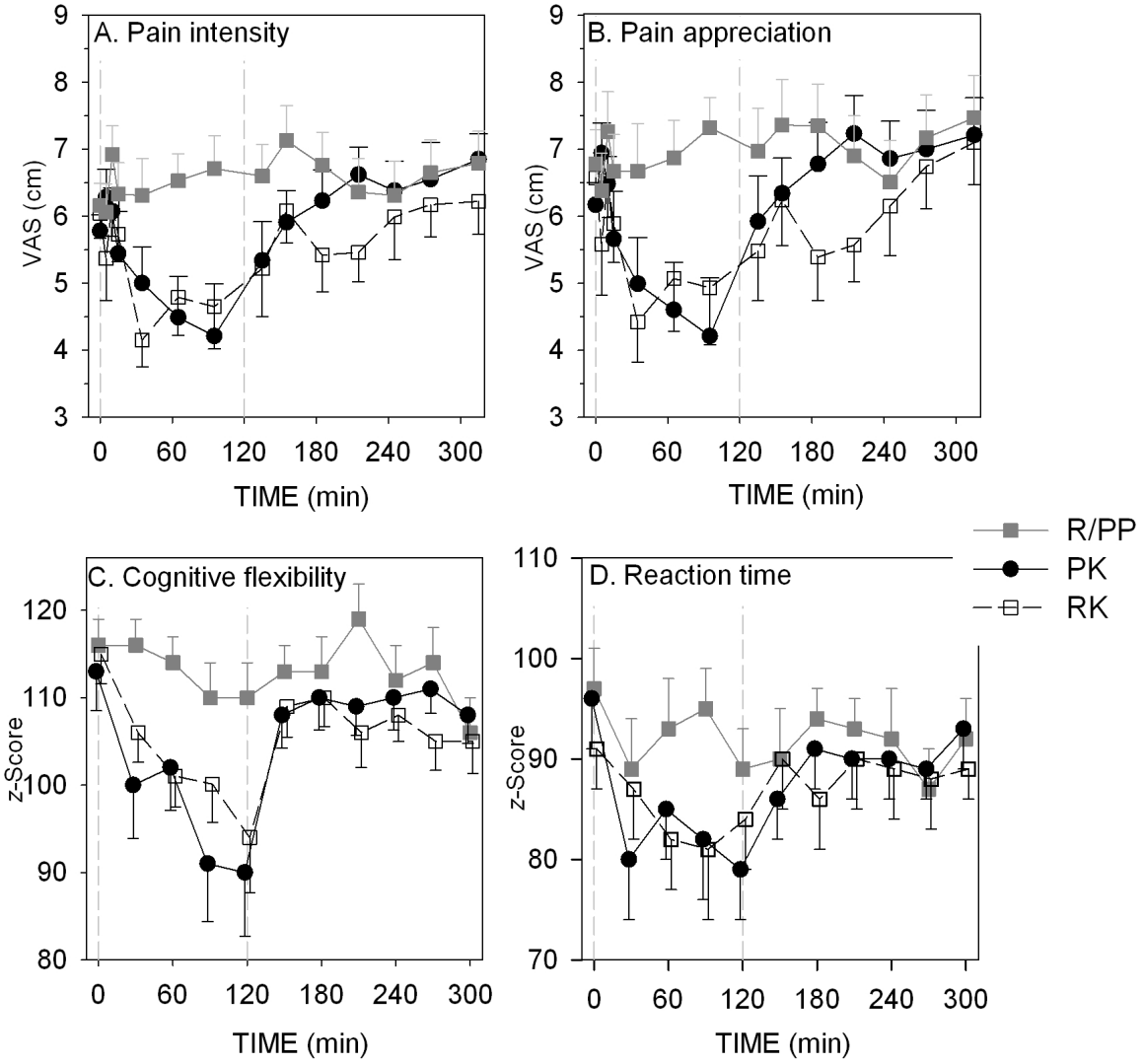

Figure 3 Average responses of the influence of rifampicin or placebo pretreatment on **A** pain intensity, **B** pain appreciation, **C** cognitive flexibility and **D** reaction time. The responses were measured during and 3 h following a 2-h S-ketamine infusion of 20 mg/h from t = 0 to t = 120 min. Grey squares are the placebo infusion data following a 5-day pretreatment with placebo or rifampicin (R/PP); black circles are the S-ketamine infusion data following a 5-day pretreatment with placebo; open squares are the S-ketamine infusion data following a 5-day pretreatment with rifampicin. Values are mean ± SEM.

Pharmacokinetic-pharmacodynamic analysis

An initial analysis was performed in which the S-norketamine contribution to S-ketamine effect was constrained to behave in a similar direction as S-ketamine (e.g., ketamine and norketamine are both analgesic and produce both drug high). This yielded no contribution of norketamine to effect in any of the tested end-points (i.e., $F_N = 0$). Since we observed that in some of the end-points the RK data following infusion remained below the PK data (e.g., pain intensity and pain

appreciation, Figure 3A and B), any constraint on F_N was removed, and F_N was allowed to have values causing an effect in the same as well opposite direction as S-ketamine.

Table 1 Descriptive analysis of the ketamine-induced pain relief and side effects (drug high).

	Rifampicin/ Placebo-Placebo	Rifampicin-Ketamine	Placebo-Ketamine
Pain intensity			
$AUC_{0 \rightarrow 300}$ (cm)	6.8 ± 0.4	5.7 ± 0.4 *	6.0 ± 0.4 *
Pain appreciation			
$AUC_{0 \rightarrow 300}$ (cm)	7.5 ± 0.6	6.0 ± 0.4 *	6.4 ± 0.5 *
Drug high			
Score at end of infusion	0 ± 0	5.2 ± 0.6 ‖ #	7.0 ± 0.4 ‖

Values are mean ± SEM.
* P < 0.05 versus Rifampicin/Placebo-Placebo; ‖ P < 0.001 versus Rifampicin/Placebo-Placebo;
# P = 0.01 versus Placebo-Ketamine.

Examples of best, median and worst data fits for effect of S-ketamine on pain intensity after placebo and rifampicin treatment are given in Figure 4. The population PD parameter estimates are given in Table 3. Goodness of fit plots for all end-points are given in Figure 5. Overall, the data were adequately described by the linear model. For pain intensity and pain appreciation the value of F_{N^*} indicates an effect of S-norketamine opposite to that of S-ketamine (i.e., an anti-analgesic effect of S-norketamine) (Table 3). For the cognitive end-points (cognitive flexibility and reaction time) no contribution of S-norketamine to effect could be estimated.

As an example we will further discuss pain intensity (Figure 6). For pain intensity the S-ketamine contribution F_K is -0.038 cm.(ng/ml)$^{-1}$. This indicates that at an effect-site S-ketamine concentration of 100 ng/ml, the effect due to just ketamine will be a 3.8 cm decrease in VAS. The S-norketamine contribution F_N is +0.03 (= $F_K \times F_{N^*}$ = -0.038) cm.(ng/ml)$^{-1}$ × -0.824, which indicates that at a S-norketamine concentration of 50 ng/ml (assuming that this is the S-norketamine effect-site concentration that coincides with an effect site S-ketamine concentration of 100 ng/ml in short-term infusion paradigms), the contribution of just S-norketamine is +1.5 cm VAS increase resulting in a total VAS change of -2.3 cm (= -3.8 + 1.5 cm).

Table 2 Descriptive analysis of the neurocognitive data.

	Rifampicin/ Placebo-Placebo	Rifampicin-Ketamine	Placebo-Ketamine
Neurocognitive Index [II]			
0 min	105.6 ± 1.9	104.6 ± 2.4	104.3 ± 3.0
120 min	99.7 ± 2.9	83.8 ± 3.3 [*]	77.6 ± 4.2 [*]
300 min	99.6 ± 2.6 [#]	98.1 ± 2.7[#]	101.0 ± 2.3 [#]
Memory [II]			
0 min	101.3 ± 5.2	106.6 ± 4.5	104.7 ± 5.0
120 min	88.9 ± 6.1	65.1 ± 4.7 [*]	55.5 ± 5.7 [*]
300 min	90.7 ± 5.3 [#]	93.8 ± 5.5 [#]	96.1 ± 4.1 [#]
Psychomotor speed [II]			
0 min	108.2 ± 5.2	107.8 ± 5.8	108.7 ± 4.8
120 min	112.6 ± 7.0	90.6 ± 3.9 [*]	86.8 ± 5.2 [*]
300 min	117.0 ± 5.8 [#]	114.8 ± 5.9 [#]	113.9 ± 5.2 [#]
Reaction time [II]			
0 min	97.4 ± 3.8	90.9 ± 4.4	95.9 ± 5.3
120 min	88.6 ± 3.6	83.8 ± 4.8 [*]	78.8 ± 4.8 [*]
300 min	91.5 ± 4.2	88.6 ± 2.6	93.1 ± 3.6
Complex attention [II]			
0 min	104.0 ± 4.4	102.9 ± 3.2	99.4 ± 4.3
120 min	97.2 ± 3.7	85.8 ± 5.4 [*]	77.3 ± 7.5 [*]
300 min	91.3 ± 2.9 [#]	88.2 ± 4.1 [#]	94.4 ± 3.5 [#]
Cognitive flexibility [III]			
0 min	116.3 ± 3.2	114.8 ± 3.3	112.6 ± 4.5
120 min	110.1 ± 4.0	94.2 ± 6.3 [§] [**]	89.5 ± 7.3 [§] [**]
300 min	106.4 ± 4.0 [**]	104.8 ± 3.7 [**]	107.6 ± 3.2 [**]

Values are mean ± SEM.
Significant main treatment, time and time[*] treatment effects at [II] $P < 0.001$ and [III] $P < 0.05$.
Post-hoc analysis: treatment: [*] $P < 0.01$ versus Rifampicin/Placebo-Placebo (at 120 min);
[§] $P < 0.05$ versus Rifampicin/Placebo-Placebo (at 120 min);
Post-hoc analysis: time: [*] $P < 0.01$ versus t = 0, [#] $P < 0.01$ versus t = 0, [**] $P < 0.05$ versus t = 0.

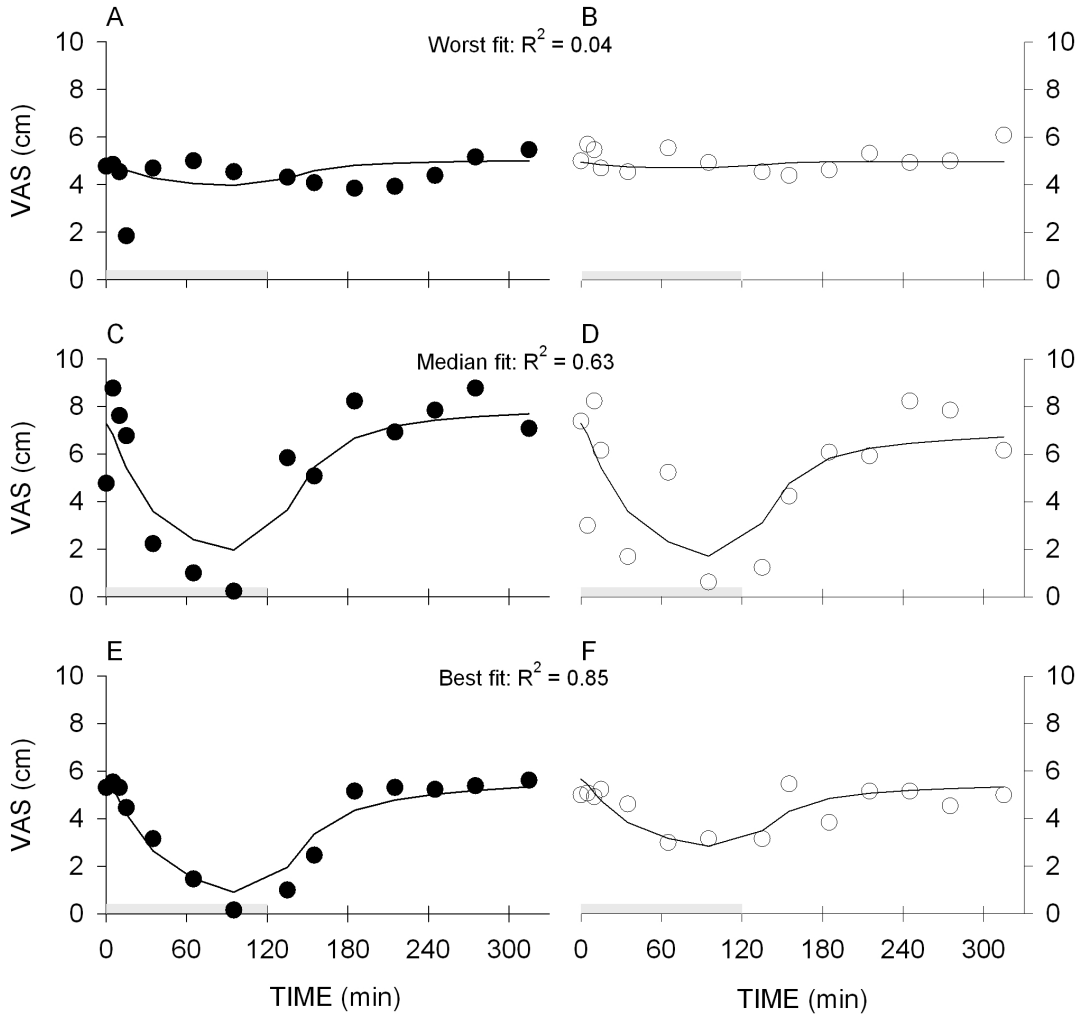

Figure 4 Examples of data fits from three subjects, showing worst (**A** and **B**), median (**C** and **D**) and best (**E** and **F**) data fits for the effect of S-ketamine on pain intensity following placebo (A, C and E) or rifampicin (B, D and F) pretreatment.

In Figure 6 the relative contributions of S-ketamine and S-norketamine to the changes in VAS score and their sum (the measured response) are simulated, using the model parameters of Table 3 for the two test conditions (placebo pretreatment, panels A and C; and rifampicin pretreatment, panels B and D). It shows the anti-analgesic effect of norketamine on the change in VAS (relative to S-ketamine's effect) with hyperalgesia following S-ketamine infusion when S-norketamine levels are high (panels A and C). When S-norketamine levels are relatively low (panels B and D) the negative effect on analgesia is less and no hyperalgesia is observed following the 2-h S-ketamine infusion.

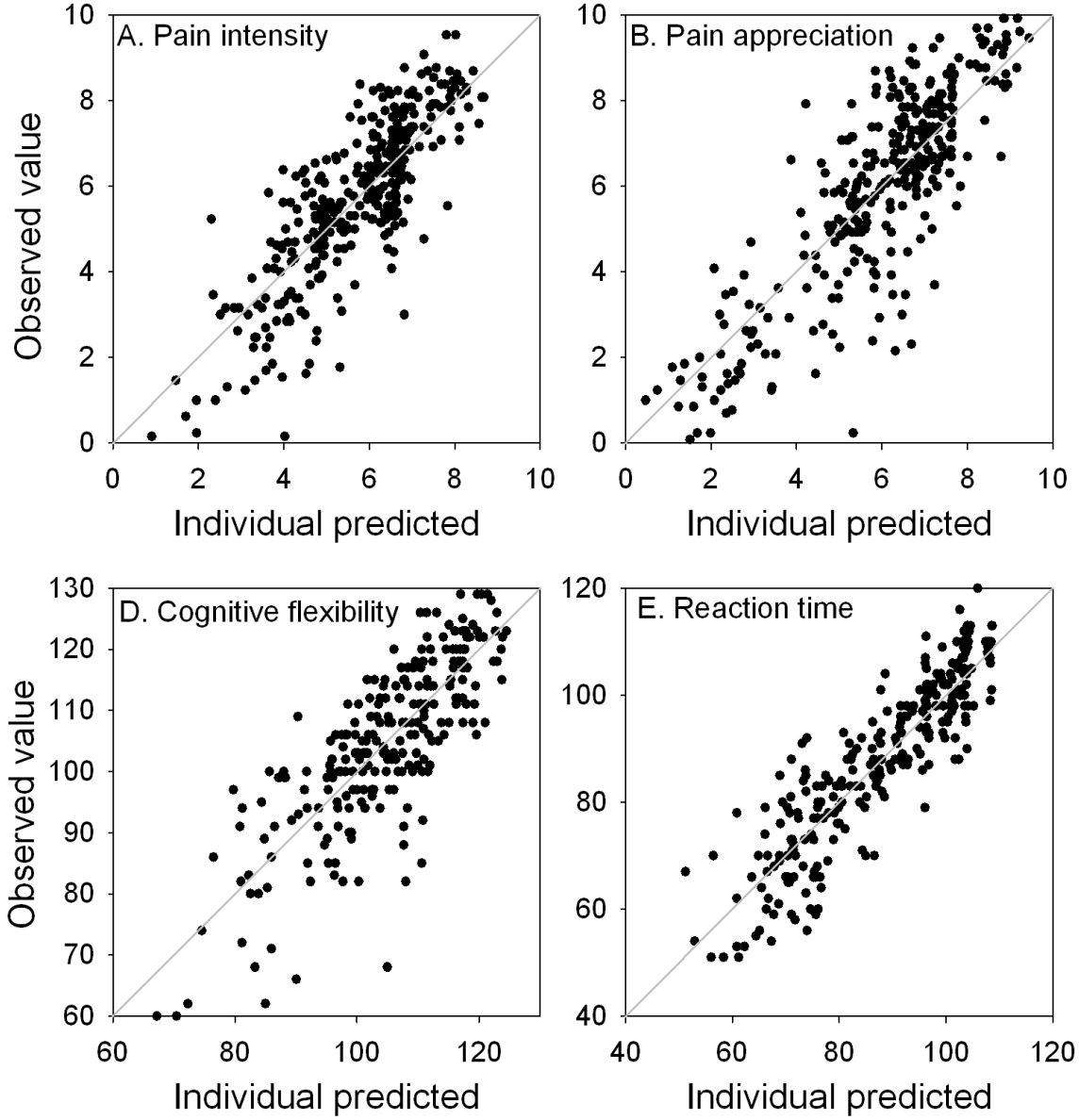

Figure 5 Goodness of fit plots for **A** pain intensity, **B** pain appreciation, **D** cognitive flexibility and **E** reaction time. Individual predicted values are plotted against the observed values. The grey lines are the lines of identity.

The blood-effect-site equilibration half-life ($t_{½}k_{e0}$) ranged from 0 min (cognitive flexibility) to 11.8 min (pain intensity). For cognitive flexibility no hysteresis between arterial plasma concentrations and effect was estimated, indicating that the effect instantaneously followed arterial plasma concentrations. The value of $t_{½}k_{e0}$ averaged across all end-points was 6.1 min.

Table 3 Pharmacodynamic model parameters.

	θ ± SEM	**ω^2 ± SEM**	**ν^2 ± SEM**
Pain intensity			
F_K (cm.(ng/ml)$^{-1}$)	$-3.80 \cdot 10^{-2} \pm 1.17 \cdot 10^{-2}$	$1.26 \cdot 10^{-2} \pm 4.94 \cdot 10^{-4}$	$2.0 \cdot 10^{-4} \pm 7.9 \cdot 10^{-5}$
F_{N^*}	-0.824 ± 0.266	$5.12 \cdot 10^{-4} \pm 4.02 \cdot 10^{-4}$	-
Y_0 (cm)	6.11 ± 0.38	1.46 ± 0.56	-
$t_{½}k_{e0}$ (min)	11.8 ± 0.2	-	-
ε	1.28 ± 0.26		
Pain appreciation			
F_K (cm.(ng/ml)$^{-1}$)	$-4.35 \cdot 10^{-2} \pm 1.20 \cdot 10^{-2}$	$1.30 \cdot 10^{-2} \pm 4.89 \cdot 10^{-4}$	$3.76 \cdot 10^{-4} \pm 1.48 \cdot 10^{-4}$
F_{N^*}	-0.785 ± 0.208	$4.95 \cdot 10^{-4} \pm 3.06 \cdot 10^{-4}$	-
Y_0 (cm)	6.55 ± 0.42	1.95 ± 1.20	-
$t_{½}k_{e0}$ (min)	10.0 ± 2.1	-	-
ε	1.71 ± 0.40		
Cognitive flexibility			
F_K (cm.(ng/ml)$^{-1}$)	$-0.245 \pm 5.67 \cdot 10^{-2}$	$3.12 \cdot 10^{-2} \pm 1.48 \cdot 10^{-2}$	$5.72 \cdot 10^{-3} \pm 2.66 \cdot 10^{-3}$
F_{N^*}	0.00 ± 0.00	-	-
Y_0 (cm)	113.0 ± 2.3	$4.17 \cdot 10^{-3} \pm 1.27 \cdot 10^{-3}$	$5.35 \cdot 10^{-4} \pm 3.24 \cdot 10^{-4}$
$t_{½}k_{e0}$ (min)	0.0[#]	-	-
ε	0.976 ± 0.175		
Reaction time			
F_K (cm.(ng/ml)$^{-1}$)	$-0.166 \pm 3.42 \cdot 10^{-2}$	$8.66 \cdot 10^{-3} \pm 5.81 \cdot 10^{-3}$	-
F_{N^*}	0.00 ± 0.00	$4.06 \cdot 10^{-2} \pm 3.68 \cdot 10^{-2}$	-
Y_0 (cm)	92.0 ± 3.8	$2.01 \cdot 10^{-4} \pm 8.86 \cdot 10^{-3}$	$1.21 \pm 9.10 \cdot 10^{-4}$
$t_{½}k_{e0}$ (min)	2.4 ± 2.2	-	-
ε	62.4 ± 9.5		

ε = a residual error term; **F_K** = the parameter that describes the contribution of ketamine to total effect; **F_{N^*}** = the fraction of F_K that describes the contribution of norketamine to total effect; **θ** = the typical parameter value; **ν^2** = interoccasion variability (in the log-domain); **$t_{½}k_{e0}$** = the blood-effects-site equilibration half-life; **Y_0** = baseline value; **ω^2** = the between-subject variability (in the log-domain).
[#] no hysteresis between blood-concentration and effect observed.

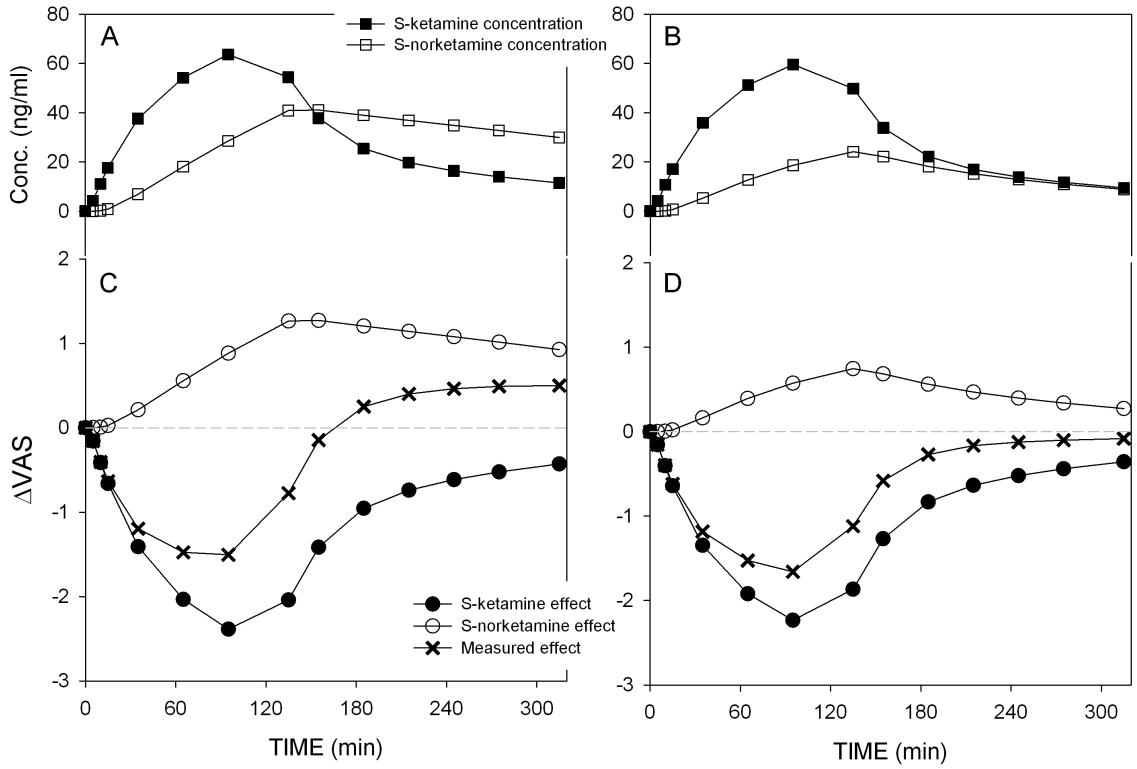

Figure 6 Simulation showing the relative contribution of S-ketamine and S-norketamine to measured effect. Simulated **A** PK and **C** PD data assuming placebo pretreatment. Simulated **B** PK and **D** PD data assuming rifampicin pretreatment.

Discussion

Many drugs used in clinical anesthesia and pain medicine are metabolized into active compounds. Often it is unknown how parent and metabolite contribute to the observed effects. One way to determine their relative contributions is to administer the metabolite and assess its potency. Next, PK-PD modeling is required to obtain a precise estimate of the relative contributions as steady-state conditions are seldom reached after infusion of the parent drug.

An illustrious example of a drug and its active compound is morphine, that is metabolized into the active morphine-6-glucuronide (and the inactive morphine-3-glucuronide). While early (descriptive) human and animal studies suggested a relative large contribution of morphine-6-glucuronide to the effects of morphine, later PK-PD studies performed in humans that combined data on the separate infusions of morphine and morphine-6-glucuronide, showed just a minor contribution of morphine-6-glucuronide to effect (at least in people with normal renal function).[13,14]

Another example of the unknown contribution of metabolites to effect is ketamine. Ketamine is metabolized by *N*-demethylation into norketamine via cytochrome P450 enzymes in the liver, and norketamine is further metabolized into hydroxynorketamine. Ketamine and norketamine are centrally acting NMDAR antagonists, hydroxynorketamine is without pharmacological activity.[4-9] Although ketamine is in use for half a century, the relative contribution of parent and active metabolite to effect remains unknown in humans. Animal studies indicate that norketamine has about 20 to 60% the potency of ketamine and is thought to contribute up to 30% of ketamine analgesia, and, to a lesser extent, to the development of psychotomimetic side effects.[5-8,15] Since norketamine is not available for human use, we assessed the contribution of S-norketamine to S-ketamine effect by measuring S-ketamine's pharmacodynamics under two specific pharmacokinetic conditions: 1 a condition in which the metabolism of S-ketamine and S-norketamine was not influenced, and 2 a condition in which the metabolism of both compounds was induced by rifampicin. These two conditions lead to variations in plasma concentration of S-ketamine (rifampicin causes a reduction in S-ketamine's C_P AUC by about 10%, Figure 1) and S-norketamine (S-norketamine C_P AUC reduced by 50%) and allow determination of their relative contributions to effect.[9]

Ketamine is a drug that causes a myriad of side effects.[16] Consequently the use of ketamine is not always without discomfort to the patient. Side effects include nausea/vomiting, cardiovascular effects and effects due to interaction of ketamine with NMDARs within the central nervous system. These latter side effects include psychotomimetic (psychedelic) effects and cognitive impairment, while animal but not human studies associate ketamine with neurotoxicity. Knowledge on the contribution of norketamine to ketamine analgesia and any of these side effects is of importance as it may lead to further drug development or adaptation of dosing regimens aimed at optimizing analgesia while minimizing side effects. Our current study was aimed at quantifying S-norketamine contribution to S-ketamine's analgesic and cognitive effects. In an initial descriptive analysis we observed that S-ketamine infusion produced analgesia, impairment of cognition and psychotomimetic effects (drug high) to a greater extent than placebo infusion (Tables 1 and 2). These findings are in close agreement with earlier studies.[17,18] As expected, the PK-PD analysis of the S-ketamine infusion data, using a linear additive model of the S-ketamine and S-norketamine contribution to effect, enabled estimation of the S-norketamine contribution. For pain intensity and pain appreciation a negative rather than a positive contribution to effect was observed (negative meaning an effect opposing the direction of the S-ketamine effect). The magnitude of these opposing effects is not easily quantified as they depend on the pertaining S-ketamine and S-norketamine concentrations. To visualize their relative contributions to measured (simulated) effect, we plotted the magnitude of

S-ketamine and S-norketamine effect versus time in Figure 6 for two conditions: placebo (Figure 6 A and C) and rifampicin (Figure 6 B and D) pretreatment. This simulation further shows that following S-ketamine infusion, when S-norketamine concentrations exceed S-ketamine concentrations, the VAS response is hyperalgesic (Figure 6 C). This observation is realistic and in close agreement with earlier studies on the effect of ketamine on pain responses in healthy volunteers and chronic pain patients.[4,19-21]

There are various indications in the literature that ketamine, under specific circumstances, is associated with pain facilitation.[4,19-23] In healthy volunteers ketamine has a dose-dependent antinociceptive effect on static nociceptive pain (repetitive noxious heat pain stimuli), while pain responses following infusion were perceived as more painful (by about 1 cm VAS) for more than 3 h compared to pretreatment pain responses.[21] In agreement with these findings, Mitchell described a cancer patient that developed severe hyperalgesia and allodynia directly following treatment with ketamine.[19] Recently we showed that endogenous modulation of pain, as assessed by the Diffuse Noxious Inhibitory Control (DNIC) paradigm, displayed pain facilitation following a 1-h infusion with S-ketamine (dose 40 mg/70 kg).[20] These findings, together with our current observations, indicate that norketamine may be anti-analgesic and produce pain facilitatory effects, especially when ketamine concentrations are low and norketamine concentrations are elevated, as occurs following a short-term infusion.

It has been argued that the hyperalgesic effects from NMDAR antagonists are related to activation of non-NMDA excitatory receptors (metabotropic or non-NMDA ionotropic glutamate receptors) activated by excitatory amino acids released from spinal or supraspinal sites, or are related to a rebound increase in NMDAR activity following the rapid decrease in ketamine concentration.[4,20-23] Our data indicate that norketamine may be an additional contributor to the hyperalgesic or anti-analgesic effects of ketamine. One possible mechanism of the excitatory behavior of norketamine on pain responses may be activation of excitatory receptors (other than the excitatory glutamate receptors), such as the σ-, κ- and muscarinic receptors.[24] For example, known agonists of the σ-receptor include the NMDAR-antagonists phencyclidine and ketamine, and σ_1-receptor activation has been associated with pronociceptive and psychotomimetic responses.[25] Assuming higher affinity and intrinsic activity of norketamine for the σ-receptor compared to ketamine, this then suggests that when norketamine concentrations are relatively low (as occurs in the rifampicin treatment group) 1 relatively more analgesia will be present (see above and Figure 6), but also that 2 psychotomimetic side effects will be of lesser intensity compared to a condition in which the norketamine concentrations are relatively higher. Indeed, in our experiments we did observe a significantly lower score for drug high at the end of

the infusion period during the RK treatment (Table 1). How much this may be attributed to the lower S-ketamine concentration or S-norketamine concentrations remains presently unknown (as no PK-PD analysis was performed on the drug high data). Our data are consistent in that they suggest that norketamine acts at a non-NMDAR that is associated with excitatory responses, including hyperalgesia, and that it enhances psychotomimetic side effects, possibly via the σ-receptor. However, no human data are available on the activity of norketamine at the σ-receptor or any of the other receptors mentioned above, and further studies are warranted to better understand our observations. The absence of effect of variations in norketamine concentration on cognitive function, suggests absence of involvement of norketamine in these ketamine-related effects. However, the changes in cognition were large and variable (Figure 3). We therefore may have missed subtle changes in cognition related to norketamine.

The PK-PD model that we applied did not make a distinction between S-ketamine and S-norketamine onset/offset times ($t_{½}k_{e0}$). The blood-effect-site equilibration half-lifes of the two compounds were assumed to be similar, as reliable estimates of ketamine's $t_{½}k_{e0}$ and that of its metabolite are not available from animal studies and separate estimations were not possible from the data we collected. The estimated values of $t_{½}k_{e0}$ ranged from 0 (absence hysteresis between plasma concentration and effect) to 11.8 min (overall mean = 6.1 min; Table 3). There are just two earlier studies that report estimates of ketamine's $t_{½}k_{e0}$. Schüttler et al. showed no hysteresis between S-ketamine plasma concentration and median frequency changes of the electroencephalogram from an anesthetic induction dose of S-ketamine in five healthy volunteers.[26] Similarly, Herd et al. estimated a value of $t_{½}k_{e0}$ of 11 s in a pediatric population during induction and recovery from general anesthesia (end-point arousal and recall memory) using racemic ketamine.[27] While these data are difficult to compare to ours (we used a much lower S-ketamine dose and measured different end-points), these data together with ours clearly point towards a rapid onset/offset of S-ketamine's effect following a short-term infusion paradigm (i.e., ketamine's pharmacodynamics is driven by its pharmacokinetics). In contrast, long-term ketamine infusion (100 h or longer) has a much more prolonged effect. In chronic pain patients we recently estimated a half-life for onset/offset of pain relief of 11 days (95% confidence interval 5-21 days).[28] These long-term effects are independent of the passage of ketamine to a postulated receptor site in the central nervous system and most probably reflect a modulatory effect of ketamine with central sensitized chronic pain pathways.

In the current study we did assess the pharmacodynamics of S-ketamine without obtaining S-ketamine and S-norketamine pharmacokinetic data. Instead, we relied on previously obtained pharmacokinetics in a similar group of volunteers that

received a similar pretreatment with rifampicin.[9] The use of simulated PK data in PK-PD modeling studies has been applied with success before when we modeled the effect of opioids on the control of breathing and recently on naloxone reversal of opioid-induced respiratory depression.[29,30] The main reason for not obtaining ketamine PK data is that frequent blood sampling from an arterial line may cause arousal and stress, which may interfere with obtaining reliable data such as pain responses and cognition. A second issue is that the ethics committee of our institution has a restrictive policy regarding the use of arterial lines when reliable PK data is available from earlier studies.[31] As indicated before, we agree that the lack of PK data is a potential drawback of our study; we do believe, however, that taken the quality of our PK data set, that our approach is valid and allows reliable assessment of the relevant PD model parameters.

The observation from our PK-PD study that S-norketamine has anti-analgesic effects opposite to its parent and co-NMDAR antagonist S-ketamine, is an intriguing finding. While it may explain some of the observations made in human studies on the development of pain facilitation following ketamine infusion[4,19-21], we believe that one has to be careful with the interpretation of these data derived from "complex" PK-PD modeling using simulated PK data. Further proof is required before we can conclude that norketamine has a negative contribution to ketamine-induced analgesia and side effects. A careful conclusion at present is that norketamine contribution to ketamine analgesia is limited and that we cannot exclude a small anti-analgesic effect from norketamine.

Appendix 1: Cognition tests

The CNS Vital Signs cognition tests have been described in full elsewhere.[11] In short:

> *Symbol digit coding*: the test consists of serial presentations of screens, each of which contains a bank of 8 symbols above and 8 empty boxes below. At the top of the screen a bank of 8 symbols is depicted with the corresponding numbers below. The subject types the number into the empty box that corresponds to the symbol that is highlighted. Each time the test is administered, the program randomly chooses eight new symbols to match to the eight digits. Scoring is the number of correct responses generated in 2 minutes.
>
> *Stroop test*: the test has three parts. **A** The words RED, YELLOW, BLUE and GREEN appear at random on the screen in black. The subject has to press a button as the word appears. **B** The words RED, YELLOW, BLUE and GREEN appear on the screen in color. The subject has to press a button when the color of the word matches the meaning of the word. **C** The words RED, YELLOW, BLUE and GREEN appear on the screen in color. The subject is asked to press a button when

the color and the meaning of the word do not match. Each test generates a separate reaction time score (part A generates a simple reaction time, parts B and C complex reaction times), which combined give an indication of information processing speed. The value of the Stroop reaction time is on average 120 ms longer than the complex reaction time generated in part B of the test (range 78-188 ms). Part C also generates an error score. The test requires about 4 minutes.

Shifting attention test (SAT): in the shifting attention test subjects are instructed to match geometric objects either by shape or color. The test measures the ability to shift from one instruction to another quickly and accurately. Three figures appear on the screen, one on top and two on the bottom. The top figure is either a square or a circle. The bottom figures are a square and a circle. These figures are either red or blue; the colors are mixed randomly. The subject is asked to match one of the bottom figures to the top figure, either by color or by shape. The rules of the matching change at random. This goes on for 90 seconds. The goal is to make as many correct matches as possible. The scores generated by SAT are: correct matches, errors and response time in ms.

Finger tapping: the test generates relevant data about fine motor control, which is based on motor speed, as well as kinesthetic and visual-motor ability. The subjects press the space bar with the index finger as many times as they can in 10 s; this test is performed 3 times with the right index finger and 3 times with the left index finger. The score is the average number of taps.

Continuous performance: this test is a measure of vigilance or sustained attention over time. The subject is asked to respond to a target stimulus, e.g. the letter B, but not to any other letter, by pressing the space bar. In 5 min, the test presents 200 letters; 40 of the letters are the target B, 160 are non-targets (any other letter). The stimuli are presented at random, although the target stimulus only appears 8 times during each minute of the test. The scores generated are: correct matches, commission errors (pressing when no B is shown, e.g., impulsive responding) and omission errors (not pressing when a B appears, e.g., inattention).

Immediate and delayed verbal memory: This is an adaptation of the Rey Auditory Verbal Learning Test. Fifteen words are presented, one by one, on the screen. A new word is presented every two seconds. The subject is asked to remember these words. Then a list of thirty words is presented. The fifteen target words are mixed randomly among 30 words of which 15 new words. When the subject recognizes a word from the original list, he or she presses the space bar. This is a recognition test, however, not a test of recall. After finishing the other tests, a delayed recognition test is performed. The 15 targets remain the same for the delayed memory testing; the 15 distractors are different between the immediate and delayed challenges.

Immediate and delayed visual memory: this test is the same as the verbal memory test, but instead of words geometric figures are used.

These tests generate scores on 5 separate domains: memory, psychomotor speed, reaction time, complex attention and cognitive flexibility.

- The Memory domain is calculated from the correct scores of the verbal and visual (immediate and delayed) memory tests.
- Psychomotor speed is derived from number of taps in the finger tapping test and number of correct answers in the symbol digit coding tests.
- The domain score for Reaction time is made up by combining two reaction time scores (B and C) of the Stroop test.
- The domain score for Complex attention is generated by adding the number of errors in the continuous performance test, the shifting attention test and the Stroop test.
- The domain score for Cognitive flexibility is generated by taking the number of the correct responses on the shifting attention test and subtracting the number of errors on the shifting attention and Stroop tests.

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