

Estimation of the Contribution of Norketamine to Ketamine-induced Acute Pain Relief and Neurocognitive Impairment in Healthy Volunteers

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ABSTRACT

Background: The *N*-methyl-D-aspartate receptor antagonist ketamine is metabolized in the liver into its active metabolite norketamine. No human data are available on the relative contribution of norketamine to ketamine-induced analgesia and side effects. One approach to assess the ketamine and norketamine contributions is by measuring the ketamine effect at varying ketamine and norketamine plasma concentrations using the CYP450 inducer rifampicin.

Methods: In 12 healthy male volunteers the effect of rifampicin *versus* placebo pretreatment on S-ketamine-induced analgesia and cognition was quantified; the S-ketamine dosage was 20 mg/h for 2 h. The relative ketamine and norketamine contribution to effect was estimated using a linear additive population pharmacokinetic-pharmacodynamic model.

Results: S-ketamine produced significant analgesia, psychotropic effects (drug high), and cognitive impairment (including memory impairment and reduced psychomotor speed, reaction time, and cognitive flexibility). Modeling revealed a negative contribution of S-norketamine to S-ketamine-induced analgesia and absence of contribution to cognitive impairment. At ketamine and norketamine effect concentrations of 100 ng/ml and 50 ng/ml, respectively, the ketamine

What We Already Know about This Topic

- Ketamine is metabolized to norketamine, and because both of these compounds block *N*-methyl-D-aspartate receptors and produce analgesia in animals, both are speculated to contribute to analgesia from ketamine administration in humans

What This Article Tells Us That Is New

- In a study of 12 healthy volunteers who received an inducer of ketamine metabolism or placebo on separate occasions to alter the ketamine/norketamine ratio, modeling of responses to S-ketamine administration suggested a mild antagonism of analgesia from ketamine by norketamine, rather than a supplement
- These data, if confirmed in more direct ways, suggest that pain facilitation, which sometimes is observed after ketamine administration ends, may reflect action of the norketamine metabolite

contribution to analgesia is -3.8 cm (visual analog pain score) *versus* a contribution of norketamine of $+1.5$ cm, causing an overall effect of -2.3 cm. The blood-effect site equilibration half-life ranged from 0 (cognitive flexibility) to 11.8 (pain intensity) min and was 6.1 min averaged across all endpoints.

Conclusions: This first observation that norketamine produces effects in the opposite direction of ketamine requires additional proof. It can explain the observation of ketamine-related excitatory phenomena (such as hyperalgesia and allodynia) upon the termination of ketamine infusions.

MANY drugs used in clinical anesthesia and pain medicine are metabolized into active compounds. Often it is unknown how the 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, pharmacokinetic-pharmacodynamic (PK/PD) modeling is required to obtain a precise estimate of the relative contributions because steady-state conditions are seldom reached after infusion of the parent drug. One such example of a drug and its active metabolite is morphine and morphine-6-glucuronide (M6G). Although early (descriptive) human and animal studies suggest a relative large contribution of M6G to morphine's effects, later studies performed in humans that combined data on the

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separate infusions of morphine and M6G, showed just a minor contribution of M6G to effect.^{1,2}

Another drug with an active metabolite is ketamine. Ketamine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, is used as an anesthetic and at low dose (to 30 mg/h) as an analgesic.^{3–5} Upon administration, ketamine is metabolized rapidly into norketamine *via* cytochrome P450 enzymes in the liver, and norketamine is further metabolized into hydroxynorketamine. Ketamine and norketamine are centrally acting *N*-methyl-D-aspartate receptor antagonists; hydroxynorketamine is without pharmacologic activity.^{6–11} Animal data indicate that norketamine has approximately 20–60% the potency of ketamine and is thought to contribute as much as 30% to the ketamine-induced analgesia and, to a lesser extent, the development of psychotropic side effects.^{7–10,12} No human data are available on norketamine's contribution to ketamine effect because norketamine is not available for human use. We showed previously that pretreating humans with rifampicin (an antibiotic that induces multiple hepatic P450s, including CYP 2B6 and 3A4, involved in the ketamine *N*-demethylation into norketamine) caused a 10% reduction in ketamine and a 50% reduction of S-norketamine concentrations.¹¹ To get an indication of the contribution of S-norketamine to the S-ketamine effect in that study, simulation studies were performed, and we predicted a 20% contribution of norketamine to ketamine effect.¹¹

In the current placebo-controlled randomized trial, we assessed the contribution of S-norketamine to S-ketamine effect by measuring S-ketamine's analgesia and cognitive impairment 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 conditions lead to variations in plasma concentration of S-ketamine and S-norketamine and allow determination of their relative contributions to effect. This design and the application of an additive ketamine-norketamine 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: to assess the effect of low-dose ketamine on pain responses and cognition during and after a 2-h infusion and to get an estimate of the contribution of norketamine to the ketamine effect. We hypothesized that in agreement with our previous simulation study, norketamine contributes as much as 20% to the ketamine-induced effect. To assess the contribution of norketamine, we performed a population PK/PD analysis using the pharmacokinetic data from our previous study.¹¹

Materials and Methods

After the protocol was approved by the local Human Ethics Committee (Commissie Medische Ethiek, Leiden, The

Netherlands) and the Central Committee on Research Involving Human Subjects (Centrale Commissie Mensgebonden Onderzoek, The Hague, The Netherlands) participants were recruited and informed consent was obtained according to the Declaration of Helsinki. The study was registered under the number NTR1328.**

Participants

Twelve healthy male volunteers aged 18–37 yr were enrolled in the study. Participants were excluded from participation in the presence of one or more of the following criteria: body mass index more than 30 kg/m²; presence or history of major heart, lung, liver, kidney, neurologic, or psychiatric disease; history of chronic alcohol or illicit drug use; medication use or allergy to study medication; use of contact lenses during the study (to prevent damage by rifampicin); and colorblindness. All participants provided a medical history and underwent physical examination before participation. Participants had to refrain from food and drinks 8 h before the start of the study day. Alcohol, coffee, and chocolate were not allowed for 24 h, and grapefruit or grapefruit juice was not allowed for 6 days before 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 3 weeks between sessions (fig. 1). In the 5 days before study occasion 1, six subjects took 600 mg rifampicin (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%) (study rifampicin/placebo-placebo). In the 5 days before study occasion 2, all 12 subjects took 600 mg rifampicin (1 tablet/day, taken before going to sleep). On the study day, all subjects received a 2-h treatment with S(+)-ketamine (S-ketamine, Pfizer BV, Capelle aan de IJssel, The Netherlands) (study rifampicin-ketamine). Finally, in the 5 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 (study placebo-ketamine). The S-ketamine intravenous infusion dose was 0.29 mg · kg⁻¹ · h⁻¹ (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 or placebo tablets and S-ketamine or saline infusion).

Before 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 (cognitive function tests, pain tests) before treatment. Next, during the 2-h treatment and 3 h after infusion, all tests and scores were performed at regular intervals.

** www.trialregister.nl. Accessed March 30, 2012.

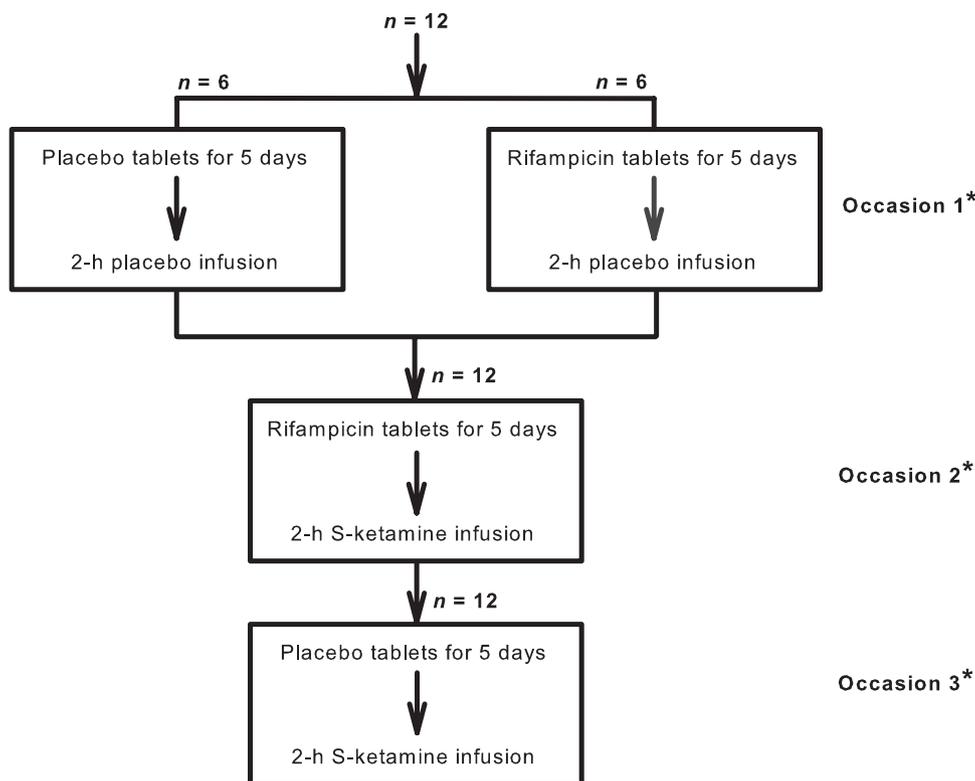


Fig. 1. Flow chart of the study. * The occasion sequence was random.

Heat Pain. Heat pain was induced with the TSA-II Neuro-Sensory Analyzer (Medoc, Ramat Yishai, Israel). A 3-cm × 3-cm thermode was placed on the skin of the volar side of the forearm. The temperature was increased (in increments of 0.5°C/second) from 32°C to the “peak temperature,” after which the temperature was rapidly returned to 32°C. After each stimulus, the visual analog 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). The peak temperature was determined for each subject individually during a test phase and was varied from 46° to 52°C at intervals of 1°C. The lowest temperature that caused a VAS of 6 or greater was used in the study. Pain tests were performed at 0 (baseline), 5, 10, and 15 min after the start of drug infusion and subsequently at 30-min intervals. To prevent sensitization of the skin, the thermode was repositioned after each stimulus.¹³

Side Effects: Drug High. Drug high was scored at the end of the S-ketamine infusion on a 10-point numerical rating scale from 0 (no effect) to 10 (maximal effect). Only integers were allowed as scores.

Cognition. Cognition was measured with a neurocognitive test battery (CNS Vital Signs, Morrisville, NC) and performed on a laptop computer.¹⁴ 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 the appendix for additional explanation of the tests. All seven tests (*i.e.*, the full battery)

were performed before drug infusion (baseline) and at 120 and 300 min after the start of infusion (the duration of the battery was 30 min). At 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. All tests were in the Dutch language. The full battery generates scores on five separate domains: memory, psychomotor speed, reaction time, complex attention, and cognitive flexibility (see appendix). The short battery generates scores on the domains of 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, SD 15).¹⁴ The average of the z-scores for the five domains generates a summary score, the NeuroCognition Index (NCI), which also is reported as a standard score. The NCI is similar to an IQ score, which 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.

Power Analysis and Statistical Analysis

Power Analysis. Taking into account our previous estimations,^{6,11} we assumed a difference in effects between rifampicin and placebo runs of 20%. With further assumptions of an SD of 20%, $\alpha = 0.05$, and β greater than 0.80, at least 11 subjects are needed per treatment (SigmaPlot version 12 for Windows; Systat Software, Inc., San Jose, CA). In the cur-

rent study, we chose (somewhat arbitrarily) to test 12 subjects (3 subjects were added to this number and served as reserve subjects in the event some subjects did not complete all three visits; consequently 15 subjects are mentioned in the trial register.

Descriptive Analysis. Before the group comparisons, the placebo-placebo and rifampicin-placebo data were compared. Because no significant differences were present, these two groups were combined in the remainder of the analysis. The area-under-the-curve divided by the 300 min duration of the study (AUC/300) of pain intensity and appreciation were calculated. These areas-under-the-curve of the three treatments were compared with an analysis of variance (and *post hoc* Bonferroni test) or Kruskal-Wallis test (and *post hoc* Dunnett's test). Drug high scores at the end of infusion were compared with an analysis of variance (and *post hoc* Bonferroni test). The NCI and the five cognition domains were analyzed with a repeated measures analysis of variance (factors: time and medication) with *post hoc* Bonferroni test. Data analysis was performed with SPSS 16.0. *P* values < 0.05 were considered significant. Data are presented as mean ± SEM unless otherwise stated.

PK/PD Analysis. Because blood sampling has stimulatory effects that may interfere with the measurement of pain, side effects, and cognition, we decided to perform this study without the drawing of blood. Under these conditions, to be able to perform a PK/PD analysis, we assumed that S-ketamine and S-norketamine concentrations are well described by previously 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.^{6,11}

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 $t_{1/2k_{e0}}$ (*i.e.*, the blood-effect site equilibration half-life). A similar value of $t_{1/2k_{e0}}$ was assumed for S-ketamine and S-norketamine.

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

$$Y_E(t) = Y_0 + F_K \cdot C_{E,K}(t) + F_N \cdot C_{E,N}(t) \quad (1)$$

where $Y_E(t)$ = the effect at time t , Y_0 = predrug baseline effect, F_K the ketamine contribution to effect, $C_{E,K}$ = the ketamine effect-site concentration, F_N the norketamine contribution to effect, and $C_{E,N}$ = the norketamine effect-site concentration. F_N is defined 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 is $1 \times 0.2 = 0.2$, indicating that norketamine contributes as much to the effect as ketamine (both cause a 20% change in effect).

The sensitivity of the pharmacodynamic parameters on the pharmacokinetic parameters was assessed as follows. First, 95% CIs of the pharmacokinetic parameters were constructed based on the interindividual and interoccasion variability available from a previous study.⁹ Next, the pharmacodynamic analyses of the pain intensity data were rerun in turn for all pharmacokinetic parameters at both endpoints of those intervals.

The PK/PD data were analyzed with the statistical package NONMEM VII (ICON Development Solutions, Ellicott City, MD).¹⁵ Model parameters were assumed to be log-normally distributed. Residual error was assumed to be additive with variance σ^2 . Model selection was based on the chi-square test with *P* values < 0.01 considered significant (to select highly significant model components).

Results

All subjects completed the protocol without unexpected side effects. The subjects' age, weight, height, and body mass index averaged to 23 ± 5 yr, 184 ± 6 cm, 75 ± 12 kg, and 22 ± 3 kg/m², respectively (values are mean ± SD).

Descriptive Analysis Comparison to Placebo

The population averages are given in figure 2. Based on the areas-under-the-curve (table 1), S-ketamine produced antinociception to a greater extent than did placebo (rifampicin/placebo-placebo). No difference in area-under-the-curve was observed for antinociception between placebo-ketamine and rifampicin-ketamine. As determined from the measurement at the end of infusion, drug high was reduced in the subjects pretreated with rifampicin (rifampicin-ketamine) compared with those treated with placebo (placebo-ketamine; table 1). S-ketamine produces cognitive impairment greater than placebo (rifampicin/placebo-placebo) for all measures at 120 min (difference ranging between 17 and 24%, except for reaction time, for which the differences ranged from 5 to 12%) with no difference between treatment groups placebo-ketamine and rifampicin-ketamine. Most indices showed a decline over time, possibly because of 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 and the results of the short battery in figure 2. These latter data were used in the PK/PD analysis.

PK/PD Analysis

An initial analysis was performed in which the S-norketamine contribution to S-ketamine effect was constrained to behave in a direction similar to that of S-ketamine (*e.g.*, ketamine and norketamine are both analgesic or produce both drug high). This yielded no contribution of norketamine to effect in any of the tested endpoints (*i.e.*, $F_N = 0$). Because we observed that in some of the endpoints the rifampicin-ketamine data after infusion remained below the pharmaco-

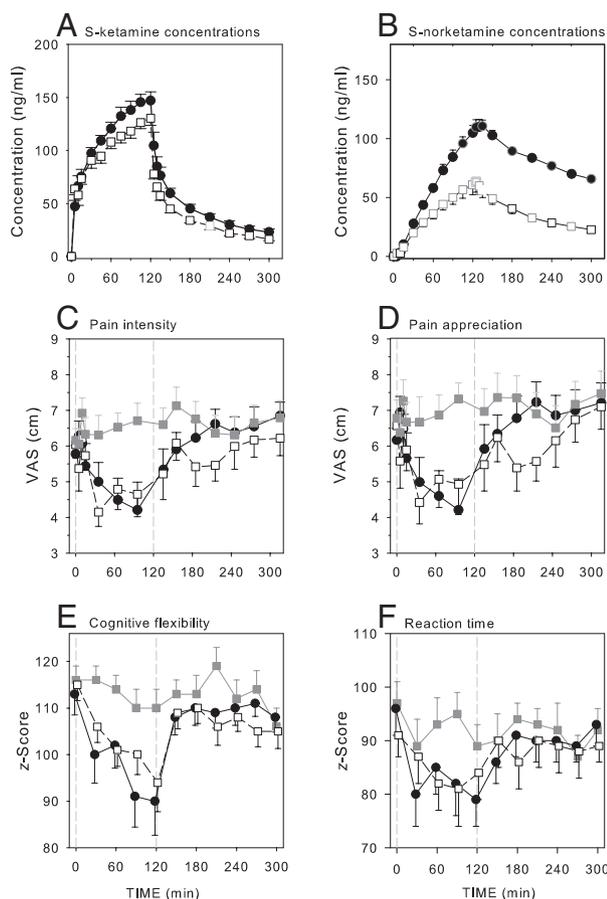


Fig. 2. Effect of placebo (closed symbols) and rifampicin (open symbols) administered before treatment (600 mg by mouth per day for 5 days) on S-ketamine (A) and S-norketamine (B) concentrations during and after a 2-h S-ketamine infusion (t = 0–120 min; dose = 20 mg/h). Values are mean ± SEM. Data are from Noppers *et al.*¹¹ Average responses of the influence of rifampicin or placebo pretreatment on pain intensity (C), pain appreciation (D), cognitive flexibility (E), and reaction time (F). The responses were measured during and 3 h after a 2-h S-ketamine infusion of 20 mg/h from t = 0 to t = 120 min. Gray squares are the placebo infusion data after a 5-day pretreatment with placebo or rifampicin (rifampicin-placebo or placebo-placebo). Black circles are the ketamine infusion data after a 5-day pretreatment with placebo. Open squares are the ketamine infusion data after a 5-day pretreatment with rifampicin. Values are mean ± SEM. VAS = visual analog score.

dynamic data (*e.g.*, pain intensity and pain appreciation, fig. 2, C and D), 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. Examples of best, median, and worst data fits for two endpoints are given in figure 3 for pain intensity. The population pharmacodynamic parameter estimates are given in table 3. Goodness of fit plots for all endpoints are given in figure 4. 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 (table 3). For

the cognitive endpoints (cognitive flexibility and reaction time) no contribution of S-norketamine to effect could be estimated.

As an example, we will discuss pain intensity in greater detail. For pain intensity, the S-ketamine contribution F_K is $-0.038 \text{ cm} \cdot (\text{ng/ml})^{-1}$. 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 $+0.03$ ($F_{N^*} \times F_K = -0.824 \times -0.038$) $\text{cm} \cdot (\text{ng/ml})^{-1}$, which indicates that at an 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 a 1.5-cm VAS increase, resulting in a total VAS change of -2.3 cm ($-3.8 + 1.5 \text{ cm}$). In figure 5, 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 negative effect of norketamine on the change in VAS (relative to S-ketamine's effect) with hyperalgesia after S-ketamine infusion when S-norketamine concentrations are high (panels A and C). When S-norketamine concentrations are relatively low (panels B and D), the negative effect on analgesia is less, and no hyperalgesia is observed after the 2-h S-ketamine infusion.

The blood-effect site equilibration half-life ($t_{1/2k_{e0}}$) ranged from 0 (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 $t_{1/2k_{e0}}$ averaged across all endpoints was 6.1 min.

Sensitivity of Pharmacodynamic Data in Response to Variations in Pharmacokinetics

The reanalysis of the pharmacodynamic data using variations in pharmacokinetic parameters (by setting the parameters at both endpoints of their 95% CIs) showed that parameter F_{N^*} was most sensitive to changes in ketamine clearance and volume of norketamine's peripheral compartment. Variations in F_{N^*} ranged from -1.2 to -0.6 (compare to value of -0.8 observed in the analysis, see table 3), with less than a 4-point change in objective function.

Discussion

Ketamine causes many side effects,¹⁶ including nausea and vomiting, hypertension, psychotropic (psychedelic) effects, and cognitive impairment. Knowledge on the contribution of norketamine to ketamine analgesia and any of these side effects is of importance because it may lead to additional drug development or adaptation of dosing regimens aimed at op-

Table 1. Descriptive Analysis of the Ketamine-induced Pain Relief and Side Effects (Drug High)

	Rifampicin/Placebo-Placebo	Placebo-Ketamine	Rifampicin-Ketamine
Pain intensity AUC/300 (cm)	6.8 ± 0.4	6.0 ± 0.4*	5.7 ± 0.4*
Pain appreciation AUC/300 (cm)	7.5 ± 0.6	6.4 ± 0.5*	6.0 ± 0.4*
Drug high Score at end of infusion	0 ± 0	7.0 ± 0.4†	5.2 ± 0.6†‡

Values are mean ± SEM.

* $P < 0.05$ vs. rifampicin/placebo-placebo. † $P < 0.05$ vs. rifampicin/placebo-placebo. ‡ $P < 0.05$ vs. placebo-ketamine.

AUC = area-under-the-curve.

timizing analgesia and minimizing side effects. Our current study was aimed at quantifying S-norketamine contribution to S-ketamine analgesia and S-ketamine cognitive effects. The descriptive analysis indicates that S-ketamine produced greater analgesia, psychotropic effects (drug high), and impairment of cognition than did placebo (tables 1 and 2), which is in agreement with the findings of previous studies on racemic ketamine.^{17,18} As expected, the PK/PD analysis of the S-ketamine data, using a linear additive model of the

S-ketamine and S-norketamine contribution, 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 because they depend on the ratio of S-ketamine and S-norketamine concentrations. To visualize their relative contributions to measured (simulated) effect, we performed

Table 2. Descriptive Analysis of the Neurocognitive Data

	Rifampicin/Placebo-Placebo	Placebo-Ketamine	Rifampicin-Ketamine
Neurocognitive index*			
0 min	105.6 ± 1.9	104.3 ± 4.1	104.6 ± 2.4
120 min	104.3 ± 4.1	77.6 ± 4.1†‡	83.8 ± 11.3†‡
300 min	104.6 ± 2.4‡	101.0 ± 2.4‡	98.1 ± 2.7‡
Memory*			
0 min	101.3 ± 5.2	104.7 ± 5.0	106.6 ± 4.5
120 min	88.9 ± 6.1	55.5 ± 5.7†	65.1 ± 4.7†
300 min	90.7 ± 5.3‡	96.1 ± 4.1‡	93.8 ± 5.5‡
Psychomotor speed*			
0 min	108.2 ± 5.2	108.7 ± 4.8	107.8 ± 5.8
120 min	112.6 ± 7.0	86.8 ± 5.2†‡	90.6 ± 3.9†‡
300 min	117.0 ± 5.8‡	113.9 ± 5.2‡	114.8 ± 5.9‡
Reaction time*			
0 min	97.4 ± 3.8	95.9 ± 5.3	90.9 ± 4.4
120 min	88.6 ± 3.6	78.8 ± 4.8†‡	83.8 ± 4.8†‡
300 min	91.5 ± 4.2	93.1 ± 3.6	88.6 ± 2.6
Complex attention*			
0 min	104.0 ± 4.4	99.4 ± 4.3	102.9 ± 3.3
120 min	97.2 ± 3.7	77.3 ± 7.5†‡	85.8 ± 5.4†‡
300 min	91.3 ± 2.9‡	94.4 ± 7.5‡	88.2 ± 4.1‡
Cognitive flexibility*			
0 min	116.3 ± 3.2	112.6 ± 4.5	114.8 ± 3.3
120 min	110.1 ± 4.0	89.5 ± 7.3†‡	94.2 ± 6.3†‡
300 min	106.4 ± 4.0‡	107.6 ± 10.9‡	104.8 ± 3.7‡

Values are mean ± SEM.

* Significant main treatment, time and time × treatment effects at $P < 0.05$. For *post-hoc* analysis: Treatment: † $P < 0.01$ vs. rifampicin/placebo-placebo (at 120 min). Time: ‡ $P < 0.05$ vs. t = 0.

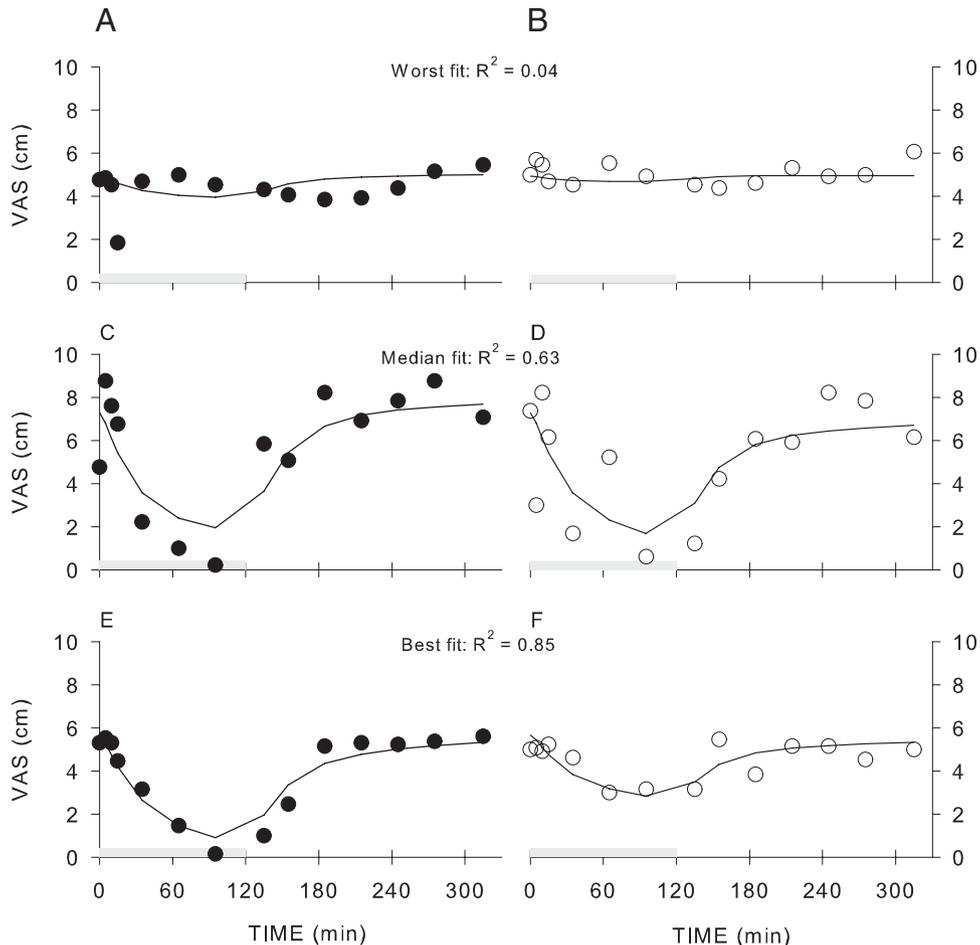


Fig. 3. Examples of data fits from three subjects showing worst (A and B), median (C and D), and best (E and F) data fits for the effect of S-ketamine on pain intensity after placebo (A, C, and E) or rifampicin (B, D, and F) pretreatment. VAS = visual analog score.

PK/PD simulations and plotted the magnitude of S-ketamine and S-norketamine effect *versus* time in figure 5 for two conditions: placebo (fig. 5, A and C) and rifampicin (fig. 5, B and D) pretreatment. This simulation shows that after S-ketamine infusion, when S-norketamine concentrations exceed S-ketamine concentrations, the VAS response is hyperalgesic (fig. 5C). This observation is realistic and in close agreement with the findings of previous studies on the effect of ketamine on pain responses in healthy volunteers and patients with chronic pain.^{6,19–21} When S-norketamine concentrations are relatively low, as occurs after rifampicin pretreatment, the VAS-response is reduced and no hyperalgesia is observed (fig. 5, B and D).

There are various observations that ketamine under specific circumstances is associated with pain facilitation.^{6,19–23} In volunteers, ketamine has a dose-dependent antinociceptive effect on experimental nociceptive pain, but pain responses after infusion were perceived as more painful compared with pretreatment responses.²¹ In agreement with these findings, Mitchell described a patient with cancer who experienced severe hyperalgesia and allodynia directly after treatment with ketamine.¹⁹ Recently, we showed that endog-

enous modulation of pain (using the conditioning pain modulation paradigm) displayed pain facilitation after a 1-h infusion with S-ketamine.²⁰ These findings, together with our current observations, indicate that ketamine may be antianalgesic and produces pain facilitatory effects, especially when ketamine concentrations are low and norketamine concentrations are increased, as occurs after a short-term infusion.

It has been argued that the hyperalgesic effects from NMDA receptor antagonists are related to activation of 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 NMDA receptor activity after the rapid decrease in ketamine concentration.^{6,19–23} Our data indicate that norketamine may be an additional contributor to the hyperalgesic or antianalgesic 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 NMDA receptor antagonists phencyclidine and ketamine,

Table 3. Pharmacodynamic Model Parameters

	θ (95% ci)	ω^2 (95% ci)	ν^2 (95% ci)
Pain Intensity			
F_K (cm·(ng/ml) ⁻¹)	-3.80×10^{-2} (-6.10×10^{-2} to -2.63×10^{-2})	1.26×10^{-2} (1.16×10^{-2} to 1.35×10^{-2})	2.0×10^{-4} (4.6×10^{-4} to 35.4×10^{-4})
F_{N^*}	-0.824 (-1.34 to -0.30)	$5.12 \cdot 10^{-4}$ (4.32×10^{-4} to 5.92×10^{-4})	
Y_0 (cm)	6.11 (5.36 to 6.80)	1.46 (0.36 to 1.66)	
$t_{1/2k_{e0}}$ (min)	11.8 (11.4 to 21.2)		
σ_ε^2 (cm) ²	1.28 (0.78 to 1.78)		
Pain appreciation			
F_K (cm·(ng/ml) ⁻¹)	-4.35×10^{-2} (-2.01×10^{-2} to -6.69×10^{-2})	1.30×10^{-2} (1.20×10^{-2} to 1.40×10^{-2})	3.76×10^{-4} (0.88×10^{-4} to 6.64×10^{-4})
F_{N^*}	-0.785 (-1.19 to -0.38)	4.95×10^{-4} (0.1×10^{-4} to 8.9×10^{-4})	
Y_0 (cm)	6.55 (5.75 to 7.35)	1.95 (0.1 to 4.29)	
$t_{1/2k_{e0}}$ (min)	10.0 (6.1 to 13.9)		
σ_ε^2 (cm) ²	1.71 (1.00 to 2.42)		
Cognitive flexibility			
F_K (ng/ml) ⁻¹	-0.245 (-0.34 to -0.14)	3.12×10^{-2} (0.1×10^{-2} to 6.0×10^{-2})	5.72×10^{-3} (0.2×10^{-3} to 10.9×10^{-3})
F_{N^*}	0.00 ± 0.00 (-)	-	-
Y_0	113.0 (108.5 to 117.5)	4.17×10^{-3} (1.7×10^{-3} to 6.7×10^{-3})	5.35×10^{-4} (0.1×10^{-4} to 13.7×10^{-4})
$t_{1/2k_{e0}}$ (min)	0.0* (-)		
σ_ε^2 (-)	0.976 (0.62 to 1.33)		
Reaction time			
F_K (ng/ml) ⁻¹	-0.166 (-0.22 to -0.10)	8.66×10^{-3} (0.1×10^{-3} to 19.9×10^{-3})	
F_{N^*}	0.00 ± 0.00 (-)	4.06×10^{-2} (0.1×10^{-2} to 11.3×10^{-2})	
Y_0	92.0 (84.6 to 99.4)	2.01×10^{-4} (0.1×10^{-4} to 19.0×10^{-4})	1.21 (1.18 to 1.24)
$t_{1/2k_{e0}}$ (min)	2.4 (0.1 to 6.4)		
σ_ε^2 (-)	62.4 (44.4 to 80.4)		

* No hysteresis between blood concentration and effect observed.

ci = confidence interval; 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; ω^2 = the between-subject variability (in the log-domain); ε = a residual error term; $t_{1/2k_{e0}}$ = the blood-effects site equilibration half-life; θ = the typical parameter value; ν^2 = interoccasion variability (in the log-domain); Y_0 = baseline value.

and σ_1 -receptor activation has been associated with pronociceptive and psychotomimetic responses.²⁵ Assuming the intrinsic activity of norketamine and its higher affinity for the σ -receptor compared with that of ketamine can explain that when norketamine concentrations are relatively low (as occurs in the rifampicin treatment group), relatively more analgesia will be present (fig. 5) compared with a condition in

which the norketamine concentrations are relatively higher. Our data are consistent in that they suggest that norketamine acts at a receptor system associated with excitatory responses, including hyperalgesia, and psychotomimetic side effects, possibly the σ -receptor. However, no human data are available on the activity of norketamine at the σ -receptor or any excitatory receptor system, and additional studies are war-

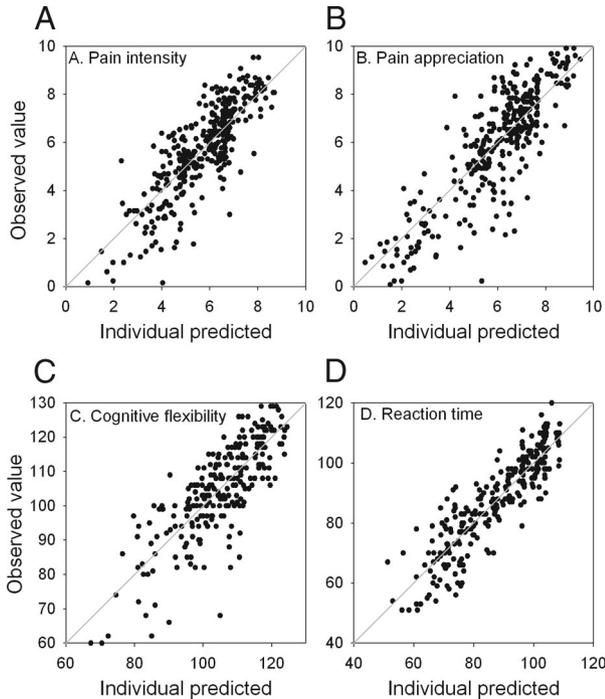


Fig. 4. Goodness of fit plots for pain intensity (A), pain appreciation (B), cognitive flexibility (C), and reaction time (D). Individual predicted values are plotted against the observed values. The gray lines are the lines of identity.

ranted 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 (fig. 2). Thus,

we 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 or offset times ($t_{1/2k_{e0}}$). The blood-effect site equilibration half-lives of the two compounds were assumed to be similar because reliable estimates of ketamine's $t_{1/2k_{e0}}$ and that of its metabolite are not available, and separate estimations were not possible from the data. The estimated values of $t_{1/2k_{e0}}$ ranged from 0 (absence of hysteresis between plasma concentration and effect) to 11.8 min (overall mean, 6.1 min; table 3). Only two previous studies report estimates of ketamine's $t_{1/2k_{e0}}$. Schüttler *et al.*²⁶ showed no hysteresis between S-ketamine plasma concentration and changes in the electroencephalogram. Similarly, Herd *et al.*²⁷ estimated a $t_{1/2k_{e0}}$ value of 11 s in a pediatric population during induction and recovery from general anesthesia (endpoint arousal and recall memory) using racemic ketamine. These data together with ours point toward a rapid onset and offset of S-ketamine's effect after a short-term infusion paradigm.

In the current study, we assessed 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 who received a similar pretreatment with rifampicin.¹¹ The use of simulated pharmacokinetic 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.^{28,29} The main reason for not obtaining ketamine pharmacokinetic data are that frequent blood sampling from an arterial-line

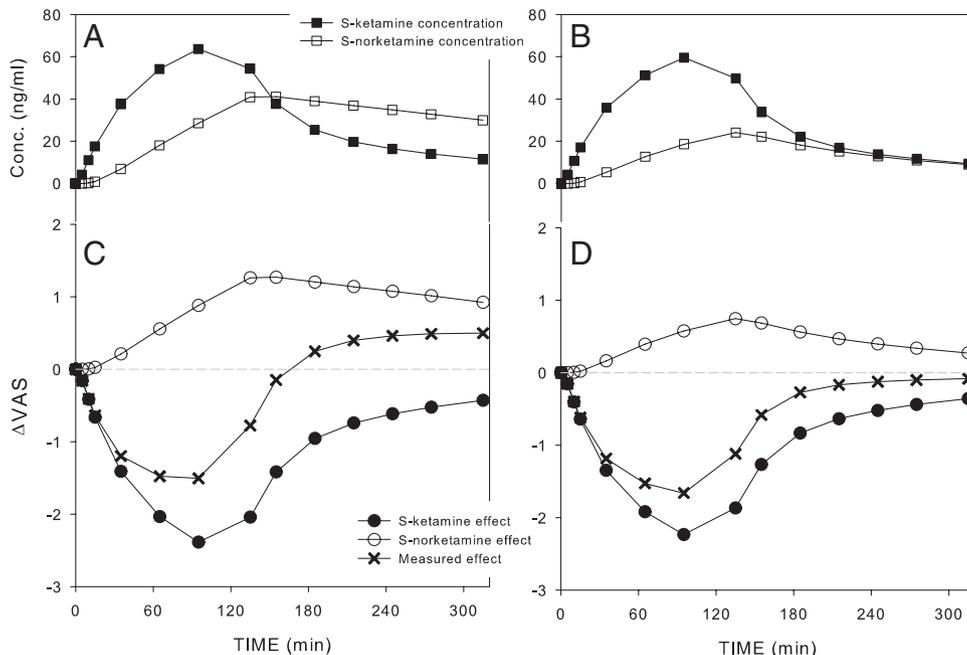


Fig. 5. Pharmacokinetic-pharmacodynamic (PK/PD) simulation showing the relative contribution of S-ketamine and S-norketamine to measured effect. Simulated pharmacokinetic (A) and pharmacodynamic (C) data assuming placebo pretreatment. Simulated pharmacokinetic (B) and pharmacodynamic data (D) assuming rifampicin pretreatment. VAS = visual analog score.

can 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 pharmacokinetic data are available from previous studies.³⁰ We performed a *post hoc* reanalysis of the data to assess the sensitivity of the pharmacodynamics on variations in plasma concentrations of S-ketamine and S-norketamine. The results indicate that it is very unlikely that the finding of a negative contribution of norketamine to effect is caused by differences in model predicted and absence of measured ketamine and norketamine concentrations. Although we agree that the lack of pharmacokinetic data is a potential drawback of our study, we believe that given the quality of our pharmacokinetic data set, our approach is valid and allows reliable assessment of the relevant pharmacodynamic model parameters.

Our results are surprising in light of previous animal studies showing that norketamine has significant antinociceptive properties.^{7–10} Our findings are similar to the observations with morphine and its active metabolite M6G.^{1,2} Although rodent data showed that M6G produces potent analgesia at already low plasma concentrations, in humans M6G-induced analgesia occurs only at high plasma concentrations. It is thought that the low M6G potency in humans is related to its very slow passage across the blood-brain barrier.^{1,2} Apart from the evident species differences, the discrepant norketamine data in humans and animals remain unexplained. Possibly different NMDA receptor subtypes in humans compared with rodents may be held responsible for the observed absence of norketamine effect.

The observation from our PK/PD study that S-norketamine has antianalgesic effects opposite to its parent and co-NMDA receptor antagonist is an intriguing finding. Although it may explain some of the observations made in human studies on the development of pain facilitation after ketamine infusion,^{6,19–21} we think one has to be careful with the interpretation of these data derived from “complex” PK/PD modeling using simulated pharmacokinetic data. Additional 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 the norketamine contribution to ketamine analgesia is limited and that we cannot exclude a small antianalgesic effect from norketamine.

Appendix: Cognition Tests

The CNS Vital Signs cognition tests have been described in full elsewhere.¹⁴ A brief description is provided here.

Symbol Digit Coding

The test consists of serial presentations of screens, each of which contains a bank of eight symbols above and eight empty boxes

below. The subject types in the number 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 min.

Stroop Test

The test has three parts: (A) The words RED, YELLOW, BLUE, and GREEN (printed in black) appear at random on the screen. The subject has to press a button as the word appears. (B) The words RED, YELLOW, BLUE, and GREEN appear on the screen printed 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 printed in color. The subject is asked to press a button when the color and word meaning do not match. Each test generates a separate reaction time score (test A generates a simple reaction time, tests 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 approximately 4 min.

Shifting Attention

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 s. The goal is to make as many correct matches as possible. The scores generated by the shifting attention test are correct matches, errors, and response time in milliseconds.

Finger Tapping

The test generates relevant data about fine motor control, which is based on motor speed and kinesthetic and visual-motor ability. The subjects press the space bar with their index fingers as many times as they can in 10 s; this test is performed three times with the right index finger and three 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 stimulus by pressing the space bar. In 5 min, the test presents 200 letters; 40 of the letters are the target B, 160 are nontargets (any other letter). The stimuli are presented at random, although the target stimulus appears only eight 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 2 s. The subject is asked to remember these words. Then a list of 30 words is presented. The 15 target words are mixed randomly among 30 words, of which 15 are new words. When the subject recognizes a word from the original list, he or she presses the space bar. This is a recognition test, 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 except instead of words, geometric figures are used.

Domains

These tests generate scores on five 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 the number of taps in the finger tapping test and the number of correct answers in the symbol digit coding test.

The domain score for reaction time is made by combining the 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 complex 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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