CHAPTER 4

EARLY STAGE DEVELOPMENT OF THE GLYCINE-1 REUPTAKE INHIBITOR SCH 900435: CENTRAL NERVOUS SYSTEM EFFECTS COMPARED TO PLACEBO IN HEALTHY MEN

M Liem-Moolenaar[1], P Peeters, MSD[2], IMC Kamerling[1], C Hogg[3], G Holder[3], HJ Kleijn, MSD[2], E Spaans, MSD[2], J Udo De Haes, MSD[2], ML de Kam[1], KL Franson[1], AF Cohen[1], JMA van Gerven[1]

1 Centre for Human Drug Research, Leiden, The Netherlands
2 Early Stage Development, Oss, The Netherlands
3 Moorfields Eye Hospital, London, UK & Institute of Ophthalmology, London, UK

SUBMITTED

Abstract

AIM To report the first three studies with SCH 900435, a highly selective glycine-1 reuptake inhibitor in development for treating schizophrenia, using systematic evaluations of pharmacodynamics to understand the observed (adverse) effects.

METHODS Three double-blind, placebo-controlled studies (single dose, visual effect analysis and multiple dose) were performed. In the single and multiple dose study SCH 900435 (0.5-30mg) was given to healthy males and frequent pharmacokinetic and pharmacodynamic measurements were performed. The visual effects study, which was performed after the single and before the multiple dose study, focussed on the visual system and incorporated visual electrophysiological measures of macular, retinal and intracranial visual pathway function.

RESULTS In the single dose study increases in smooth pursuit eye movements (8, 12, 30mg), pupil/iris ratio (20 and 30mg), VAS colour perception (30mg) and changes in spontaneous reports of short-lasting visual disturbance were found, while FSH (8, 12, 20mg), LH (8-30mg) and EEG alpha$_2$ activity decreased (12, 20, 30mg). A subsequent dedicated visual effects study demonstrated that visual effects were transient without underlying electrophysiological changes. This provided enough safety information for starting a multiple ascending dose study, which showed rapid development of tolerance for visual effects.

CONCLUSIONS Several CNS effects and gonadotropic changes resulted from administration of 8mg and higher, providing evidence for CNS penetration and pharmacological activity of SCH 900435. Transient visual symptoms were reported to which rapid tolerance occurred. It remains to be established whether these effects are specific for this pharmacological class and whether this class of drugs offers any antipsychotic activity in patients.

Introduction

Traditional models of schizophrenia emphasize the importance of dopaminergic (DA) dysregulation, particularly with regard to the positive symptoms [1,2]. An alternative model is based on the effects of non-competitive antagonists of N-methyl-D-aspartate (NMDA) glutamate receptors, which induce psychotic symptoms in healthy subjects and exacerbate symptoms in schizophrenic patients. These effects seem to resemble schizophrenia more closely than those induced by dopamine activation [3-6]. Moreover, dysfunction of glutamatergic neuronal systems would be consistent with the dopamine hypothesis of schizophrenia [7-11]. Due to major concerns over potentially serious adverse events of indiscriminate glutamatergic stimulation, which could affect key functions such as learning, memory and neuronal excitation and cell death, research has focused on alternative strategies to augment NMDA receptor function [12]. One alternative pathway is through the glycine receptor site, an obligatory co-agonist at the NMDA receptor [3]. Direct glycine agonists appear to show some effect in treatment-resistant schizophrenia [3], but this requires gram-level doses. Inhibition of presynaptic reuptake of GlyT$_1$ transporters can also increase local endogenous glycine levels [13,14]. As reviewed by Javitt [3], glycine reuptake inhibitors (e.g. D-cycloserine, glycine and D-serine) have been effective in a variety of rodent schizophrenia models [15-19] and in schizophrenic patients [20-22], but these agents are neither potent nor selective. SCH 900435 is a selective and highly potent GlyT$_1$-reuptake inhibitor (Figure 1 - structural formula), which effectively increases extracellular glycine levels in rat brain regions and spinal cord, but does not affect strychnine-sensitive GlyT$_2$-transporters. Based on its general facilitation of NMDA-receptor activity throughout the nervous system, its effects could be expected to influence a wide range of CNS-effects. Lastly, preclinical studies suggested that SCH 900435 could have a steep dose-effect relationship for adverse events. Therefore, a strategy was chosen for the early development of SCH 900435 that allowed for careful stepwise assessment of the pharmacological and clinical characteristics of the

drug. During the first administration of SCH 900435 in human, each dose escalation step was based on a detailed evaluation of the interim analyses of pharmacokinetics (PK), pharmacodynamics (PD) and clinical effects of the preceding doses. A multimodal test battery was used to frequently measure a wide range of effects, covering most drug sensitive CNS-domains and vital functions. This article describes the three studies in human volunteers performed to determine a dosing regimen of SCH 900435, that were expected to be safe and therapeutically relevant for subsequent patient dose finding studies. Each study was approved by an independent Ethics Review Board and full written informed consent was obtained from all subjects.

Single ascending dose (SD) study

Subjects and methods

Sixteen healthy male volunteers between 18 and 45 years were recruited for the first administration of single ascending doses of SCH 900435 at the Centre for Human Drug Research in Leiden, The Netherlands. Exclusion criteria and study restrictions included the use of agents known to affect CNS performance (including nicotine, alcohol or drugs) and evidence of relevant clinical or psychiatric abnormalities. Subjects remained in house until 72 hours after the last study drug administration. The study was double-blind, placebo controlled and randomized. Each subject was assigned to one of four dosing groups in a four-way crossover study (three active ascending doses and one randomized placebo dose), with a minimum wash-out period of one week (table 1a). Based on preclinical evaluations, the original dosing regimen was planned to cover a range from 0.5 mg (the human starting dose based on animal safety data) to 135 mg (predicted to lead to plasma levels in the anticipated therapeutic range). According to the protocol, doses could be adapted based on investigator-blinded interim assessments.

Adverse events, laboratory safety parameters, ECG, oral body temperature, blood pressure and heart rate measurements were performed regularly throughout the study. Blood samples were obtained pre-dose and frequently up to 72 hours after SCH 900435 administration. Frequent measurements of a multimodal CNS test battery containing a wide range of drug-responsive CNS-domains up to eight hours after dosing were obtained: Visual Analogue Scales (VAS measuring subjective alertness, mood, calmness, psychedelic effects, sleep quality), pharmaco-electroencephalography (pharmaco-EEG), saccadic pursuit eye movements (measure of alertness), smooth pursuit eye movements (measure of motor coordination), adaptive tracking (visuo-motor coordination), body sway (postural stability), pupillometry (pupil-iris ratio) and neuroendocrine effects (serum prolactin, LH, FSH and testosterone levels).

Both a general treatment effect and a simple linear effect was tested for the PD parameters. AUEs (Area Under Effect-time curves) were calculated per subject and divided by the corresponding time span, resulting in a weighted average response used for ANCOVA analysis. If the general treatment effect was significant, contrasts of all treatments versus placebo were calculated. Differences between treatments were defined as statistically significant at a p-value of 0.05 or lower.

If the treatment effect was significant and the linear effect suggested a dose effect relation, an additional regression analysis of the relevant parameter on log dose was performed. The slope of the regression was considered significantly different from zero at a p-value of 0.05 or lower. Conclusions about PK with respect to dose proportionality/ dose independence were primarily based on descriptive statistics of PK parameters and on summary plots. An additional exploratory Analysis of Variance (ANOVA) was performed to test for dose proportionality and dose independence. Detailed methods (pharmacodynamic measurements and statistics) are described in appendix I.

Results and discussion

Sixteen healthy males were included in the single dose study. Two subjects were withdrawn due to visual symptoms (described in more detail below)

and 14 subjects completed the study. The mean (range) age of the subjects was 24 (19-32) years.

After interim assessment of the first dose, it was observed that exposure in terms of plasma AUC was 10-fold higher in humans than that which was allometrically predicted. No pharmacodynamic or adverse effects were noted after the starting dose, but the original anticipated dose range was reduced from 0.5-135 mg to 0.5-30 mg (table 1b).

No statistically significant changes in vital signs, respiratory function, physical examination or laboratory parameters were observed during the study. There were no serious adverse events (SAEs). The most frequently reported adverse events (AEs) during the in-house study period were dizziness, somnolence, headache, fatigue and abnormal vision (table 2). All were mild to moderate and self-limiting. At 3 mg, one subject developed anxiety and other psychological effects in addition to visual changes, which led to his withdrawal. In the subsequent two dosing groups, four of 12 subjects reported visual changes (one each at 3 and 8 and two at 12 mg), often described as spots of enhanced contrast or intensity accompanied by blurred vision and dizziness. These symptoms occurred around 30 minutes after drug administration, and disappeared within minutes to a few hours after the start of the symptoms. They were considered to be drug-related, but were of limited duration and intensity. All four subjects in the fourth dosing group reported spots in the visual fields (starting at 20 or 30 mg) similar to those observed in previous groups. It was decided to withdraw one of these subjects as he reported recurrent symptoms, which, although moderate, increased in the third visit (30 mg) compared to the second visit (20 mg). Ophthalmologic examinations did not reveal any subjective or objective visual system abnormalities, either during the study or at follow-up.

Peak concentrations of SCH 900435 26,7 ng/mL (for 0.5 mg) to 1170 ng/ml (for 30 mg) and were reached between 30 and 50 minute after dosing. Maximal drug plasma concentrations were higher in subjects who reported visual AEs than those who did not (approximately 770 ng/mL versus 140 ng/mL). Peak concentrations of 26.7 ng/mL (for 0.5 mg) to 1170 ng/mL (for 30 mg) SCH 900435 were reached 0.5-0.8 hours after dosing.

Pharmacokinetics (PK) were dose-linear over the tested dose range. The mean concentration-versus time profiles (figure 2) showed that the last parts of the curves for all doses ran essentially parallel and that the terminal elimination phase started approximately 16 hours after dosing. The terminal half-lives varied from 6.6 to 7.8 hours, and clearance from 4.5 to 6.1 L/h.

In agreement with the adverse event reports, significant increases were observed in the 'colours' item of the Bowdle visual analogue scale (table 3). The increases did not exhibit a linear dose-response relationship across all doses (table 4) but were consistent at 30 mg (figure 3). After a maximum at approximately 40 minutes, all effects had disappeared by 3 hours following dosing (figure 4). Pupillometry demonstrated an increase of pupil/iris ratio at 20 mg SCH 900435 (table 3), with the largest effect at 30 mg (table 3, figure 5).

Smooth pursuit (0-4 hours) showed a statistically significant increase at 8, 12, and 30 mg (largest difference) (table 3). The regression analysis showed a statistically significant dose-response relationship for smooth pursuit eye movements (table 4). It should be noted that the smaller number of subjects in the higher dose groups (the 20 mg dose group for smooth pursuit eye movements) may have prevented the detection of a treatment effect from reaching statistical significance (the same could be true for the 30 mg dose group for FSH levels).

Both LH and FSH decreased statistically significantly compared to placebo (table 3, figure 5). LH started to diminish after 12 mg and FSH after 8 mg; the effect was more pronounced with higher doses. Testosterone showed a decrease at 8 and 20 mg (table 3). The LH level decrease followed SCH 900435 administration within one hour and was virtually normalized after ten hours. FSH levels diminished after five to six hours, and remained reduced during the full observation period. Regression analysis showed indications for linear dose response relationships for all three hormones (table 4).

Signs of pharmacological activity in this single ascending dose study were evident from doses starting at 8 mg. Clear increases in pupil size and subjective changes of visual perception were noted after 20 and

30 mg. The effects resolved rapidly and were no more than mild to moderate, but were initially disconcerting and were not anticipated. Therefore, a more specific study on the effect of the drug on visual system function was therefore initiated before a multiple dosing was started.

Visual effects study

Based on the conjecture that the visual symptoms were more likely to reflect a retinal rather than central origin, consultation with ophthalmology experts and a review of the literature revealed that the retina is rich in glycine receptors. It appeared that although CNS-studies of glycinergic or glutamatergic agents did not specifically describe visual disturbances [18,23,24], such effects have been reported with high-dose intravesicular glycine administration in men, following prostate surgery [25,26]. Based on the combination of drug-induced pupil dilation and abnormal vision, direct retinal effects were considered more probable than autonomically induced mydriasis or central visual impairment. Detailed assessments of retinal architecture and function in rats and dogs exposed to SCH 900435 did not demonstrate any electrophysiological or structural abnormalities of the retina or other components of the visual system after repeated dosing (data on file). In the first-in-human study, all symptoms were mild and rapidly reversible without any sequelae, both subjectively and during follow-up. Moreover, the resolution of visual symptoms was faster than the reduction of plasma concentrations, suggesting that the visual effects may not have functional consequences. This was investigated in a dedicated ophthalmologic study with SCH 900435 at Moorfields Eye Hospital in London, UK.

Subjects and methods

The visual effects study was a double-blind, placebo-controlled, four-period crossover study involving the administration of three single oral doses of SCH 900435 (5, 13 and 20 mg) in which the placebo was randomized in an ascending order in 24 healthy male subjects. The exclusion and inclusion criteria and study restrictions were similar to the single ascending dose study. Inclusion criteria for the study included a normal eye examination and good visual acuity (6/9 or better in each eye). Each treatment period lasted one day and was separated by a wash-out of at least 3 days. Subjects were further randomized into one of three groups of eight subjects in which PK, PD and several PD eye assessments were performed. In group 1 (fully dark adapted with dilated pupils; duration: approximately 12 minutes, in cycles of 25 minutes) the following PD tests were performed: dark adaptation - measuring the threshold of light detection without electrophysiology, rod specific single flash, red flash, intermediate, International Society for Clinical Electrophysiology of Vision (ISCEV) standard and ISCEV + 0.6 LV (see appendix II). In group 2 (light conditions with dilated pupils; duration: approximately 20 minutes, in cycles of 25 minutes) the following tests were taken: single flash Cone response - 2 Hz, flicker response - 30 Hz, on-/off- response, S-Cone response and Photopic Negative Response (see appendix II). In group 3 (light conditions with undilated pupils; duration: approximately 45 minutes) the following tests were performed: VEP - pattern reversal/ Flash, pattern ERG, Color Contrast Sensitivity, visual Acuity, pupillometry (see appendix II) and neuroendocrine effects. PK, PD and statistics are described in more detail in appendix II. Endocrinologic investigations (performed in groups 1 and 2 only) included repeated measurements of LH, FSH and testosterone. AEs, laboratory safety parameters, ECG, blood pressure and heart rate measurements were performed regularly during the study. SCH 900435 blood samples were obtained predose and frequently up to 24 h post-dose (for PK methods see single ascending dose study appendix I).

Results and discussion

Twenty-four (three groups of eight) subjects were included. There were no SAEs or clinically relevant changes in laboratory safety parameters, vital signs, and ECG data. The majority of the AEs were considered to be of mild intensity (102 out of 109 AEs), four AEs were of moderate intensity

and three were of severe intensity. One visual AE followed after 13 mg SCH 900435 and two following 20 mg and were similar to the events that were also described in the SD study. The symptoms were transient and resolved without intervention.

Pharmacokinetics and hormone results were also similar to those observed in the SD study (data not shown), with the exception of the FSH profile which showed no decrease in this study. Overall, there was no consistent change in any ophthalmologic test result in any subject, other than those that may be expected from normal variation. The visual symptoms questionnaire was the most sensitive and specific determination of visual disturbance (table 5). Although the results should be treated with caution because of the well documented consequences of multiple hypothesis testing (only unadjusted 95% confidence intervals were calculated) and influence of extraneous factors (e.g. subject tiredness and dizziness, adaptation/intolerance to the testing), the data provided no indication for clinical concerns to perform a multiple (ascending) dose study. This could possibly be an explanation of the reporting of blurred vision and apparent film over the eyes pre-dose and following administration of placebo.

Multiple (ascending) dose (md) study

Subjects and methods

The multiple ascending dose study was performed at Guys Drug Research Unit, London, UK. Selection criteria and study restrictions were similar to the visual effects study. The study design of the multiple dose (MD) study was double-blind, placebo-controlled and parallel group. Multiple (ascending) oral doses between 4 and 30 mg were given once or twice daily to 40 (five groups of eight) healthy male subjects for 13 days (table 6).

Similar to the SD and visual effects study, PK samples were obtained frequently for up to 72 hours after the last SCH 900435 dose, and AEs, laboratory safety parameters, ECG, oral body temperature, blood pressure and heart rate measurements were followed up regularly (see for the study design table 7). PD and safety assessments in the MD study were based on the results of the SD and visual effects study: Visual Analogue Scales (VAS), pharmaco-EEG, pupillometry and neuroendocrine effects (LH, FSH, testosterone) (table 7). In addition, the cognitive effects of multiple dose treatment with SCH 900435 were examined with the following tests from the Cognitive Drug Research (CDR) test battery: simple reaction time, digit vigilance, choice reaction time, spatial working memory, numeric working memory, immediate word recall, word recognition, postural stability and tracking. At screening and follow-up, visual examinations were performed at Moorfields Eye Hospital, London, and subjects were given a questionnaire consisting of eight questions to ascertain visual symptoms. The primary aim of this MD study was to examine the clinical course of the events that had been observed during single dosing. Descriptive and summary statistics for the VAS and AEs were used as statistical models were considered to have a chance of obstructing the identification of isolated but relevant clinical observations. The statistical analyses performed on PK, pharmaco-EEG and results of the CDR test battery are described in more detail in appendix III.

Results and discussion

Forty healthy male subjects, with a mean (range) age of 25.9 (18-42) years, were included in the study. There were no clinically relevant abnormal findings in the laboratory safety parameters, vital signs or ECG data. The most common AEs reported by subjects receiving SCH 900435 were eye disorders and CNS disorders (table 8). These symptoms were mostly noted between 0.5 and 1 hours post-dose after both single and multiple doses and did not appear to be prolonged. None of the AEs were severe and no SAEs were reported during the study. One subject was withdrawn from the study on day 12 (before the morning administration of SCH 900435) due to a persistent focussing difficulty; those symptoms disappeared by the evening of day 12. Notably, the number of reported visual symptoms following SCH 900435 12 mg twice daily (after dose

titration) were lower than following 16 mg once daily (table 8). Similarly, the incidence of lethargy, dizziness and somnolence was less during dose titration (table 8).

No clinically relevant deviations in PD effects were shown. Mean VAS data seemed to show increases in dizziness, decreases in alertness and in activeness, sleepiness and nausea and drowsiness on day four, while these effects had diminished or cleared a few days later, suggesting some adaptation to treatment. Although a decrease in pupil diameter was seen (as opposed to the dilation seen in the single dose study), this change was not sustained, not evident under the other conditions or in the other dose groups and it can not exclude that this finding was coincidental in this small group of subjects. There were also no clear results on EEG power and the cognitive data.

Similarly to the SD study, FSH, LH and testosterone significantly decreased during multiple dosing (see figure 7 for LH; the figures for the other hormones were similar and were therefore not shown). There was no difference in hormone reductions between the first and last days of dosing. The effect was generally largest following administration of 16 mg SCH 900435.

The PK parameters of the multiple (ascending) dose study were as expected from the single dose study and the visual effects study (data therefore not shown). Steady state concentrations were reached between 2 and 6 days of daily dosing. Mean accumulation of SCH 900435 exposure (AUC) at steady state after once daily dosing ranged between 5% and 11% and was 22% after 12 mg twice daily treatment. The elimination half-life was increased 20% after 10 days of daily dosing. PK of SCH 900435 was dose-proportional at steady state in the dose range 4 - 16 mg once daily and 12 mg twice daily.

In summary, the incidence of central nervous and visual symptoms measured by AE reporting increased when the dose of SCH 900435 was higher than 8 mg once daily. The other PD measurements did not show relevant or consistent changes in the doses tested in this study, except limited but statistically significant decreases in gonadotrophic hormones. Clinically, the results indicate that dose titration and twice daily (compared to once daily) dosing of SCH 900435 improve tolerance to the subjective visual disturbances that primarily occur shortly after the initiation of treatment.

Discussion

The changes in visual VAS scales, the reported visual symptoms and the pupil dilation that were observed in the SCH 900435 studies are all compatible with a direct retinal effect of SCH 900435. Visual spots can be a manifestation of cortical spreading depression such as that which occurs during a migraine aura, and it cannot be excluded that a similar phenomenon caused the visual symptoms that occurred with SCH 900435. This would be consistent with a potential role of enhanced glutamatergic activity, which among other biochemical derangements has been postulated to play a role in cortical spreading depression [27]. On the other hand, visual auras typically migrate and this was not reported by participants in our studies. Also, no other migrating neurological deficits or headaches occurred. Furthermore, pupillary abnormalities are usually not observed in occipital visual disturbance. A transient change in retinal function may then explain the visual symptoms, even in the absence of any detectable changes in retinal function as measured electrophysiologically.

Glycine is one of the essential neurotransmitters modulating visual signals in the retina [28]. The retinal amacrine cells appear to possess a specific uptake mechanism for glycine [29] and contribute to the generation of the oscillatory potentials (OPs) in the electroretinogram. In different animal models these potentials were found to be blocked by glycine [29,30]. In a recent study with a glycine reuptake inhibitor in healthy humans a high incidence of visual effects was described [31]. Visual effects included visual disturbance, blurred vision, photophobia, diplopia, and photopsia and were only reported in the higher dose ranges. The time course of the effects in the current study was also compatible with an acute pharmacological effect. In the SD study, the reported visual symptoms and pupil dilation were both clearly related to

peak plasma concentrations. Although no formal concentration-effect analyses were performed, it seemed that the subjective visual changes (assessed with the Bowdle VAS colours) resolved more rapidly than the plasma concentrations, perhaps as a result of habituation. In addition, high peak concentrations could be avoided by a slow release formulation which has been developed for this reason. The visual system is highly adaptive, so the question remained whether symptoms faded because of (retinal) adaptation to ongoing functional impairments. Preclinical multiple-dose studies showed no morphological abnormalities in the eyes of experimental animals and the dedicated ophthalmologic study found no electrophysiological or functional indications of significant retinal impairment. In the MD study, the incidence of AEs related to eye disorders increased with ascending doses of SCH 900435. However, reports of visual disturbances were less frequent with dose titration than without. This provided further confidence that the rapid resolution of the visual symptoms after a single dose or during dose titration reflects the development of pharmacological tolerance without further functional consequences. Similarly, in the VAS data there was an indication for tolerance to dizziness, alertness, sleepiness, nausea, activeness and drowsiness.

In all three studies decreases in sex hormones were observed after SCH 900435 (with the exception of FSH in the visual effects study), and the relation between these effects and the dose was linear. Literature on the relationship between NMDA receptor stimulation and FSH, LH or testosterone production in humans is sparse, and most studies show no clear association [32,33] or contradict the present findings [34]. In preclinical experiments, an increase of FSH was found [35,36]. The testosterone reductions found in this study are compatible with the diminished gonadotrophic hormone concentrations, particularly of LH. The differences in the response of LH and FSH after SCH 900435 administration are partly related to the differences in half-life. LH has an elimination half-life of around one to two hours [37], while that of FSH is in the region of five hours [38]. Although a direct effect of SCH 900435 on the expression of any of these hormones individually cannot be excluded, the time-courses of the hormonal effects and the linear relation between dose and effect agree better with a common effect on LH and FSH release, most likely due to reduction of GnRH release [39], and suggesting the site of action of SCH 900435 to be the hypothalamus. These gonadotrophic responses are dose dependent and quite consistent, which makes these hormones (and LH in particular), the most reliable and sensitive pharmacodynamic indicators of a central SCH 900435 effect that were identified in these studies.

In general, animal studies of glycine reuptake inhibitors and glycine agonists showed central nervous system depression (i.e. reduction in motor activity) [7,40,41]. In apparent contrast, SCH 900435 produced slight improvements of smooth pursuit eye movements in the SD study, which seemed to be dose dependent, and hence to be related to the pharmacological activity of the drug. Improved smooth pursuit could point to a mild stimulant effect of SCH 900435. On the other hand, the EEG demonstrated small statistically significant reductions in alpha$_2$ power (10.5 - 12.5 Hz), which are more in line with CNS depression. However, this alpha reduction could be secondary to the visual symptoms caused by SCH 900435, since EEG alpha rhythm is particularly sensitive to visual input (e.g. eye opening or closure) and subjective well being (e.g. suppression by nausea and dizziness). These findings should be replicated before firm conclusions can be drawn.

Since experience with indirect NMDA-receptor activators in humans was limited, a cautious and adjustable approach to development of these drugs in humans is required. The present paper describes how a novel glycine reuptake inhibitor, which had a steep in dose-response curve for adverse events in test animals and showed unanticipated and initially disconcerting visual symptoms in humans, was safely introduced in healthy volunteers using an adaptive research strategy consisting of frequent interim analyses of PK characteristics and of data-intensive PD effects, and a return to dedicated animal studies before the drug was reintroduced in humans. Unanticipated visual effects were detected from adverse event reports, and could be interpreted and pursued more reliably using frequent measurements of visual analogue scales for a broad range

of subjective symptoms, and pharmacodynamic measures of multiple systemic and CNS effects, including pupil size. This strategy is particularly useful for chemical entities with novel mechanisms of action, which have a higher chance of showing unanticipated effects.

Intensive pharmacodynamic measurements can also provide indications for CNS-penetration and dose-related pharmacological activity, especially since the early studies in healthy volunteers almost always cover a large dosage range. The establishment of dose-related changes provides strong evidence for a drug-related effect. This is clearly demonstrated by the consistency of almost all effects that were observed during the first study, even though they were unexpected and may even have initially seemed spurious. It cannot be ascertained whether the indications for CNS penetration and pharmacological activity of SCH 900435 are due to $GlyT_1$ reuptake inhibition, although this is suggested by the high potency and selectivity of SCH 900435 at this site. The retina, which is particularly rich in glycine- and glutamate-containing cells [42], has a structure similar to the blood brain barrier. The observed visual spots in the SD study together with the dose-responsive hormone changes and neurophysiological effects could indicate that SCH 900435 passes the blood retinal barrier and penetrates into the CNS. Long-term studies and investigations with other glycine reuptake inhibitors are needed to confirm whether retinal and gonadotropic changes are pharmacological effects of this novel drug class. Although these studies provided indications that SCH 900435 has a pharmacological effect in the CNS at doses that are well tolerated after titration, the antipsychotic effects and the therapeutically active doses of SCH 900435 and of glycine enhancement strategies in schizophrenia in general [43,44] remain to be established.

Appendix I

Description of pharmacodynamic tests and statistics single ascending dose study

Different measures of the effects of SCH 900435 on the central nervous and visual systems were used in this study. A multimodal test battery was performed regularly, to keep close track of any potential drug-related changes in CNS-functionality.

VISUAL ANALOGUE SCALES (VAS)

Subjective effects were quantified using a Dutch translation of the 16 visual analogue scales (VAS) originally described by Norris [1] and applied to drug effect by Bond and Lader [2]. The Dutch translation showed effects on many different CNS active drugs, including sedative agents [3,4], dopaminergic drugs [5], scopolamine [6], and THC [7]. From the set of 16 scales, three factors corresponding to alertness, mood and calmness were derived.

Bowdle described 13 VAS's to quantify the psychotomimetic effects of ketamine [8]. A translated version of the Bowdle VAS showed concentration-related effects in THC [7], scopolamine [6], and zolpidem [9].

The Leeds Sleep Evaluation Questionnaire (LSEQ) contains 14-item visual analogue scales related to the subjective aspects of sleep and waking [10].

PHARMACO-ELECTROENCEPHALOGRAPHY

Pharmaco-EEG was performed as a general measure of CNS activity [11]. The literature suggests that antipsychotics show distinct profiles of EEG-changes [12,13]. EEG recordings were made using silver-silver chloride electrodes, fixed with collodion according to the international 10/20 system. The electrode resistances were kept below 5 kOhm. The duration of one EEG measurement was 3 minutes. The signals were amplified and stored on a Vitaport-8® system. A/D converted data were subjected to a windowed Fast Fourier transform analysis giving a frequency resolution

of 0.25 Hz. Summary parameters were calculated using the frequency bands as defined by Hermann from a factor analysis of human EEG [14].

SACCADIC AND SMOOTH PURSUIT EYE MOVEMENTS

Saccadic and smooth pursuit eye movements were recorded as described previously [3,15,15-17] and have shown dose- and concentration-related effects on many different CNS active drugs, including GABA-ergic [4,5,18], serotonergic [19], noradrenergic [20-22], and dopaminergic drugs [23].

ADAPTIVE TRACKING

The adaptive tracking test has proved to be useful for measurement of CNS effects of alcohol, various psychoactive drugs and sleep deprivation [3,17]. The adaptive tracking test was performed as described earlier and can be divided into two tasks: the PURSUIT and the FITTS task [24-26]. During the PURSUIT task, the subject was instructed to keep a dot inside a moving circle by operating a joystick. If this effort was successful, the speed of the moving circle was increased. Conversely, the velocity was reduced if the test subject could not maintain the dot inside the circle. The FITTS task was to measure quick motor responses and the subject was asked to aim at targets with a pen as quickly as possible without missing the target.

BODY SWAY

The body sway meter records body movements in a single plane, providing a measure of postural stability. Changes in body sway were seen for many different CNS active drugs, including GABA-ergic drugs [4,18,27], and THC [7] and was performed as previously described [23].

PUPILLOMETRY

Pupil size was included as a measure of autonomic tone and ocular function. In the SD study, exploratory pupillometry was performed using digital photometry. Twa *et al* showed that estimation of pupil size by digital photography was more repeatable and accurate than estimates by common clinical techniques over a wide range of illumination [28].

The subject was instructed to look into the lens of a digital camera after at least five minutes adaptation in ambient lighting. A picture of the eyes was taken using a single flash. All pictures were imported and stored in Adobe Illustrator 9, to determine the diameters of the pupil and the iris in millimetres. For each eye, these values were recorded, and the pupil/iris ratio was calculated as a measure of pupil size.

NEUROENDOCRINE EFFECTS

In humans LH has proven to be a sensitive marker of NMDA activity [29,30]. Prolactin was also measured as an indication of (indirect) dopamine activity [5].

After collection, blood samples for LH, FSH, testosterone, and prolactin were kept at room temperature for about 30 minutes to allow coagulation. Serum was separated by centrifugation (2,000´g at 4°C for 15 minutes) and stored in a deep freezer at - 40 °C. The hormones were performed according to Good Laboratory Practice analysed by Organon Development GmbH using a validated fluoro-immunoassay.

Reference values for men were 2 - 80 U/L for LH, 2-10 U/L for FSH, 10-40 nmol/L for testosterone and < 15 µg/L for prolactin. For cortisol reference values were time dependent: 0,20 - 0,60 µmol/L (8 AM), 0,10 - 0,30 µmol/L (11 PM) and < 0,18 µmol/L (11 PM).

The assays had LLQs of 0.25 mU/mL (FSH), 0.6 mU/mL (LH), 0.14 ng/mL (testosterone), 0.25 ng/mL (prolactin), an intra-assay precision (expressed as coefficient of variation) of 0.8-1.8% (FSH), 1.4-2.2% (LH), 3.3-5.2% (testosterone), 2.8-3.2% (prolactin) and inter-assay precision of 1.4-1.6% (FSH), 1.8-2.6% (LH), 2.7-8.6% (testosterone), and 4.5-6.3% (prolactin).

Pharmacokinetics

Plasma (K-EDTA) was centrifuged within 15 minutes after sampling and stored at -40 degrees Celsius. Plasma samples were assayed for SCH 900435 using a validated liquid chromatographic method with mass spectrometric detection with a lower limit of quantification of 0.1 ng/mL.

The assay had an LLQ of 0.1 ng/mL, an intra-assay precision (expressed as coefficient of variation) of 1.4-6.5% and inter-assay precision of 2.0-24.6%.

Statistical analysis

For the PD parameters both a general treatment effect and a simple linear effect was tested.

To calculate the treatment effect, AUEs (Area Under Effect-time curves) per subject were calculated with the linear trapezoidal rule using protocol times, and divided by the corresponding time span, resulting in a weighted average response. AUEs of the whole time period, of the period 0-4 hours and 0-90 minutes (only for pupil size) of the PD variables were analyzed with an analysis of variance with fixed factors treatment and visit, random factor subject and pre-value as covariate (ANCOVA analysis).

If the treatment effect was significant, contrasts of all treatments versus placebo were calculated. The different time spans of the AUCs were chosen as there were very fast, short lasting effects and slower, more long lasting effects.

If the treatment effect was significant and the linear effect suggested a dose effect relation, an additional regression analysis of the concerning parameter on log dose was performed. This was performed by calculating the regression of relationship between the change from baseline AUEs and the log10 dose with subject, intercept and slope as random factors and visit as fixed factor. Slopes being different from zero were tested with an alpha of 0.05 two sided.

Conclusions with respect to dose proportionality/dose independence were primarily based on descriptive statistics of PK parameters and on summary plots of (dose-normalised-(dn))C_{MAX} and (dn-)AUC versus dose. An additional exploratory Analysis of Variance (ANOVA) was performed on dn-C_{MAX}, dn-AUC, $T_{1/2}$, (weight-normalised-(wn))Cl_{APP}, and (wn-)$V_{Z,APP}$ and an appropriate non-parametric test on T_{MAX} and t_{LAG} to test for dose proportionality/dose independence.

Appendix II

Description of pharmacodynamic tests and statistics visual effects study

VISUAL ELECTROPHYSIOLOGY

A series of electrophysiological tests were performed to obtain a comprehensive overview of various retinal and pupillary functions. Subjects were divided in different groups, to allow investigations under different lighting conditions. All eyes were dilated before full field testing using tropicamide (1%) and/or phenylephrine hydrochloride (2.5%). Measurements were performed according to the standards of the International Society for Clinical Electrophysiology of Vision (ISCEV), as described by Marmor and Zrenner (2004) [31] unless cited otherwise.

One group of subjects was tested under dark conditions, with a number of tests that lasted approximately 12 minutes and was repeated every 25 minutes. Subjects who were examined under dark conditions were adapted to the dark for twenty minutes after pupillary dilation, before the minimum luminance of a test spot required to produce a visual sensation was determined [31]. The full field ('Ganzfeld') ERG protocol incorporated the ISCEV standard and used a commercial system (RETIscan System; Roland Consult, Wiesbaden, Germany). Small disc electrodes were placed on the skin at the side of the subject's eye, and corneal soft gold foil recording electrodes were placed under red light. Rod specific ERG was recorded after a single flash. The rod response was identified as the first signal measured after dark adaptation. Additional recordings included the use of a red flash stimulus [32]. Maximal ERGs were recorded after a brighter maximal flash (Standard + 0.6LU).

Another group was examined under light conditions. The duration of testing was approximately 20 minutes, in cycles of 25 minutes. These subjects also underwent standard ISCEV ERGs after pupillary dilation and a standard period and intensity of light adaptation [31]. Cone responses

were measured after a single white flash preceded by background illumination to suppress rod function. Under the same conditions 30 Hz flicker responses were obtained with repeated single flash stimuli.

Long duration photopic stimulation was used to record ON and OFF ERG responses using previously described techniques [33]. In brief, an amber stimulus of 120 ms or 200 ms duration (luminance 560 cd/m2) was presented upon a bright green background (luminance 160 cd/m2) close to peak rod spectral sensitivity and thus suitable to suppress rod function. A- and b-waves occur at onset of the flash, and a d-wave at light offset. These responses represent different functional pathways of retinal rods and cones. Human S-cones mediate the signals of short wavelength sensitive cones [34]. S-cone responses were measured using a chromatic stimulus ERG as described by Yamamoto *et al* [35].

Under photopic conditions, the ERG shows an a-wave that arises from cones and off-bipolar cells, and a b-wave that originates from on- and off-bipolar cells with a possible contribution from Mueller cells. The late negative component subsequent to the b-wave is called the photopic negative response. This measurement was performed as described by Holder *et al* [36].

A third group of subjects was investigated under light conditions with undilated pupils. These tests lasted approximately 45 minutes. Flash visual evoked potentials (VEPs) were performed according to the ISCEV standard for clinical VEP testing [36]. Pattern ERG was recorded using a black and white checkerboard stimulus, with 98% contrast and a mean luminance of 80 cd/m2. The stimulus frequency was 4.5 reversals/sec. The field size was 15˚ x 11˚, with a check size of 45 min. The amplifier gain was set to 200•103, band pass filter 1-100 Hz, with a sampling rate of 2 kHz. Optimum spectacle correction was used. Fixation was enabled by a red central fixation target and the patient's fixation was monitored using CCTV (closed circuit television). The amplitude and latency of the PERG P50 and N95 components were analysed. The p1 and n1 components of the first order kernel of the mfERG were analysed using the RETIscan software. Trace arrays were divided into 5 concentric rings centred on the fovea (Ring 1, 0-2.1˚; Ring 2, 1.4-6.7˚; Ring 3, 5.7-12.0˚; Ring 4, 9.5-19.8˚; Ring 5, 15.1-28.5˚). The traces within the ring were averaged and the amplitude and latency of p1 and n1 from each ring summation was analysed.

Colour contrast sensitivity was measured psychophysically by determining thresholds along isoluminant protan, deutan, and tritan colour confusion axes using the Arden colour contrast sensitivity system [37]. Pupillometry was performed using a similar method as described in the single ascending dose study (see Appendix I).

NEUROENDOCRINE EFFECTS

See Appendix I single ascending dose study.

VISUAL ANALOGUE SCALES (VAS)

See Appendix I single ascending dose study. This Bond and Lader VAS was extended with a Bastani mood rating scale [1,2].

Additionally, subjects were given a visual questionnaire to ascertain visual symptoms. The questionnaire consisted of eight questions and subjects answered whether they agreed or not and if they agreed then whether the symptoms were mild or severe. The eight questions were as follows:

Your vision is blurred
There appears to be a film over your eyes
You have difficulty assessing how far away objects are
You are seeing flashes of light
You are seeing dark patches
You are having more difficulty than usual focusing
You would not feel safe driving a car with your vision as it is
Do you have any other visual symptoms or disturbance?

The final question had space for a description of other symptoms if present.

Pharmacokinetics

See Appendix I single ascending dose study.

Statistical analysis

Only descriptive and summary statistics were performed for the electrophysiological parameters, hormones, laboratory tests (hematology, serum blood chemistry, urinalysis and any urine microscopy) and pharmacokinetics. An Analysis of Variance (ANOVA) on log-transformed pharmacokinetic parameters (for T_{MAX} after ranking) with factor group; all tests at the 5% level of significance.

Appendix III

Based on the outcomes of the single ascending dose trial, a selection of tests was made for the multiple (ascending) dose study. These measurements were partly targeted at cognitive effects, which were difficult to assess in great detail after single doses and to the visual effects that were observed after the first administration in man.

VISUAL ANALOGUE SCALES (VAS)

See Appendix II visual effects study.

PHARMACO-ELECTROENCEPHALOGRAPHY

During the multiple ascending dose study, more extensive EEG examinations were performed than when the drug was first administered to humans. A Walter Graphtek Polygraph System running validated PL Windsor acquisition and Fourier analysis software was used to collect full 21-lead EEGs, including three minutes eyes closed vigilance control (primary condition), three minute resting, hyperventilation and photic stimulation. Clinical neurophysiologist excluded subjects with clinically significant abnormalities at screening, and recordings with persistent artefacts. Electrodes were attached according to the international 10-20 system using similar materials and methods described for the single ascending dose study. Data from each subject's dominant side was extracted for the frontal (F3 or F4 channel), central (C3 or C4 channel), parietal (P3 or P4 channel) and occipital (O1 or O2 channel) positions at pre-dose, 0.5, 1, 2, 4 and 6 h post-dose on Days 5 and 13 and matching time points on Day -1. The following variables were recorded:total power [μV^2], peak power [μV^2]; peak frequency [Hz]; mean frequency (frequency (1-30 Hz) below which 50 % of total power is located) [Hz]; spectral edge (frequency (1-30 Hz) below which 90 % of total power is located) [Hz]; vigilance index: ($alpha_1$ + $alpha_2$)/(theta + delta) [ASI]; and power [μV^2] and centroid frequency [Hz] for the delta (1-4 Hz), theta (4-8.5 Hz), $alpha_1$ (8.5-10.5 Hz), $alpha_2$ (10.5-12.5 Hz), beta1 (12.5-18.5 Hz) and beta3 (21-30 Hz) frequency bands.

COGNITIVE TESTING

A battery of tasks from the Cognitive Drug Research (CDR, Goring-On-Thames, UK) was administered and consisted of the following tests (described in [38]): simple reaction time, digit vigilance, choice reaction time, spatial working memory, numeric working memory, immediate word recall, word recognition, postural stability and tracking. Postural stability measures the ability to stand upright without moving waist assessed using the CDR meter that was modelled on the Wright Ataxiameter [13]. A cord from the meter was attached to the subject who was required to stand as still as possible with feet apart and eyes closed for one minute. During the tracking test the subject used a joystick to track a randomly moving target on the screen for 1 minute. The distance off-target per second was recorded.

PUPILLOMETRY

See Appendix I single ascending dose study. In the multiple (ascending) dose study, the pupil size was measured more extensively than in the single (ascending) dose study in three different conditions (0.04 lux/scotopic, 0.4 lux/low mesopic, 4.0 lux/high mesopic) after the subject was dark adapted to the room for 5 minutes using a Procyon P2000SA Pupillometer (Keeler Instruments, Broomall, Pa.).

Neuroendocrine effects

See Appendix I single ascending dose study (prolactin concentrations were not determined in this study).

Statistical analysis

Only descriptive and summary statistics were performed for the parameters of this study except for pharmacokinetics, pharmaco-EEG parameters (total power, delta, theta, alpha$_1$, alpha$_2$ and vigilance index) and CDR parameters (simple reaction time, digit vigilance, choice reaction time, spatial working memory, numeric working memory, immediate word recall, word recognition, postural stability and tracking).

SCH 900435 concentrations in plasma and derived PK parameters were summarized using descriptive statistics. Analysis of variance, to determine time to reach steady state during multiple dosing, was conducted using the Helmert contrast transformation on pre-dose concentrations. Testing on dose proportionality/independence and regimen effects was done using analyses of variance on PK parameters. Food-effect testing was based on C_{MAX} and $AUC_{0\text{-}12}$ of SCH 900435.

Only the primary EEG parameters in both vigilance controlled and rested conditions were statistically analysed. Analysis was performed separately at each electrode location (frontal, central, parietal and occipital). The mixed model described below was used to estimate the effect of each dosing regimen (as ratio of placebo, averaged over time on each day separately) with 95 % CI.

For each log-transformed primary pharmaco-EEG parameter, day and location separately, a repeated measures analysis model was fitted using PROC MIXED in SAS® Version 8.2 with time-matched log-transformed value on Day -1 as baseline covariate and fixed effects terms for time, dose and time x dose interaction term. The repeated nature of the data on each subject was modelled with autoregressive covariance structure [AR(1)]. Data for placebo were pooled across all cohorts (Groups I, II, III and IV). Since only two subjects received placebo in each cohort, no attempt was made to adjust for cohort. Data were included in the model from all available time points (0, 0.5, 1, 2, 4 and 6 h) and the difference in the average value over post dose time points (i.e. excluding 0 time point) of each dosing regimen relative to placebo was estimated with 95 % CI. These estimates and confidence limits were back transformed to give the effect as a ratio of placebo with 95 % CI. No adjustments for multiple comparisons were made since these analyses are considered exploratory. The influence of any outliers was explored on the statistical significance of results, where necessary.

For the CDR parameters repeated measures analysis of variance (ANOVA) was conducted on the 'difference from baseline' scores using SAS® PROC MIXED. A pooled placebo treatment was used. Fixed terms were fitted to the model for treatment (up to 6 levels), day (2 levels), time (4 levels) and the 2-way and 3-way interactions. A random effect of subjects-within-sequence was fitted to the model. Significance of the interactions was tested at the $p<0.05$ level. The interactions were assessed sequentially, highest order first, and non-significant interaction terms were removed from the model. Pairwise comparisons of the active treatments and placebo were performed to clarify any statistically significant interaction effects.

Table 1 Dosing groups with corresponding SCH 900435 (mg) and placebo (P) treatment in single ascending dose study

A. Dosing as originally planned in protocol

Group I				Group II				Group III				Group IV				Group V			
0.5	2.0	8.0	P	8.0	12	18	P	18	27	40	P	40	60	90	P	90	135	P	
0.5	2.0	P	8.0	8.0	12	P	18	18	27	P	40	40	60	P	90	90	P	135	
0.5	P	2.0	8.0	8.0	P	12	18	18	P	27	40	40	P	60	90	P	90	135	
P	0.5	2.0	8.0	P	8.0	12	18	P	18	27	40	P	40	60	90				

B. Actual dosing during study

Group I				Group II				Group III				Group IV			
0.5	1.0	2.0	P	2.0	3.0	5.0	P	5.0	8.0	12	P	12	20	30	P
0.5	1.0	P	2.0	2.0	3.0	P	5.0	5.0	8.0	P	12	12	20	P	30
0.5	P	1.0	2.0	2.0	P	3.0	5.0	5.0	P	8.0	12	12	P	20	30
P	0.5	1.0	2.0	P	2.0	3.0	5.0	P	5.0	8.0	12	P	12	20	30

Table 2 Most frequent Adverse Events single ascending dose study

Reported AE[A]	SCH 900435 dose	Placebo (%)		0.5 mg (%)		30 mg (%)		0.5-30 mg (%)		0.5-30 mg + placebo (%)	
	N of subjects	16		4		4		16		32	
	N of AEs	10		5		15		85		95	
Dizziness		1	(10.0)	0	(0.0)	3	(20.0)	14	(16.5)	15	(15.8)
Somnolence		2	(20.0)	1	(20.0)	3	(20.0)	13	(3.5)	15	(15.8)
Headache		1	(10.0)	0	(0.0)	0	(0.0)	12	(14.1)	13	(13.7)
Fatigue		2	(20.0)	1	(20.0)	1	(6.7)	8	(9.4)	10	(10.5)
Abnormal vision		0	(0.0)	0	(0.0)	3	(20.0)	6	(7.1)	6	(6.3)

A. Not all AEs from each dose are shown for reasons of clarity; there were no relevant differences in these omitted groups

Table 3 Contrasts with placebo on dose groups ANCOVA analysis single ascending dose study (reported for doses SCH 900435 with statistically significant contrasts and higher)

Parameter	Unitc	Dose (mg)	LSM Estimate placebo	LSM Estimate SCH 900435	Estimate of difference	p-value[A]	95% Confidence Interval
Pupil/iris ratio right eye (0-90 minutes)[B]	NA	20	0.41	0.44	-0.030	**0.0145**	-0.05 - -0.01
		30		0.48	-0.065	**<0.0001**	-0.09 - -0.04
VAS colours (0-4 hours)	mm	30	1.45	10.94	-9.48	**<0.0001**	-13.05 - -5.91
VAS dizzy (0-4 hours)	mm	12	43.72	49.57	-5.85	**0.0170**	-10.59 - -1.10
		20		50.34	-6.62	**0.0388**	-12.88 - -0.36
		30		52.14	-8.42	**0.0118**	-14.89 - -1.95
LH (0-final hours)	U/L	8	3.04	2.21	0.82	**0.0025**	0.31 – 1.34
		12		2.69	0.35	0.0864	-0.05 – 0.74
		20		2.10	0.93	**0.0009**	0.41 – 1.46
		30		1.90	1.14	**0.0002**	0.58 – 1.70
LH (0-4 hours)	U/L	8	2.93	2.17	0.76	**0.0335**	0.06 – 1.45
		12		2.15	0.78	**0.0062**	0.24 – 1.32
		20		1.77	1.16	**0.0017**	0.46 – 1.86
		30		1.58	1.35	**0.0003**	0.65 – 2.05
FSH (0-final hours)	U/L	8	2.91	2.50	0.42	**0.0015**	0.18 – 0.66
		12		2.67	0.25	**0.0131**	0.06 – 0.45
		20		2.63	0.28	**0.0301**	0.03 – 0.54
		30		2.68	0.24	0.1068	-0.05 – 0.52
Testosterone (0-final hours)	nmol/L	8	5.88	4.93	0.95	**0.0110**	0.24 – 1.66
		12		5.45	0.43	0.1269	-0.13 – 0.99
		20		4.66	1.22	**0.0024**	0.47 – 1.98
		30		5.26	0.62	0.1440	-0.22 – 1.46
EEG alpha2 (0-4 hours)	µV	12	3.45	3.35	0.11	**0.0449**	0.00– 0.21
		20		3.28	0.17	**0.0099**	0.044 – 0.30
		30		3.18	0.27	**0.0002**	0.14 – 0.41
Smooth pursuit (0-final hours)	%	8	50.94	58.00	-7.06	**0.0172**	-12.80 - -1.32
		12		54.43	-3.49	0.1195	-7.93 – 0.95
		20		51.16	-0.22	0.9397	-6.01 – 5.57
		30		57.26	-6.32	**0.0433**	-12.45 - -0.20
Smooth pursuit (0-4 hours)	%	8	50.281	56.17	-5.89	**0.0289**	-11.14 - -0.64
		12		56.37	-6.09	**0.0042**	-10.14 - -2.04
		20		53.00	-2.71	0.3086	-8.01 – 2.59
		30		57.50	-7.22	**0.0130**	-12.85 - -1.60

A. Significant p-values are indicated in bold (lower than 0.05); B. only results for the right eye were shown as these were similar to the left eye; C. mm = millimetre; U/L = units / liter; µV = microvolt.

Table 4 Regression analysis table of dose-linearity single ascending dose study

Parameter	Unit[B]	p-value slope[A]	Estimate of the slope	95% CI
Log pupil/iris ratio right eye (0-90 minutes)	NA	0.573	0.004	0.017 / -0.010
Log VAS colours (0-4 hours)	NA	0.157	0.081	0.198 / -0.035
VAS dizzy (0-4 hours)	mm	0.062	2.302	4.722 / -0.119
LH (0-final hours)	U/L	**0.032**	-0.356	-0.031 / -0.682
LH (0-4 hours)	U/L	**0.004**	-0.501	-0.164 / -0.838
FSH (0-final hours)	U/L	**0.020**	-0.193	-0.042 / -0.344
Testosterone (0-final hours)	nmol/L	**0.012**	-0.424	-0.101 / -0.747
EEG alpha2 (0-4 hours)	µV	0.142	-0.053	0.020 / -0.126
Smooth pursuit (0-4 hours)	%	**0.001**	3.305	5.125 / 1.485

A. Statistically significant p-values (lower than 0.05) are indicated in bold; B. NA = not applicable; mm = millimetre; U/L = units / liter; µV = microvolt.

Table 5 Results of visual analogue scales in visual effects study - number of subjects responding 'yes' to the question [number of subjects considering the effect as severe]

Question/Study time	Placebo	SCH 900435		
		5 mg	13 mg	20 mg
N	24	24	24	24
Your vision is blurred				
Pre-dose	9 [2]	11 [3]	12 [2]	12 [2]
1.5 hours post-dose	7	10 [1]	16 [1]	14 [1]
8 hours post-dose	0	1	0	1
There appears to be a film over your eyes				
Pre-dose	1	2	2	1
1.5 hours post-dose	1	2	3	3 [1]
8 hours post-dose	0	0	0	0
You have difficulty assessing how far away objects are				
Pre-dose	2	1	2	2
1.5 hours post-dose	2	2	3 [1]	8 [1]
8 hours post-dose	0	0	0	0
You are seeing flashes of light				
Pre-dose	0	0	0	0
1.5 hours post-dose	0	0	5 [1]	7 [1]
8 hours post-dose	0	0	0	0
You are seeing dark patches				
Pre-dose	0	0	0	0
1.5 hours post-dose	0	0	2 [1]	3 [1]
8 hours post-dose	0	0	0	0
You are having more difficulty than usual focusing				
Pre-dose	7 [1]	11 [2]	8	7 [1]
1.5 hours post-dose	6	7 [1]	14 [1]	10 [1]
8 hours post-dose	1	2	1	1
You would not feel safe driving a car with your vision as it is				
Pre-dose	5 [1]	4	3	5
1.5 hours post-dose	4	6	11 [1]	12 [1]
8 hours post-dose	3	1	0	1
Do you have any other visual symptoms or disturbance?				
Pre-dose	0	0	0	0
1.5 hours post-dose	0	0	2 [1]	3 [1]
8 hours post-dose	1	1	0	0

Table 6 Treatment groups multiple (ascending) dose study

Dose group	Treatment [A,C]	Study day [B]												
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	4 mg o.d.	x			x	x	x	x	x	x	x	x	X	x
2	8 mg o.d.	x			x	x	x	x	x	x	x	x	X	x
3	16 mg o.d.	x			x	x	x	x	x	x	x	x	X	x
4	12 mg o.d.	x												
	12 mg b.i.d				x	x	x	x	x	x	x	x	X	
	16mg o.d.													x
5 (dose titration)	8 mg b.i.d	x	x	x										
	12 mg b.i.d				x	x	x							
	16 mg b.i.d							x	x	x				
	20 mg b.i.d										x	x	X	
	30 mg o.d.													x

A o.d. = once daily; B.i.d. = twice daily; B x = dose administered; C doses for groups 4 and 5 were determined following blinded review of the data for groups 1 to 3

Table 7 Study design pharmacodynamic measurements multiple (ascending) dose study

	Days										
Assessment	-1	1	2	3	4	5,7,9,11	6,8,10	12	13	14	15
Visual assessments	On medical need only										
SCH 900435 dosing		X	X	X	X	X	X	X	X		
Pharmaco EEG	X [A]					Day 5 [A]			X [D]		
LSEQ					X		X		X		X
VAS, pupillometry [A]				X	X	Day 7		X			X
Visual questionnaire [A]		X		X	X	X	X	X	X		X
LH, FSH, testosterone [B]		X		X			X		X		X
PK sampling [C]		X	X	X	X	X	X	X	X	X	X
Cognitive Test battery [D]	X				X			X			

A Pre-dose and 0.5, 1, 2, 4 and 6 hours post-dose; B Groups I-IV only: Days 1, 3, 6, 8, 10, 13: pre-dose, 2, 6 and 12 h post-dose; Day 15:pre-dose; C PK sampling times were frequent pre-dose and post-dose and were different depending on the treatment group; D Pre-dose and 1, 4 and 6 hours post-dose (in the morning).

Table 8 Number of subjects (%) with AEs possible or probably related to SCH 900435 for 'eye disorders' and 'CNS disorders' in the multiple (ascending) dose study

	Treatment [A]					
	Placebo	4mg q.d.	8mg q.d.	16mg q.d.	12mg b.i.d.	Dose titration
	n=10	n=6	n=6	n=6	n=6	n=6
Eye disorder						
Accommodation disorder	0	0	0	0	1 (17)	0
Eye pain	0	0	0	0	0	2 (33)
Eyelid irritation	0	0	0	1 (17)	0	0
Ocular discomfort	0	0	0	0	1 (17)	0
Photopsia	0	0	0	3 (50)	0	1 (17)
Vision blurred	0	0	1 (17)	5 (83)	2 (33)	4 (67)
Visual disturbance	0	0	1 (17)	3 (50)	2 (33)	1 (17)
Central Nervous System disorders						
Balance disorders	0	0	0	0	1 (17)	0
Coordination abnormal	0	0	0	2 (33)	0	0
Dizziness	1 (10)	0	1 (17)	3 (50)	2 (33)	5 (83)
Dizziness postural	0	0	1 (17)	3 (50)	3 (50)	2 (33)
Headache	2 (20)	0	0	1 (17)	3 (50)	2 (33)
Lethargy	0	0	0	2 (33)	2 (33)	6 (100)
Memory impairment	0	0	0	0	0	1 (17)
Somnolence	1 (10)	0	0	4 (67)	1 (17)	3 (50)
Tremor	1 (10)	0	0	0	0	0
Tunnel vision	0	0	0	1 (17)	0	0

A. q.d. = once daily; b.i.d. = twice daily

Figure 1 Structural formula of SCH 900435

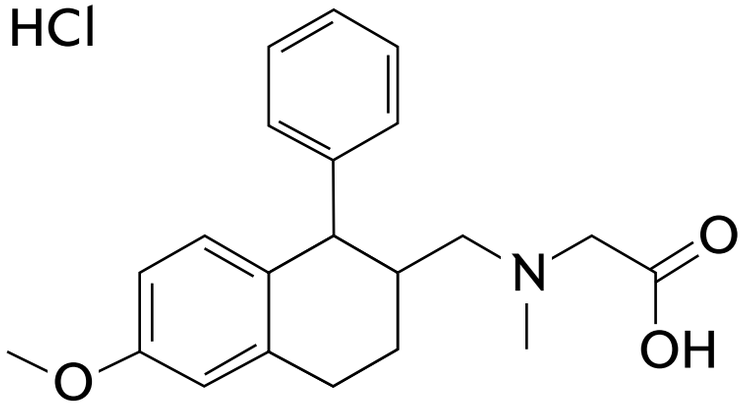


Figure 2 Mean Concentration-versus-Time plot in single ascending dose study

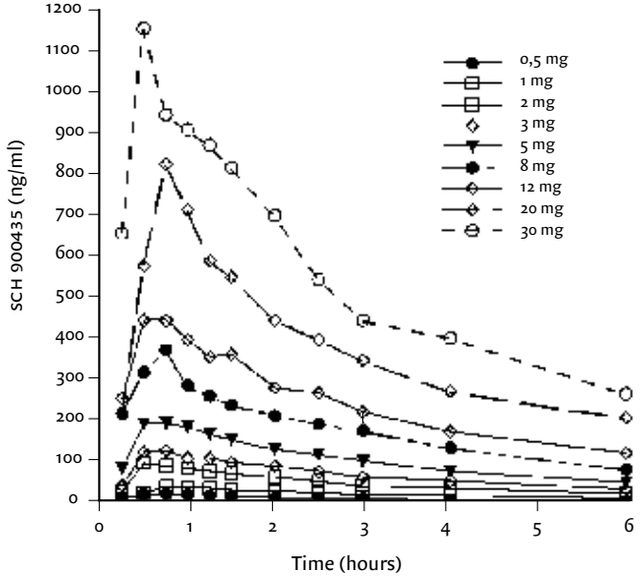


Figure 3 Average graph of AUE change from baseline 0-4 hours VAS Colours by log dose (standard deviations as error bars) in single ascending dose study

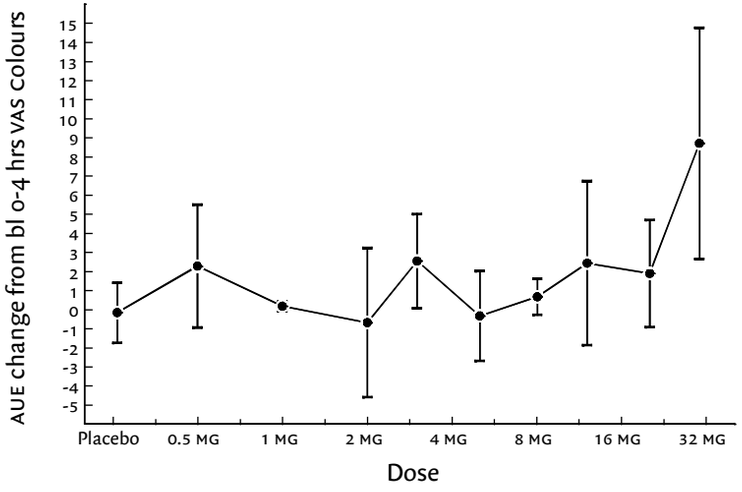


Figure 4 Adjusted (for baseline) mean time profile of different doses of VAS Colours with 95% CI for highest dose and lower 95% CI for lowest dose in single ascending dose study

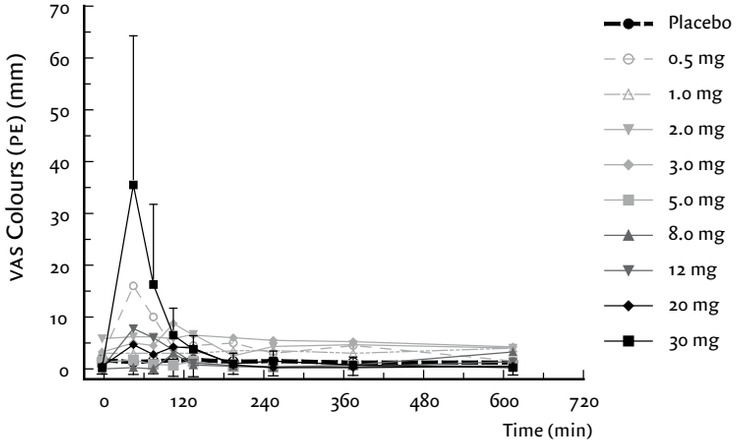


Figure 5 Adjusted (for baseline) mean time profile of different doses of LH with 95% CI for highest dose and lower 95% CI for lowest dose in single ascending dose study.

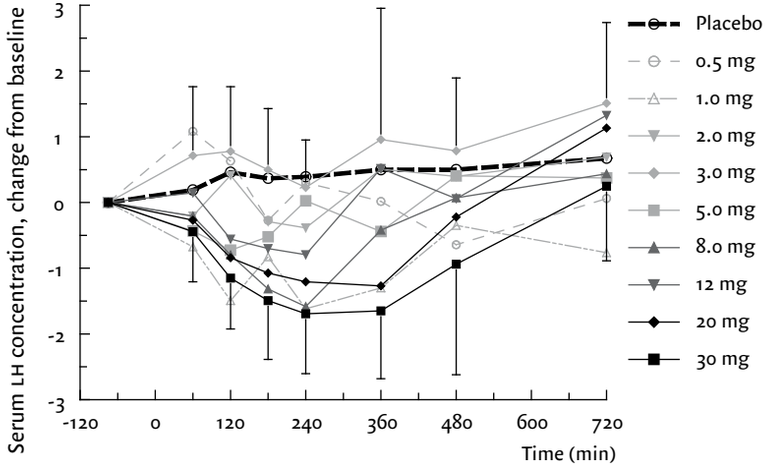


Figure 6 Average graph of AUE change from baseline 0-90 minutes pupil/iris ratio right eye by log dose (standard deviations as error bars) in single ascending dose study

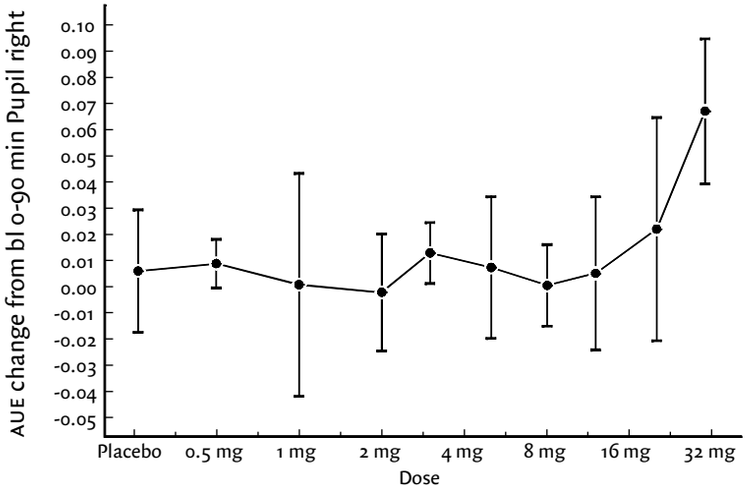
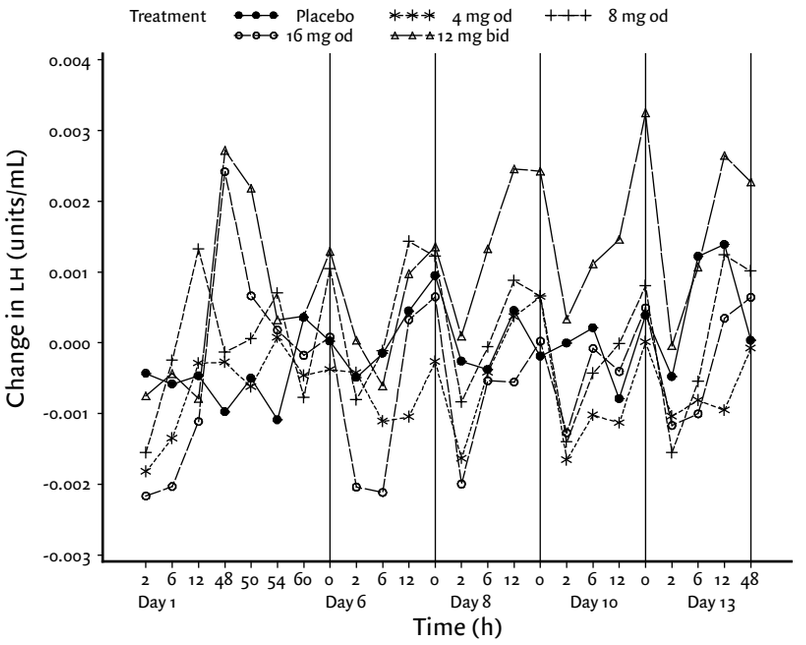


Figure 7 Mean changes from baseline LH values against time following administration of SCH 900435 or placebo in multiple ascending dose study



REFERENCES

1 Creese I, Burt DR, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 1976; 192: 481-3.
2 Seeman P, Chau-Wong M, Tedesco J, Wong K. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. Proc Natl Acad Sci U S A 1975; 72: 4376-80.
3 Javitt DC. Is the glycine site half saturated or half unsaturated? Effects of glutamatergic drugs in schizophrenia patients. Curr Opin Psychiatry 2006; 19: 151-7.
4 Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Jr., Charney DS. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994; 51: 199-214.
5 Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991; 148: 1301-8.
6 Krystal JH, D'Souza DC, Petrakis IL, Belger A, Berman RM, Charney DS, Abi-Saab W, Madonick S. NMDA agonists and antagonists as probes of glutamatergic dysfunction and pharmacotherapies in neuropsychiatric disorders. Harv Rev Psychiatry 1999; 7: 125-43.
7 Millan MJ. N-Methyl-D-aspartate receptors as a target for improved antipsychotic agents: novel insights and clinical perspectives. Psychopharmacology (Berl) 2005; 179: 30-53.
8 Schmidt WJ, Kretschmer BD. Behavioural pharmacology of glutamate receptors in the basal ganglia. Neurosci Biobehav Rev 1997; 21: 381-92.
9 Javitt DC, Hashim A, Sershen H. Modulation of striatal dopamine release by glycine transport inhibitors. Neuropsychopharmacology 2005; 30: 649-56.
10 Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry 1995; 52: 998-1007.
11 Adams B, Moghaddam B. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. J Neurosci 1998; 18: 5545-54.
12 Javitt DC. Glycine modulators in schizophrenia. Curr Opin Investig Drugs 2002; 3: 1067-72.
13 Smith KE, Borden LA, Hartig PR, Branchek T, Weinshank RL. Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. Neuron 1992; 8: 927-35.
14 Aragon C, Lopez-Corcuera B. Glycine transporters: crucial roles of pharmacological interest revealed by gene deletion. Trends Pharmacol Sci 2005; 26: 283-6.
15 Chen L, Muhlhauser M, Yang CR. Glycine tranporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons in vitro and in vivo. J Neurophysiol 2003; 89: 691-703.
16 Depoortere R, Dargazanli G, Estenne-Bouhtou G, Coste A, Lanneau C, Desvignes C, Poncelet M, Heaulme M, Santucci V, Decobert M, Cudennec A, Voltz C, Boulay D, Terranova JP, Stemmelin J, Roger P, Marabout B, Sevrin M, Vige X, Biton B, Steinberg R, Francon D, Alonso R, Avenet P, Oury-Donat F, Perrault G, Griebel G, George P, Soubrie P, Scatton B. Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-1 SSR504734, a potential new type of antipsychotic. Neuropsychopharmacology 2005; 30: 1963-85.
17 Harsing LG, Jr., Gacsalyi I, Szabo G, Schmidt E, Sziray N, Sebban C, Tesolin-Decros B, Matyus P, Egyed A, Spedding M, Levay G. The glycine transporter-1 inhibitors NFPS and Org 24461: a pharmacological study. Pharmacol Biochem Behav 2003; 74: 811-25.
18 Javitt DC, Balla A, Sershen H, Lajtha A. A.E. Bennett Research Award. Reversal of phencyclidine-induced effects by glycine and glycine transport inhibitors. Biol Psychiatry 1999; 45: 668-79.
19 Le Pen G, Kew J, Alberati D, Borroni E, Heitz MP, Moreau JL. Prepulse inhibition deficits of the startle reflex in neonatal ventral hippocampal-lesioned rats: reversal by glycine and a glycine transporter inhibitor. Biol Psychiatry 2003; 54: 1162-70.
20 Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 2004; 55: 452-6.
21 Lane HY, Chang YC, Liu YC, Chiu CC, Tsai GE. Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. Arch Gen Psychiatry 2005; 62: 1196-204.
22 Tsai GE, Yang P, Chang YC, Chong MY. D-alanine added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 2006; 59: 230-4.
23 Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA, Hayden DL, McCarley R, Coyle JT. A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. Arch Gen Psychiatry 1999; 56: 21-7.
24 Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 2001; 158: 1367-77.
25 Mizutani AR, Parker J, Katz J, Schmidt J. Visual disturbances, serum glycine levels and transurethral resection of the prostate. J Urol 1990; 144: 697-9.
26 Hahn RG, Andersson T, Sikk M. Eye symptoms, visual evoked potentials and EEG during intravenous infusion of glycine. Acta Anaesthesiol Scand 1995; 39: 214-9.
27 Dreier JP. The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. Nat Med 2011; 17: 439-47.
28 Shen W, Jiang Z. Characterization of glycinergic synapses in vertebrate retinas. J Biomed Sci 2007; 14: 5-13.
29 Korol S, Leuenberger PM, Englert U, Babel J. In vivo effects of glycine on retinal ultrastructure and averaged electroretinogram. Brain Res 1975; 97: 235-51.
30 Wachtmeister L, Dowling JE. The oscillatory potentials of the mudpuppy retina. Invest Ophthalmol Vis Sci 1978; 17: 1176-88.
31 Ouellet D, Sutherland S, Wang T, Griffini P, Murthy V. First-Time-in-Human Study With GSK1018921, a Selective $GlyT_1$ Inhibitor: Relationship Between Exposure and Dizziness. Clin Pharmacol Ther 2011; 90: 597-604.
32 van Berckel BN, Lipsch C, Timp S, Gispen-de Wied C, Wynne H, van Ree JM, Kahn RS. Behavioral and neuroendocrine effects of the partial NMDA agonist D-cycloserine in healthy subjects. Neuropsychopharmacology 1997; 16: 317-24.
33 van Berckel BN, Oranje B, van Ree JM, Verbaten MN, Kahn RS. The effects of low dose ketamine on sensory gating, neuroendocrine secretion and behavior in healthy human subjects. Psychopharmacology (Berl) 1998; 137: 271-81.
34 van Berckel BN, Lipsch C, Gispen-de Wied C, Wynne HJ, Blankenstein MA, van Ree JM, Kahn RS. The partial NMDA agonist D-cycloserine stimulates LH secretion in healthy volunteers. Psychopharmacology (Berl) 1998; 138: 190-7.
35 Bonavera JJ, Swerdloff RS, Sinha Hakim AP, Lue YH, Wang C. Aging results in attenuated gonadotropin releasing hormone-luteinizing hormone axis responsiveness to glutamate receptor agonist N-methyl-D-aspartate. J Neuroendocrinol 1998; 10: 93-9.
36 Bettendorf M, de Zegher F, Albers N, Hart CS, Kaplan SL, Grumbach MM. Acute N-methyl-D,L-aspartate administration stimulates the luteinizing hormone releasing hormone pulse generator in the ovine fetus. Horm Res 1999; 51: 25-30.
37 Tornoe CW, Agerso H, Senderovitz T, Nielsen HA, Madsen H, Karlsson MO, Jonsson EN. Population pharmacokinetic/pharmacodynamic (PK/PD) modelling of the hypothalamic-pituitary-gonadal axis following treatment with GnRH analogues. Br J Clin Pharmacol 2007; 63: 648-64.
38 le Cotonnec JY, Loumaye E, Porchet HC, Beltrami V, Munafo A. Pharmacokinetic and pharmacodynamic interactions between recombinant human luteinizing hormone and recombinant human follicle-stimulating hormone. Fertil Steril 1998; 69: 201-9.
39 Brann DW, Mahesh VB. Glutamate: a major neuroendocrine excitatory signal mediating steroid effects on gonadotropin secretion. J Steroid Biochem Mol Biol 1995; 53: 325-9.
40 Lechner SM. Glutamate-based therapeutic approaches: inhibitors of glycine transport. Curr Opin Pharmacol 2006; 6: 75-81.
41 Eulenburg V, Armsen W, Betz H, Gomeza J. Glycine transporters: essential regulators of neurotransmission. Trends Biochem Sci 2005; 30: 325-33.
42 Davanger S, Storm-Mathisen J, Ottersen OP. Colocalization of glutamate and glycine in bipolar cell terminals of the human retina. Exp Brain Res 1994; 98: 342-54.
43 Buchanan RW, Javitt DC, Marder SR, Schooler NR, Gold JM, McMahon RP, Heresco-Levy U, Carpenter WT. The Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST): The Efficacy of Glutamatergic Agents for Negative Symptoms and Cognitive Impairments. Am J Psychiatry 2007; 164: 1593-602.
44 Duncan EJ, Szilagyi S, Schwartz MP, Bugarski-Kirola D, Kunzova A, Negi S, Stephanides

M, Efferen TR, Angrist B, Peselow E, Corwin J, Gonzenbach S, Rotrosen JP. Effects of D-cycloserine on negative symptoms in schizophrenia. Schizophr Res 2004; 71: 239-48.

REFERENCES APPENDICES

1 Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 1971; 10: 181-91.
2 Bond A, Lader M. The use of analogue scales in rating subjective feelings. Br J Med Psychol 1974; 47: 211-8.
3 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. Clin Pharmacol Ther 1991; 50: 172-80.
4 de Haas SL, de Visser SJ, van der Post JP, de Smet M, Schoemaker RC, Rijnbeek B, Cohen AF, Vega JM, Agrawal NG, Goel TV, Simpson RC, Pearson LK, Li S, Hesney M, Murphy MG, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_{(A)}$ alpha(2,3) subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. J Psychopharmacol 2007; 21: 374-83.
5 de Visser SJ, van der PJ, Pieters MS, Cohen AF, van Gerven JM. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 2001; 51: 119-32.
6 Liem-Moolenaar M, de BP, Timmers M, Schoemaker RC, van Hasselt JG, Schmidt S, van Gerven JM. Pharmacokinetic-pharmacodynamic relationships of central nervous system effects of scopolamine in healthy subjects. Br J Clin Pharmacol 2011; 71: 886-98.
7 Zuurman L, Roy C, Schoemaker RC, Hazekamp A, Den Hartigh J, Bender JC, Verpoorte R, Pinquier JL, Cohen AF, van Gerven JM. Effect of intrapulmonary tetrahydrocannabinol administration in humans. J Psychopharmacol 2008; 22: 707-16.
8 Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology 1998; 88: 82-8.
9 de Haas S, Schoemaker R, van GJ, Hoever P, Cohen A, Dingemanse J. Pharmacokinetics, pharmacodynamics and the pharmacokinetic/pharmacodynamic relationship of zolpidem in healthy subjects. J Psychopharmacol 2009.
10 Zisapel N, Laudon M. Subjective assessment of the effects of CNS-active drugs on sleep by the Leeds sleep evaluation questionnaire: a review. Hum Psychopharmacol 2003; 18: 1-20.
11 Cohen AF, Ashby L, Crowley D, Land G, Peck AW, Miller AA. Lamotrigine (BW430C), a potential anticonvulsant. Effects on the central nervous system in comparison with phenytoin and diazepam. Br J Clin Pharmacol 1985; 20: 619-29.
12 Sobczak S, Honig A, Schmitt JA, Riedel WJ. Pronounced cognitive deficits following an intravenous L-tryptophan challenge in first-degree relatives of bipolar patients compared to healthy controls. Neuropsychopharmacology 2003; 28: 711-9.
13 Wright BM. A simple mechanical ataxia-meter. J Physiol 1971; 218: 27P-8P.
14 Hermann WM. Development and critical evaluation of an objective procedure for the electroencephalographic classification of psychotropic drugs. 1982; 249-351.
15 van Steveninck A. Methods of assessment of central nervous system effects of drugs in man. Thesis Leiden University 1994.
16 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 1999; 13: 10-7.
17 van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MS, Kroon JM, Breimer DD, Cohen AF. Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. Psychopharmacology (Berl) 1993; 110: 471-8.
18 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003; 55: 39-50.
19 Gijsman HJ, van Gerven JM, Verkes RJ, Schoemaker RC, Pieters MS, Pennings EJ, Hessing TJ, Cohen AF. Saccadic peak velocity and EEG as end-points for a serotonergic challenge test. Hum Psychopharmacol 2002; 17: 83-9.
20 de Visser SJ, van Gerven JM, Schoemaker RC, Cohen AF. Concentration-effect relationships of two infusion rates of the imidazoline antihypertensive agent rilmenidine for blood pressure and development of side-effects in healthy subjects. Br J Clin Pharmacol 2001; 51: 423-8.
21 Kemme MJ, vd Post JP, Schoemaker RC, Straub M, Cohen AF, van Gerven JM. Central nervous system effects of moxonidine experimental sustained release formulation in patients with mild to moderate essential hypertension. Br J Clin Pharmacol 2003; 55: 518-25.
22 van der Post JP, de Visser SJ, Schoemaker RC, Cohen AF, van Gerven JM. Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. J Psychopharmacol 2004; 18: 221-7.
23 Liem-Moolenaar M, Gray F, de Visser S, Franson K, Schoemaker R, Schmitt J, Cohen A, van Gerven J. Psychomotor and cognitive effects of a single oral dose of talnetant (SB223412) in healthy volunteers compared with placebo or haloperidol. J Psychopharmacol 2008.
24 Cutmore TR, Beninger RJ. Do neuroleptics impair learning in schizophrenic patients? Schizophr Res 1990; 3: 173-86.
25 FITTS PM. The information capacity of the human motor system in controlling the amplitude of movement. J Exp Psychol 1954; 47: 381-91.
26 Fitts PM, Peterson JR. Information capacity of discrete motor responses. J Exp Psychol 1964; 67: 103-12.
27 de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, Murphy MG, de Smet M, Vessey LK, Ramakrishnan R, Xue L, Cohen AF, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of MK-0343, a gaba(a) $alpha_{2,3}$ subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacol 2008; 22: 24-32.
28 Twa md, Bailey MD, Hayes J, Bullimore M. Estimation of pupil size by digital photography. J Cataract Refract Surg 2004; 30: 381-9.
29 van Berckel BN, Lipsch C, Gispen-de Wied C, Wynne HJ, Blankenstein MA, van Ree JM, Kahn RS. The partial NMDA agonist D-cycloserine stimulates LH secretion in healthy volunteers. Psychopharmacology (Berl) 1998; 138: 190-7.
30 van Berckel BN, Oranje B, van Ree JM, Verbaten MN, Kahn RS. The effects of low dose ketamine on sensory gating, neuroendocrine secretion and behavior in healthy human subjects. Psychopharmacology (Berl) 1998; 137: 271-81.
31 Marmor MF, Zrenner E. Standard for clinical electroretinography (1999 update). International Society for Clinical Electrophysiology of Vision. Doc Ophthalmol 1998; 97: 143-56.
32 Holder GE. The importance of dark adapted cone function in ERG interpretation. Acta Ophthalmologica 2011; 89.
33 Downes SM, Holder GE, Fitzke FW, Payne AM, Warren MJ, Bhattacharya SS, Bird AC. Autosomal dominant cone and cone-rod dystrophy with mutations in the guanylate cyclase activator 1A gene-encoding guanylate cyclase activating protein-1. Arch Ophthalmol 2001; 119: 96-105.
34 Gouras P. The role of S-cones in human vision. Doc Ophthalmol 2003; 106: 5-11.
35 Yamamoto S, Hayashi M, Tsuruoka M, Ogata K, Tsukahara I, Yamamoto T, Takeuchi S. Selective reduction of S-cone response and on-response in the cone electroretinograms of patients with X-linked retinoschisis. Graefes Arch Clin Exp Ophthalmol 2002; 240: 457-60.
36 Holder GE, Brigell MG, Hawlina M, Meigen T, Vaegan, Bach M. ISCEV standard for clinical pattern electroretinography--2007 update. Doc Ophthalmol 2007; 114: 111-6.
37 Arden G, Gunduz K, Perry S. Color vision testing with a computer graphics system: preliminary results. Doc Ophthalmol 1988; 69: 167-74.
38 Tildesley NT, Kennedy DO, Perry EK, Ballard CG, Wesnes KA, Scholey AB. Positive modulation of mood and cognitive performance following administration of acute doses of Salvia lavandulaefolia essential oil to healthy young volunteers. Physiol Behav 2005; 83: 699-709.