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CHAPTER 4

Central nervous system effects of ethanol at a pseudo-steady state concentration using ethanol clamping in healthy volunteers

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ABSTRACT

BACKGROUND In determining the acute effects of alcohol, it is helpful if alcohol concentrations are maintained at stable levels, to facilitate the interpretation of the results. Recently, an alcohol clamping method was developed that resulted in stable alcohol concentrations for hours. In this study, a range of central nervous system (CNS) effects were tested under pseudo-steady state conditions.

METHODS To achieve a pseudo-steady state of 0.6 $g{\cdot}L^{-1}$, breath alcohol concentrations (BrAC) were frequently measured, and fed back into a spreadsheet-based program to guide intravenous dosing. CNS-effects were frequently measured throughout the clamp.

RESULTS The clamping paradigm resulted in a pseudo-steady state BrAC of 0.61 $g{\cdot}L^{-1}$ (CV: 6.2%). A plateau was maintained from 25 - 300 min. and caused significant effects on smooth pursuit eye movements (-9.7% (95% CI: -12.4, -7.1)), adaptive tracking (-3.4% (95% CI: -4.5, -2.2)), VAS alertness (-13 mm (95% CI: -20, -6)), VAS alcohol effects (16 mm (95% CI: 7, 25)) and body sway (21.3% (95% CI: 1.8, 45)). Some effects (like smooth pursuit eye movements) closely followed the relatively stable alcohol concentrations, whereas others (such as body sway and VAS alcohol effects) fluctuated during the plateau-phase.

CONCLUSIONS Most CNS-effects of alcohol showed a trend to change over time, despite stable concentrations. Other variables remained stable under pseudo-steady state conditions. The intravenous clamping method provides careful control over BrAC levels and allows frequent repetition of different CNS-measurements. These features make this technique eminently suitable to study the complex pharmacodynamic effects of acute alcohol administration.

INTRODUCTION

To determine the effects of alcohol or to assess alcohol-drug interactions, it is helpful to maintain alcohol plasma concentration at reasonably steady state levels, since most of the effects of alcohol are concentration dependent. Nonetheless, few studies of alcohol effects or drug interactions are actually performed under (pseudo) steady state conditions. In many cases, alcohol exposure is controlled by adaptation of the dose to weight, and sometimes to sex or other demographic variables (Wilkinson, 1980). Measurements of the alcohol effects are usually performed at fixed time intervals after intake. Often, breath alcohol levels are measured, but plasma levels vary after administration and efforts to maintain constant levels are rare (Wilkinson, 1980).

Based on the principles described by O'Connor *et al.* (O'Connor *et al.*, 1998), we recently developed a method, which is able to maintain constant levels of alcohol for hours by clamping the breath alcohol concentration (BrAC). This new clamping procedure demonstrated a stable and reliable pseudo-steady state alcohol concentration profile for five hours, with low variability. The procedure is described elsewhere in more detail (Zoethout *et al.*, 2008). The pharmacodynamic results that were obtained during this procedure are reported here.

Alcohol has many measurable CNS-effects in healthy subjects. Numerous test methods have been developed to study these parameters like alertness, oculomotor function, (visio-)motor control, attention and subjective effects (de Visser *et al.*, 2001; Heishman *et al.*, 1997; Shamsi *et al.*, 2001). All of these CNS-domains are covered in the 'Neurocart' test battery that has been developed at the Centre for Human Drug Research (CHDR) to examine different kinds of CNS-active drugs (de Haas *et al.*, 2007; van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1996). In the current exploratory study, these methods were used to determine the pharmacodynamic CNS-effects of alcohol at a pseudo-steady state level of 0.6 $g{\cdot}L^{-1}$ for five hours, and to investigate whether this intensive battery of CNS-measurements could be performed during clamping, without affecting its concomitant execution or vice versa.

METHODS

Design

The study was a randomised, double-blind, placebo-controlled, two-way cross-over study in 12 healthy volunteers, with a minimum washout period of two days.

Subjects

Six healthy female and six healthy male volunteers, between 18 and 39 years of age gave oral and written informed consent after approval of the study protocol by the Medical Ethics Review Board of the Leiden University Medical Centre (LUMC). Before inclusion, subjects were screened by medical history and physical examination and participated in a pharmacodynamic training session. All 12 subjects were found eligible to participate and entered the study. Subjects were familiar with the effects of alcohol and were instructed not to use more than two alcohol consumptions a day, for at least two days prior to the study occasions. Twelve hours prior to each study start, the use of alcohol was prohibited. The oral use of alcohol and xanthine containing beverages was not allowed on study days. On the evening following each test day, driving and the handling of machines were not permitted.

General Procedure

All subjects reported at the research unit at 08:00 in the morning of each test day. After a short introduction, EEG- and eye-electrodes were mounted. Around 09:00 a standard breakfast was provided. The alcohol or placebo infusion started between 09:30 and 10:00. Alcohol breath samples and pharmacodynamic measurements were obtained at regular time points. A standardised lunch was given around 3.5 hours after starting the infusion. The administration of alcohol or placebo ended five hours after the start of the infusion. All subjects were taken home by taxi at the end of the study day. The second study occasion was performed in an identical way.

Study Treatments

An ethanol solution (10% w/v in 5% glucose) was prepared and was infused on study days. Infusion was based on an alcohol breath clamping paradigm to achieve a target level of 0.6 $g{\cdot}L^{-1}$ (Zoethout *et al.*, 2008). Glucose 5% was used as a placebo, employing a sham-procedure including adaptations of placebo infusion rates based on a simulated spreadsheet. The infusion rates between 0 and 5 minutes and between 5 and 10 minutes were fixed at 72 $g{\cdot}hr^{-1}$ and 60 $g{\cdot}hr^{-1}$, respectively. Hereafter, rates were modified at 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240 and 270 minutes, according to the obtained BRAC samples, which were measured at the same timepoints. During study days, a parallel glucose infusion (glucose 5%) was administered to all subjects during the first ten minutes post-start, to prevent pain or discomfort in the infusion arm that may be caused by the high-flow alcohol infusion during the loading phase.

ALCOHOL INFUSION

During the intravenous infusion procedure, online adjustments in infusion rates were made using BRAC samples as a guideline to maintain a pseudo-steady state alcohol level of 0.6 $g{\cdot}L^{-1}$, according to a recently introduced infusion paradigm, which was adapted from a method originally described by O'Connor (O'Connor *et al.*, 1998). The O'Connor method is based upon the theory that for substances with marked saturable elimination in the relevant concentration range (like alcohol), an approximately linear relationship exists between the applied infusion rate and the resulting change in alcohol concentration. When alcohol elimination is fully saturated, it is excreted at a constant ($g{\cdot}min^{-1}$) rate, independent of concentration. Therefore, when the input ($g{\cdot}min^{-1}$) is changed, this will result in a proportional change in alcohol

concentration. The change in alcohol level required to achieve the target concentration can then be used to back-extrapolate the infusion rate that corresponds with, and should hence lead to this necessary change. Although accurate results were obtained by O'Connor, an increase in sampling interval to 30 minutes (after steady state was reached) would improve the feasibility of other pharmacodynamic or functional assessments, as a ten minute interval (used by O'Connor) may interfere with the required measurements. However, simulations indicated that the original O'Connor procedure would result in infusion rate suggestions that would be too large, and that the increase to a 30 minute measurement interval would result in alcohol increases that would be difficult to recover from (even by shutting down the alcohol infusion for the next 30 minutes). As a result, an adapted, spreadsheet-based regime was empirically determined consisting of a time dependent reduction of the suggested O'Connor infusion rate. This new BrAC clamping method is described in detail elsewhere (Zoethout *et al.*, 2008). In the current study, it was tested for its feasibility to perform an intensive battery of CNS measurements concomitantly.

ALCOHOL CONCENTRATIONS Both breath alcohol concentrations and plasma alcohol levels were obtained for pharmacokinetic analysis during each study day at t = 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450 and 480 minutes.

BREATH ALCOHOL CONCENTRATIONS (BrAC) Online adaptation of the BrAC was based on measurements with a hand-held Alco-Sensor IV meter (Honac, Apeldoorn, the Netherlands), which had a lower limit of quantification (LLQ) of 0.01 $g{\cdot}L^{-1}$. The BrAC was entered into a spreadsheet, which calculated the corresponding infusion rate to maintain the BrAC at 0.6 $g{\cdot}L^{-1}$. Because sampling intervals shorter than five minutes (occurring during the initial part of the infusion) cause the BrAC meter to show fatigue, two different measurement devices were alternated. A pilot study was performed prior to the start of the study, in which no fatigue was observed

during alternation of both BrAC devices according to the sampling scheme of the study. A research assistant, who was not involved in any other activity was made responsible for the BrAC measurements and the execution of the clamp (or the sham procedure (during placebo administration)), to maintain blinding of the study participant and the research team members during the investigation. The BrAC devices were calibrated prior to the start of the study.

BLOOD ALCOHOL CONCENTRATIONS (BAC) Blood samples were collected in 8.5 ml SST® Gel and Clot Activator tubes (Becton and Dickinson) and were allowed to clot for at least 30 minutes (max. 60 minutes) at room temperature. Subsequently, samples were centrifuged at 2800G for ten minutes at 4°C. Tubes were then stored refrigerated until alcohol concentrations were determined within 24 hours after collection, in the Central Laboratory for Clinical Chemistry of the LUMC. An enzymatic assay using a Modular P analyzer (Roche, Switzerland) was employed, where ethyl alcohol and NAD were converted to acetaldehyde and NADH by alcohol dehydrogenase. NADH formed during the reaction was measured photometrically as a rate of change in absorbance, which is directly proportional to the ethyl alcohol concentration. The analyzer's LLQ was 0.1 $g{\cdot}L^{-1}$.

CNS-pharmacodynamics

The 'Neurocart' is a battery of sensitive tests for a wide range of CNS-domains that has been developed at CHDR to examine different kinds of CNS-active drugs (de Haas *et al.*, 2007; van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1996). The following tests were performed twice at baseline, and repeated hourly during the plateau and washout phases in a quiet room with ambient illumination, in the following order:

BODY SWAY Body sway was measured with an apparatus similar to the Wright ataxiameter (Wright, 1971). The body sway meter allows measurement of body movements in a single plane, providing a measure of postural

stability. During sway measurements, subjects were instructed to keep their eyes closed for two minutes. Body sway measurements were obtained twice pre-dose and at t = 5, 15, 25, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450 and 480 minutes.

SYMBOL-DIGIT SUBSTITUTION TEST (SDST) The symbol-digit (SDST) and digit-symbol substitution tests (DSST) have frequently been used in human psychopharmacology for establishing the effects of a wide range of psychotropic drugs on CNS-functioning (Magliozzi *et al.*, 1989; Jalava *et al.*, 1995). During SDST measurements, selected symbols appear in random order on a computer screen. Subjects were instructed to reproduce the digits associated with the symbols by using a symbol-digit code. The numbers of attempted and correct responses were recorded to assess attention and executive functions, for a period of 90 seconds. SDST measurements were obtained twice pre-dose and at t = 60, 120, 180, 240, 300, 360, 420 and 480 minutes.

VISUAL ANALOGUE SCALES (VAS) Visual analogue scales, as originally described by Norris (Norris, 1971) have been used previously to quantify subjective effects of a variety of sedative agents (van Steveninck *et al.*, 1991; van Steveninck *et al.*, 1996; van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1999). In the current study alertness, mood and calmness were derived from a VAS previously described by Bond and Lader (Bond and lader, 1974). These factors were used to quantify subjective drug effects. The specific subjective effects of alcohol were assessed by a VAS alcohol effects, asking the subject to indicate 'how large is the effect of alcohol that you feel?' All scales consist of 10 cm line segments. Subjects mark a point on the line that best represents their subjective state corresponding to the condition tested. The result is a distance calculated from the mark on the line. The VAS measurements were obtained twice pre-dose and at t = 60, 120, 180, 240, 300, 360, 420 and 480 minutes.

ADAPTIVE TRACKING The adaptive tracking test was performed as originally described by Borland and Nicholson (Borland and Nicholson, 1984), using customised equipment and software (Hobbs, 2000, Hertfordshire, UK). Adaptive tracking is a pursuit tracking task that has proved to be useful for measurement of CNS-effects of alcohol, various psychoactive drugs and sleep deprivation (van Steveninck *et al.*, 1991; van Steveninck *et al.*, 1999). Each test was preceded by a run-in period. Performance was scored after a fixed period of five minutes and reflected visuo-motor control and vigilance. The average performance scores were used in the analysis. Adaptive tracking measurements were obtained twice pre-dose and at t = 60, 120, 180, 240, 300, 360, 420 and 480 minutes.

SACCADIC EYE MOVEMENTS Saccadic eye movements were recorded using a microcomputer-based system, customised CED software for data sampling and analysis (Cambridge Electronics Design, Cambridge, UK), Nihon Kohden equipment for stimulus display, signal collection and amplification (Nihon Kohden Corporation, Tokyo, Japan), and disposable surface electrodes for registration of the electro-oculographic signals (Medicotest N-OO-S, Olstykke, Denmark). Average values of latency (reaction time), saccadic peak velocity and inaccuracy (difference between stimulus angle and corresponding saccade in percentages) were calculated for all artefact-free saccades. Saccadic peak velocity has been validated as one of the most sensitive parameters for sedation (de Haas *et al.*, 2007; van Steveninck *et al.*, 1996; van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1991; van Steveninck *et al.*, 1999). Saccadic eye movements were performed twice pre-dose and at t = 60, 120, 180, 240, 300, 360, 420 and 480 minutes.

SMOOTH PURSUIT EYE MOVEMENTS For smooth pursuit eye movements, the target moves sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5 degrees eyeball rotation to both sides. Four cycles were recorded for each

stimulus frequency. The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as a parameter. This parameter can be used as an accurate biomarker for oculomotor function and attention (Lehtinen *et al.*, 1982). The method has been validated earlier (van Steveninck *et al.*, 1989; van Steveninck, 1993) based on the work of Bittencourt *et al.* (Bittencourt *et al.*, 1983) and the original description of Baloh *et al.* (Baloh *et al.*, 1975). Smooth pursuit eye movements were performed twice pre-dose and at t = 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420 and 480 minutes.

ELECTROENCEPHALOGRAPHY (EEG) Pharmaco-electroencephalography was measured to provide non-specific measures of CNS-functions (Cohen *et al.*, 1985). EEG signals were obtained from leads F_Z - C_Z and P_Z - O_Z. The signals were amplified using a Nihon Kohden AB-621G bioelectric amplifier (Nihon Kohden Corporation, Tokyo, Japan) with a time constant of 0.3 seconds and a low pass filter at 100 Hz. For the fast Fourier analysis, data collection and analysis were performed using customised CED software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 laboratory interface (Cambridge Electronics Design, Cambridge, UK). Data blocks containing artefacts were identified by visual inspection and these were excluded from the analysis. Fast Fourier transform analysis was performed to obtain the sum of amplitudes in the delta (0.5 - 3.5 Hz), theta (3.5 - 7.5 Hz), alpha (7.5 - 11.5 Hz) and beta (11.5 - 30 Hz) frequency ranges. The duration of EEG measurements was two minutes per session. EEG measurements were obtained twice pre-dose and at t = 60, 120, 180, 240, 300, 360, 420 and 480 minutes.

Statistical analysis

The pharmacodynamic endpoints were analysed by mixed model analyses of variance (using SAS PROC MIXED) with subject, subject by treatment and subject by time as random effects, with treatment, occasion, time and treatment by time as fixed effects, and the average baseline value was included as covariate. Time and treatment by time effects were only included for graphical representation of the baseline corrected data; the contrast between alcohol and placebo was calculated within the statistical mixed model. All statistical analyses were performed using SAS version 8.2 (SAS Institute Inc., Cary, NC., USA).

The different elements in our mixed model were implemented to explain part of the variation and to estimate the correct residual variation to test the fixed effect variation against. The only tested factor of interest though, was the treatment effect; all other factors were only used to attribute the correct amount of variation to the treatment and to calculate the right residual term to test the treatment effect against. The statistical hypothesis tested in this study was 'there is no difference between alcohol and placebo'.

RESULTS

Subjects

Subjects participating in the study were on average 22 years old (range: 18 - 39 years old), had an average weight of 76 kg (range: 56 - 89 kg) and an average height of 173 cm (range: 161 - 195 cm).

One subject dropped out after the first occasion, because of personal reasons. This subject was replaced by another healthy volunteer, who received the same order of treatments. Another subject repeated the first occasion, because this subject turned out not to have slept the night before the occasion, despite instructions to maintain a regular diurnal rhythm during the study period. All other subjects completed the study according to the protocol.

Safety

No serious adverse reactions occurred during the study. Frequently reported adverse events after alcohol treatment included inebriation, a painful arm at the start of the infusion, sleepiness and headache. These symptoms were all transient and mild in severity.

Alcohol concentrations

The average BrAC and BAC profiles are presented in figure 1. A pseudo-steady state level of approximately 0.6 $g{\cdot}L^{-1}$ for both the BrAC and the BAC measurements can be observed from the figure. This level was reached within approximately 25 minutes after the start of the alcohol infusion and was maintained until the end of the infusion at 300 minutes. The intravenous loading regimen did not produce an overshoot. The mean BrAC level during the pseudo-steady state phase (i.e. from 25 to 300 minutes) was found to be 0.606 $g{\cdot}L^{-1}$ (SD: 0.038 $g{\cdot}L^{-1}$, range: 0.54 - 0.67 $g{\cdot}L^{-1}$), with a coefficient of variance (CV) of 6.2%. The mean BAC level during this period was 0.628 $g{\cdot}L^{-1}$ (SD: 0.042 $g{\cdot}L^{-1}$, range: 0.56 - 0.69 $g{\cdot}L^{-1}$) with a CV of 6.7%. The BrAC curve slightly but consistently exceeded the BAC curve on the ascending limb of the concentration-time figure. In contrast, during the pseudo-steady state phase and the descending limb, all parts of the BAC curve exceeded the BrAC curve. However the difference was too small in view of the variability to reach statistical significance (p = 0.18). Rejection of the null hypothesis would have provided support for the possibility that BrAC and BAC results are not necessarily identical at the same sampling timepoint.

The applied infusion rates during clamping may serve as an indirect parameter for alcohol clearance. This parameter can be used to compare alcohol metabolism between certain subgroups. Differences in alcohol metabolism between sex and race or changes in alcohol pharmacokinetics due to drug-alcohol interactions can be assessed and quantified, using infusion rates as a marker. In this study the mean individual infusion rates needed to maintain a stable BrAC during the plateau phase were somewhat higher for males than for females (143.5 $mL{\cdot}hr^{-1}$ (SD: 36.1) and 108.3 $mL{\cdot}hr^{-1}$ (SD: 23.7) respectively), but the difference between men and women did not reach significance in this small group (p = 0.08).

CNS-pharmacodynamics

Pharmacodynamic test results are summarised in table 1.

VISUAL ANALOGUE SCALES (VAS) Figure 2 shows a significant average reduction of 13 mm (95% CI: -20, -6) on the VAS alertness after the administration of alcohol, compared to placebo. The VAS alcohol effects increased significantly after alcohol treatment compared to placebo (16 mm (95% CI: 7, 25)), as can be observed from figure 3. Both the VAS alertness and the VAS alcohol effects showed variations in effect over time, despite stable alcohol levels. VAS mood and VAS calmness were not significantly affected by alcohol.

BODY SWAY The body sway results were log-transformed prior to the analysis to meet the ANOVA requirements. The results are presented in figure 4. A significant mean increase in body sway of 21.3% (95% CI: 1.8%, 45%) was observed after alcohol treatment compared to placebo. The effects of alcohol on body sway appeared to vary despite pseudo-steady state BrAC levels.

ADAPTIVE TRACKING Alcohol reduced adaptive tracking scores significantly by 3.4% (95% CI: -4.5%, -2.2%) compared to placebo. These results are presented in figure 5. Tracking scores seemed to fluctuate during the plateau phase. The effect of alcohol on adaptive tracking showed a profile that was reminiscent of an inverted body sway profile.

SMOOTH PURSUIT EYE MOVEMENTS Alcohol produced a significant reduction in percentage smooth pursuit of 9.7% (95% CI: -12.4%, -7.1%)

compared to placebo (figure 6). Throughout the plateau phase, average smooth pursuit reductions fluctuated between -5.4% and -12.6%, but these reductions in smooth pursuit performance remained relatively stable throughout the clamp.

SACCADIC EYE MOVEMENTS Saccadic peak velocity seemed to decrease somewhat under alcohol treatment, but no significant effects were observed on any of the saccadic eye movement outcome parameters.

ELECTROENCEPHALOGRAPHY (EEG) No significant alcohol effects on the electroencephalography measurements were observed.

SYMBOL-DIGIT SUBSTITUTION TEST (SDST) The symbol-digit substitution test was not significantly affected by alcohol.

Time- and concentration-related changes in CNS-effects

Most CNS-effects of alcohol (like body sway and VAS alcohol effects) showed some variations despite stable concentrations, indicating that the concentration dependence of these effects varies with time. Other parameters (such as smooth pursuit eye movements) remained stable under pseudo-steady state conditions. Since this study was not primarily designed as such, no detailed analyses of concentration-effect relationships were performed. Formal pharmacokinetic/pharmacodynamic (PK/PD) analyses would have required more frequent pharmacodynamic measurements during the ascending part of the concentration-time curve, and/or more fluctuations at different alcohol levels. With these limitations, these changes were examined *post hoc* in an exploratory fashion, by plotting average alcohol levels against the various concomitant average CNS-effects to describe PK/PD relations. The average blood alcohol levels at the times of CNS-testing were calculated by linear interpolation from adjacent measurements. Figure 7-10 show the relationships between the average blood alcohol levels and the different CNS-measurements. The shapes of these time-effect-curves confirm the observation that most CNS-effects did not remain as stable as the alcohol levels during the plateau phase of the alcohol clamp.

DISCUSSION

The alcohol breath clamping method, which is described elsewhere in more detail (Zoethout *et al.*, 2008), produced an accurate and stable alcohol serum concentration of 0.6 $g \cdot L^{-1}$ over a five hour period. The procedure resulted in constant alcohol levels, and did not produce any relevant concentration over- or undershoot in any individual. Although the exact cause remains unknown, BrAC curve deviations from the BAC curve were observed in this study. This phenomenon has already been reported in literature (Jones and Andersson, 2003) and is explained by intra- and inter-individual differences in e.g. body temperature (Fox and Hayward, 1987; Fox and Hayward, 1989) and breathing technique (Jones, 1982; Mulder and Neuteboom, 1987). These factors change the course of the BrAC profile, leaving the BAC profile unaffected. Moreover, ethanol is able to induce changes in these physiological factors that may affect the BrAC curve even more. However, we would like to stress that the differences between the breath and serum estimates were only relatively small.

Authors like Jones (Jones, 1997) and Ramchandani (Ramchandani *et al.*, 2001) clearly describe the effect of food on alcohol metabolic rates. They argue that the feeding state influences hepatic blood flow and that this may consequently alter hepatic (alcohol) elimination capacity. Although this is a widely accepted phenomenon, we do not think that the meal given around 3.5 hours after the start of the alcohol infusion can explain the declining trend in alcohol levels, which can be observed after approximately 150 minutes (figure 1). First of all, in our study the apparent clearance, estimated from the infusion rates during the plateau phase was not clearly affected by the light, standardized meal that we provided to our subjects. But, even if this meal would have influenced alcohol metabolic rates, we sincerely doubt whether this would have affected the shape of the concentration curve shown in figure 1.

The pharmacokinetic feedback mechanism incorporated in the infusion paradigm automatically corrects for any change in the system (e.g. a change in alcohol metabolism rate) to keep alcohol serum concentrations close to their intended target level. In our opinion, any apparent change in alcohol metabolic rate would not have affected the concentration curve as presented here.

The alcohol clamp resulted in significant changes in body sway, visual analogue scales, adaptive tracking and smooth pursuit eye movements. These results correspond to literature findings. Effects on postural stability, subjective assessments, (visuo-)motor control and oculomotor coordination or attention are frequently observed after alcohol administration (van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1996). Some of these effects fluctuated significantly during the plateau phase, despite relatively constant alcohol levels. The causes for these fluctuations are not immediately apparent, but there are several possibilities. First, subtle BrAC/BAC-changes during the clamp could have resulted in exaggerated response fluctuations. Second, the rapid increase of alcohol levels during the loading phase could have caused some delay in the development of CNS-responses. Third, acute tolerance to the effects of alcohol may have ensued during the plateau phase. And fourth, some parameters may have exhibited effect thresholds. The different time profiles for the various effects indicate that these processes vary between CNS-effects, and combinations of these processes are also possible. In all, this could produce quite complicated relationships between concentrations and effects, which cannot be easily investigated with this relatively simple clamp, where one alcohol level was achieved rapidly with a loading phase that allowed for only a few CNS-measurements when alcohol levels changed. Consequently, the range of concentrations and concomitant measurements was too limited for detailed PK/PD-analyses, which would be necessary to dissect the complex concentration-effect relationships that were observed.

With these limitations to a more detailed interpretation, it seems that various processes underlie the different time-effect profiles. The body sway profile (figure 4) showed a rather sudden increase after pseudo steady state levels were reached, followed by an equally fast decrease at the end of the plateau phase was. This curve might be explained by a slow development of the responses (hysteresis) combined with an acute tolerance to the effects of alcohol (proteresis). The initial slow development of the body sway impairments could also be due to a threshold effect, but the measurements during the loading phase were not frequent enough to allow this conclusion. Similar to the body sway responses, tracking performance, VAS alertness and VAS alcohol effects decreased significantly throughout the course of the clamp. This may have been due to acute tolerance, or to an exaggerated response to a slight decrease in BrAC BrAC-levels, which graphically seemed to diminish slightly towards the end of the plateau phase of the clamp (figure 1). Again, the number of assessments over time was not frequent enough to allow a distinction between hysteresis, proteresis or threshold effects as an explanation for the course of the effects over time for these measurements. Other effects like smooth pursuit eye movements remained more constant.

Apparently, time-related changes in alcohol sensitivity differ between the various CNS-systems that are affected by alcohol. The literature shows several attempts to investigate these phenomena. Acute changes in the effects of alcohol over time (acute tolerance) have been demonstrated in many single-dose studies, but this phenomenon has been questioned for alcohol at steady state (Kaplan *et al.*, 1985). Other research showed indications for acute tolerance to stable alcohol levels in a small subject sample (Hiltunen *et al.*, 2000), but the obtained average alcohol level was less constant than the pseudo-steady state concentrations presented here. A few groups were able to generate accurate and stable alcohol levels, by clamping the BrAC according to the original O'Connor procedure (O'Connor *et al.*, 1998), and reported acute tolerance to constant alcohol levels, particularly for subjective measurements (Morzorati *et al.*, 2002; Ramchandani *et al.*, 2002; Ramchandani *et al.*, 1999b). However, these findings were based on only two measurements throughout the steady state periods. The results presented here show acute changes in CNS-effects of alcohol at pseudo-steady state on multiple functional CNS-domains and were based on intensive

pharmacodynamic sampling. The causes and practical implications of these observations could not be established from this single-level clamping experiment. Most pharmacodynamic measurements were obtained at relatively stable alcohol concentrations, and hardly any during the increasing part of the clamp. Alcohol clamping experiments at several different levels or different rates of increase of alcohol concentrations should be performed to examine the concentration- and time-effect relationships of alcohol on different functional domains. In principle, the alcohol clamp presented here could also be used to maintain several different alcohol levels.

Neither the symbol digit substitution test nor the visual analogue scales for mood and calmness or the electroencephalography measurements were affected by the stable clamp level. Alcohol decreased saccadic peak velocity, but not significantly. Other research did show effects of alcohol on saccadic eye movements (Nutt *et al.*, 2007) and electroencephalography (Ehlers *et al.*, 1999) at higher doses. The clear effects on the visual analogue scale for alcohol effects indicate that subjective parameters seem more sensitive to a stable alcohol level than objective measurements that reflect alertness indirectly. In this respect, the effects of alcohol, which is an indirect $GABA_A$-agonist (Santhakumar *et al.*, 2007) differ considerably from those of benzodiazepines, for which saccadic peak velocity and EEG beta power are particularly sensitive (de Visser *et al.*, 2003).

Although significant CNS-changes on multiple domains were observed in this study, the artificial pseudo-steady state condition does not resemble alcohol consumption in real life. Fluctuations in alcohol levels that occur during social drinking, may result in different CNS-effects compared to the effects at pseudo-steady state, at similar BrAC levels. Moreover, in contrast to the changing pharmacodynamic effects under an experimental pseudo-steady condition, acute tolerance in a social drinking situation may be counteracted by increases in the oral intake of alcohol, to maintain the desired effect level.

The alcohol clamp has distinct advantages to study the acute effects of alcohol. The pseudo-steady state levels with very little inter- and intra-variability allow the separation of concentration- and time-dependent changes in alcohol effects. In the current study, the results show indications for a delayed development of the effects of alcohol, followed by an acute reduction of the effects relative to the plasma concentrations. Under less well controlled circumstances, such observations are often difficult to make because of fluctuating alcohol concentrations, although successful efforts have been made to by-pass this problem (Martin and Moss, 1993; O'Connor *et al.*, 1998; Ramchandani *et al.*, 1999a).

The present study demonstrated that the responsiveness to constant alcohol levels changes over time. Concentration-effect modelling would be needed to examine these phenomena in more detail, but this would require frequent measurements at different levels of alcohol. This could be achieved with step-wise increases and decreases of target alcohol levels that can also be attained with the alcohol clamp. Such studies are needed to help understand the functional and medico-legal implications of alcohol's concentration-effect relationships, which apparently are diverse and time-dependent. A time component in the risk assessment of blood alcohol concentrations might be necessary.

In summary, significant CNS-effects were observed during a pseudo-steady state alcohol serum concentration of 0.6 $g{\cdot}L^{-1}$ for five hours. The course of alcohol effects differed among CNS-measurements. Indications for both hysteresis and acute tolerance were found, but these findings were not equally distributed among the performed CNS-tests and could not completely be confirmed. Some of the questions rising from the current exploratory study may be answered by future research (e.g. clamping at multiple alcohol levels during one session to facilitate PK-PD modeling, or acute tolerance development during pseudo-steady state conditions). Our clamping paradigm has not only shown to be a practical and accurate way to maintain stable serum levels of alcohol, it has also proved to be a suitable method to study the pharmacodynamic CNS-effects of constant alcohol serum concentrations concomitantly. The clamping procedure might be a useful tool in future alcohol interaction studies or in studies investigating the complex pharmacodynamic effects of alcohol.

FIGURE 1 SERUM AND BREATH ALCOHOL CONCENTRATION PROFILES WITH THEIR SD'S AS ERROR BARS
Alcohol was infused between t=0 and t=300 min. The plateau-phase is marked by the two vertical lines. The set-point is marked by the horizontal line.

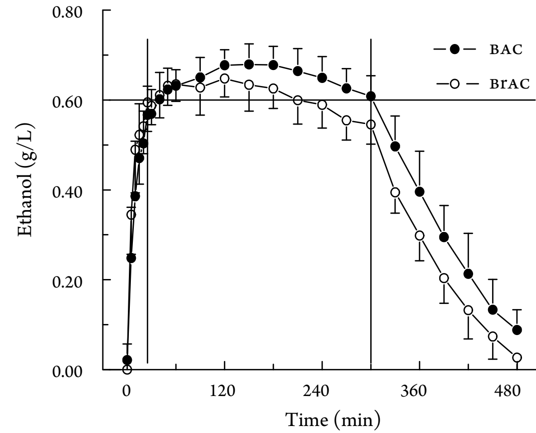

FIGURE 2 LS MEANS GRAPH OF VAS ALERTNESS (MM): CHANGE FROM BASELINE WITH 95% CI ERROR BARS
The plateau-phase is marked by the two vertical lines.

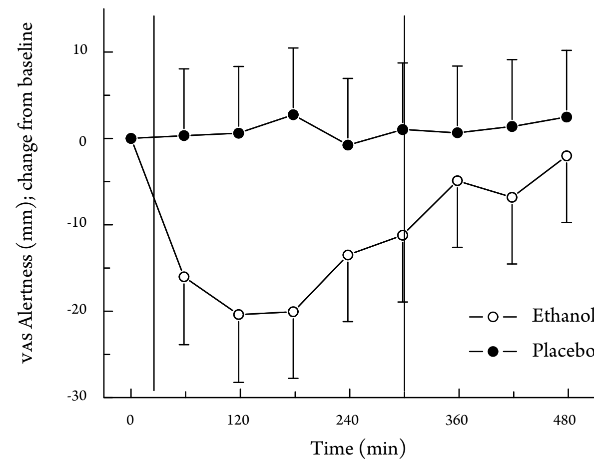

FIGURE 3 LS MEANS GRAPH OF VAS ALCOHOL EFFECTS (MM): CHANGE FROM BASELINE WITH 95% CI ERROR BARS
The plateau-phase is marked by the two vertical lines.

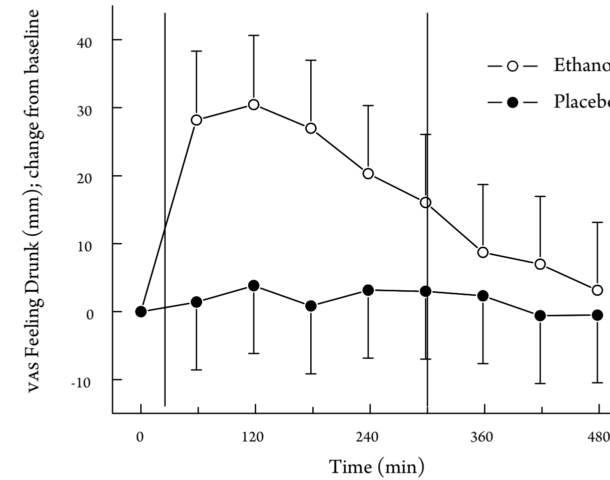

FIGURE 4 LS MEANS GRAPH OF BODY SWAY (MM): % CHANGE FROM BASELINE WITH 95% CI ERROR BARS
The plateau-phase is marked by the two vertical lines.

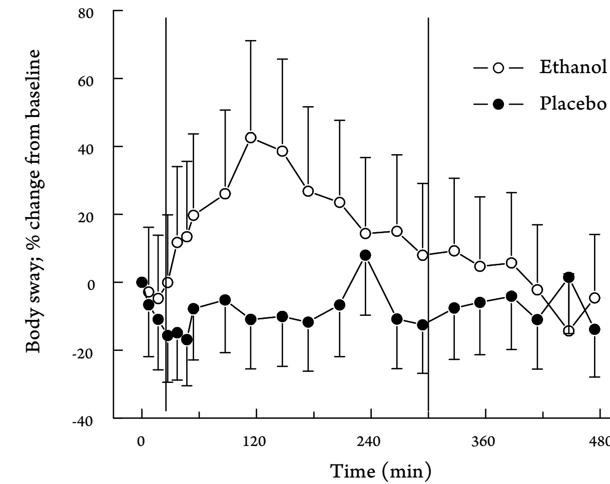

FIGURE 5 LS MEANS GRAPH OF TRACKING (%): CHANGE FROM BASELINE WITH 95% CI ERROR BARS
The plateau-phase is marked by the two vertical lines.

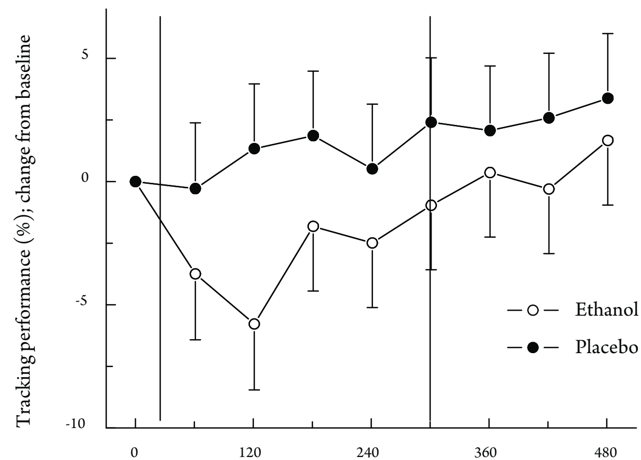

FIGURE 6 LS MEANS GRAPH OF SMOOTH PURSUIT
Change from baseline with 95% CI error bars. The plateau-phase is marked by the two vertical lines.

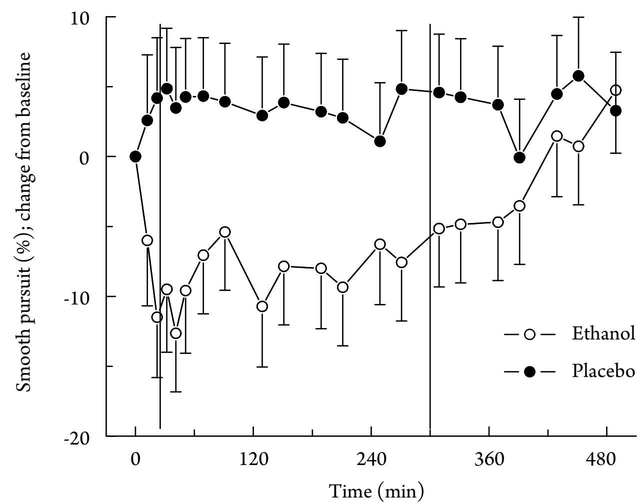

FIGURE 7 EXPLORATORY PK/PD RELATIONSHIP BETWEEN AVERAGE SERUM ALCOHOL AND THE MEAN EFFECTS ON BODY SWAY

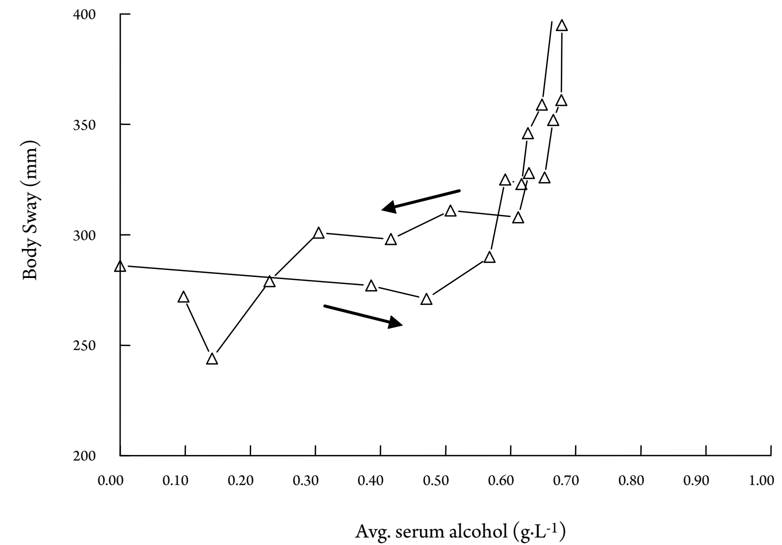

FIGURE 8 EXPLORATORY PK/PD RELATIONSHIP BETWEEN AVERAGE SERUM ALCOHOL AND THE MEAN EFFECTS ON VAS ALERTNESS

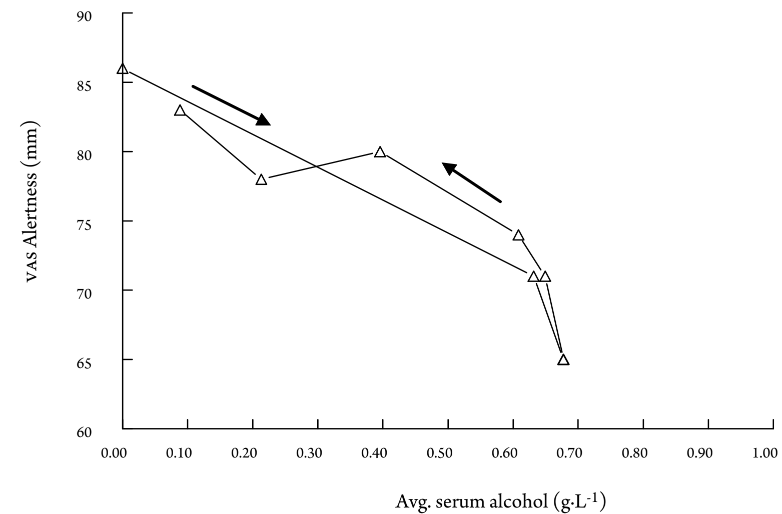

FIGURE 9 EXPLORATORY PK/PD RELATIONSHIP BETWEEN AVERAGE SERUM ALCOHOL AND THE MEAN EFFECTS ON VAS ALCOHOL EFFECTS

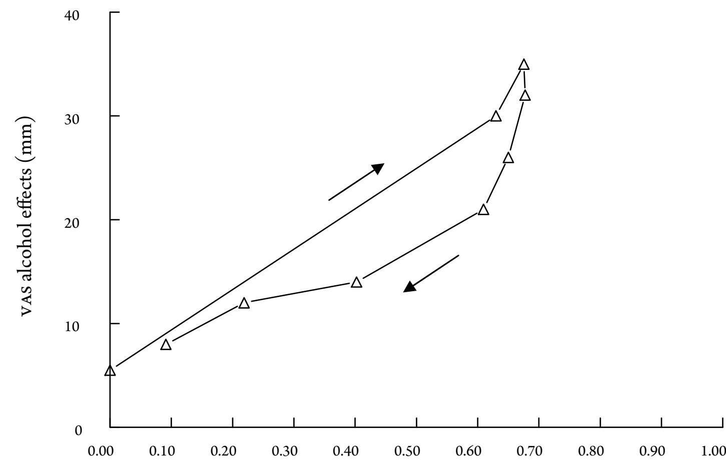

FIGURE 10 EXPLORATORY PK/PD RELATIONSHIP BETWEEN AVERAGE SERUM ALCOHOL AND THE MEAN EFFECTS ON TRACKING PERFORMANCE

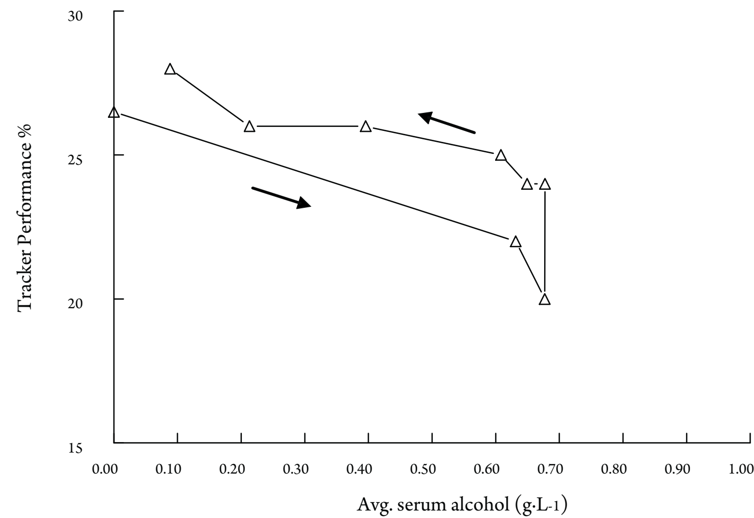

TABLE 1 SUMMARY OF PHARMACODYNAMIC EFFECTS OF ALCOHOL COMPARED TO PLACEBO

Average Alcohol and Placebo effect					
Parameter	**Least Square Means for Alcohol**	**Least Square Means for Placebo**	**Estimate of difference**	**p-value**	**95% CI**
Saccadic peak velocity (deg/s)	433.4	455.4	-22.1	0.2006	(-58, 13.8)
Saccadic latency (s)	0.228	0.221	0.006	0.2660	(-.01, .019)
Saccadic inaccuracy (%)	9.9	7.4	2.5	0.3272	(-3.0, 8.1)
Smooth pursuit (%)	46.0	55.7	-9.7	<.0001	(-12.4, -7.1)
SDST number correct	78	81	-3	0.6159	(-16, 10)
SDST number incorrect	1.2	0.9	0.3	0.0883	(-0.1, 0.6)
Body Sway (mm)	318	263	21.3%	0.0347	(1.8%, 45%)
Tracking (%)	24.3	27.7	-3.4	0.0004	(-4.5, -2.2)
SD of Tracking (%)	3.8	4.1	-0.2	0.3364	(-0.7, 0.3)
VAS alertness (mm)	71	84	-13	0.0022	(-20, -6)
VAS mood (mm)	84	85	-1	0.3396	(-5, 2)
VAS calmness (mm)	83	86	-3	0.1197	(-6, 1)
VAS alcohol effects (mm)	24	8	16	0.0029	(7, 25)
EEG Alpha FzCz (μV)	3.203	3.032	5.6%	0.1572	(-3%, 15%)
EEG Alpha PzOz (μV)	5.668	5.618	0.9%	0.8501	(-9%, 12%)
EEG Beta FzCz (μV)	1.745	1.673	4.3%	0.3085	(-5%, 14%)
EEG Beta PzOz (μV)	2.087	2.145	-2.7%	0.5180	(-11%, 6.7%)
EEG Delta FzCz (μV)	2.404	2.479	-3.0%	0.4986	(-12%, 7.1%)
EEG Delta PzOz (μV)	2.354	2.200	7.0%	0.1029	(-2%, 16%)
EEG Theta FzCz (μV)	2.996	2.819	6.3%	0.1941	(-4%, 17%)
EEG Theta PzOz (μV)	3.129	2.946	6.2%	0.3679	(-8%, 23%)

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