CHAPTER 5

A comparison of the central nervous system effects of alcohol at pseudo-steady state in Caucasian and expatriate Japanese healthy male volunteers

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ABSTRACT

In general, Japanese and Caucasians differ in their response to alcohol. To investigate these differences the alcohol clamping method can be used. This strictly controlled infusion regimen provides a reliable tool to study contrasts in central nervous system (CNS) effects and/or alcohol disposition. In this study twelve Japanese and twelve Caucasian healthy volunteers received two concentrations of intravenous alcohol or placebo using the alcohol clamp. Infusion rates during the steady state phase were used to compare alcohol clearance between the subgroups. Central nervous system (CNS) effects were frequently measured throughout the clamp. On average, significantly lower amounts of alcohol were needed to maintain similar stable concentrations in the Japanese group However, these differences disappeared when values were corrected for lean body mass. The most pronounced pharmacodynamic differences between the groups were observed on body sway and on the visual analogue scale (VAS) for subjective alcohol effects, mainly at the highest dose level. The alcohol clamp seems a useful method to compare differences in alcohol metabolism between groups. Some CNS-effects of alcohol differed clearly between Japanese and Caucasians, but others did not, even though alcohol levels were stable and similar between the two groups.

INTRODUCTION

Japanese people are more sensitive to alcohol compared to Caucasians (Shibuya *et al.*, 1989). This is at least partly related to genetic differences in pharmacokinetics, since a high proportion of Japanese have a relative deficiency for alcohol dehydrogenase (ADH) and/or aldehyde dehydrogenase (ALDH) causing higher blood levels of alcohol and/or acetaldehyde (Chan, 1986). Differences in body size and in lifestyle (food, use of medication) may further influence the kinetics of alcohol (Duranceaux *et al.*, 2008). Repeated exposure to alcohol may also induce tolerance to its effects (Bennett *et al.*, 1993), and avoidance could therefore contribute to increased sensitivity. It is less clear however, whether Japanese and Caucasian subjects differ in their sensitivity to the various central nervous system (CNS) effects of alcohol.

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- and time-dependent. In an earlier study, stable breath alcohol concentrations (BrAC) were maintained for hours, using a breath alcohol clamping paradigm in a Caucasian population, with different body postures and metabolic profiles (Zoethout *et al.*, 2008). Since this clamping method is based on the feedback of individual alcohol concentrations, it is likely that the clamp will also be able to overcome major differences in alcohol kinetics between certain ethnic subgroups, with potentially distinct metabolic activities or body compositions. In this way, alcohol elimination rates can be compared relatively easily. Moreover, ethnic differences in pharmacodynamic effects of alcohol can be accurately compared using the alcohol clamp, since alcohol levels are kept at comparable levels.

To investigate differences in CNS drug effects a transportable CNS measurement battery used for on site assessment of drug effects was used. This has been used in numerous studies with different kinds of CNS drugs at the Centre for Human Drug Research (CHDR) (de Haas *et al.*, 2006; de Visser *et al.*, 2001; van der Post *et al.*, 2004), including alcohol (Zoethout *et al.*, 2009). The Neurocart consists of a series of measurements that were chosen for their frequent repeatability, low variability, and their sensitivity to a wide range of drug-induced CNS-effects. Elements of the Neurocart have previously also been used in a comparative study of the effects of nitrazepam in Caucasians and Japanese (van Gerven *et al.*, 1998).

In the current study, the pharmacokinetic and CNS-pharmacodynamic effects of two levels of alcohol were examined and compared between healthy Japanese and Caucasian volunteers, using the multimodal CNS-test battery and an intravenous alcohol clamp.

METHODS

Design

This was a randomised, double-blind, placebo-controlled, three-way cross-over study with a washout period of two weeks, in which the effects of two levels of alcohol and placebo were examined in healthy Caucasian and Japanese male volunteers.

Subjects

Twelve healthy Caucasian male subjects and twelve healthy Japanese males (expatriates living in the Netherlands), aged 18-40 years, gave their 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 for general health by medical history and physical examination and participated in a pharmacodynamic training session. Subjects who used more than four units of alcohol per day on average were excluded from study participation.

All 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.

General procedure

Subjects reported at the CHDR at 08:00hr in the morning of the test day in fasted condition, after which a short introduction was given. Two intravenous cannulae (one cannula for blood sampling and another for infusion of ethanol or placebo) were inserted and electroencephalography- (EEG) and eye-electrodes were mounted. Before the start of the infusion subjects were provided with a light breakfast. The alcohol infusion started between 09:30hr and 10:00hr and ended five hours later, followed by an infusion-free washout period of three hours. Blood alcohol samples, breath alcohol measurements and pharmacodynamic measurements were obtained at regular time intervals indicated below, until eight hours after the start of the infusion. A standardised lunch was given at around 3.5 hours post-dose. Subjects were taken home by taxi, after dinner.

Alcohol clamping method

Ethanol 10% w/v solution in 5% glucose (for the 0.6 $g \cdot L^{-1}$ occasion), ethanol 5% w/v solution in 5% glucose (for the 0.3 $g \cdot L^{-1}$ occasion) and glucose 5% (placebo) were administered intravenously on three different occasions, according to the study design previously mentioned. Ethanol 5% and ethanol 10% were used to achieve alcohol levels of 0.3 and 0.6 $g \cdot L^{-1}$, respectively. Glucose 5% was used during placebo sessions. 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 might be caused by the high-flow alcohol infusion during the loading phase. The infusion rates for the first and the second five minutes were determined individually for each subject (before the start of the infusion), based on demographic data (weight, height, age and gender) and on the target level (0.3 or 0.6 $g \cdot L^{-1}$) to avoid overshoots, using Watson's estimates of body water (Watson *et al.*, 1981). After the loading phase, infusion rates during the plateau were adapted to BrAC changes to maintain one of the two predefined pseudo-steady state levels, according to a recently introduced spreadsheet-based infusion paradigm that was adapted from a method originally described by O'Connor (O'Connor *et al.*, 1998). This method resulted in stable alcohol levels for hours and is described in more detail elsewhere (Zoethout *et al.*, 2008). Infusion rates were adapted to BrAC changes, based on measurements obtained at 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min post-dose, when the infusion was stopped. Additional BrAC levels were determined at 330, 360, 390, 420, 450 and 480 minutes. At the same times, samples for measurements

of blood alcohol concentrations were obtained, which were processed and analyzed according to methods described previously (Zoethout *et al.*, 2009). Unfortunately, we were unable to set up reliable acetaldehyde measurements or to determine aldehyde dehydrogenase polymorphisms.

BrAC measurements were performed 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 infusion rate predicted to maintain or reach a BrAC-level at 0.6 $g \cdot L^{-1}$ or at 0.3 $g \cdot L^{-1}$. Because sampling intervals shorter than five minutes (as required during the initial part of the infusion) cause the BrAC meter to show fatigue, two different devices were alternated. A pilot study showed that no fatigue was observed during alternation of both BrAC devices according to the sampling scheme of the study. Both BrAC devices were calibrated prior to the start 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 throughout the study.

CNS-pharmacodynamics

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 The body sway meter allows the determination of body movements in a single plane, providing a measure of postural stability. Body sway was measured with an apparatus similar to the Wright ataxia meter (Wright, 1971). With a string attached to the waist, all body movements over a period of two minutes were integrated and expressed as millimeter (mm) sway on a digital display. Measurements were performed with closed eyes.

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.*, 1996; van Steveninck *et al.*, 1991; van Steveninck *et al.*, 1999; van Steveninck *et al.*, 1993). From these measurements, three factors were derived as described by Bond and Lader (Bond and lader, 1974), corresponding to alertness, mood and calmness. The Bond and Lader VAS have been extensively used at CHDR and were performed electronically and according to CHDR standard operating procedures. In addition, to this VAS-list a separate 100 mm-line was added, asking the subject to indicate 'how large is the effect of alcohol that you feel?' (VAS alcohol effects). Both Japanese and Dutch versions were available and all subjects completed the scales in their own native language. The Japanese version was tested and validated prior to the start of the study, to avoid semantic issues.

ADAPTIVE TRACKING Adaptive tracking is a pursuit tracking task. A circle moves randomly on a computer screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the subject cannot maintain the dot inside the circle. Performance was scored after a fixed period. Each test is preceded by a run-in period. After 4 to 6 practice sessions, learning effects are limited. The adaptive tracking test is more sensitive to impairment of eye-hand coordination and vigilance by drugs than compensatory pursuit tasks or other pursuit tracking tasks, such as the pursuit rotor. The adaptive tracking test 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). 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). The average performance and the standard deviation of scores over a 3.5 minutes period were used for analysis.

SACCADIC EYE MOVEMENTS Saccadic peak velocity is one of the most sensitive parameters for sedation (van Steveninck *et al.*, 1996; van Steveninck *et al.*, 1991; van Steveninck *et al.*, 1999; van Steveninck *et al.*, 1993). Recording and analysis of saccadic eye movements was conducted with a microcomputer-based system for sampling and analysis of eye movements. The equipment used for stimulus display, signal collection and amplification was from Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan). Disposable silver-silver chloride electrodes (Medicotest N-00-S, Olstykke, Denmark) were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. Skin resistance was reduced to less than 5 kOhm before application of the electrodes. Head movements were restrained using a fixed head support. The target consisted of an array of light emitting diodes on a bar, fixed at 50 cm in front of the head support. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of latency (reaction time), saccadic peak velocity and inaccuracy of all artefact-free saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

SMOOTH PURSUIT EYE MOVEMENTS The same system as used for saccadic eye movements was also used for measurement of smooth pursuit. For smooth pursuit eye movements, the target moved sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponded to 20 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 parameter. Prior studies show that 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) based on the work of Bittencourt *et al.* (Bittencourt *et al.*, 1983) and the original description of Baloh *et al.* (Baloh *et al.*, 1975).

PHARMACO-ELECTROENCEPHALOGRAPHY (PHEEG) phEEG was used to monitor any drug effects, which can be interpreted as evidence of penetration and activity in the brain (van Steveninck *et al.*, 1993; Cohen *et al.*, 1985). Electroencephalography (EEG) provides non-specific measures of CNS functions. EEG recordings were obtained at times specified in the study flow chart. EEG recordings were made using gold electrodes, fixed with EC2 paste (Astromed) at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). The signal was amplified by use of a Grass 15LT series Amplifier Systems with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis was performed using customized CED and Spike2 for Windows 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 Power (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artefacts were identified by visual inspection and these were excluded from analysis. For each lead, 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 64 seconds per session.

Statistical analysis

The pharmacodynamic endpoints were analyzed by a mixed model analyses of variance (using SAS PROC MIXED) with treatment, group, period, time, treatment by time, treatment by group and treatment by time by group as

fixed effects and subject, subject by treatment and subject by time as random effects and the average baseline value was included as covariate. Body sway and EEG values were log-transformed prior to analysis to correct for the expected log-normal distribution of the data. Least square means (LSM) were obtained and were used to compare the effects of alcohol on the different pharmacodynamic parameters. All calculations were performed using SAS for windows V9.1.2 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Subjects

Twelve healthy Caucasian subjects were included in the study and completed the study per protocol. Eleven Japanese subjects completed the study per protocol. One Japanese subject had to unexpectedly return to Japan for personal reasons, and hence did not complete his third occasion. The complete data of eleven Japanese subjects and the two completed occasions of the Japanese dropout were included in the analysis.

Caucasian subjects participating in the study were on average 26 years old (range: 18 - 39 years old), had a weight of 85 kg (range: 63 - 103 kg) and an average height of 186 cm (range: 178 - 197 cm). On average, they used 1.75 units (range 1 - 3 units) of alcohol per day. Japanese subjects were on average 28 years old (range: 20 - 34 years old), weighed 67 kg (range: 56 - 94 kg) and had a height of 174 cm (range: 164 - 182 cm). The Japanese habitually used somewhat less alcohol than the Caucasians (1.33 units (range 0-4) per day), but the difference was not significant (p=0.283).

Adverse events

No serious adverse reactions occurred during the study. Signs of poor alcohol tolerability or intoxication (i.e. nausea and vomiting, rashes and heavy perspiration) only occurred in the Japanese subpopulation and were more frequently observed at the highest ethanol level (0.6 $g \cdot L^{-1}$). All symptoms were transient and mild to moderate in severity. An overview of all the adverse events observed after alcohol treatment is presented in table 2.

Alcohol concentrations

Average BrAC profiles of both Caucasian and Japanese volunteers for both target levels are shown in figure 1. Pseudo-steady state alcohol concentrations were obtained for both ethnic subgroups at both target levels. The set-points were achieved within approximately 30 minutes after the start of the infusion. Following a minor overshoot, pseudo-steady state levels can be observed throughout the infusion from 30 – 300 minutes. After the alcohol infusion was stopped, BrAC levels returned to baseline. No clinically meaningful differences between the BrAC profiles of the two groups were observed, at both the 0.3 $g \cdot L^{-1}$ level (0.003 $g \cdot L^{-1}$ (95% CI: -0.01, 0.01)) and the 0.6 $g \cdot L^{-1}$ level (0.012 $g \cdot L^{-1}$ (95% CI: -0.01, 0.03)).

Alcohol disposition

During the loading phase, alcohol infusion rates were based on anthropometric estimates of body water (Watson *et al.*, 1981). They were hence closely correlated with age, height and weight and on average lower in the Japanese subjects than in the larger Caucasians. The total amount of alcohol infused during the steady state plateau phase may serve as an indirect parameter for alcohol clearance, which is expected to be also related to metabolic differences. The mean infusion rates required to maintain both set-points per subgroup are presented in figure 2. For both levels, lower rates are required to maintain pseudo-steady state concentrations in Japanese compared to Caucasians (since the Japanese curves are entirely located below the Caucasian curves). Japanese required 35.9 g of alcohol on average to maintain the 0.3 set-point for 5 hours, compared to 46.8 g in Caucasians ($p < 0.00008$). However, when these values were corrected for lean body

mass, according to a formula described by Hallynck (Hallynck *et al.*, 1981), this difference was no longer significant (p = 0.14). Japanese required 50.1 g of alcohol to maintain the 0.6 set-point for 5 hours, which differed significantly from the larger amount of 62.5 g needed in Caucasians (p = 0.0011). This difference was also abolished after correction for lean body mass (p = 0.73).

CNS pharmacodynamics

VISUAL ANALOGUE SCALES (VAS) The VAS results are graphically presented in figure 3 and 4. The only significant effect in the Caucasian subgroup was a significant increase of VAS alcohol effects at the highest dose level (i.e. 11.9 mm (95% CI: 0.6, 23.2) compared to placebo). For the Japanese volunteers, both a reduction in alertness during clamping at 0.6 $g \cdot L^{-1}$ (10.7 mm (95% CI: -15.9, -5.4)) and an increase in subjective alcohol effects on both alcohol levels were found (13.9 mm (95% CI: 2.4, 25.4) at 0.3 $g \cdot L^{-1}$ and 43.0 mm (95% CI: 31.4, 54.6) during the 0.6 $g \cdot L^{-1}$ session). Despite similar BrAC levels of 0.6 $g \cdot L^{-1}$, Japanese volunteers rated their alertness on average 5.9 mm (95% CI: -11.2, -0.6) lower than Caucasian volunteers. Also, Japanese subjects rated themselves 30.6 mm (95% CI: 17.0, 44.2) 'more drunk' compared to the Caucasians.

BODY SWAY The body sway results are presented in figure 5. Neither treatment caused significant effects on the body sway in the Caucasian group. However, in the Japanese group, body sway measurements increased 46.5% (95% confidence interval (CI): 25.7, 70.7) during clamping at 0.6 $g \cdot L^{-1}$ compared to placebo. No significant differences from placebo or between ethnic groups were observed during the 0.3 $g \cdot L^{-1}$ level. The difference between Caucasian and Japanese subjects was significant at the 0.6 $g \cdot L^{-1}$ level. Japanese had 51.2% (95% CI: 17.7, 94.2) higher scores than Caucasians

ADAPTIVE TRACKING The results of the adaptive tracking task are presented in figure 6. Reduced scores were only found under high alcohol doses for both populations (-2.7% (95% CI: -4.4, -1.0) and -3.1% (95% CI: -4.9, -1.4) for the Caucasians and the Japanese, respectively). There were no ethnic differences.

SACCADIC EYE MOVEMENTS The only significant effect in the Caucasian group was found during clamping at 0.6 $g \cdot L^{-1}$. At this level, peak velocity was decreased by 20.9 deg/s (95% CI: -33.7, -8.2) compared to placebo (figure 7). In the Japanese group, neither alcohol level showed effects on saccadic eye movements. There were no significant differences between the two groups.

SMOOTH PURSUIT EYE MOVEMENTS In Caucasians, smooth pursuit decreased after the low alcohol dose compared to placebo (-3.9% (95% CI: -7.5, -0.2)), and even more during clamping at the higher level (-11.1 (95% CI: -14.8, -7.5)). The Japanese volunteers were only significantly impaired at the higher alcohol level (-6.0% (95% CI: -9.8, -2.3)). Furthermore, at the low alcohol dose, the Caucasian volunteers were on average 5.0% (95% CI: -9.8, -0.3) more impaired on this test compared to the Japanese volunteers. No differences between the groups were observed at the higher clamp level. All these effects are presented in figure 8.

PHARMACO-EEG The only (minor) EEG-effect found in the Caucasian group was a decrease in EEG delta (Fz-Cz) of 10.3% (95% CI: -19.2, -0.5) at the 0.6 level, compared to placebo. Some other EEG-changes were found in Japanese, which showed an increase in EEG beta (Fz-Cz) at the 0.3 level (12.3% (95% CI: 2.2, 23.5)) and an increase in EEG theta (Pz-Oz) at the 0.6 level (13.1% (95% CI: 2.5, 24.8), compared to placebo. This EEG theta increase in Japanese was 19.8% (95% CI: 0.1, 43.5) larger than in Caucasians. No other EEG-effects were found. Pharmacodynamic test results are summarized in table 1.

DISCUSSION

Our study indicated that Japanese were more sensitive to the subjective effects of alcohol compared to Caucasians, who seemed to be subjectively more resilient. Somewhat unexpectedly, strictly regulated alcohol levels did not cause much more pronounced objective impairments in Japanese than in Caucasians. There were some differences between the two populations in EEG changes (where a bit more slowing in Japanese was found) and in smooth pursuit (which were somewhat more impaired in Caucasians), but these effects do not signify major ethnic differences. The adaptive tracking effects were in fact quite consistent. Apart from the much larger subjective effects of alcohol, the Japanese only showed a considerably larger impairment of body sway at the 0.6 $g \cdot L^{-1}$ level.

These pharmacodynamic differences between Japanese and Caucasians cannot be explained by differences in concentration, since comparable alcohol levels were obtained. The small disparity in habitual alcohol exposure (on average 1.33 *vs* 1.75 units per day, respectively) is also an inadequate explanation for the variations in sensitivity. The fact that subjective effects were more pronounced in Japanese could be related to cultural differences, for instance in the acceptability of alcohol effects or the expression of feelings of inebriation; or even to linguistic differences in the interpretation of visual analogue scales (van Gerven *et al.*, 1998). It is also important to realize that lifestyle differences (food, repeated exposure to alcohol) between indigenous and expatriate Japanese are known to influence the kinetics of alcohol (Duranceaux *et al.*, 2008).

Acetaldehyde may also have played a role, particularly for differences in body sway effects. Many of the observed side-effects in Japanese (headache, dizziness, nausea, vomiting) were compatible with higher serum concentrations of acetaldehyde in this population (Eriksson, 2001). Accumulating acetaldehyde levels may not only have accounted for the observed adverse events, but may also have directly caused some of the CNS-differences, notably on postural imbalance. This would also explain the gradual increase of postural instability during the plateau phase of the ethanol clamp (figure 5). Contrary to the fairly stable elevations of most other pharmacodynamic parameters that roughly followed the BrAC timeprofile, average body sway increased from 515 mm one hour after the start of the infusion to 727 mm two hours later (figure 5). A delayed development of postural imbalance was also found in a previous alcohol clamping study in Caucasian subjects (Zoethout *et al.*, 2009). Clearly, determination of the concentration profiles of alcohol and acetaldehyde and their relationships to the different pharmacodynamic parameters is needed to show which effects are related to alcohol and which to the metabolite or both. Unfortunately, we were unable to measure acetaldehyde concentrations or aldehyde-dehydrogenase polymorphisms in this study. However, table 2 suggests that approximately 25% of the Japanese population suffered from high acetaldehyde levels.

Our findings show that the subjective alcohol scale was a very sensitive tool to detect alcohol effects in both Caucasians and Japanese subjects. This is confirmed by earlier literature findings (Zoethout *et al.*, 2010). The VAS alcohol effects seemed to be even more responsive in Japanese, because effects on this scale were not only found at the highest alcohol level (like with the Caucasians), but also with the lowest dose. In Japanese, the subjective alcohol effect scale was the only measurement that was affected by both alcohol levels.

There were clear ethnic distinctions between the postural and subjective effects of alcohol, but it is perhaps more surprising that we found only a few other and relatively small pharmacodynamic differences between the two groups. In Caucasians, only smooth pursuit eye movements were affected by both alcohol levels. Smooth pursuit performance was reduced at doses that did not result in any subjective effects, and this test can therefore be considered as the most sensitive test to detect alcohol effects in Caucasians in this setup. In Japanese, smooth pursuit performance was only significantly affected by the highest alcohol dose.

Postural stability was not significantly influenced by ethanol in the Caucasian volunteers. This differs from other research groups that demonstrated effects of ethanol on body sway at comparable doses (Jones, 1993; Martin

et al., 1981; Lukas *et al.*, 1989; Zoethout *et al.*, 2009; Zoethout *et al.*, 2010). In contrast, body sway did show pronounced effects in Japanese, but mainly at the highest dose level. Body sway was somewhat less sensitive to alcohol than subjective alcohol scales in Japanese or smooth pursuit performance in Caucasians, which both showed significant effects at the 0.3 $g \cdot L^{-1}$ level. Adaptive tracking showed a similar sensitivity i.e. only to the higher alcohol level in both ethnic groups. This finding is in accordance with prior research.

In the Caucasian group, we found no reductions of VAS alertness but significant decreases of saccadic eye movements. Subjective scores of alertness and (even more so) saccadic eye movements are both described as sensitive biomarkers for the sedative effects of benzodiazepines (de Visser *et al.*, 2003). Nonetheless, ethanol (as an indirect non-competitive agonist at the $GABA_A$-receptor (Santhakumar *et al.*, 2007)) only affected the eye movements in this study. In Caucasians, saccadic eye movements thus seem more sensitive to ethanol level than subjective measurements of alertness. In contrast, the (non-significant) impairment in saccadic eye movements in the Japanese group was confirmed by effects on the VAS alertness.

In Japanese some small EEG effects were observed (i.e. an increase in EEG Theta) that were in line with the sedative effects observed with other tests. However EEG does not seem to be a very specific biomarker for the effects of other sedative drugs (e.g. neuroleptics (de Visser *et al.*, 2001) or cannabinoids (Zuurman *et al.*, 2009)). The only EEG effect in Caucasians was a decrease in delta waves.

Besides these pharmacodynamic effects and differences, this study shows that constant alcohol concentrations could be maintained for several hours at two different levels in both Japanese and Caucasian healthy male volunteers, using the alcohol clamping paradigm. The alcohol clamping method allows frequent measurements of CNS-effects concomitantly and serves as an ideal procedure to compare different (ethnic) groups in their alcohol clearance abilities as well as in their individual CNS responses to stable levels of alcohol.

In summary, we found considerable subjective differences in the effects of alcohol between Japanese and Caucasians, at levels that were stable and similar between the two groups. Japanese also developed significantly more postural instability than Caucasian healthy volunteers. We hypothesize that acetaldehyde might play a role in these differences. Other pharmacodynamic measurements like adaptive tracking and eye movements did not differ quite as much between the two groups, which could be an indication that some CNS-functions are influenced more by alcohol itself than by its major metabolite. More research is required to confirm this hypothesis.

FIGURE 1 BREATH ALCOHOL CONCENTRATION PROFILES

With standard deviations (SD) as error bars. Square: ethanol 0.3 g·L^{-1}; triangle: ethanol 0.6 g·L^{-1}; open symbols: Caucasians; closed symbols: Japanese.

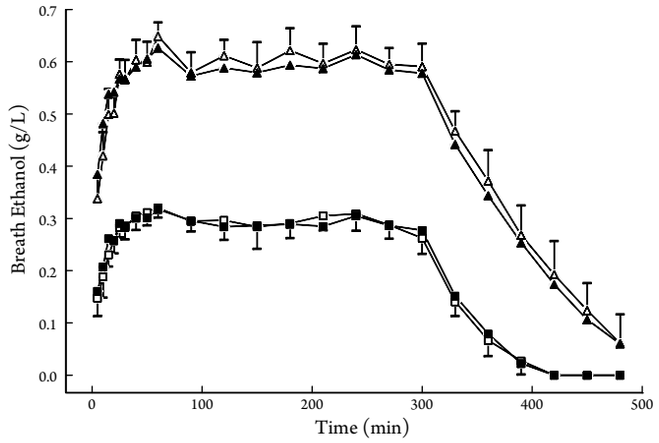

FIGURE 2 MEAN INFUSION RATES PER SUBGROUP PER DOSE LEVEL

With SD's as error bars. The plateau phase is marked by the two vertical lines.

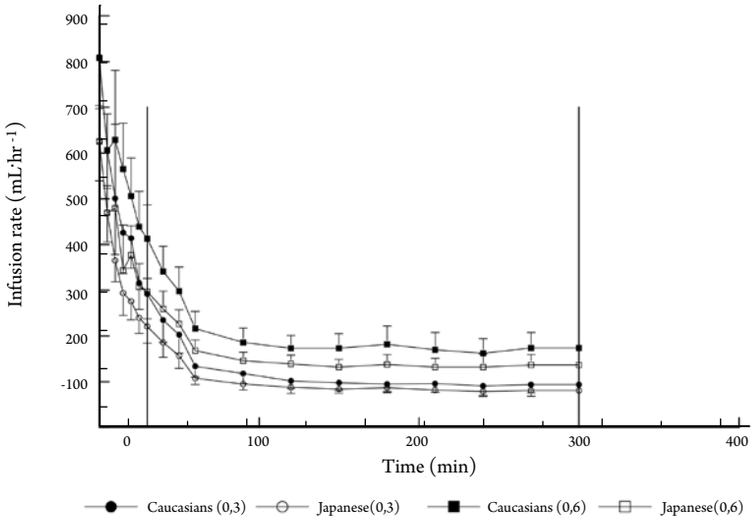

FIGURE 3 LEAST SQUARE MEANS OF VAS ALCOHOL EFFECTS

Change from baseline with 95% confidence intervals (CI) as error bars. Circle: placebo; square: ethanol 0.3 g·L^{-1}; triangle: ethanol 0.6 g·L^{-1}; open symbols: Caucasians; closed symbols: Japanese.

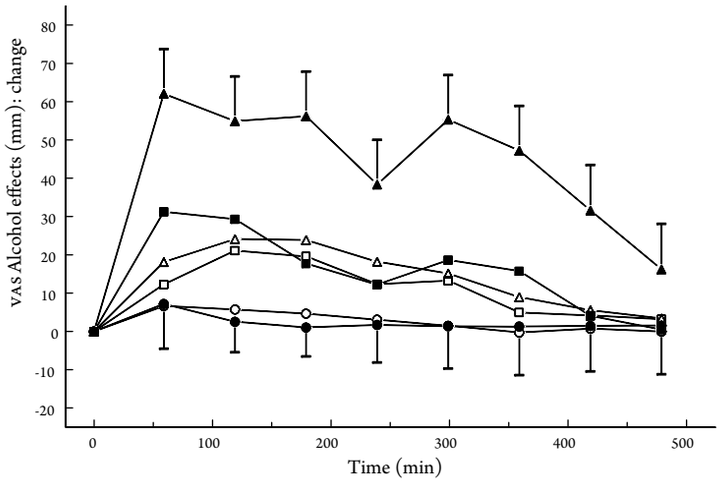

FIGURE 4 LEAST SQUARE MEANS OF VAS ALERTNESS

Change from baseline with 95% confidence intervals (CI) as error bars. Circle: placebo; square: ethanol 0.3 g·L^{-1}; triangle: ethanol 0.6 g·L^{-1}; open symbols: Caucasians; closed symbols: Japanese.

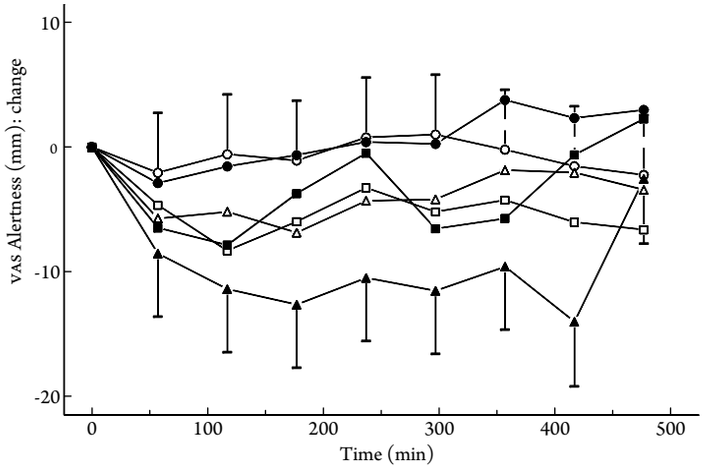

FIGURE 5 LEAST SQUARE MEANS OF BODY SWAY

Change from baseline with 95% confidence intervals (CI) as error bars. Circle: placebo; square: ethanol 0.3 g·L^{-1}; triangle: ethanol 0.6 g·L^{-1}; open symbols: Caucasians; closed symbols: Japanese.

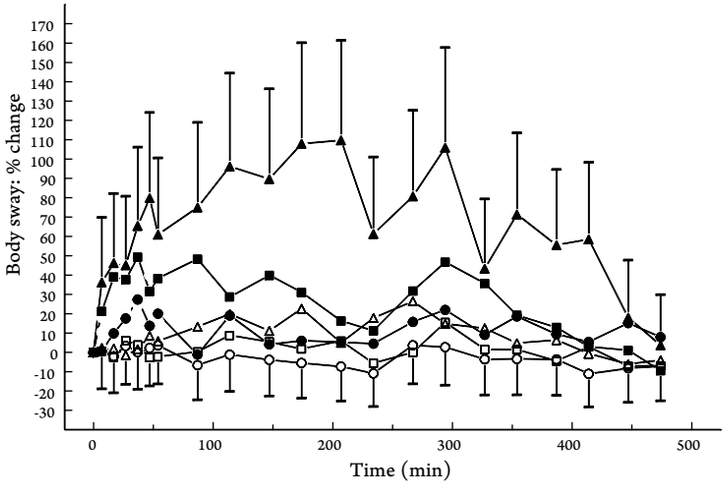

FIGURE 6 LEAST SQUARE MEANS OF ADAPTIVE TRACKING

Change from baseline with 95% confidence intervals (CI) as error bars. Circle: placebo; square: ethanol 0.3 g·L^{-1}; triangle: ethanol 0.6 g·L^{-1}; open symbols: Caucasians; closed symbols: Japanese.

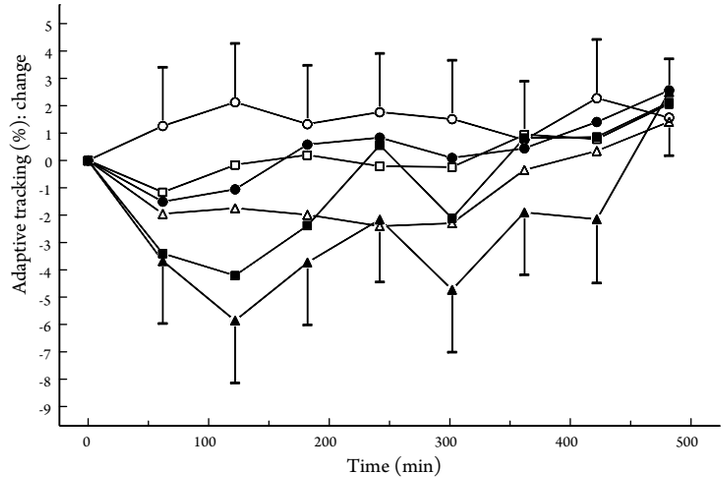

FIGURE 7 LEAST SQUARE MEANS OF SACCADIC PEAK VELOCITY IN DEGREES PER SECOND

Change from baseline with 95% confidence intervals (CI) as error bars. Circle: placebo; square: ethanol 0.3 g·L^{-1}; triangle: ethanol 0.6 g·L^{-1}; open symbols: Caucasians; closed symbols: Japanese.

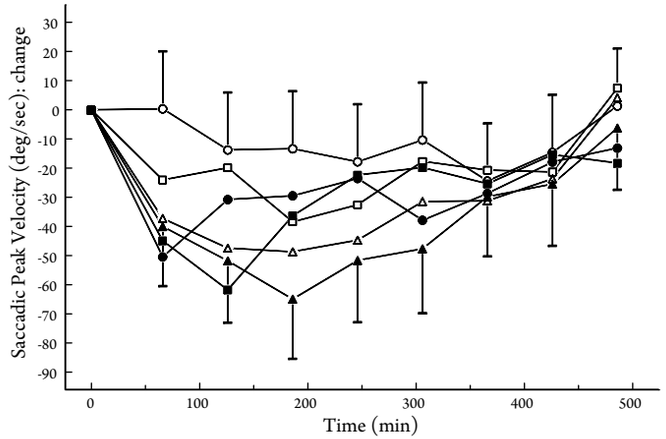

FIGURE 8 LEAST SQUARE MEANS OF SMOOTH PURSUIT EYE MOVEMENTS

Change from baseline with 95% confidence intervals (CI) as error bars. Circle: placebo; square: ethanol 0.3 g·L^{-1}; triangle: ethanol 0.6 g·L^{-1}; open symbols: Caucasians; closed symbols: Japanese.

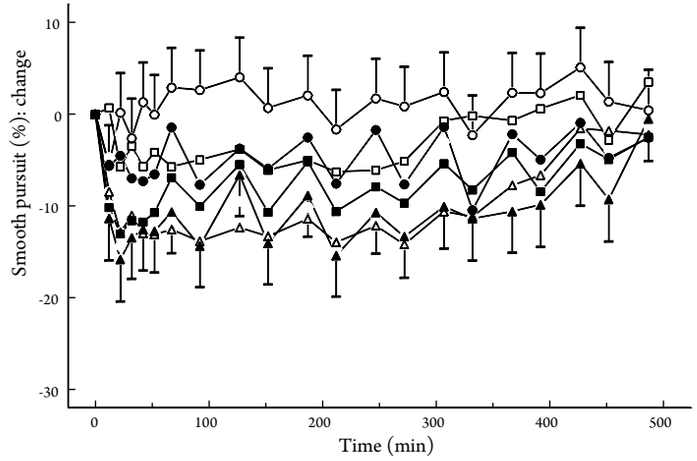

TABLE 1 CONTRASTS BETWEEN TREATMENTS AND SUBGROUPS FOR EACH PHARMACODYNAMIC PARAMETER

95% confidence intervals are provided between brackets (significant results in bold).

	placebo vs. alcohol 0.3 g·L^{-1} (Caucasians)	**placebo vs. alcohol 0.6 g·L^{-1} (Caucasians)**	**placebo vs. alcohol 0.3 g·L^{-1} (Japanese)**	**placebo vs. alcohol 0.6 g·L^{-1} (Japanese)**	**Caucasians vs. Japanese (alcohol 0.3 g·L^{-1})**	**Caucasians vs. Japanese (alcohol 0.6 g·L^{-1})**
Body sway (%)	4.1 (-10.2, 20.7)	11.1 (-4.3, 29.0)	12.8 (-2.7, 30.9)	**46.5 (25.7, 70.7)**	24.3 (-2.7, 58.7)	**51.2 (17.7, 94.2)**
Saccadic Inaccuracy (%)	**-0.2 (-0.8, 0.3)**	-0.0 (-0.6, 0.5)	0.2 (-0.3, 0.8)	-0.5 (-1.2, 0.1)	0.5 (-0.5, 1.4)	-0.5 (-1.5, 0.4)
Saccadic Peak Velocity (deg/sec)	-9.3 (-22.1, 3.5)	**-20.9 (-33.7, -8.2)**	-1.6 (-14.4, 11.2)	-10.7 (-24.0, 2.6)	-9.6 (-28.8, 9.5)	-7.2 (-27.3, 13.0)
Saccadic Reaction Time (msec)	0.9 (-5.9, 7.7)	2.2 (-4.6, 9.0)	**7.7 (0.9, 14.5)**	**12.9 (5.7, 20.1)**	1.0 (-12.3, 14.4)	4.9 (-8.6, 18.4)
Smooth pursuit (%)	**-3.9 (-7.5, -0.2)**	**-11.1 (-14.8, -7.5)**	-3.2 (-6.9, 0.4)	**-6.0 (-9.8, -2.3)**	**-5.0 (-9.8, -0.3)**	-0.6 (-5.4, 4.1)
Adaptive tracking (%)	-1.3 (-3.0, 0.4)	**-2.7 (-4.4, -1.0)**	-1.39 (-3.1, 0.3)	**-3.1 (-4.9, -1.4)**	-1.2 (-3.9, 1.4)	-1.6 (-4.3, 1.1)
VAS Alcohol effects (mm)	8.6 (-2.7, 19.9)	**11.9 (0.6, 23.2)**	**13.9 (2.4, 25.4)**	**43.0 (31.4, 54.6)**	4.8 (-8.5, 18.1)	**30.6 (17.0, 44.2)**
VAS Alertness (mm)	-4.8 (-9.9, 0.3)	-3.5 (-8.6, 1.7)	-4.2 (-9.4, 0.9)	**-10.7 (-15.9, -5.4)**	1.9 (-3.3, 7.1)	-5.9 (-11.2, -0.6)
VAS Calmness (mm)	1.5 (-5.7, 8.7)	3.1 (-4.1, 10.3)	6.4 (-0.9, 13.6)	1.1 (-6.2, 8.5)	6.2 (-0.9, 13.3)	-0.7 (-7.9, 6.6)
VAS Mood (mm)	-0.0 (-4.7, 4.7)	0.8 (-4.0, 5.5)	0.1(-4.6, 4.9)	-3.5 (-8.3, 1.4)	1.0 (-3.5, 5.5)	-3.3 (-7.9, 1.3)
EEG Alpha Fz-Cz (µV)	1.5 (-9.2, 13.5)	2.9 (-8.0, 15.1)	-0.3 (-10.8, 11.6)	1.4 (-9.6, 13.8)	-5.2 (-16.1, 7.2)	-4.9 (-16.1, 7.8)
EEG Alpha Pz-Oz (µV)	-1.3 (-11.9, 10.7)	2.8 (-8.3, 15.3)	-6.4 (-16.4, 5.0)	3.0 (-8.7, 16.3)	-7.6 (-33.7, 28.8)	-2.4 (-30.1, 36.3)
EEG Beta Fz-Cz (µV)	1.4 (-7.7, 11.5)	-2.4 (-11.2, 7.3)	**12.3 (2.2, 23.5)**	2.8 (-6.7, 13.3)	2.6 (-7.0, 13.1)	-2.5 (-11.8, 7.8)
EEG Beta Pz-Oz (µV)	-4.1 (-13.4, 6.3)	-0.8 (-10.4, 10.0)	-3.7 (-13.1, 6.7)	7.4 (-3.5, 19.5)	3.3 (-14.5, 24.9)	11.4 (-8.1, 34.9)
EEG Delta Fz-Cz (µV)	-6.7 (-15.9, 3.5)	**-10.3 (-19.2, -0.5)**	3.9 (-6.4, 15.4)	-1.3 (-11.3, 9.9)	9.4 (-2.3, 22.4)	8.1 (-3.7, 21.4)
EEG Delta Pz-Oz (µV)	-2.7 (-11.0, 6.3)	-1.2 (-9.5, 8.0)	1.1 (-7.4, 10.5)	3.4 (-5.7, 13.4)	13.2 (-2.5, 31.3)	13.9 (-2.0, 32.4)
EEG Theta Fz-Cz (µV)	0.7 (-7.7, 9.9)	-4.4 (-12.4, 4.3)	2.9 (-5.7,12.2)	-4.0 (-12.3, 5.01)	0.6 (-9.5, 11.8)	-1.1 (-11.3, 10.13)
EEG Theta Pz-Oz (µV)	1.1 (-8.0, 11.0)	3.6 (-5.7, 13.8)	4.0 (-5.3, 14.2)	**13.1 (2.5, 24.8)**	13.0 (-5.7, 35.4)	**19.8 (0.1, 43.5)**

TABLE 2 ADVERSE EVENTS AFTER ETHANOL TREATMENT

** Nausea and vomiting, rashes and heavy perspiration*

0.3 g·L^{-1}	**Japanese**	**Caucasians**
Inebriation	7 (58%)	5 (42%)
Sleepiness	5 (42%)	4 (33%)
Dizziness	3 (25%)	2 (17%)
Headache	3 (25%)	0 (0%)
Poor alcohol tolerability*	1 (8%)	0 (0%)
0.6 g·L^{-1}	**Japanese**	**Caucasians**
Inebriation	9 (75%)	6 (50%)
Painful infusion	5 (42%)	6 (50%)
Sleepiness	5 (42%)	3 (25%)
Dizziness	3 (25%)	3 (25%)
Poor alcohol tolerability*	3 (25%)	0 (0%)
Dry mouth	0 (0%)	3 (25%)
Headache	2 (17%)	0 (0%)
Feeling hot	2 (17%)	0 (0%)

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