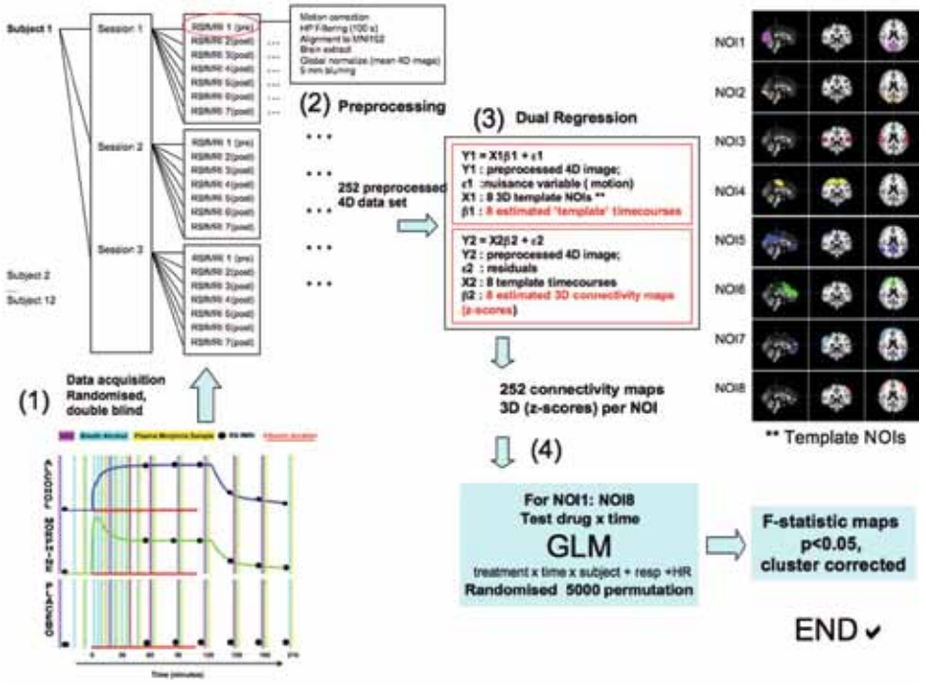
REMCO ZOETHOUT

# APPLICATIONS OF ALCOHOL CLAMPING IN EARLY DRUG DEVELOPMENT

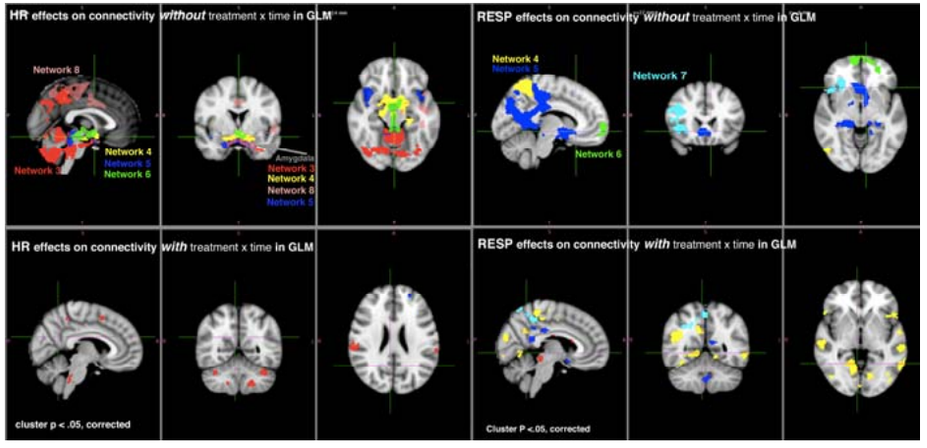
**CHAPTER 6 * FIGURE 1**

SCHEMATIC PRESENTATION OF STUDY DESIGN AND DATA COLLECTION




**CHAPTER 6 * FIGURE 4**

OVERLAYING MAPS OF VOXEL-WISE REGRESSION OF RESPIRATION (RESP) AND HEART RATE (HR) WITH CONNECTIVITY TO THE 8 TEMPLATE NETWORKS



# APPLICATIONS OF ALCOHOL CLAMPING IN EARLY DRUG DEVELOPMENT

**PROEFSCHRIFT**

ter verkrijging van de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus Prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties,
te verdedigen op woensdag 27 juni 2012,
klokke 11:15 uur

**DOOR**

Remco Wiebe Martijn Zoethout
geboren te Hoorn
in 1979

**PROMOTIECOMMISSIE**

PROMOTORES

Prof. dr. J.M.A. van Gerven

Prof. dr. A.F. Cohen

OVERIGE LEDEN

Prof. dr. A. Dahan

Prof. dr. M. Danhof

Dr. R.J. Verkes, psychiater, Radboud MC,
Radboud Universiteit Nijmegen

DESIGN

Caroline de Lint, Voorburg (caro@delint.nl)

ACKNOWLEDGEMENTS

The publication of this thesis was financially supported by the foundation
Centre for Human Drug Research (CHDR), Leiden, the Netherlands

# CHAPTER 1

*Introduction*

Alcohol (or ethanol) is probably the most commonly used drug worldwide (Jang and Harris, 2007), and is mainly consumed for its mild euphoric and disinhibitory effects. For the most part, alcohol is a central nervous system depressant, although it may have biphasic effects (Pohorecky, 1982). Low doses of alcohol often have stimulant properties and increase sociability. In the non-alcoholic, typical effects of low alcohol levels (50 mg/dl) include talkativeness, relaxation and tension reduction. Higher blood levels (above 100 mg/dl) significantly impair mental and cognitive ability including judgement, and there is depression of sensory-motor functioning. As blood ethanol levels exceed 200 mg/dl sensory and cognitive functioning is markedly impaired and at 300 mg/dl most individuals would be stuporous. At higher concentrations ($LD_{50}$ = 400 mg/dl), alcohol is lethal due to severe depression of respiratory function or other complications, such as aspiration of vomit (Pohorecky and Brick, 1988).

The effects of alcohol can partly be explained by its increasing effect on membrane fluidity and its subsequent disturbance of otherwise strictly regulated ion channels and electrolyte balances (Pohorecky and Brick, 1988). Additionally (or consequently), alcohol's central nervous system (CNS) effects are mediated through actions on a variety of neurotransmitters. Dopamine, serotonine, glutamate, noradrenaline and gamma-aminobutyric acid (GABA) are primarily involved. $GABA_A$-receptors are among the most widely distributed neurotransmitter systems in the brain, and alcohol causes an allosteric enhancement of these inhibitory ion channels. This could explain why many of the effects of alcohol are related to CNS-depression. Research shows that alcohol also tends to interfere with opioid receptor binding (Pohorecky and Brick, 1988). There is a complex interplay between these excitatory and inhibitory systems. The numerous transmitters involved in alcohol's action explain its diverse effects and the large number of drug interactions with both prescribed and illicit drugs (McIntosh and Chick, 2004).

These acute CNS-effects have already been extensively investigated. Numerous tests and methods are currently used in such studies to investigate the different effects of alcohol. This diversity reflects the wide range of effects caused by alcohol and its large biomedical and psychosocial impact, but does not explain or justify why so many different tests are used even to study the same effect. The sensitivity of these tests to the effects of alcohol has often not been completely ascertained, and concentration- or dose-effect relationships have only rarely been systematically reported. An overview of the sensitivity and dose responsiveness of different CNS-tests to the effects of alcohol would be useful for future studies focusing on acute alcohol effects or drug-alcohol interaction studies, and could constitute a useful collection of tests to evaluate the acute effects of alcohol on the CNS. Chapter 2 of this thesis contains a systematic review, which attempts to determine the alcohol sensitivity for the large number of CNS tests that is described in the literature.

Another methodological problem in acute alcohol studies is the huge variability in the alcohol exposure profiles that are encountered in such studies. Individuals vary as much as 3- to 4-fold in systemic concentrations and metabolic rates after alcohol administration (Ramchandani *et al.*, 2006). Many factors contribute to this variability, including doses and administration modes, the amount and rate of prior alcohol exposure, drinking history and food intake, differences in gastric emptying, liver volume, blood flow, race, age and gender (Ramchandani *et al.*, 2006). Besides, most of the effects of alcohol are concentration- and time-dependent (Hiltunen *et al.*, 2000; Vogel-Sprott, 1979), and interpretation of the results can be complex if plasma levels change over time, particularly when this is not recorded accurately. This is a problem for many types of alcohol research, including drug interaction studies. A major part of this thesis is devoted to the development and validation of an alcohol administration mode that allows the investigators to attain stable plasma levels of alcohol at a predetermined level in each individual. Few studies of alcohol effects or drug interactions are performed at pseudo-steady state levels, or under other conditions in which these complexities are regulated. In many cases, alcohol exposure is merely controlled by adaptation of the dose to weight, and sometimes to gender or other demographic variables (Wilkinson, 1980). Measurements of the alcohol effects are usually performed at fixed time intervals after intake, and the number of samples is small. Often, breath alcohol levels (BrAC) are measured, but efforts to maintain constant levels are rare. A major reason for this lack of methodological stringency is the complexity and variability of alcohol's pharmacokinetic characteristics.

In an attempt to reduce experimental variance during alcohol studies, O'Connor *et al.* developed a method to keep alcohol plasma levels within close limits using an oral loading dose followed by an intravenous infusion (O'Connor, 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 or BrAC. When alcohol elimination is fully saturated, it is excreted at a constant rate, independent of concentration (Michaelis-Menten kinetics). Therefore, when the input 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 to, and should hence lead to this necessary change. This method was described as the Indiana BrAC clamp.

In this thesis, the original clamping paradigm was modified, with the aim of creating a comparably reliable technique, but one that would be less intensive for the operator and the study subject. In this way, the alcohol administration mode would be more feasible for concomitant CNS research in larger numbers of subjects. The oral loading dose was replaced by an intravenous loading dose to increase experimental control. Also, the time interval between breath samples was increased to facilitate the integration of multiple CNS-measurements into the course of the clamp. Simulations were used to optimize the different phases of the infusion regimen. Additionally, to simplify the execution of the procedure for a larger number of operators after minimal training, the new paradigm was converted into a spreadsheet-based program. These alterations resulted in a new alcohol clamping paradigm, which was tested and simultaneously compared to another procedure for maintaining alcohol at a pre-set plasma level (chapter 3).

Subsequently, a study was performed to investigate whether an intensive battery of CNS measurements could be performed during the course of the new clamping method without interference with or by the alcohol clamping procedure and with the stability of the alcohol levels (chapter 4). This battery consisted of CNS-tests that were highly sensitive to the effects of alcohol and its selection was largely based on our own literature findings (i.e. chapter 2). The CNS-effects of alcohol under pseudo-steady state conditions were quantified in this study, to investigate the profile and time-dependence of CNS-effects during stable (pseudo-steady state) alcohol levels.

The clamping method might also be an appropriate tool to compare groups. Both pharmacodynamic differences and differences in alcohol metabolism can be explored between certain subgroups (e.g. male/female; alcoholics/non-alcoholics; Caucasians/Japanese) without confounding differences in alcohol levels. It is generally assumed that 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 aldehyde dehydrogenase (ALDH) deficiency (Chan, 1986). Differences in body posture and in lifestyle (food and/or 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 of alcohol or other differences in habitual use patterns could contribute to an increased sensitivity. Since the alcohol clamp by-passes most of these factors, we tried to directly compare alcohol metabolism and CNS-effects between Caucasians and Japanese subjects in a subsequent study (chapter 5). Besides, efforts were made in this study to expand and to fine-tune the clamping procedure: steady-states were clamped at multiple levels (on different occasions) and initial infusion rates were customized to individual demographic characteristics, to avoid overshoots.

In CNS drug research it is rarely possible to reliably characterize and differentiate drugs on the basis of one single functional test. Profiles of CNS-effects using a test battery usually provide more distinctive information, but this clearly depends on the breadth of scope of the test battery. The information is only reliable if the battery covers the CNS functions that are primarily affected by one drug and much less so or differently by the other. However, CNS-functions are hardly ever determined by a single neuropharmacological mechanism. Hence, functional assessments are bound to show overlap between different drugs classes, and to be affected by non-pharmacological factors like motivation, fatigue and other aspects of

psychological and physical well-being. A generally applicable methodology for repeated measurements of direct drug effects on the entire CNS, without task-related interactions and a priori models, would constitute a major improvement in CNS drug development. Resting-state functional magnetic resonance imaging could satisfy many of these requirement, and was hence hypothesized to be a promising technique for pharmacological research. We investigated whether different psychoactive substances cause drug-specific effects in functional brain connectivity during resting-state (chapter 6). Both alcohol and morphine were selected for this purpose, because of their well-known CNS-effects, which show functional similarities (on attention and mood) as well as distinctions (on respiration, heart rate, motor function and others). Stable drug levels are preferred in this proof-of-concept study to assist in the calibration of the resting state technique. Therefore, the alcohol clamp was used here to produce stable and prolonged alcohol levels with minimal variability, as well as a target-controlled morphine infusion.

Alcohol is a well-know drug in medicine and in society. The effects of alcohol can therefore be used as a frame of reference for compounds that share some functional aspects with alcohol. Some drugs affect performance, and it could be useful to compare these impairments to the effects of alcohol, at a level with a recognized functional impact - for instance based on legal driving limits. Another example is to use a well-known therapeutic effect of alcohol as a benchmark for the effects of a novel drug with a similar putative therapeutic effect. This approach was chosen for the development of two new drugs for essential tremor (ET), which in many patients is improved by alcohol. ET is one of the most common neurological disorders among adults, and is the most common tremor disorder (Louis *et al.*, 1998; Louis, 2001; Louis, 2005). Current pharmacological treatment act symptomatically and have variable effectiveness (Chen and Swope, 2003). Moreover, the occurrence of side effects, like sedation, weight gain and cognitive impairment, limit their use. Estimations indicate that alcohol is effective in approximately 70% of ET patients (Lou and Jankovic, 1991; Koller *et al.*, 1994). This finding is confirmed by controlled studies where alcohol was administered acutely (Growdon *et al.*, 1975; Koller and Biary, 1984). It most likely acts via a reduction of central overactivity, which results in reduced tremor amplitude, whereas the frequency remains unaffected (Koller and Biary, 1984; Koller, 1991). In a search for novel pharmacological treatment options a $GABA_A$ α2,3 subtype-selective partial agonist and a histamine-3 receptor inverse agonist were investigated in two different studies for their efficacy to reduce tremor symptoms (chapter 7 and 8). In both studies, the alcohol clamp was introduced and served as a positive control. In contrast to most controlled trials in which the effect of alcohol on ET is being studied, we attempted to obtain fixed alcohol levels for a prolonged period of time to induce a stable stimulus that would minimize the variability.

An obvious application of the alcohol clamp in drug development, is its use during ethanol interaction studies. The stability and predictability of the clamp reduces the variability that is inherent to most other modes of ethanol administration. This increases the reliability of the statistical analyses of an interaction, and reduces the risks of unexpectedly high pharmacokinetic levels and pharmacodynamic effects in the presence of other drugs with metabolic or central nervous system drug effects. In chapter 9, we employed the alcohol clamp for an interaction study with a novel $D_3$-antagonist.

This thesis describes the development of a novel alcohol clamp (chapter 3), a new method to obtain stable plasma levels of alcohol and its application in CNS-research. The method might have several advantages that were explored in subsequent studies described in this thesis. The stability of the alcohol clamp was used to examine functional effect profiles and time-dependence of different CNS-effects (chapter 4). The tests to examine these effects were chosen based upon a prior review of the literature, during which the most sensitive CNS-tests were selected (chapter 2). Hereafter, we studied the alcohol clamping method as a tool to compare alcohol disposition capacities between different (ethnic) populations and as a tool to compare their different CNS-responses to *multiple* stable alcohol levels. We also investigated whether the clamping method could be useful as a future benchmarking entity in CNS-research, based on its fMRI effects on the brain at rest (chapter 6) and its efficacy on tremor symptoms (chapter 7 and 8). Finally, we employed the method in an interaction study with a compound that is in development for addictive disorders including alcoholism (chapter 9).

Bennett RH, Cherek DR, Spiga R (1993). Acute and chronic alcohol tolerance in humans - effects of dose and consecutive days of exposure. Alcohol Clin Exp Res 17:740-745
Chan AW (1986). Racial differences in alcohol sensitivity. Alcohol Alcohol 21:93-104.
Chen J J, Swope D M (2003). Essential tremor: diagnosis and treatment. Pharmacotherapy 23: 1105-1122
Duranceaux NC, Schuckit MA, Luczak SE, Eng MY, Carr LG, Wall TL (2008). Ethnic differences in level of response to alcohol between Chinese Americans and Korean Americans. J Stud Alcohol Drugs 69:227-234
Growdon JH, Shahani BT, Young R R (1975). The effect of alcohol on essential tremor. Neurology 25: 259-262
Hiltunen AJ, Saxon L, Skagerberg S, Borg S (2000). Acute tolerance during intravenous infusion of alcohol: comparison of performance during ascending and steady state concentrations – a pilot study. Alcohol 22:69–74
Jang GR, Harris RZ (2007). Drug interactions involving ethanol and alcoholic beverages. Expert Opinion on Drug Metabolism & Toxicology 3:719-731
Koller WC, Biary N (1984). Effect of alcohol on tremors: comparison with propranolol. Neurology 34: 221-222
Koller WC (1991). A new drug for treatment of essential tremor? Time will tell. Mayo Clin Proc 66: 1085-1087
Koller WC, Busenbark K, Miner K (1994). The relationship of essential tremor to other movement disorders: report on 678 patients. Essential Tremor Study Group. Ann Neurol 35: 717-723
Lou JS, Jankovic J (1991). Essential tremor: clinical correlates in 350 patients. Neurology 41: 234-238
Louis ED, Ottman R, Hauser WA (1998). How common is the most common adult movement disorder? estimates of the prevalence of essential tremor throughout the world. Mov Disord 13: 5-10
Louis ED (2001). Essential tremor. New England Journal of Medicine 345: 887-891
Louis ED (2005). Essential tremor. Lancet Neurology 4: 100-110
Mukherjee S, Das SK, Vaidyanathan K, Vasudevan DM (2008). Consequences of alcohol consumption on neurotransmitters -an overview. Curr Neurovasc Res. 5(4):266-72
McIntosh C, Chick J (2004). Alcohol and the nervous system. J Neurol Neurosurg Psychiatry 75(Suppl III):16–21
O'Connor S, Morzorati S, Christian J, Li TK (1998). Clamping breath alcohol concentration reduces experimental variance: application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcohol Clin Exp Res. 22(1):202-10
Pohorecky LA (1982). Influence of alcohol on peripheral neurotransmitter function. Fed Proc. 41(8):2452-5
Pohorecky LA, Brick J (1988). Pharmacology of ethanol. Pharmac. Ther. 36:335-427
Ramchandani VA, O'Connor S, Neumark Y, Zimmermann US, Morzorati SL, de Wit H (2006). The Alcohol Clamp: Applications, Challenges, and New Directions – An RSA 2004 Symposium Summary. Alcoholism: Clinical and Experimental Research 30(1):155-164
Shibuya A, Yasunami M, Yoshida A (1989). Genotypes of alcohol-dehydrogenase and aldehyde dehydrogenase loci in Japanese alcohol flushers and nonflushers. Human Genetics 82:14-16.
Vogel-Sprott MD (1979). Acute recovery and tolerance to low doses of alcohol: differences in cognitive and motor skill performance. Psychopharmacology (Berl) 61:287–91
Wilkinson PK (1980). Pharmacokinetics of ethanol–review. Alcohol Clin Exp Res. 4:6–21



# CHAPTER 2

## *Functional biomarkers for the acute effects of alcohol on the central nervous system in healthy volunteers*

*Br J Clin Pharmacol 2011, 71(3), 331-350*

R.W.M. Zoethout, W.L. Delgado, A.E. Ippel, A. Dahan, J.M.A. van Gerven

## ABSTRACT

*BACKGROUND* The central nervous system (CNS) effects of acute alcohol administration have been frequently assessed. Such studies often use a wide range of methods to study each of these effects. Unfortunately, the sensitivity of these tests has not completely been ascertained.

*METHODS* A literature search was performed to recognize the most useful tests (or biomarkers) for identifying the acute CNS-effects of alcohol in healthy volunteers. All tests were grouped in clusters and functional domains. Afterwards, the effect of alcohol administration on these tests was scored as improvement, impairment or as no effect. Furthermore, dose-response relationships were established.

*RESULTS* A total number of 218 studies, describing 342 different tests (or test variants) were evaluated. Alcohol affected a wide range of CNS-domains. Divided attention, focused attention, visuo-motor control and scales of feeling high and of subjective drug effects were identified as the most sensitive functional biomarkers for the acute CNS-effects of alcohol.

*CONCLUSIONS* The large number of CNS-tests that are used to determine the effects of alcohol interferes with the identification of the most sensitive ones and of drug-response relationships. Our results may be helpful in selecting rational biomarkers for studies investigating the acute CNS-effects of alcohol or for future alcohol-interaction studies.


## INTRODUCTION

Ethyl alcohol, or ethanol causes dose-dependent central nervous system (CNS) depression, which culminates in a state of general unconsciousness at high plasma concentrations (Little, 1991). Prior investigations indicate that the predominant mechanism of CNS depression involves selective alcohol interactions with ion channels that include allosteric enhancement of inhibition mediated by gamma-aminobutyric acid A (GABA-A) receptors, antagonism of excitation by N-methyl-D-aspartic acid (NMDA) glutamate receptors and possibly inhibition of central L-type $Ca^{+2}$ channels (Little, 1991; Weight *et al.*, 1992). Although alcohol is classified as a sedative drug, it can also have stimulant effects (Charness *et al.*, 1989; Little, 1991; Samson and Harris, 1992). The concentration- and time-dependence of its inhibitory and stimulatory properties in humans has not yet been fully elucidated, partly due to the complicated and variable pharmacokinetics of alcohol, but also to the lack of standarised tests for the CNS-effects of ethanol.

Alcoholic beverages are used commonly and worldwide (Jang and Harris, 2007), and the CNS-effects of acute alcohol administration have been frequently quantified, a wide range of methods are used in such studies to study the different effects of alcohol. The sensitivity of these tests to the effects of alcohol has often not been completely ascertained, and concentration- or dose-effect relationships have only rarely been systematically reported. An overview of the sensitivity and dose-responsiveness of different CNS-tests to the effects of alcohol would be useful for future studies focusing on acute alcohol effects or drug-alcohol interaction studies, and would constitute a useful collection of tests to evaluate the acute effects of alcohol on the CNS.

A biomarker is described as a characteristic that is measured and evaluated as an indicator of normal or pathologic biological processes or pharmacologic responses to a therapeutic intervention (Colburn, 2003). A biomarker can be any response measure that shows a clear, consistent response to meaningful doses, across studies from a sufficient number of different research groups. A dose-response relationship and a plausible relationship between the biomarker and the pharmacology of alcohol provide indications that a biomarker reflects pharmacological activity. Previously, these criteria were used to evaluate the usefulness of CNS-tests (or functional biomarkers) for the effects of antipsychotic drugs (de Visser *et al.*, 2001), benzodiazepines (de Visser *et al.*, 2003), selective serotonin reuptake inhibitors (SSRI's) (Dumont *et al.*, 2005), 3,4-methyleendioxymethamfetamine (MDMA) (Dumont and Verkes, 2006) and $\Delta^9$-tetrahydrocannabinol (THC) (Zuurman *et al.*, 2008) in healthy subjects. In general, these systematic reviews showed that only a small number of tests actually display proper characteristics for a meaningful

effect biomarker, that these tests differ between the various drug classes, and that most of these biomarkers belong to a small number of functional CNS-domains: attention, memory, visuomotor and motor performance, subjective effects and certain neurophysiological tests (eye movements, electroencephalography). In addition, some drug classes cause specific neuroendocrine responses.

In an attempt to structure and subsequently evaluate the wide diversity of functional biomarkers for the CNS-effects of ethanol, an extensive literature search was performed. Because of an apparent lack of standardization between the studies (even for the same tests), a structured procedure described previously was adopted, which includes progressive condensation of the tests into clusters of related tests and into domains of CNS-functions, prior to the analyses (de Visser *et al.*, 2001; de Visser *et al.*, 2003; Dumont *et al.*, 2005; Dumont and Verkes, 2006). The criteria mentioned for meaningful biomarkers were eventually applied to the results. All effects of alcohol other than on the CNS (e.g. on the liver) were excluded, except neuro-endocrine responses. The primary objective of the current review is to present a systemic overview of the usefulness of the different CNS-tests described in the literature, which allow a reliable assessment of the acute CNS-effects of alcohol in healthy adult volunteers. Accurate tests to measure the acute effect of alcohol on the CNS are vital when the effect of alcohol in combination with a CNS-drug is being studied. The results of this review may also be useful to rationally select sensitive CNS-test for drug/alcohol interaction studies, which are often required for registration of new CNS drugs.

## METHODS

### *Structured literature evaluation*

'Ethanol' (MESH), 'effect' and 'CNS' were used as pivotal keywords to construct a MedLine search. This search included a large number of studies that were irrelevant for the specific primary objective of this review. Therefore, a wide range of specific CNS-functions was added to these keywords to ensure a comprehensive CNS-effect profile. Subsequently, inappropriate terms (e.g. 'in vitro', 'withdrawal' or 'deaths') were excluded from the search by using the '*not*' search option. To obtain a manageable data-set, the search was limited to 'adult: 19-44 years', 'English', 'publication date from 1980 to 2008' and 'humans'. The complete search query, which yielded 1263 publications, is provided in table 1.

All publications obtained using this strategy underwent a thorough selection process. Initially, all articles were manually screened by title. Articles with irrelevant titles, given the selected search terms were discarded. Remaining articles were carefully studied and those that did not comply with the main objectives of this review (e.g. studies describing chronic alcohol effects) were discarded. In addition, studies investigating alcohol effects under specific artificial circumstances or conditions (e.g. sleep restriction, hypoxemia or anxiety paradigms), and studies dealing with more drugs or substances than alcohol alone (i.e. interaction studies), were not selected for further analysis, even if part of the design complied with the requirements of this review. Also, studies investigating a specific group of subjects other than regular healthy adult volunteers (e.g. heavy drinkers, patients or certain professionals) were disregarded. Studies in which such populations were discussed have been excluded from our analysis, as in our opinion such populations exhibit different responses to similar doses of alcohol compared to 'healthy volunteers' and thus may negatively bias our results (e.g. pilots are supposed to have faster baseline reaction times in tests that measure reaction time speed and alcoholics probably show less effects in studies measuring subjective effects). Thereby, we only excluded tests that were also frequently reported in healthy volunteers (but with different results) rather than tests that were specifically used in these special populations. Furthermore, studies with fewer than twelve participating healthy volunteers were also disregarded. Finally, papers that only mentioned the dose of alcohol instead of the blood alcohol concentration (BAC) or the equivalent breath alcohol concentration (BrAC) were excluded, since these studies are less suitable for accurate analysis of the relationships between alcohol levels and effects.

At the end of this process, 218 titles were found eligible for review, which were subsequently evaluated based on the items summarized in table 2. The

results were captured into a Microsoft Excel® database. During this process the effect of alcohol on every individual test was scored, tests were grouped and alcohol levels were categorized.

## *Individual test results*

Based on previous reviews (de Visser *et al.*, 2001; de Visser *et al.*, 2003; Dumont *et al.*, 2005; Dumont and Verkes, 2006), it was anticipated that in most cases no consistent quantitative results could be obtained from individual tests, because of the large diversity of methods, parameters and treatments. Therefore, the ability of a test to detect a statistically significant difference from placebo or baseline was scored as '+' (improvement/increase), '=' (no significant effect) or '-' (impairment/decrease). Subjective assessments of effects that were signs of improved CNS-function or that most users would consider pleasurable (e.g. increase of a high or drug effect scale) were scored as an improvement/increase; symptoms of CNS-depression or less desirable, adverse effects (e.g. increase of sedation or reduction of alertness) as an impairment/decrease. For physiological responses like changes in hormone levels or EEG-power a functional interpretation was not always obvious, and these results were scored as increases (+), decreases (-) or as no change (=), according to the direction of the reported effects. The total amounts of (+), (-) and (=) were determined within each cluster and percentages were calculated. Afterwards, these percentages were visually inspected to detect whether alcohol mainly impaired, improved or had no effect on a certain cluster. Subsequently, the sensitivity of each domain to alcohol was evaluated by inspection of the number of clusters within a certain domain that was clearly affected by alcohol.

Some studies explicitly reported the use of several different tests in the methods section, without presentation of the results, for no apparent reason, such as a separate publication. To avoid bias due to underreporting of negative results, it was assumed that these tests had not shown any significant effects and were scored accordingly. In some studies with different drug doses, overall significances were reported for drug effects, without (post hoc) quantifications of the statistical significance levels for each individual dose. In these cases, efforts were made to estimate the individual dose effects from graphs or tables provided in the article. If this was impossible, only the largest average effect was assumed to be significant (in case of overall statistical significance) and smaller effects were considered non-significant.

## *Grouping of individual test results*

Because of an apparent lack of standardization between the studies even for the same tests, a structured procedure was adopted as described previously (de Visser *et al.*, 2001; de Visser *et al.*, 2003; Dumont *et al.*, 2005; Dumont and Verkes, 2006) in order to obtain a meaningful overview. This approach allowed the preservation of individual study data in early stages, followed by a progressive condensation of results into logical test clusters and functional (CNS) domains. For example, all tests that determine the ability to discriminate flash- or flicker frequencies were grouped as the test cluster flicker discrimination and were subsequently categorized under the corresponding CNS-domain attention. A compendium of neuropsychological tests from Spreen *et al.* (Spreen and Stretton, 1998) was primarily consulted to group functional tests into clusters of related tests or test variants. Additionally, the compendium of Lezak *et al.* (Lezak *et al.*, 2004) was frequently consulted. Occasionally, a specific test was not described in these compendia. In these cases, the author's classification was followed or if necessary the test was clarified using other literature sources and classified by consensus. Tests and clusters were grouped further into domains that represent higher aggregates of integration of subjective, neuropsychological, neuroendocrine and neurophysiological functions. The neuropsychological domain is generally subdivided into executive functions, memory, attention, motor functions, language and perception (Spreen and Stretton, 1998). For each test (or cluster), the compendia were also used to determine which CNS-function was principally assessed by the test. Some tests provided different parameters with information on more than one functional domain. The results of the effects of a single test on different domains were scored separately, and the secondary effects were marked.

The effects of alcohol on multifactorial assessments like the Profiles of Mood States (POMS) (McNair *et al.*, 1971), the Addiction Research Center Inventory (ARCI) (Haertzen, 1965) or the Bond & Lader Visual Analogue Scales (VAS) (Bond and lader, 1974) were frequently reported. Subscales of such inventories were grouped together if they fell in the same cluster. Sometimes, individual subjective scales (and variants) were reported that could be regarded as variants from a basic form (e.g. subscales that are also part of more comprehensive tools like the Bond & Lader VAS). Such scales were clustered according to the original scale with which they corresponded (e.g. into scale alertness, scale mood or scale calmness in case of a Bond & Lader subscale). Within such clusters, all scales showing a significant effect were grouped, whereas all scales showing no effect were grouped separately. In this way, scales within the same cluster that showed mixed results were also scored equivocally.

Comprehensive scoring instruments like the ARCI, the POMS or the Drug Effect Questionnaire (DEQ) (Mintzer and Griffiths, 1999) can be subdivided into different subjective clusters (e.g. scale craving or scale alertness), but these subscales were not always reported separately. In these cases, the results of the composite scores were presented as part of the overall scale drug effect cluster for the DEQ and ARCI and the scale mood cluster for the POMS. A similar procedure was followed for driving tests. In some cases the effects on a driving task were reported as effects on separate CNS-functions like divided attention, reaction time and motor function. These driving tasks were grouped accordingly. However, when an overall composite driving score was reported, driving tests were grouped under the cluster driving.

## *Categorization of alcohol levels*

The chance that a test will detect a difference from placebo is expected to increase with the alcohol level (measured through BAC and/or BrAC). To investigate this possibility, it was determined for each individual test whether the proportion of statistically significant effects increased with BAC/BrAC.



In this way, the most frequently used tests and alcohol dosages could be compared for dose-dependency. For individual tests, the number of studies or the variability of alcohol levels were too small to determine meaningful dose-effect relationships. To obtain an overview of dose-effects, alcohol levels were pooled into 'lower', 'medium' and 'higher' levels. The levels were determined after inspection of the reported alcohol levels, but before relationships with pharmacodynamic tests were examined. The 'medium' level was chosen to be a BAC or BrAC of 0.5 $g \cdot L^{-1}$ - 0.7 $g \cdot L^{-1}$, because this resulted in an even distribution of studies across alcohol levels. This mid-range also included the legal driving limits for most western countries, and it would be useful to show which functional biomarkers are able to detect alcohol effects at this legal level. The 'lower' and 'higher' levels were all BAC/BrAC's outside the medium range. This approach allowed the identification of tests or clusters that showed a consistent response across studies and alcohol concentrations.

Several studies reported the effects of both ascending or descending alcohol levels. In these cases, we considered a certain test to be effective in detecting alcohol effects, when at least one of both limbs was significantly affected. In addition, it was the intention to evaluate the effect of increasing or decreasing alcohol levels on individual tests that showed a consistent dose-response relationship *and* were measured frequently enough.

# RESULTS

## *Study characteristics*

General study characteristics are reported in table 3.

## *Subject characteristics*

The mean number of participants was 31 (23 males and 8 females). The mean age of participants was not reported in 35% of the cases. The mean age for all remaining articles was 24.8 years (range: 19 – 42 years).

## *Alcohol characteristics*

In the majority of the cases (98%), alcohol was orally administered. In only 2% of all studies an intravenous administration procedure was described. Doses were not reported in 18 articles (8%). The calculated mean dose that was administered during trials was 0.69 $g \cdot kg^{-1}$ (SD: 0.25 $g \cdot kg^{-1}$). BrAC was measured in 199 studies (91%), whereas BAC was used as a parameter in only 13 studies (6%). Six studies (3%) reported both values jointly. BAC and BrAC were pooled together to calculate the mean alcohol concentration, which was 0.65 $g \cdot L^{-1}$ (SD: 0.20 $g \cdot L^{-1}$).

### *CNS tests*

This review yielded 342 different tests to assess the acute CNS-effects of alcohol. Table 4 shows a distribution of the test frequencies across the literature search, indicating that a sizeable majority of all the described tests was used only once (69.3%) or no more than five times (89.2%). Tests that were used more than 10 times in the overall data-set are summarized in table 5. This arbitrary cut-off was used to get an indication of the most frequently used tests. The results of such solitary tests cannot be used to draw general conclusions about acute alcohol effects. Only scale intoxication was used frequently enough (26 times) to allow an individual analysis of alcohol responsiveness, but in all other cases tests needed to be clustered to increase numbers sufficiently for a more general interpretation.

## *Clustered alcohol effects*

Individual tests were grouped into predefined clusters in an attempt to facilitate the general interpretation of the results. Table 6 summarizes the progressive condensation of all individual tests into clusters with their corresponding CNS-domains. Overall calculated significant alcohol effects (i.e. impairment/decrease (-), no significant change (=) or improvement/increase (+)) on each cluster are shown together with the publications in which these effects were described. Table 6 shows that alcohol mainly caused either no effect or functional impairments. Impairments were most pronounced in the clusters divided attention, digit symbol substitution test-like (DSST-like), motor control, postural stability, visuo-motor control, scale performance and in auditory/verbal memory: immediate recall. These clusters were reported frequently enough (>10 times) to allow some general interpretations. Saccadic eye movements were also impaired in 90% of all cases, but these were described only seven times.

Individual memory tests sometimes showed improvements in delayed recall or recognition (between 10 and 33%, table 6), but never on tests of immediate or short term memory. In each of these studies, alcohol had been administered prior to the presentation of learning material. This is in line with the literature, which suggest that memory for information learned before the consumption of alcohol can be retroactively facilitated (Parker *et al.*, 1980; Parker *et al.*, 1981).

Overall increases in effects were mainly found on the domain subjective experience. The clusters scale high and drug effect showed distinct increases of their subjective scores. In contrast, several different clusters of the subjective experience domain did not change much (marked as (=) in table 6) following alcohol administration (e.g. scales aggression, alertness, calmness, mood, and fatigue). The clusters production and semantics (language domain) were also hardly affected by alcohol. Because the effects of alcohol on the functional domain (neuro)endocrine and on several different clusters like production, scale depression, sleep, visual perception and electro-encephalography alpha (EEG alpha) were reported in only a small number of studies (<10), solid conclusions cannot be drawn on the sensitivity of these functional biomarkers.

### *Dose-response relationships*

The ability to show clear dose-response relationships is an important requirement for a meaningful drug-effect biomarker (de Visser *et al.*, 2001; de Visser *et al.*, 2003; Dumont *et al.*, 2005; Dumont and Verkes, 2006). The dose also determines the sensitivity of a test for a drug, and the chance to detect an effect. Therefore, tests and clusters were examined for potential relationships to ethanol dose. An arbitrary cut-off of ten reports per dose level for at least two levels was used to study the dose-response relationships for the most frequently reported clusters (table 7). Divided attention, scale high and scale drug effect were among the most sensitive clusters to detect alcohol effects, since a majority of tests were affected at the lowest alcohol dose levels. Many test clusters showed an increased proportion of significant drug effects at higher dose levels (table 7). The most convincing dose-response impairments were found for focused attention (7% of the tests within this cluster were impaired in the <0.5 $g \cdot L^{-1}$ level, increasing to 62% at 0.5 – 0.7 $g \cdot L^{-1}$ and 74% with levels >0.7 $g \cdot L^{-1}$), divided attention, reaction time, inhibition, working memory and visuo-motor control. Clear dose-related effects were also found for the cluster scale drug effect.

We made efforts to evaluate the effect of increasing and decreasing alcohol levels on individual tests that showed a consistent dose-response relationship and were reported frequently enough. Unfortunately, only few of these studies did address this issue. We therefore restricted our review to the main objective of creating an overview of the most sensitive CNS-tests to measure the acute effects of alcohol.

All 15 scales of the cluster subjective high that were tested at the 0.5 - 0.7 $g \cdot L^{-1}$ level increased after alcohol administration. Although they were only tested 9 times under high dose circumstances throughout the studies, all observed subjective high scales were affected by alcohol at this level. The effects on other frequently reported clusters described in table 7 either increased hardly with dose (e.g. evoked potential and scale craving) or were not clearly dose-related (scale calmness, scale mood and scale alertness). While visual perception was only occasionally reported across different research groups, a mild dose-related deterioration was observed at the highest dose level.

## DISCUSSION

A large number of tests are used in the literature to measure the acute CNS effects of alcohol, even for the same effect. As with similar reviews for other drug classes (de Visser *et al.*, 2001; de Visser *et al.*, 2003; Dumont *et al.*, 2005; Dumont and Verkes, 2006; Zuurman *et al.*, 2008), there were even more tests than articles: 342 in 218 publications. Almost 70% of all reported tests were only used once, and close to 90% was used five times or less. This lack of standardisation limits the comprehension of the effects of drugs on the CNS. For alcohol, this is complicated further by complex (saturable) pharmacokinetics (with large intersubject variability related to induction of clearance, sex and other genetic factors), tolerance and withdrawal effects, drug and food interactions and differences between patient populations (alcoholics, drug abusers, social anxiety disorder, etc.). Understanding these complexities, and their functional consequences for social and problematic drinking, would be facilitated by the use of a limited number of well-characterised biomarkers for different alcohol effects, reflecting a range of relevant functions. With this background, tests were grouped into test clusters and functional domains. Prior reviews indicate that this technique served as a helpful tool in evaluating functional biomarkers for other drug effects (de Visser *et al.*, 2001; de Visser *et al.*, 2003; Dumont *et al.*, 2005; Dumont and Verkes, 2006; Zuurman *et al.*, 2008). Although this methodology inevitably led to the loss of certain information, it resulted in a structured and comprehensive overview of the CNS-effects of alcohol.

As far as possible, we used neuropsychological compendia (Spreen and Stretton, 1998; Lezak *et al.*, 2004) ill, some categorization we undertook might seem arbitrary. The short memory test for example, was captured under executive functions instead of memory (as one might expect from the test name). Although this seems confusing at first sight, it is completely in line

with the neuropsychological compendia we used. These authors state that the short memory test should be considered as a working memory (or executive) task rather than a pure memory task, because it is governed by brain areas that are also related to planning, organizing and time orientation. Longer-term memory tests in a stricter sense require much more hippocampal activity.

Tests within the most sensitive clusters as shown in table 6, which also show a clear dose-response relationship as shown in table 7 are considered most valuable. Thus, divided attention tests (i.e. attention domain), visuo-motor control tests (i.e. motor domain) and subjective drug effect tests (i.e. domain subjective effects) are the most sensitive functional biomarkers for the acute effects of alcohol on the CNS in healthy volunteers (at least according to the results of our review). Most clusters of the attention domain were clearly affected by alcohol. The cluster divided attention showed a higher sensitivity to alcohol compared to clusters like reaction time and focused attention, since these tests could detect lower alcohol levels. Tests within the divided attention cluster are more complex than those measuring simple reaction time or focused attention, and it is likely that lower doses of alcohol will have a larger impact on a more complex task than on a simpler version. Tests within the cluster reaction time can still be useful biomarkers, since 73% showed impairments at higher alcohol levels (>0.7 $g \cdot L^{-1}$), but they are less suitable to measure the impact of lower exposure. Similarly, executive clusters like working memory and inhibition are also quite capable of detecting alcohol effects at high doses (on average in 75% and 64% of the cases respectively).

Alcohol clearly impaired the three clusters of the motor domain, but only visuo-motor control tests were reported frequently enough at the different dose levels to allow a dose-response analysis. Although the effects on motor control and postural stability look promising, these tests can only be considered validated CNS-biomarkers for alcohol effects if dose-response relationships are established. Alcohol effects on visuo-motor control were identified at concentrations >0.5 $g \cdot L^{-1}$ and a dose-dependent impairment was observed. The cluster visuo-motor control fulfilled the criteria as a useful functional biomarker. Scales of subjective high and drug effects were by far the most sensitive clusters in the subjective experience domain. Both scales increased dose-dependently, and showed effects in over 90% of the cases in the highest dose category. Many different subjective scales (or scale variants) are currently used in literature to measure subjective alcohol experience, but this review shows that only a few of those scales (subjective high and drug effects) are actually able to accurately measure the subjective effects of alcohol. Scales of calmness, mood and alertness seem to be less sensitive to alcohol.

The sensitivity of many clusters could not be assessed, because they were not reported frequently enough to allow an accurate evaluation (e.g. critical flicker fusion, visual perception and all the (neuro)endocrine clusters), although some of these uncommon clusters showed promising results (e.g. saccadic eye movements, EEG alpha and EEG theta). Clusters like semantics and scale aggression do not seem to be valuable biomarkers for alcohol effects, because the majority of the tests show no effect after alcohol administration in healthy volunteers. Some clusters showed significant overall alcohol effect in only a modest proportion of studies, like inhibition (50%) and working memory (53%). These executive tasks were measured frequently enough to allow a subdivision according to dose levels, which revealed larger percentages in the highest dose category of >0.7 $g \cdot L^{-1}$ (64 and 75% respectively). This indicates that alcohol effects (particularly at higher doses) can be masked for clusters that do not contain enough tests across the different doses to allow dose categorization. An important issue concerning tests within clusters like immediate recall (auditory/verbal memory), working memory and visuo-motor control is that all these functions may be affected by attention and concentration (Spreen and Stretton, 1998). Attention tasks show an effect in 73-79% of cases at higher alcohol doses. Divided attention was even more sensitive, yielding significant results at low doses in over half the cases. The strong influence of alcohol on attention should be taken into account when looking at the results of other test clusters and functional domains that rely on attention.

Despite its infrequent appearance throughout our search, all tests assessing overall driving performance (i.e. cluster driving) were impaired under alcohol. Driving performance is an executive task that to a large extent relies on (visuo-)motor control and focused/divided attention. The most

sensitive functional biomarkers to detect alcohol effects at the average legal driving level (i.e. the medium dose level) include tests of visuo-motor control as well as scales of subjective high and drug effect, followed by focused and divided attention. For visuo-motor control, the pursuit rotor task (a tracking task) was the most appropriate method to detect alcohol effects around the driving limit, at least in a laboratory setting. It is not surprising therefore, that all of the ten driving tests included in our review showed an effect of alcohol, including the two cases that studied the effects of a low dose. Reaction time is another aspect of driving, but individual reaction time tests only showed an impairment at medium levels in only half of the cases. This function seems less suitable to demonstrate the impact of alcohol on driving proficiency in a medico-legal setting.

In summary, the most sensitive functional biomarkers for the acute CNS-effects of alcohol that were identified in this review are divided attention, focused attention, visuo-motor control, scale high and scale drug effect. Furthermore, reaction time, working memory and inhibition are also considered useful, but only at higher alcohol doses. Driving tasks also seemed to be sensitive to even low levels of alcohol, but this complicated setup was not used very frequently in the literature. The impairing effects of alcohol on the clusters DSST-like, motor control, postural stability and immediate recall (auditory/verbal memory) are noteworthy, but clear dose-effect relationships could not be established.

This review describes a systemic literature search aimed to assess the sensitivity and usefulness of functional biomarkers to demonstrate acute CNS-effects of alcohol in healthy volunteers. The results of this review may be helpful in selecting rational biomarkers for studies investigating the acute CNS-effects of alcohol or for future alcohol-interaction studies. The results also show that many different biomarkers are currently used, when a certain CNS effect of alcohol is studied, and that such studies would greatly benefit from a certain degree of standardization.



**TABLE 1** SEARCH QUERY

| Search query |
|---|
| (**effect OR effects**) AND (**'ethanol'**[MESH] NOT (patient OR patients OR genetic OR genetics OR disease OR diseases OR preclinical OR 'in vitro' OR death OR deaths OR traffic OR law OR laws OR injury OR injuries OR hangover OR withdrawal OR chronic OR sexual OR sexuality OR aggressive OR aggression OR MRI OR fMRI)) AND (**'central nervous system'**[MESH] OR neurophysiology OR neuroendocrine OR neuropsychology OR subjective OR behaviour OR cognitive OR cognition OR performance OR executive OR attention OR visuomotor OR psychomotor OR motor OR memory OR sensory OR auditory OR visual OR language OR perception)<br>*limits: entrez date from 1980 to 2008, humans, English, adult: 19-44 years* |

**TABLE 2** CRITERIA USED FOR STUDY EVALUATION

| Evaluation Criteria |
|---|
| Number of males |
| Number of females |
| Age (mean/range) |
| Blinding (open/single-blind/double-blind) |
| Randomization (randomized/non-randomized) |
| Design (parallel/cross-over) |
| Control (baseline controlled/placebo controlled) |
| Dose |
| Route (oral/intravenous) |
| BAC/BrAC |
| Test |
| Test item |
| Primary/secondary outcome parameter |
| Cluster |
| Domain |
| Effect (+/=/-) |

**TABLE 3** SUMMARY OF GENERAL STUDY CHARACTERISTICS

| **Blinding** | | | | **Randomization** | |
|---|---|---|---|---|---|
| double-blind | single-blind | open | un-known | randomized | non-randomized |
| 31% | 46% | 22% | 1% | 86% | 14% |
| **Design** | | | | **Control** | |
| cross-over | | parallel | | baseline-controlled | placebo-controlled |
| 56% | | 44% | | 23% | 77% |

**TABLE 4** FREQUENCY DISTRIBUTION OF 342 TESTS USED IN 218 ACUTE ALCOHOL STUDIES

| Frequency of use | Number of tests | % |
|---|---|---|
| >20 | 1 | 0.3 |
| 11-20 | 13 | 3.8 |
| 6-10 | 23 | 6.7 |
| 2-5 | 68 | 19.9 |
| 1 | 237 | 69.3 |

**TABLE 5** TESTS USED MORE THAN 10 TIMES

| Test name | Number |
|---|---|
| Visual Analogue Scale (VAS): Scale Intoxication | 26 |
| Choice Reaction Time Task | 16 |
| Go/No Go Test | 15 |
| Profile of Mood States (POMS): Scale Depression/Dejection/Elation/(Positive) Mood | 14 |
| Digit Symbol Substitution Test (DSST) | 13 |
| Profile of Mood States (POMS): Scale Anxiety/Confidence/Unsure/Tension/Arousal/Composed | 13 |
| Pursuit Rotor Task | 13 |
| Beverage Rating Scale Scale Intoxication | 12 |
| Biphasic Alcohol Effects Scale (BAES): Scale Sedation | 11 |
| Biphasic Alcohol Effects Scale (BAES): Scale Stimulation | 11 |
| Profile of Mood States (POMS): Scale Anger/Friendliness/Hostility/Agreeable | 11 |
| Profile of Mood States (POMS): Scale Confusion/Vigour/Bewilderment/Activity/Clearheadedness | 11 |
| Profile of Mood States (POMS): Scale Fatigue/Inertia/Tired/Energetic | 11 |
| Subjective Intoxication Level | 11 |



**TABLE 6** PROGRESSIVE CONDENSATION OF ALL REPORTED TESTS, INTO THEIR CORRESPONDING CLUSTERS AND DOMAINS (IN BOLD)

*The overall cluster effects are reported together with the articles in which they are reported. "+" reflects an improvement or increase, "=" reflects no significant effect and "-" reflects an impairment or decrease as measured by the corresponding test. Whenever tests provided different parameters with information on more than one functional domain, effects were scored separately, and the secondary effects were marked (*). Some tests were reported more than once within the same reference (e.g. at several dose levels).*

| **Language** | | | | | |
|---|---|---|---|---|---|
| *Cluster* | *Test* | *(-)* | *(=)* | *(+)* | *References (frequency)* |
| Production | Speech Performance, Speech Test, Voice Onset Time | 33 | 67 | 0 | (Clarici *et al.*, 1995), (Clarici *et al.*, 1993), (Hollien *et al.*, 2001), (Swartz, 1992) (n=4) |
| Semantics | Backwards Reading Task (semantic priming), Lexical Decision Task, Mill Hill Vocabulary Test, Multiple Selective Vocabulary Test, Semantic Priming Task, Sentence Verification Task, Verbal Memory Task*, WAIS III: Vocabulary Test, Word Categorization Task | 31 | 69 | 0 | (Cameron *et al.*, 2001), (Lister *et al.*, 1991), (Maylor and Rabbitt, 1988), (Maylor *et al.*, 1988), (Maylor *et al.*, 1987a), (McKee *et al.*, 2006), (Moulton *et al.*, 2005), (Nagoshi *et al.*, 1992), (Ray and Bates, 2006), (Ray *et al.*, 2004), (Breitmeier *et al.*, 2007) (n=11) |
| **Attention** | | | | | |
| *Cluster* | *Test* | *(-)* | *(=)* | *(+)* | *References (frequency)* |
| Divided Attention | Choice Reaction Time Task (fixed sequences)*, Choice Reaction Time Task (random sequences)*, Choice Reaction Time Task integrated in Pursuit Rotor Task, Choice Reaction Time Task*, Critical Tracking Test (divided attention), Divided Attention Task, Driving Simulation Test (dual task), Primary Tracking Task with Secondary Visual Reaction Time, Pursuit Rotor Task combined with Visual Stimulus-Response Task, Saccadic Interference Task, Switching Attention Task, Tracking Input Manipulator*, Verbal-Manual Task, Visual Vigilance Task, VisuoSpatial Attention Task | 68 | 29 | 3 | (Abroms *et al.*, 2006), (Antebi, 1982), (Clarici *et al.*, 1995), (Clarici *et al.*, 1993), (Connors and Maisto, 1980), (Degia *et al.*, 2005), (Grant *et al.*, 2000), (Knowles and Duka, 2004), (Landauer and Howat, 1983), (Maylor *et al.*, 1989), (Maylor and Rabbitt, 1987b), (Maylor *et al.*, 1987b), (McKee *et al.*, 2006), (Millar *et al.*, 1999), (Mills *et al.*, 1996), (Mills and Bisgrove, 1983a), (Mills and Bisgrove, 1983b), (Niaura *et al.*, 1987), (Schulte *et al.*, 2001), (Sommer *et al.*, 1993), (Tiplady *et al.*, 2001), (Zwyghuizen-Doorenbos *et al.*, 1990), (Breitmeier *et al.*, 2007), (do Canto-Pereira *et al.*, 2007), (Rupp *et al.*, 2007b) (n=25) |
| DSST-like | Code Substitution Task, Digit Symbol Substitution Test (DSST), Digit Symbol Yes/No Test, Simple Letter Verification Task, Symbol Digit Substitution Test (SDST), Symbol Matching Task (reference key present during task), Verification Task | 61 | 39 | 0 | (Cameron *et al.*, 2001), (de Wit *et al.*, 1987), (DeWit *et al.*, 1989), (Farquhar *et al.*, 2002), (Gengo *et al.*, 1990), (Holdstock *et al.*, 2006), (Holdstock and de Wit, 1999), (Holdstock and de Wit, 1998), (Kennedy *et al.*, 1993), (Kennedy *et al.*, 1992), (Lukas *et al.*, 1989), (McKee *et al.*, 2006), (Moulton *et al.*, 2005), (Richards *et al.*, 1999), (Schweizer *et al.*, 2006), (Schweizer *et al.*, 2005), (Schweizer *et al.*, 2004), (Tiplady *et al.*, 2001), (Tzambazis and Stough, 2000) (n=19) |
| Flicker Discrimination | Apparent Movement, Backward Masking, Bistable Stroboscopic Motion, Critical Flicker Fusion Test, Simultaneity Task, Velocity Perception Task | 55 | 45 | 0 | (Baker *et al.*, 1985), (Jansen *et al.*, 1985), (Jones *et al.*, 1998), (Liguori *et al.*, 1999), (MacCarthy and Tong, 1980), (Rammsayer, 1995) (n=6) |

| | | | | | |
|---|---|---|---|---|---|
| Focused/ Selective Attention | Auditory Discrimination Task, Continuous Performance Task, Digit Span Task (forward), Go/No-Go Task, Go/No-Go Task*, Inspection Time Task, Letter Cancellation Task, Memory Scanning Test, Pattern Comparison Task, Perceptual Speed Task, Rapid Information Processing Task, Rapid Visual Information Processing Task, Signal Detection Task, Spatial Frequency Discrimination Test, Tachistoscopic Perception Task, Test d2, Vernier Discrimination Task, Visual Search Task, Visual Stimulus-Response Task (complex), Visual Stimulus-Response Task (complex), Visual Stimulus-Response Task (simple) | 50 | 48 | 2 | (Baker *et al.*, 1985), (Davidson *et al.*, 1997), (Farquhar *et al.*, 2002), (Fillmore and Vogel-Sprott, 1997), (Fogarty and Vogel-Sprott, 2002), (Jansen *et al.*, 1985), (Kennedy *et al.*, 1993), (Kennedy *et al.*, 1992), (Lex *et al.*, 1988), (Linnoila *et al.*, 1980), (Maylor and Rabbitt, 1988), (Maylor *et al.*, 1987b), (Maylor *et al.*, 1987a), (McKee *et al.*, 2006), (Mills *et al.*, 1996), (Moulton *et al.*, 2005), (Nagoshi *et al.*, 1992), (Ortner *et al.*, 2003), (Reynolds *et al.*, 2006), (Schweizer *et al.*, 2005), (Schweizer *et al.*, 2004), (Tiplady *et al.*, 2001), (Tzambazis and Stough, 2000), (Watten *et al.*, 1998), (Wegner and Fahle, 1999a), (Hoyer *et al.*, 2007) (n=26) |
| Reaction Time | Anticipation Timing Task, Auditory Discrimination Task*, Auditory Vigilance Task*, Choice Reaction Time Task, Choice Reaction Time Task (fixed sequences), Choice Reaction Time Task (random sequences), Complex Reaction Time Task*, Complex Reaction Time Test, Concept Identification Task, Concurrent Set Memory Task*, Continuous Performance Task (delayed memory)*, Continuous Performance Task (immediate memory)*, Continuous Performance Task*, Critical Tracking Test*, Digit Symbol Substitution Test (DSST), Digit Symbol Substitution Test (DSST)*, Digit Symbol Yes/No Test*, Divided Attention Task*, Driving Simulation Test (reaction time), Driving Simulation Test (dual task)*, Eriksen Flanker Test*, Fixed Set Memory Task*, Go/No Go Test (cued)*, Go/No-Go Task (high cognitive load)*, Go/No-Go Task (low cognitive load)*, Go/No-Go Task *, Go/No-Go Task*, Lexical Decision Task*, Memory Scanning Test*, Mill Hill Vocabulary Test*, Number Matching Pair Test*, Offset Reaction Time Task, Omitted Auditory Stimulus Task, Onset Reaction Time Task, Pattern Recognition Task*, Psychomotor Task, Pursuit Tracking Task (stressalyser)*, Rapid Visual Information Processing Task, Rapid Visual Information Processing Task*, Reaction Time Test, Reaction Time Test (acoustic stimulus), Reaction Time Test (optical sequence of stimuli) , Reaction Time Test (optical stimulus) , Reaction Time Test (optical/ aucoustic stimuli), Reaction Time Test to Omitted Auditory Stimuli, Reaction Time Test to Omitted Tactile Stimuli, Reaction Time Test to Omitted Visual Stimuli, Repetition-Alternation Task (shape, color, location), Saccadic Interference Task, Signal Detection Test*, Simple Auditory RT Test, Simple Letter Verification Task*, Simple Reaction Time Task, Spatial Recognition Task*, Stop Signal Task*, Stroop Task*, Symbol Matching Task (reference key present during task)*, Tracking | 53 | 47 | 0 | (Abroms *et al.*, 2006), (Antebi, 1982), (Baker *et al.*, 1985), (Bartholow *et al.*, 2003a), (Beirness and Vogel-Sprott, 1982), (Cameron *et al.*, 2001), (Colzato *et al.*, 2004), (Degia *et al.*, 2005), (Dougherty *et al.*, 2000), (Drake *et al.*, 2003), (Easdon *et al.*, 2005), (Easdon and Vogel-Sprott, 2000), (Farquhar *et al.*, 2002), (Feely and Wood, 1982), (Fillmore *et al.*, 2005), (Fillmore, 2004), (Fillmore and Blackburn, 2002), (Fillmore and Vogel-Sprott, 2000), (Galbraith, 1986), (Gengo *et al.*, 1990), (Grant *et al.*, 2000), (Gustafson and Kallmen, 1990), (Gustafson, 1986), (Hernandez *et al.*, 2006), (Kennedy *et al.*, 1993), (Kennedy *et al.*, 1992), (Knowles and Duka, 2004), (Landauer and Howat, 1983), (Landauer and Howat, 1982), (Liguori *et al.*, 1999), (Linnoila *et al.*, 1980), (Marczinski *et al.*, 2005), (Marczinski and Fillmore, 2005b), (Marczinski and Fillmore, 2005a), (Marczinski and Fillmore, 2003), (Maylor *et al.*, 1989), (Maylor and Rabbitt, 1988), (Maylor *et al.*, 1988), (Maylor and Rabbitt, 1987b), (Maylor *et al.*, 1987b), (McKee *et al.*, 2006), (Mulvihill *et al.*, 1997), (Nagoshi *et al.*, 1992), (Nicholson *et al.*, 1992b), (Nicholson *et al.*, 1992a), (Pishkin *et al.*, 1983), (Post *et al.*, 1996), (Rammsayer, 1995), (Reynolds *et al.*, 2006), (Roehrs *et al.*, 1992), (Rohrbaugh *et al.*, 1988), (Ross and Pihl, 1988), (Ryan *et al.*, 1996), (Schulte *et al.*, 2001), (Schweizer *et al.*, 2006), (Schweizer *et al.*, 2005), (Schweizer *et al.*, 2004), (Sommer *et al.*, 1993), (Tiplady *et al.*, 2001), (Tzambazis and Stough, 2000), (Versavel *et al.*, 2005), (Wang *et al.*, 1992), (Weissenborn and Duka, 2003), (Young and Pihl, 1982), (Zwyghuizen-Doorenbos *et al.*, 1990), (Breitmeier *et al.*, 2007), (do Canto-Pereira *et al.*, 2007) (Hernandez *et al.*, 2007), (Khan and Timney, 2007), (Rupp *et al.*, 2007b) (n=70) |



| | | | | | |
|---|---|---|---|---|---|
| | Input Manipulator*, Varied Set Memory Task*, Verification Task*, Vigilance Task (acoustic stimulus)*, Vigilance Task (optical stimulus)*, Vigilance Task*, Visual Reaction Time test, Visual Search Task*, Visual Stimulus-Response Task (during tracking), Visual Sustained Attention Task*, Visual Vigilance Task, Visual Vigilance Task*, VisuoSpatial Attention Task, Word Categorization Task*, Work Performance Series* | | | | |
| Sustained Attention (Vigilance) | Auditory Vigilance Task, Continuous Attention Task, Continuous Performance Task (delayed memory), Continuous Performance Task (delayed memory)*, Continuous Performance Task (immediate memory)*, Number Matching Pair Test, Psychomotor Vigilance Task, Rapid Visual Information Processing Task, Serial Sevens Test, Signal Detection Test, Sustained Attention Task, Vigilance Task, Vigilance Task (acoustic stimulus), Vigilance Task (optical stimulus), Visual Sustained Attention Task, Visual Vigilance Task, Work Performance Series | 43 | 57 | 0 | (Cameron *et al.*, 2001), (Dougherty *et al.*, 2000), (Drake *et al.*, 2003), (Duka *et al.*, 1998), (Farquhar *et al.*, 2002), (Linnoila *et al.*, 1980), (Millar *et al.*, 1999), (Roehrs *et al.*, 1992), (Rohrbaugh *et al.*, 1988), (Versavel *et al.*, 2005), (Breitmeier *et al.*, 2007), (Rupp *et al.*, 2007b) (n=12) |
| **Executive** | | | | | |
| *Cluster* | *Test* | *(-)* | *(=)* | *(+)* | *References (frequency)* |
| Creativity | Purdue Creativity Test | 0 | 100 | 0 | (Gustafson, 1991) (n=1) |
| Driving | Driving Simulation Test, Driving Simulation Test (drive only), Simulated Automobile Driving Task, Tracking Input Manipulator | 100 | 0 | 0 | (Dalrymple-Alford *et al.*, 2003), (Gawron and Ranney, 1988), (Gengo *et al.*, 1990), (Landauer and Howat, 1983), (Rupp *et al.*, 2007b) (n=5) |
| Inhibition | Anticipation Timing Task, Antisaccadic Eye Movement Test, Balloon Analogue Risk Task, Barratt Impulsiveness Scale, Continuous Performance Task (delayed memory)*, Continuous Performance Task (immediate memory)*, Continuous Performance Task*, Covert Shift of Attention Task, Delay Discounting Test, Delayed Ocular Response Task, Eriksen Flanker Test, Experimental Discounting Task, General Knowledge Test, Gibson Spiral Maze test*, Go/No Go Test (cued), Go/No-Go Task, Go/No-Go Task , Go/No-Go Task (high cognitive load), Go/No-Go Task (low cognitive load), Iowa Gambling Task, Newman Perseveration Task, Pattern Comparison Task*, Question-based Delay Discounting Task, Recognition Task, Recognition Task (with context cues)*, Recognition Task (without context cues)*, Rectangular Maze Test*, Repetition-Alternation Task (shape, color, location), Risk-Taking Task, Signal Detection Task*, South Oaks Gambling Screen, Startle Response, Stop Signal Task, Stroop Task, Stroop Task: Negative Primes, Stroop Task: Positive Primes, Sustained Attention Task*, Tower of London*, Tracking Input Manipulator*, Visual Sustained Attention Task* | 50 | 46 | 4 | (Abroms *et al.*, 2006), (Baker *et al.*, 1985), (Balodis *et al.*, 2006), (Bartholow *et al.*, 2003a), (Birnbaum *et al.*, 1980), (Blekher *et al.*, 2002), (Colzato *et al.*, 2004), (Davidson *et al.*, 1997), (Degia *et al.*, 2005), (Dougherty *et al.*, 2000), (Drake *et al.*, 2003), (Easdon *et al.*, 2005), (Easdon and Vogel-Sprott, 2000), (Farquhar *et al.*, 2002), (Fillmore *et al.*, 2005), (Fillmore, 2004), (Fillmore and Blackburn, 2002), (Fillmore *et al.*, 2000b), (Fillmore *et al.*, 2000a), (Fillmore and Vogel-Sprott, 2000), (Finn *et al.*, 1999), (Gustafson and Kallmen, 1990), (Hutchison *et al.*, 1997), (Jansen *et al.*, 1985), (Landauer and Howat, 1983), (Lane *et al.*, 2004), (Marczinski *et al.*, 2005), (Marczinski and Fillmore, 2005b), (Marczinski and Fillmore, 2005a), (Marczinski and Fillmore, 2003), (Maylor *et al.*, 1987a), (McKee *et al.*, 2006), (Millar *et al.*, 1999), (Mulvihill *et al.*, 1997), (Ortner *et al.*, 2003), (Reynolds *et al.*, 2006), (Richards *et al.*, 1999), (Rohrbaugh *et al.*, 1988), (Schulte *et al.*, 2001), (Schweizer *et al.*, 2006), (Tiplady *et al.*, 2001), (Weissenborn and Duka, 2003), (Donohue *et al.*, 2007), (Phillips and Ogeil, 2007) (n=44) |
| Judgement | Choice Reaction Time Task* | 50 | 50 | 0 | (Maylor *et al.*, 1987b) (n=1) |

| | | | | | |
|---|---|---|---|---|---|
| Planning | Tower of London | 100 | 0 | 0 | (Weissenborn and Duka, 2003) (n=1) |
| Reasoning/ Association | Block Design Task, Categorization Task, Concept Identification Task, Conditional Associative Learning Task, Grammatical Reasoning Task, Logical Reasoning Task, Mathematical Processing Task, Mathematical Reasoning Task, Picture Arrangement Task, Picture Completion Task, Sentence Completion Task | 55 | 45 | 0 | (De Cesarei *et al.*, 2006), (Duka *et al.*, 1998), (Finn *et al.*, 1999), (Kennedy *et al.*, 1993), (Kennedy *et al.*, 1992), (Lex *et al.*, 1988), (Pishkin *et al.*, 1983), (Tzambazis and Stough, 2000) (n=8) |
| Set Shifting | Go/No-Go Task*, Wisconsin Card Sorting Test | 33 | 67 | 0 | (Easdon and Vogel-Sprott, 2000), (Lyvers and Maltzman, 1991a), (Nagoshi *et al.*, 1992) (n=3) |
| Spatial Orientation | Card Rotations Task , Little Man Task, Manikin Task, Manikin Test | 50 | 50 | 0 | (Farquhar *et al.*, 2002), (Kennedy *et al.*, 1993), (Kennedy *et al.*, 1992), (Lex *et al.*, 1988) (n=4) |
| Time/ Distance Estimation | Choice Reaction Time Task*, Drawing Squares Test, Kinaesthetic Distance Estimation Test, Size Estimation Test, Speed Estimation Task (without speedometer), Temporal Discrimination Task, Time Estimation Task, Time Perception Task, Time Production Task, Visual Distance Estimation Task | 46 | 46 | 8 | (Duka *et al.*, 1998), (Farquhar *et al.*, 2002), (Kearney and Guppy, 1988), (Lapp *et al.*, 1994), (McNamee *et al.*, 1980), (Rammsayer, 1995), (Tiplady *et al.*, 2005) (n=7) |
| Working Memory/ Immediate Recognition | Auditory Memory Task, Backwards Reading Task, Choice Reaction Time Task*, Complex Reaction Time Test, Concentration Test, Concurrent Set Memory Task, Continuous Performance Task (delayed memory), Continuous Performance Task (immediate memory), Design Memory Task, Digit Span Task (backward), Fixed Set Memory Task, Letter-Number Test, Pattern Memory Test, Pattern Recognition Task, Recognition Task, Short Term Memory Task, Short-Term Memory for Words and Figures, Short-Term Memory Test, Spatial Memory Span Test, Spatial Recognition task, Spatial Working Memory Task, Sternberg Memory Scanning Task, Symbol Matching Task (reference key absent during task), Symbol Matching Task (reference key absent during task)*, Three Letters Task, Varied Set Memory Task, Visual Memory Task, WAIS-R: Digit Span Test (backward), Word Discrimination Task (immediate), Word Repetition Priming Task, X's and O's Task | 53 | 47 | 0 | (Baker *et al.*, 1985), (Dougherty *et al.*, 2000), (Drake *et al.*, 2003), (Finn *et al.*, 1999), (Grattan-Miscio and Vogel-Sprott, 2005), (Kennedy *et al.*, 1993), (Kennedy *et al.*, 1992), (Krause *et al.*, 2002), (Landauer and Howat, 1982), (Lister *et al.*, 1991), (Marinkovic *et al.*, 2004), (Maylor and Rabbitt, 1987a), (McKee *et al.*, 2006), (Moulton *et al.*, 2005), (Ryan *et al.*, 1996), (Schweizer *et al.*, 2006), (Tzambazis and Stough, 2000), (Versavel *et al.*, 2005), (Weissenborn and Duka, 2003), (Saults *et al.*, 2007) (n=20) |
| **Memory** | | | | | |
| Cluster | Test | (-) | (=) | (+) | References (frequency) |
| Auditory/ Verbal Memory: Delayed Recall | Delayed Free Recall Test (words), Delayed Recall Task (words), Delayed Word Recall Task (24h), Free Recall Task (words), Hopkins Verbal Learning Test (words), Logical Memory Test (story), Memory for Profile of Mood States (POMS) scores, Memory Task (8 letter-word pairs), Memory Task for Verbally Presented Words, Prose Memory Task, Recall for Mood States, Recall Task (names), Retroactive Interference Task (words), Wilde Intelligence Test (memory subset), Word List Learning (40 min. prior to alcohol intake), Word List Learning (immediately before alcohol intake) | 40 | 30 | 30 | (Acheson *et al.*, 1998), (Bruce *et al.*, 1999), (Cowan, 1983), (Erblich and Earleywine, 1995), (Mann *et al.*, 1984), (McKee *et al.*, 2006), (Moulton *et al.*, 2005), (Mueller *et al.*, 1983), (Ray *et al.*, 2004), (Schandler *et al.*, 1984), (Tyson and Schirmuly, 1994), (Weissenborn and Duka, 2000), (Breitmeier *et al.*, 2007) (n=13) |



| | | | | | |
|---|---|---|---|---|---|
| Auditory/ Verbal Memory: Delayed Recognition | Delayed Recognition Task (words), Hopkins Verbal Learning Test (words), Memory Task for Verbally Presented Words, Recognition of Depressive Mood States, Recognition of Elating Mood States, Recognition of Neutral Mood States, Recognition Task (names), Sentence Recognition Task (with context cues), Sentence Recognition Task (without context cues), Wilde Intelligence Test (memory subset), WMS-R: Paired Associates Learning (difficult), WMS-R: Paired Associates Learning (easy), Word Discrimination Task (delayed), Word List Learning (before alcohol intake), Word Recognition Memory Task, Word Recognition Task, Word Stem Completion Task (cued) | 43 | 48 | 10 | (Acheson *et al.*, 1998), (Birnbaum *et al.*, 1980), (Bruce and Pihl, 1997), (Cowan, 1983), (Duka *et al.*, 2001), (Erblich and Earleywine, 1995), (McKee *et al.*, 2006), (Mueller *et al.*, 1983), (Ray and Bates, 2006), (Schweizer *et al.*, 2006), (Tyson and Schirmuly, 1994), (Williams and Rundell, 1984), (Breitmeier *et al.*, 2007) (n=13) |
| Auditory/ Verbal Memory: Immediate Recall | Dichotic Listening Test, Free Recall Task (words), Immediate Recall Acquisition Task (words), Immediate Recall Task/Acquisition Task (words), Memory Task (words), Memory Task for Verbally Presented Words, Paired Associate Word Learning Task, Prose Memory Task, Verbal Immediate Memory Task (words), Verbal Short-term Memory Task (words) | 67 | 33 | 0 | (Clarici *et al.*, 1993), (Duka *et al.*, 2001), ( Jones and Jones, 1980), (Lister *et al.*, 1991), (McKee *et al.*, 2006), (Moulton *et al.*, 2005), (Niaura *et al.*, 1987), (Ray *et al.*, 2004), (Tracy and Bates, 1999), (Weintraub and Goldman, 1983), (Weissenborn and Duka, 2000), (Williams and Rundell, 1984) (n=12) |
| Implicit Memory | Cued Incidental Learning (after alcohol intake), Cued Incidental Learning (before alcohol intake), Event Frequency Task, Frequency of Word Occurrence Task, Judgement of Frequency Task, Recall of Depressive Mood Sates, Recall of Elating Mood States, Recall of Neutral Mood States, Stem Completion Task (automatic processes), Word-Stem Completion Task, Word-Stem Completion Task (controlled processes ) | 33 | 40 | 27 | (Birnbaum *et al.*, 1987), (Bruce and Pihl, 1997), (Duka *et al.*, 2001), (Fillmore *et al.*, 1999), (Grattan and Vogel-Sprott, 2001), (Kirchner and Sayette, 2003), (Lister *et al.*, 1991), (Ray and Bates, 2006), (Tracy and Bates, 1999), (Tyson and Schirmuly, 1994) (n=10) |
| Learning | Verbal Memory Task* | 100 | 0 | 0 | (McKee *et al.*, 2006) (n=1) |
| Visual/ Spatial Memory: Delayed Recall | Complex Figure Task, Delayed Memory for Geographical Map (with route), Emotional Memory Task | 40 | 40 | 20 | (Acheson *et al.*, 1998), (Knowles and Duka, 2004), (Lowe, 1983) (n=3) |
| Visual/ Spatial Memory: Delayed Recognition | Design Memory Task, Face Recognition Task, Picture Recognition Task | 0 | 67 | 33 | (Cowan, 1983), (Parker *et al.*, 1981), (Schweizer *et al.*, 2006) (n=3) |
| Visual/ Spatial Memory: Immediate Recall | Benton Visual Retention Test, Complex Figure Task, Immediate Memory for Geographical Map (with route) | 25 | 75 | 0 | (Acheson *et al.*, 1998), (Lowe, 1983), (Wegner and Fahle, 1999a) (n=3) |
| **Motor** | | | | | |
| *Cluster* | *Test* | *(-)* | *(=)* | *(+)* | *References (frequency)* |
| Motor Control | Balance Beam Test, Choice Reaction Time Task*, Hand Steadiness Test (eyes closed), One Leg Stand Test, Reaction Time Test (acoustic stimulus)*, Reaction Time Test (optical sequence of stimuli)*, Reaction | 61 | 39 | 0 | (Clarici *et al.*, 1995), (Clarici *et al.*, 1993), (Galbraith, 1986), (Kennedy *et al.*, 1993), (Kennedy *et al.*, 1992), (Landauer and Howat, 1982), (Lukas *et al.*, 1989), (McKee *et al.*, 2006), (Mundt *et al.*, 1997), (Rammsayer, 1995), |

| | | | | | |
|---|---|---|---|---|---|
| | Time Test (optical stimulus)*, Reaction Time Test (optical/aucoustic stimuli)*, Standing Steadiness, Tapping Task, Tapping Task (non-dominant hand), Tapping Task (two hands) | | | | (Vogel-Sprott and Barrett, 1984), (Breitmeier *et al.*, 2007) (n=12) |
| Postural Stability | Body Sway, Body Sway (closed eyes), Body Sway (open eyes), One Leg Stand Test, Physical Activity Measurement (actiwatch), Sensory Organization Posturography Test, Steadiness Test, Walk and Turn Test | 86 | 14 | 0 | (Galbraith, 1986), (Jones and Neri, 1994), (Jones, 1993), (Ledin and Odkvist, 1991), (Liguori *et al.*, 1999), (Lukas *et al.*, 1989), (Martin *et al.*, 1981), (Martinez and Martinez, 2003), (Mills and Bisgrove, 1983a), (Mundt *et al.*, 1997), (Nagoshi *et al.*, 1992), (Niaura *et al.*, 1987), (O'Malley and Maisto, 1984), (Addicott *et al.*, 2007) (n=14) |
| Visuo-Motor Control | Bead String Test, Character Writing Test, Continuous Tracking Task (continuous instability), Continuous Tracking Task (frequent instability), Continuous Tracking Task (minor instability), Coordination Test Steering, Critical Tracking Test, Divided Attention Task*, Gibson Spiral Maze Task, Gibson Spiral Maze test, Hand Steadiness Test, Hand-Eye Coordination Test, Handwriting Analysis, Object Assembly Task, Pegboard Test, Primary Tracking Task, Pursuit Rotor Task, Pursuit Rotor Task Modified, Pursuit Tracking Task (stressalyser), Rectangular Maze Test, Signature Task, Speed Estimation Task (with speedometer), Spiral Maze Task, Tracking Input Manipulator*, Tracking Test, Tracometer, Visuomotor Integration Task | 78 | 22 | 0 | (Asicioglu and Turan, 2003), (Beirness and Vogel-Sprott, 1984), (Beirness and Vogel-Sprott, 1982), (Cameron *et al.*, 2001), (Connors and Maisto, 1980), (Degia *et al.*, 2005), (Farquhar *et al.*, 2002), (Fillmore, 2003), (Fillmore and Vogel-Sprott, 1998), (Fillmore and Vogel-Sprott, 1996a), (Fillmore and Vogel-Sprott, 1996b), (Fillmore and Vogel-Sprott, 1995), (Fogarty and Vogel-Sprott, 2002), (Galbraith, 1986), (George *et al.*, 1990), (Grant *et al.*, 2000), (Harrison and Fillmore, 2005a), (Haubenreisser and Vogel-Sprott, 1983), (Jones and Neri, 1994), (Jones, 1993), (Kearney and Guppy, 1988), (Landauer and Howat, 1983), (Linnoila *et al.*, 1980), (Lukas *et al.*, 1989), (McKee *et al.*, 2006), (Millar *et al.*, 1999), (Nagoshi *et al.*, 1992), (Niaura *et al.*, 1987), (Roehrs *et al.*, 1992), (Thomson and Newlin, 1988), (Tiplady *et al.*, 2005), (Tiplady *et al.*, 2001), (Tzambazis and Stough, 2000), (Versavel *et al.*, 2005), (Vogel-Sprott and Fillmore, 1993), (Vogel-Sprott and Barrett, 1984), (Vogel-Sprott *et al.*, 1984), (Young and Pihl, 1982), (Zack and Vogel-Sprott, 1993), (Zwyghuizen-Doorenbos *et al.*, 1990) (n=40) |

**Neurophysiologic**

| *Cluster* | *Test* | *(-)* | *(=)* | *(+)* | *References (frequency)* |
|---|---|---|---|---|---|
| EEG Alpha | Electro-encephalography (EEG), Magneto-encephalography (MEG) | 6 | 38 | 56 | (Cohen *et al.*, 1993), (Ehlers *et al.*, 1999), (Ehlers *et al.*, 1989), (Lukas and Mendelson, 1988), (Lukas *et al.*, 1986b), (Nikulin *et al.*, 2005), (Stenberg *et al.*, 1994) (n=7) |
| EEG Beta | Electro-encephalography (EEG), Magneto-encephalography (MEG) | 9 | 64 | 27 | (Cohen *et al.*, 1993), (Ehlers *et al.*, 1999), (Nikulin *et al.*, 2005), (Stenberg *et al.*, 1994) (n=4) |
| EEG Delta | Electroencephalography (EEG) | 0 | 100 | 0 | (Stenberg *et al.*, 1994) (n=1) |
| EEG Theta | Electro-encephalography (EEG), Magneto-encephalography (MEG) | 0 | 22 | 78 | (Ehlers *et al.*, 1999), (Ehlers *et al.*, 1989), (Lukas *et al.*, 1986b), (Nikulin *et al.*, 2005), (Stenberg *et al.*, 1994) (n=5) |
| Evoked Potential | Alternating Check Task, Categorization Task*, Choice Reaction Time Task*, Eriksen Flanker Test*, Event Related Potentials (ERP), Go/No-Go Task*, Memory Scanning Test, Odd Ball Paradigm, Vestibular Evoked Myogenic Potential Test, Visual Evoked Potentials, Visual Sustained Attention Task*, Watching 'Emotional' Pictures, Word Repetition Priming Task | 52 | 21 | 27 | (Bartholow *et al.*, 2003a), (Bartholow *et al.*, 2003b), (Chiang and Young, 2007), (De Cesarei *et al.*, 2006), (Easdon *et al.*, 2005), (Erwin and Linnoila, 1981), (Feely and Wood, 1982), (Krause *et al.*, 2002), (Marinkovic *et al.*, 2004), (Quintyn *et al.*, 1999), (Rohrbaugh *et al.*, 1987), (Skalka *et al.*, 1986), (Sommer *et al.*, 1993), (Teo and Ferguson, 1986), (Zuzewicz, 1981), (Franken *et al.*, 2007) (n=16) |
| Eye Movements | Binocular Balance Test, Oculomotor Functioning | 100 | 0 | 0 | (McNamee *et al.*, 1981), (Mundt *et al.*, 1997) (n=2) |
| Eye Movements - Blink Rates | Electro-oculography (EOG) | 0 | 100 | 0 | (Rohrbaugh *et al.*, 1988) (n=1) |
| Eye Movements - Nystagmus | Caloric and Visual Supression Test, Electro-oculography (EOG), Horizontal Gaze Nystagmus, Vestibulo-Ocular Nystagmus Test | 100 | 0 | 0 | (Chiang and Young, 2007), (Harder and Reker, 1995), (Jones and Neri, 1994), (Jones, 1993), (Ledin and Odkvist, 1991), (Martinez and Martinez, 2003) (n=6) |
| Eye Movements - Pursuit | Oculomotor Functioning, Smooth Pursuit Eye Movement Task | 67 | 33 | 0 | (Holdstock and de Wit, 1999), (Mundt *et al.*, 1997) (n=2) |
| Eye Movements - Saccadic | Delayed Ocular Response Task, Oculomotor Functioning, Saccadic Eye Movement Test, Visuomotor Reading Task | 90 | 10 | 0 | (Abroms *et al.*, 2006), (Blekher *et al.*, 2002), (Holdstock and de Wit, 1999), (Mundt *et al.*, 1997), (Nyberg *et al.*, 2004), (Watten and Lie, 1997), (Wegner and Fahle, 1999b) (n=7) |
| Sleep | Multiple Sleep Latency Test (MSLT), Repeated Test of Sustained Wakefulness (RTSW), Sleep Latency, Sleep Test | 40 | 60 | 0 | |

**Perception**

| *Cluster* | *Test* | *(-)* | *(=)* | *(+)* | *References (frequency)* |
|---|---|---|---|---|---|
| Olfactory Perception | Odor Discrimination/Odor Memory Test, Olfactory Ethanol Detection Test, Phenyl Ethyl Alcohol Detection Threshold Tests, University of Pennsylvania Smell Identification Test | 50 | 50 | 0 | (Patel *et al.*, 2004) (n=1) |
| Visual Perception | Accomodation Task, Arden Plates, Binocular Vision Task, Contrast Sensitivity Chart, Depth Perception Task, Goldmann Visual Fields , IR Refractometer Task, Perceptual Vision Task, Rod and Frame Test (visual field dependence), Rotating Laser Drum Task, Snellen Acuity, Stereo Optical Stereo Tests, Stereoscopic Depth Perception, Subjective Vertical/Horizontal Test, TV Grating Contrast Sensitivity, Vision Test, Visual Contrast Sensitivity Test, Visual Field Test | 52 | 48 | 0 | (Nicholson *et al.*, 1992a), (Quintyn *et al.*, 1999), (Skalka *et al.*, 1986), (Wang *et al.*, 1992), (Watten and Lie, 1996), (Wegner and Fahle, 1999a), (Hafstrom *et al.*, 2007) (n=7) |

**Subjective Experience**

| *Cluster* | *Test* | *(-)* | *(=)* | *(+)* | *References (frequency)* |
|---|---|---|---|---|---|
| Scale Aggression | Affect Inventory (Scale Anger/Sympathetic), Profile of Mood States (POMS) (Scale Agreeable/Hostile), Profile of Mood States (POMS) (Scale Anger), Profile of Mood States (POMS) (Scale Anger/Friendliness), Profile of Mood States (POMS) (Scale Anger/Hostility), Profile of Mood States (POMS) (Scale Friendliness), Subjective Effects of Alcohol Scale (SAES) (Scale Positive Social Influences), Visual Analogue Scale (VAS) (Scale Irritability) | 8 | 75 | 17 | (DeWit *et al.*, 1989), (Holdstock and de Wit, 1998), (Knowles and Duka, 2004), (McKee *et al.*, 2006), (Mundt *et al.*, 1997), (Nagoshi *et al.*, 1992), (O'Malley and Maisto, 1984), (Pierucci-Lagha *et al.*, 2006), (Reynolds *et al.*, 2006), (Soderlund *et al.*, 2005), (Wilkie and Stewart, 2005), (Zeichner *et al.*, 1993) (n=12) |

| | | | | | |
|---|---|---|---|---|---|
| Scale Alertness | Affect Inventory (Scale Powerfulness/ Energy), Biphasic Alcohol Effects Scale (BAES) (Scale Sedation), Profile of Mood States (POMS) (Scale Clearheaded/ Confused), Profile of Mood States (POMS) (Scale Confusion), Profile of Mood States (POMS) (Scale Confusion/Bewilderment/ Vigor/Activity), Profile of Mood States (POMS) (Scale Confusion/Vigour), Profile of Mood States (POMS) (Scale Vigour), Profile of Mood States (POMS) (Scale Vigour/Activity/ Confusion/Bewilderment), Profile of Mood States (POMS) (Scale Vigour/Confusion), Sleep Questionnaire, Stanford Sleepiness Scale (SSS), Subjective Effects of Alcohol Scale (SAES) (Scale Sedation), Subjective Rating Scale (Scale Activity/Drowsiness), Subjective Rating Scale (Scale Alertness), Visual Analogue Scale (VAS) (Scale Alertness), Visual Analogue Scale (VAS) (Scale Drowsiness), Visual Analogue Scale (VAS) (Scale Sedation), Visual Analogue Scale (VAS) (Scale Sleepiness) | 35 | 60 | 5 | (Bartholow *et al.*, 2003b), (Bruce *et al.*, 1999), (Davidson *et al.*, 1997), (DeWit *et al.*, 1989), (Fillmore *et al.*, 2005), (Holdstock *et al.*, 2006), (Holdstock and de Wit, 1999), (Holdstock and de Wit, 1998), (Hutchison *et al.*, 1997), (Knowles and Duka, 2004), (Landauer and Howat, 1983), (Liguori *et al.*, 1999), (McKee *et al.*, 2006), (Mundt *et al.*, 1997), (Nagoshi *et al.*, 1992), (Nyberg *et al.*, 2004), (O'Malley and Maisto, 1984), (Pierucci-Lagha *et al.*, 2006), (Rammsayer, 1995), (Reynolds *et al.*, 2006), (Soderlund *et al.*, 2005), (Walsh *et al.*, 1991), (Wilkie and Stewart, 2005), (Zeichner *et al.*, 1993), (Zwyghuizen-Doorenbos *et al.*, 1990), (Addicott *et al.*, 2007), (Rupp *et al.*, 2007c), (Saults *et al.*, 2007) (n=28) |
| Scale Calmness | Affect Inventory (Scale Afraidness), Affect Inventory (Scale Relaxedness), Biphasic Alcohol Effects Scale (BAES) (Scale Stimulation), Estimation of Mood Change Test (Scale Tense/Relaxed), Profile of Mood States (POMS) (Scale Anxiety), Profile of Mood States (POMS) (Scale Arousal), Profile of Mood States (POMS) (Scale Composed/ Anxious), Profile of Mood States (POMS) (Scale Confident/Unsure), Profile of Mood States (POMS) (Scale Tension ), Profile of Mood States (POMS) (Scale Tension), Profile of Mood States (POMS) (Scale Tension/ Anxiety), Subjective Rating Scale (Scale Relaxation), Taylor's Manifest Anxiety Scale, Visual Analogue Scale (VAS) (Scale Nervousness), Visual Analogue Scale (VAS) (Scale Relaxedness), Visual Analogue Scale (VAS) (Scale Stimulatedness) | 23 | 76 | 2 | (Bartholow *et al.*, 2003b), (Bruce *et al.*, 1999), (Davidson *et al.*, 1997), (DeWit *et al.*, 1989), (Duka *et al.*, 2001), (Fillmore *et al.*, 2005), (Gustafson and Kallmen, 1990), (Holdstock *et al.*, 2006), (Holdstock and de Wit, 1999), (Holdstock and de Wit, 1998), (Hutchison *et al.*, 1997), (Knowles and Duka, 2004), (Liguori *et al.*, 1999), (Lindman, 1985), (McKee *et al.*, 2006), (Nagoshi *et al.*, 1992), (O'Malley and Maisto, 1984), (Pierucci-Lagha *et al.*, 2006), (Rammsayer, 1995), (Reynolds *et al.*, 2006), (Soderlund *et al.*, 2005), (Weissenborn and Duka, 2003), (Weissenborn and Duka, 2000), (Wilkie and Stewart, 2005), (Zeichner *et al.*, 1993), (Addicott *et al.*, 2007), (Rupp *et al.*, 2007c), (Saults *et al.*, 2007) (n=28) |
| Scale Craving | Drug Effects Questionnaire (DEQ) (Scale Like), Drug Effects Questionnaire (DEQ) (Scale Like/Want More), Drug Effects Questionnaire (DEQ) (Scale Want More), Drug Effects Questionnaire (DEQ) Scale Like/Want More), Visual Analogue Scale (VAS) (Scale Desire), Visual Analogue Scale (VAS) (Scale Like), Visual Analogue Scale (VAS) (Scale Like/Dislike) | 50 | 50 | 0 | (Balodis *et al.*, 2006), (Davidson *et al.*, 1997), (Duka *et al.*, 1998), (Fillmore, 2004), (Fillmore and Blackburn, 2002), (Fillmore, 2001), (Holdstock *et al.*, 2006), (Holdstock and de Wit, 1999), (Holdstock and de Wit, 1998), (Liguori *et al.*, 1999), (Marczinski and Fillmore, 2005b), (Marczinski and Fillmore, 2005a), (Marczinski and Fillmore, 2003), (Ortner *et al.*, 2003), (Pierucci-Lagha *et al.*, 2006), (Reynolds *et al.*, 2006), (Richards *et al.*, 1999), (Franken *et al.*, 2007) (n=18) |
| Scale Depression | Affect Inventory (Scale Depression), Profile of Mood States (POMS) (Scale Depression), Profile of Mood States (POMS) (Scale Depression/Dejection), Profile of Mood States (POMS) (Scale Elated/Depressed) | 8 | 85 | 8 | (Nagoshi *et al.*, 1992), (O'Malley and Maisto, 1984), (Pierucci-Lagha *et al.*, 2006), (Soderlund *et al.*, 2005), (Wilkie and Stewart, 2005), (Zeichner *et al.*, 1993) (n=6) |



| | | | | | |
|---|---|---|---|---|---|
| Scale Dizziness | Visual Analogue Scale (VAS) (Scale Dizziness), Visual Analogue Scale (VAS) (Scale Lightheadedness) | 100 | 0 | 0 | (Knowles and Duka, 2004), (Liguori *et al.*, 1999) (n=2) |
| Scale Drug Effect | Addiction Research Center Inventory (ARCI) (Scale A), Addiction Research Center Inventory (ARCI) (Scale A/BG/MBG/LSD), Addiction Research Center Inventory (ARCI) (Scale A/BG/MBG/LSD/PCAG), Addiction Research Center Inventory (ARCI) (Scale A/ BG/MBG/PCAG/LSD), Addiction Research Center Inventory (ARCI) (Scale A/BG/PCAG/ LSD), Addiction Research Center Inventory (ARCI) (Scale A/M/MBG), Addiction Research Center Inventory (ARCI) (Scale A/M/MBG/PCAG), Addiction Research Center Inventory (ARCI) (Scale A/MBG/ PCAG), Addiction Research Center Inventory (ARCI) (Scale BG/LSD ), Addiction Research Center Inventory (ARCI) (Scale BG/LSD), Addiction Research Center Inventory (ARCI) (Scale MBG), Addiction Research Center Inventory (ARCI) (Scale PCAG), Addiction Research Center Inventory (ARCI) (Scale PCAG/A/LSD), Beverage Rating Scale, Drug Effects Questionnaire (DEQ) (Scale Drug Effect), Drug Effects Questionnaire (DEQ) (Scale Feel), Drug Effects Questionnaire (DEQ) (Scale Intoxication), Drunkenness Scale, Likert "how drunk scale", Profile of Mood States (POMS) (Scale Intoxication), Subjective Effects Questionnaire (Scale Intoxication), Subjective Estimations of Intoxication, Subjective Intoxication Level, Subjective Judgement of Intoxication, Subjective Rating Scale (Scale Inebriation), Visual Analogue Scale (VAS) (Scale Drunkenness), Visual Analogue Scale (VAS) (Scale Feel), Visual Analogue Scale (VAS) (Scale Intoxication) | 0 | 20 | 80 | (Abroms *et al.*, 2006), (Balodis *et al.*, 2006), (Bartholow *et al.*, 2003a), (Bartholow *et al.*, 2003b), (Cameron *et al.*, 2001), (Davidson *et al.*, 1997), (De Cesarei *et al.*, 2006), (Duka *et al.*, 1998), (Farquhar *et al.*, 2002), (Field and Duka, 2002), (Fillmore *et al.*, 2005), (Fillmore, 2004), (Fillmore and Blackburn, 2002), (Fillmore, 2001), (Fillmore and Vogel-Sprott, 1998), (Fillmore and Vogel-Sprott, 1995), (Gengo *et al.*, 1990), (Holdstock *et al.*, 2006), (Holdstock and de Wit, 1999), (Holdstock and de Wit, 1998), (Jones, 1993), (Kirchner and Sayette, 2003), (Landauer and Howat, 1983), (Lane *et al.*, 2004), (Lapp *et al.*, 1994), (Liguori *et al.*, 1999), (Lukas and Mendelson, 1988), (Lukas *et al.*, 1986a), (Lyvers and Maltzman, 1991b), (Lyvers and Maltzman, 1991a), (MacDonald *et al.*, 1995), (Marczinski *et al.*, 2005), (Marczinski and Fillmore, 2005b), (Marczinski and Fillmore, 2005a), (Marczinski and Fillmore, 2003), (Marinkovic *et al.*, 2004), (Mulvihill *et al.*, 1997), (Mundt *et al.*, 1997), (Nagoshi *et al.*, 1992), (Niaura *et al.*, 1987), (Nicholson *et al.*, 1992b), (Nyberg *et al.*, 2004), (O'Boyle *et al.*, 1994), (O'Malley and Maisto, 1984), (Ortner *et al.*, 2003), (Pierucci-Lagha *et al.*, 2006), (Reynolds *et al.*, 2006), (Richards *et al.*, 1999), (Rohrbaugh *et al.*, 1988), (Ross and Pihl, 1988), (Schweizer *et al.*, 2006), (Schweizer *et al.*, 2005), (Schweizer *et al.*, 2004), (Sher, 1985), (Soderlund *et al.*, 2005), (Stenberg *et al.*, 1994), (Tiplady *et al.*, 2001), (Wegner and Fahle, 1999a), (Wilkie and Stewart, 2005), (Zack and Vogel-Sprott, 1993), (Zeichner *et al.*, 1993), (Franken *et al.*, 2007), (Hoyer *et al.*, 2007), (Saults *et al.*, 2007) (n=64) |
| Scale Fatigue | Profile of Mood States (POMS) (Scale Energetic/Tired), Profile of Mood States (POMS) (Scale Energy), Profile of Mood States (POMS) (Scale Fatigue), Profile of Mood States (POMS) (Scale Fatigue/Inertia) | 14 | 81 | 5 | (DeWit *et al.*, 1989), (Holdstock and de Wit, 1998), (Knowles and Duka, 2004), (McKee *et al.*, 2006), (Nagoshi *et al.*, 1992), (Pierucci-Lagha *et al.*, 2006), (Reynolds *et al.*, 2006), (Soderlund *et al.*, 2005), (Wilkie and Stewart, 2005), (Zeichner *et al.*, 1993) (n=10) |
| Scale High | Drug Effects Questionnaire (DEQ) (Scale High), Profile of Mood States (POMS) (Scale High), Subjective High Assessment Scale, Subjective High Assessment Scale (Scale High), Visual Analogue Scale (VAS) (Scale High) | 0 | 13 | 87 | (Ehlers *et al.*, 1989), (Fillmore, 2004), (Fillmore and Blackburn, 2002), (Fillmore, 2001), (Holdstock *et al.*, 2006), (Holdstock and de Wit, 1999), (Holdstock and de Wit, 1998), (Lex *et al.*, 1988), (Liguori *et al.*, 1999), (Lukas *et al.*, 1989), (Marczinski and Fillmore, 2005b), (Marczinski and Fillmore, 2005a), (Marczinski and Fillmore, 2003), (Marinkovic *et al.*, 2004), (Parker *et al.*, 1981), (Pierucci-Lagha *et al.*, 2006), (Reynolds *et al.*, 2006), (Richards *et al.*, 1999), (Soderlund *et al.*, 2005) (n=19) |

| | | | | | |
|---|---|---|---|---|---|
| Scale Mood | Affect Inventory (Scale Surprisedness/Affection/Digust/Guilt/Sexuality/Humor/Happiness), Beck-Depression-Inventory (Scale Depression), Estimation of Mood Change Test (Scale Elated/Depressed), Mood Scale (Scale Pleasure/Activity/Dominance), Mood Sorting Test, Personality-Trait Presentation Task, Positive Affect/Negative Affect Schedule (Scale Negative Affect), Positive Affect/Negative Affect Schedule (Scale Positive Affect), Profile of Mood States (POMS) (Scale Depression), Profile of Mood States (POMS) (Scale Depression/Elation), Profile of Mood States (POMS) (Scale Depression/Elation/Positive Mood), Profile of Mood States (POMS) (Scale Elation/Positive Mood), Profile of Mood States (POMS) (Scale Mood), Profile of Mood States (POMS) (Scale Positive Mood), Profile of Mood States (POMS) (Scale Tension/Anxiety/Depression/Dejection/Anger/Hostility/Vigour/Fatigue/Confusion/Bewilderment), Rotter's Internal-External Scale, Subjective Effects of Alcohol Scale (SAES) (Scale Negative Affect), Subjective Mood States (Scale Mood), Subjective Rating Scale (Scale Joyfulness), Subjective Rating Scale (Scale Positive Mood), Visual Analogue Scale (VAS) (Scale Contentedness), Visual Analogue Scale (VAS) (Scale Mood Changes), Visual Analogue Scale (VAS) (Scale Mood), Visual Analogue Scale (VAS) (Scale Pleasantness) | 5 | 65 | 30 | (Bruce *et al.*, 1999), (Cameron *et al.*, 2001), (Cowan, 1983), (De Cesarei *et al.*, 2006), (DeWit *et al.*, 1989), (Duka *et al.*, 2001), (Duka *et al.*, 1998), (Farquhar *et al.*, 2002), (Gustafson and Kallmen, 1990), (Gustafson and Kallmen, 1989), (Holdstock and de Wit, 1998), (Hutchison *et al.*, 1997), (Knowles and Duka, 2004), (Liguori *et al.*, 1999), (Lindman, 1985), (Lukas *et al.*, 1986b), (McKee *et al.*, 2006), (Mundt *et al.*, 1997), (O'Malley and Maisto, 1984), (Rammsayer, 1995), (Reynolds *et al.*, 2006), (Sher, 1985), (Stenberg *et al.*, 1994), (Tiplady *et al.*, 2001), (Weissenborn and Duka, 2003), (Weissenborn and Duka, 2000), (Zeichner *et al.*, 1993), (Breitmeier *et al.*, 2007) (n=28) |
| Scale Morality | Subjective Attitudes and Intentions to Drink and Drive | 100 | 0 | 0 | (MacDonald *et al.*, 1995) (n=1) |
| Scale Performance | Affect Inventory (Scale Intelligentness), Alcohol Sensation Scale (Scale Impairment), Driving Questionnaire, Self-Evaluation of Performance , Subjective Estimations of Ability, Subjective Impairment Scale, Visual Analogue Scale (VAS) (Scale Driving), Visual Analogue Scale (VAS) (Scale Functional Integrity) | 67 | 33 | 0 | (Cameron *et al.*, 2001), (Davidson *et al.*, 1997), (Farquhar *et al.*, 2002), (Harrison and Fillmore, 2005b), (Harrison and Fillmore, 2005a), (Landauer and Howat, 1983), (MacDonald *et al.*, 1995), (Millar *et al.*, 1999), (Mills and Bisgrove, 1983a), (Mills and Bisgrove, 1983b), (Mundt *et al.*, 1997), (O'Malley and Maisto, 1984), (Tiplady *et al.*, 2001), (Young and Pihl, 1982) (n=14) |
| Scale Symptoms | Alcohol Sensation Scale (Scale Anesthesia), Alcohol Sensation Scale (Scale Central/Warm/Dynamic/Periphery/Nausea), Alcohol Sensation Scale (Scale Sensations), Alcohol Sensation Scale (Scale Somatic Sensations), Drug Effects Questionnaire (DEQ) (Scale Nausea), Sensation Scale (Scale Physical Sensations), Sensation Scale (Scale Symptoms), Von Zerssen's List of Complaints (Scale Complaints) | 42 | 58 | 0 | (Davidson *et al.*, 1997), (Holdstock *et al.*, 2006), (O'Malley and Maisto, 1984), (Pierucci-Lagha *et al.*, 2006), (Sher, 1985), (Breitmeier *et al.*, 2007) (n=6) |



**(Neuro)Endocrine**

| *Cluster* | *Test* | *(-)* | *(=)* | *(+)* | *References (frequency)* |
|---|---|---|---|---|---|
| Catecholamines | 3-methoxy-4-hydroxy ethyleneglycol (MOPEG) | 0 | 0 | 100 | (Borg *et al.*, 1983) (n=1) |
| Cortisol/ACTH | ACTH, Cortisol | 0 | 43 | 57 | (Holdstock *et al.*, 2006), (Inder *et al.*, 1995), (Lukas and Mendelson, 1988), (Pierucci-Lagha *et al.*, 2006), (Sarkola *et al.*, 1999) (n=5) |
| Other Neuroendocrine Substances | AVP, Beta-Endorphin, Cholecystokinin, Dopamine, Melatonin, Prolactine, Serotonin | 14 | 71 | 14 | (Boyer *et al.*, 2004), (Inder *et al.*, 1995), (Sarkola *et al.*, 1999), (Rupp *et al.*, 2007a) (n=4) |
| Sex Hormones | 3α-hydroxy-5α-pregnan-20-one-like progesterones, Allopregnanolone, DHEA, DHEA-S, Estradiol, Estradiol/Estrone/Progesterone, Luteinizing Hormone (LH), Pregnenolone, Progesterone, Testosterone | 27 | 64 | 9 | (Holdstock *et al.*, 2006), (Pierucci-Lagha *et al.*, 2006), (Sarkola *et al.*, 1999) (n=3) |

**TABLE 7** DOSE RESPONSE RELATIONSHIPS

*For clusters tested at least 10 times (except for values between brackets) with at least two dose levels. Results are in % per alcohol dose level. "+" reflects an improvement or increase, "=" reflects no significant effect and "-" reflects an impairment or decrease.*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Executive** | | | | | | | | | |
| *Cluster* | *< 0.5 g·L⁻¹* | | | *0.5 - 0.7 g·L⁻¹* | | | *> 0.7 g·L⁻¹* | | |
| | (-) | (=) | (+) | (-) | (=) | (+) | (-) | (=) | (+) |
| Inhibition | 32 | 65 | 3 | 56 | 41 | 3 | 64 | 29 | 7 |
| Working Memory | 18 | 82 | 0 | 39 | 61 | 0 | 75 | 25 | 0 |
| **Attention** | | | | | | | | | |
| *Cluster* | *< 0.5 g·L⁻¹* | | | *0.5 - 0.7 g·L⁻¹* | | | *> 0.7 g·L⁻¹* | | |
| | (-) | (=) | (+) | (-) | (=) | (+) | (-) | (=) | (+) |
| Divided Attention | 58 | 33 | 8 | 67 | 33 | 0 | 79 | 21 | 0 |
| Focused/Selective Attention | 7 | 86 | 7 | 62 | 38 | 0 | 74 | 26 | 0 |
| Reaction Time | 23 | 77 | 0 | 51 | 49 | 0 | 73 | 27 | 0 |
| **Motor** | | | | | | | | | |
| *Cluster* | *< 0.5 g·L⁻¹* | | | *0.5 - 0.7 g·L⁻¹* | | | *> 0.7 g·L⁻¹* | | |
| | (-) | (=) | (+) | (-) | (=) | (+) | (-) | (=) | (+) |
| Visuo-Motor Control | 43 | 57 | 0 | 85 | 15 | 0 | 89 | 11 | 0 |
| **Subjective Experience** | | | | | | | | | |
| *Cluster* | *< 0.5 g·L⁻¹* | | | *0.5 - 0.7 g·L⁻¹* | | | *> 0.7 g·L⁻¹* | | |
| | (-) | (=) | (+) | (-) | (=) | (+) | (-) | (=) | (+) |
| Scale Alertness | 19 | 78 | 4 | 53 | 42 | 5 | 41 | 53 | 6 |
| Scale Calmness | 20 | 80 | 0 | 21 | 75 | 4 | 31 | 69 | 0 |
| Scale Craving | 50 | 50 | 0 | 58 | 42 | 0 | (38) | (63) | (0) |
| Scale Drug Effect | 0 | 41 | 59 | 0 | 7 | 93 | 0 | 9 | 91 |
| Scale High | 0 | 36 | 64 | 0 | 0 | 100 | (0) | (0) | (100) |
| Scale Mood | 7 | 71 | 21 | 0 | 64 | 36 | 8 | 58 | 33 |
| **Perception** | | | | | | | | | |
| *Cluster* | *< 0.5 g·L⁻¹* | | | *0.5 - 0.7 g·L⁻¹* | | | *> 0.7 g·L⁻¹* | | |
| | (-) | (=) | (+) | (-) | (=) | (+) | (-) | (=) | (+) |
| Visual Perception | - | - | - | 36 | 64 | 0 | 60 | 40 | 0 |
| **Neurophysiologic** | | | | | | | | | |
| *Cluster* | *< 0.5 g·L⁻¹* | | | *0.5 - 0.7 g·L⁻¹* | | | *> 0.7 g·L⁻¹* | | |
| | (-) | (=) | (+) | (-) | (=) | (+) | (-) | (=) | (+) |
| Evoked Potential | 50 | 25 | 25 | 54 | 23 | 23 | (50) | (13) | (38) |





Abroms BD, Gottlob LR, Fillmore MT (2006). Alcohol effects on inhibitory control of attention: distinguishing between intentional and automatic mechanisms. Psychopharmacology (Berl) 188: 324-334

Acheson SK, Stein RM, Swartzwelder HS (1998). Impairment of semantic and figural memory by acute ethanol: age-dependent effects. Alcohol Clin Exp Res 22: 1437-1442

Addicott MA, Marsh-Richard DM, Mathias CW, Dougherty DM (2007). The biphasic effects of alcohol: Comparisons of subjective and objective measures of stimulation, sedation, and physical activity. Alcoholism-Clinical and Experimental Research 31: 1883-1890

Antebi D (1982). The effects of alcohol on four choice serial reaction time. Med Sci Law 22: 181-188

Asicioglu F, Turan N (2003). Handwriting changes under the effect of alcohol. Forensic Sci Int 132: 201-210

Baker SJ, Chrzan GJ, Park CN, Saunders JH (1985). Validation of human behavioral tests using ethanol as a cns depressant model. Neurobehav Toxicol Teratol 7: 257-261

Balodis IM, MacDonald TK, Olmstead MC (2006). Instructional cues modify performance on the Iowa Gambling Task. Brain Cogn 60: 109-117

Bartholow BD, Pearson M, Sher KJ, Wieman LC, Fabiani M, Gratton G (2003a). Effects of alcohol consumption and alcohol susceptibility on cognition: a psychophysiological examination. Biol Psychol 64: 167-190

Bartholow BD, Pearson MA, Gratton G, Fabiani M (2003b). Effects of alcohol on person perception: a social cognitive neuroscience approach. J Pers Soc Psychol 85: 627-638

Beirness D, Vogel-Sprott M (1984). The development of alcohol tolerance: acute recovery as a predictor. Psychopharmacology (Berl) 84: 398-401

Beirness DJ, Vogel-Sprott MD (1982). Does prior skill reduce alcohol-induced impairment? J Stud Alcohol 43: 1149-1156

Birnbaum IM, Johnson MK, Hartley JT, Taylor TH (1980). Alcohol and elaborative schemas for sentences. J Exp Psychol (Hum Learn ) 6: 293-300

Birnbaum IM, Taylor TH, Johnson MK, Raye CL (1987). Is event frequency encoded automatically? The case of alcohol intoxication. J Exp Psychol Learn Mem Cogn 13: 251-258

Blekher T, Beard JD, O'Connor S, Orr WE, Ramchandani VA, Miller K, Yee RD, Li TK (2002). Response of saccadic eye movements to alcohol in African American and non-Hispanic white college students. Alcohol Clin Exp Res 26: 232-238

Block AJ (1984). Alcohol ingestion does not cause sleep-disordered breathing in premenopausal women. Alcohol Clin Exp Res 8: 397-398

Bond A, lader M (1974). The use of analogue scales in rating subjective feelings. Br J Med Psychol 211-218

Borg S, Kvande H, Mossberg D, Valverius P, Sedvall G (1983). Central nervous system noradrenaline metabolism and alcohol consumption in man. Pharmacol Biochem Behav 18 Suppl 1: 375-378

Boyer JC, Bancel E, Perray PF, Pouderoux P, Balmes JL, Bali JP (2004). Effect of champagne compared to still white wine on peripheral neurotransmitter concentrations. Int J Vitam Nutr Res 74: 321-328

Breitmeier D, Seeland-Schulze I, Hecker H, Schneider U (2007). The influence of blood alcohol concentrations of around 0.03% on neuropsychological functions - a double-blind, placebo-controlled investigation. Addiction Biology 12: 183-189

Bruce KR, Pihl RO (1997). Forget 'drinking to forget': enhanced consolidation of emotionally charged memory by alcohol. Exp Clin Psychopharmacol 5: 242-250

Bruce KR, Shestowsky JS, Mayerovitch JI, Pihl RO (1999). Motivational effects of alcohol on memory consolidation and heart rate in social drinkers. Alcohol Clin Exp Res 23: 693-701

Cameron E, Sinclair W, Tiplady B (2001). Validity and sensitivity of a pen computer battery of performance tests. J Psychopharmacol 15: 105-110

Charness ME, Simon RP, Greenberg DA (1989). Ethanol and the nervous system. New England Journal of Medicine 321: 442-454

Chiang HH, Young YH (2007). Impact of alcohol on vestibular function in relation to the legal limit of 0.25 mg/l breath alcohol concentration. Audiology and Neuro-Otology 12: 183-188

Clarici A, Fabbro F, Bava A (1993). Effects of ethyl alcohol on hemispheric specialization of language and on hand movements. Percept Mot Skills 77: 1259-1264

Clarici A, Fabbro F, Bava A (1995). Effects of moderate doses of ethyl alcohol on cerebral lateralization of language and on hand movements. I: A dual-task paradigm study. Boll Soc Ital Biol Sper 71: 213-220

Cohen HL, Porjesz B, Begleiter H (1993). Ethanol-induced alterations in electroencephalographic activity in adult males. Neuropsychopharmacology 8: 365-370

Colburn WA (2003). Biomarkers in drug discovery and development: From target identification through drug marketing. Journal of Clinical Pharmacology 43: 329-341

Colzato LS, Erasmus V, Hommel B (2004). Moderate alcohol consumption in humans impairs feature binding in visual perception but not across perception and action. Neurosci Lett 360: 103-105

Connors GJ, Maisto SA (1980). Effects of alcohol, instructions and consumption rate and motor performance. J Stud Alcohol 41: 509-517

Cowan JD (1983). Testing the escape hypotheses. Alcohol helps users to forget their feelings. J Nerv Ment Dis 171: 40-48
Dalrymple-Alford JC, Kerr PA, Jones RD (2003). The effects of alcohol on driving-related sensorimotor performance across four times of day. J Stud Alcohol 64: 93-97
Davidson D, Camara P, Swift R (1997). Behavioral effects and pharmacokinetics of low-dose intravenous alcohol in humans. Alcohol Clin Exp Res 21: 1294-1299
De Cesarei A, Codispoti M, Schupp HT, Stegagno L (2006). Selectively attending to natural scenes after alcohol consumption: an ERP analysis. Biol Psychol 72: 35-45
de Visser SJ, van der Post J, Pieters MSM, Cohen AF, van Gerven JMA (2001). Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 51: 119-132
de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM (2003). Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 55: 39-50
de Wit H, Uhlenhuth EH, Pierri J, Johanson CE (1987). Individual differences in behavioral and subjective responses to alcohol. Alcohol Clin Exp Res 11: 52-59
Degia A, Meadows R, Johnsen S, Hindmarch I, Boyle J (2005). Development of a portable psychometric testing device for use in the field: an alcohol investigation. Percept Mot Skills 101: 383-392
DeWit H, Pierri J, Johanson CE (1989). Assessing individual differences in ethanol preference using a cumulative dosing procedure. Psychopharmacology (Berl) 98: 113-119
do Canto-Pereira LHM, David IDA, Machado-Pinheiro W, Ranvaud RD (2007). Effects of acute alcohol intoxication on visuospatial attention. Human & Experimental Toxicology 26: 311-319
Donohue KF, Curtin JJ, Patrick CJ, Lang AR (2007). Intoxication level and emotional response. Emotion 7: 103-112
Dougherty DM, Marsh DM, Moeller FG, Chokshi RV, Rosen VC (2000). Effects of moderate and high doses of alcohol on attention, impulsivity, discriminability, and response bias in immediate and delayed memory task performance. Alcohol Clin Exp Res 24: 1702-1711
Drake CL, Roehrs T, Turner L, Scofield HM, Roth T (2003). Caffeine reversal of ethanol effects on the multiple sleep latency test, memory, and psychomotor performance. Neuropsychopharmacology 28: 371-378
Duka T, Stephens DN, Russell C, Tasker R (1998). Discriminative stimulus properties of low doses of ethanol in humans. Psychopharmacology (Berl) 136: 379-389
Duka T, Weissenborn R, Dienes Z (2001). State-dependent effects of alcohol on recollective experience, familiarity and awareness of memories. Psychopharmacology (Berl) 153: 295-306
Dumont GJH, de Visser SJ, Cohen AF, van Gerven JMA (2005). Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects. British Journal of Clinical Pharmacology 59: 495-510
Dumont GJH, Verkes RJ (2006). A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers. Journal of Psychopharmacology 20: 176-187
Easdon C, Izenberg A, Armilio ML, Yu H, Alain C (2005). Alcohol consumption impairs stimulus- and error-related processing during a Go/No-Go Task. Brain Res Cogn Brain Res 25: 873-883
Easdon CM, Vogel-Sprott M (2000). Alcohol and behavioral control: impaired response inhibition and flexibility in social drinkers. Exp Clin Psychopharmacol 8: 387-394
Ehlers CL, Garcia-Andrade C, Wall TL, Cloutier D, Phillips E (1999). Electroencephalographic responses to alcohol challenge in Native American Mission Indians. Biol Psychiatry 45: 776-787
Ehlers CL, Wall TL, Schuckit MA (1989). eeg spectral characteristics following ethanol administration in young men. Electroencephalogr Clin Neurophysiol 73: 179-187
Erblich J, Earleywine M (1995). Distraction does not impair memory during intoxication: support for the attention-allocation model. J Stud Alcohol 56: 444-448
Erwin CW, Linnoila M (1981). Effect of ethyl alcohol on visual evoked potentials. Alcohol Clin Exp Res 5: 49-55
Farquhar K, Lambert K, Drummond GB, Tiplady B, Wright P (2002). Effect of ethanol on psychomotor performance and on risk taking behaviour. J Psychopharmacol 16: 379-384
Feely J, Wood AJ (1982). Effects of cimetidine on the elimination and actions of ethanol. JAMA 247: 2819-2821
Field M, Duka T (2002). Cues paired with a low dose of alcohol acquire conditioned incentive properties in social drinkers. Psychopharmacology (Berl) 159: 325-334
Fillmore MT (2001). Cognitive preoccupation with alcohol and binge drinking in college students: alcohol-induced priming of the motivation to drink. Psychol Addict Behav 15: 325-332
Fillmore MT (2003). Reliability of a computerized assessment of psychomotor performance and its sensitivity to alcohol-induced impairment. Percept Mot Skills 97: 21-34
Fillmore MT (2004). Environmental dependence of behavioral control mechanisms: effects of alcohol and information processing demands. Exp Clin Psychopharmacol 12: 216-223
Fillmore MT, Blackburn J (2002). Compensating for alcohol-induced impairment: alcohol expectancies and behavioral disinhibition. J Stud Alcohol 63: 237-246



Fillmore MT, Dixon MJ, Schweizer TA (2000a). Alcohol affects processing of ignored stimuli in a negative priming paradigm. J Stud Alcohol 61: 571-578
Fillmore MT, Dixon MJ, Schweizer TA (2000b). Differential effects of alcohol on responses to negatively and positively primed stimuli. J Stud Alcohol 61: 872-880
Fillmore MT, Marczinski CA, Bowman AM (2005). Acute tolerance to alcohol effects on inhibitory and activational mechanisms of behavioral control. J Stud Alcohol 66: 663-672
Fillmore MT, Vogel-Sprott M (1995). Expectancies about alcohol-induced motor impairment predict individual differences in responses to alcohol and placebo. J Stud Alcohol 56: 90-98
Fillmore MT, Vogel-Sprott M (1996a). Evidence that expectancies mediate behavioral impairment under alcohol. J Stud Alcohol 57: 598-603
Fillmore MT, Vogel-Sprott M (1996b). Social drinking history, behavioral tolerance and the expectation of alcohol. Psychopharmacology (Berl) 127: 359-364
Fillmore MT, Vogel-Sprott M (1997). Resistance to cognitive impairment under alcohol: the role of environmental consequences. Exp Clin Psychopharmacol 5: 251-255
Fillmore MT, Vogel-Sprott M (1998). Behavioral impairment under alcohol: cognitive and pharmacokinetic factors. Alcohol Clin Exp Res 22: 1476-1482
Fillmore MT, Vogel-Sprott M (2000). Response inhibition under alcohol: effects of cognitive and motivational conflict. J Stud Alcohol 61: 239-246
Fillmore MT, Vogel-Sprott M, Gavrilescu D (1999). Alcohol effects on intentional behavior: dissociating controlled and automatic influences. Exp Clin Psychopharmacol 7: 372-378
Finn PR, Justus A, Mazas C, Steinmetz JE (1999). Working memory, executive processes and the effects of alcohol on Go/No-Go learning: testing a model of behavioral regulation and impulsivity. Psychopharmacology (Berl) 146: 465-472
Fogarty JN, Vogel-Sprott M (2002). Cognitive processes and motor skills differ in sensitivity to alcohol impairment. J Stud Alcohol 63: 404-411
Franken IHA, Nijs IMT, Muris P, Van Strien JW (2007). Alcohol selectively reduces brain activity during the affective processing of negative information. Alcoholism-Clinical and Experimental Research 31: 919-927
Galbraith NG (1986). Alcohol: its effect on handwriting. J Forensic Sci 31: 580-588
Gawron VJ, Ranney TA (1988). The effects of alcohol dosing on driving performance on a closed course and in a driving simulator. Ergonomics 31: 1219-1244
Gengo FM, Gabos C, Straley C, Manning C (1990). The pharmacodynamics of ethanol: effects on performance and judgment. J Clin Pharmacol 30: 748-754
George WH, Raynor J O, Nochajski TH (1990). Resistance to alcohol impairment of visual-motor performance. II: Effects for attentional set and self-reported concentration. Pharmacol Biochem Behav 36: 261-266
Grant SA, Millar K, Kenny GN (2000). Blood alcohol concentration and psychomotor effects. Br J Anaesth 85: 401-406
Grattan K E, Vogel-Sprott M (2001). Maintaining intentional control of behavior under alcohol. Alcohol Clin Exp Res 25: 192-197
Grattan-Miscio KE, Vogel-Sprott M (2005). Effects of alcohol and performance incentives on immediate working memory. Psychopharmacology (Berl) 181: 188-196
Gustafson R (1986). Effect of small doses of alcohol and signal intensity on simple auditory reaction time in a monotonous test situation. Percept Mot Skills 63: 539-543
Gustafson R (1991). Effect of alcohol on quantity of creative production using the Purdue tests. Psychol Rep 69: 83-90
Gustafson R, Kallmen H (1989). Alcohol effects on cognitive and personality style in women with special reference to primary and secondary process. Alcohol Clin Exp Res 13: 644-648
Gustafson R, Kallmen H (1990). Effects of alcohol on prolonged cognitive performance measured with Stroop's Color Word Test. Psychol Rep 67: 643-650
Haertzen CA (1965). Addiction Research-Center Inventory (Arci) - Development of A General Drug Estimation Scale. Journal of Nervous and Mental Disease 141: 300-307
Hafstrom A, Modig F, Karlberg M, Fransson PA (2007). Increased visual dependence and otolith dysfunction with alcohol intoxication. Neuroreport 18: 391-394
Harder T, Reker U (1995). Influence of low dose alcohol on fixation suppression. Acta Otolaryngol Suppl 520 Pt 1: 33-36
Harrison EL, Fillmore MT (2005a). Social drinkers underestimate the additive impairing effects of alcohol and visual degradation on behavioral functioning. Psychopharmacology (Berl) 177: 459-464
Harrison EL, Fillmore MT (2005b). Transfer of learning to compensate for impairment by alcohol and visual degradation. Psychopharmacology (Berl) 182: 461-467
Haubenreisser T, Vogel-Sprott M (1983). Tolerance development in humans with task practice on different limbs of the blood-alcohol curve. Psychopharmacology (Berl) 81: 350-353
Hernandez OH, Vogel-Sprott M, Huchin-Ramirez TC, Ake-Estrada F (2006). Acute dose of alcohol affects cognitive components of reaction time to an omitted stimulus: differences among sensory systems. Psychopharmacology (Berl) 184: 75-81
Hernandez OH, Vogel-Sprott M, Ke-Aznar VI (2007). Alcohol impairs the cognitive

component of reaction time to an omitted stimulus: A replication and an extension. Journal of Studies on Alcohol and Drugs 68: 276-281
Holdstock L, de Wit H (1998). Individual differences in the biphasic effects of ethanol. Alcohol Clin Exp Res 22: 1903-1911
Holdstock L, de Wit H (1999). Ethanol impairs saccadic and smooth pursuit eye movements without producing self-reports of sedation. Alcohol Clin Exp Res 23: 664-672
Holdstock L, Penland SN, Morrow AL, de Wit H (2006). Moderate doses of ethanol fail to increase plasma levels of neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one-like immunoreactivity in healthy men and women. Psychopharmacology (Berl) 186: 442-450
Hollien H, DeJong G, Martin CA, Schwartz R, Liljegren K (2001). Effects of ethanol intoxication on speech suprasegmentals. J Acoust Soc Am 110: 3198-3206
Hoyer WJ, Semenec SC, Buchler NEG (2007). Acute alcohol intoxication impairs controlled search across the visual field. Journal of Studies on Alcohol and Drugs 68: 748-758
Hutchison KE, Rohsenow D, Monti P, Palfai T, Swift R (1997). Prepulse inhibition of the startle reflex: preliminary study of the effects of a low dose of alcohol in humans. Alcohol Clin Exp Res 21: 1312-1319
Inder WJ, Joyce PR, Wells JE, Evans MJ, Ellis MJ, Mattioli L, Donald RA (1995). The acute effects of oral ethanol on the hypothalamic-pituitary-adrenal axis in normal human subjects. Clin Endocrinol (Oxf) 42: 65-71
Jang GR, Harris RZ (2007). Drug interactions involving ethanol and alcoholic beverages. Expert Opinion on Drug Metabolism & Toxicology 3: 719-731
Jansen AA, de Gier JJ, Slangen JL (1985). Alcohol effects on signal detection performance. Neuropsychobiology 14: 83-87
Jones AW (1993). Pharmacokinetics of ethanol in saliva: comparison with blood and breath alcohol profiles, subjective feelings of intoxication, and diminished performance. Clin Chem 39: 1837-1844
Jones AW, Neri A (1994). Age-related differences in the effects of ethanol on performance and behaviour in healthy men. Alcohol Alcohol 29: 171-179
Jones MB, Chronister JL, Kennedy RS (1998). Effects of alcohol on perceptual speed. Percept Mot Skills 87: 1247-1255
Jones MK, Jones BM (1980). The relationship of age and drinking habits to the effects of alcohol on memory in women. J Stud Alcohol 41: 179-186
Kearney SA, Guppy A (1988). The effects of alcohol on speed perception in a closed-course driving situation. J Stud Alcohol 49: 340-345
Kennedy RS, Turnage JJ, Dunlap WP (1992). The use of dose equivalency as a risk assessment index in behavioral neurotoxicology. Neurotoxicol Teratol 14: 167-175
Kennedy RS, Turnage JJ, Wilkes RL, Dunlap WP (1993). Effects of graded dosages of alcohol on nine computerized repeated-measures tests. Ergonomics 36: 1195-1222
Khan SA, Timney B (2007). Alcohol slows interhemispheric transmission, increases the flash-lag effect, and prolongs masking: Evidence for a slowing of neural processing and transmission. Vision Research 47: 1821-1832
Kirchner TR, Sayette MA (2003). Effects of alcohol on controlled and automatic memory processes. Exp Clin Psychopharmacol 11: 167-175
Knowles SK, Duka T (2004). Does alcohol affect memory for emotional and non-emotional experiences in different ways? Behav Pharmacol 15: 111-121
Krause CM, Aromaki A, Sillanmaki L, Astrom T, Alanko K, Salonen E, Peltola O (2002). Alcohol-induced alterations in ERD/ERS during an auditory memory task. Alcohol 26: 145-153
Landauer AA, Howat P (1983). Low and moderate alcohol doses, psychomotor performance and perceived drowsiness. Ergonomics 26: 647-657
Landauer AA, Howat PA (1982). Alcohol and the cognitive aspects of choice reaction time. Psychopharmacology (Berl) 78: 296-297
Lane sd, Cherek DR, Pietras CJ, Tcheremissine OV (2004). Alcohol effects on human risk taking. Psychopharmacology (Berl) 172: 68-77
Lapp WM, Collins RL, Zywiak WH, Izzo CV (1994). Psychopharmacological effects of alcohol on time perception: the extended balanced placebo design. J Stud Alcohol 55: 96-112
Ledin T, Odkvist LM (1991). Effect of alcohol measured by dynamic posturography. Acta Otolaryngol Suppl 481: 576-581
Lex BW, Greenwald NE, Lukas SE, Slater JP, Mendelson JH (1988). Blood ethanol levels, self-rated ethanol effects and cognitive-perceptual tasks. Pharmacol Biochem Behav 29: 509-515
Lezak MD, Howieson DB, Loring DW (2004). Neurophychological Assesment (4th ed). New York: Oxford University Press.
Liguori A, D'Agostino RB, Jr., Dworkin SI, Edwards D, Robinson JH (1999). Alcohol effects on mood, equilibrium, and simulated driving. Alcohol Clin Exp Res 23: 815-821
Lindman R (1985). On the direct estimation of mood change. Percept Psychophys 37: 170-174
Linnoila M, Erwin CW, Ramm D, Cleveland WP (1980). Effects of age and alcohol on psychomotor performance of men. J Stud Alcohol 41: 488-495
Lister RG, Gorenstein C, Fisher-Flowers D, Weingartner HJ, Eckardt MJ (1991). Dissociation of the acute effects of alcohol on implicit and explicit memory processes. Neuropsychologia 29: 1205-1212
Little HJ (1991). Mechanisms that may underlie the behavioral-effects of ethanol. Progress in Neurobiology 36: 171-194



Lowe G (1983). Alcohol and state-dependent learning. Subst Alcohol Actions Misuse 4: 273-282
Lukas SE, Lex BW, Slater JP, Greenwald NE, Mendelson JH (1989). A microanalysis of ethanol-induced disruption of body sway and psychomotor performance in women. Psychopharmacology (Berl) 98: 169-175
Lukas SE, Mendelson JH (1988). Electroencephalographic activity and plasma ACTH during ethanol-induced euphoria. Biol Psychiatry 23: 141-148
Lukas SE, Mendelson JH, Benedikt RA (1986a). Instrumental analysis of ethanol-induced intoxication in human males. Psychopharmacology (Berl) 89: 8-13
Lukas SE, Mendelson JH, Benedikt RA, Jones B (1986b). eeg alpha activity increases during transient episodes of ethanol-induced euphoria. Pharmacol Biochem Behav 25: 889-895
Lyvers MF, Maltzman I (1991a). Selective effects of alcohol on Wisconsin Card Sorting Test performance. Br J Addict 86: 399-407
Lyvers MF, Maltzman I (1991b). The balanced placebo design: effects of alcohol and beverage instructions cannot be independently assessed. Int J Addict 26: 963-972
MacCarthy F, Tong JE (1980). Alcohol and velocity perception: 11. Stimulus discrimination. Percept Mot Skills 51: 968-970
MacDonald TK, Zanna MP, Fong GT (1995). Decision making in altered states: effects of alcohol on attitudes toward drinking and driving. J Pers Soc Psychol 68: 973-985
Mann R E, Cho-Young J, Vogel-Sprott M (1984). Retrograde enhancement by alcohol of delayed free recall performance. Pharmacol Biochem Behav 20: 639-642
Marczinski CA, Abroms BD, Van Selst M, Fillmore MT (2005). Alcohol-induced impairment of behavioral control: differential effects on engaging vs. disengaging responses. Psychopharmacology (Berl) 182: 452-459
Marczinski CA, Fillmore MT (2003). Preresponse cues reduce the impairing effects of alcohol on the execution and suppression of responses. Exp Clin Psychopharmacol 11: 110-117
Marczinski CA, Fillmore MT (2005a). Alcohol increases reliance on cues that signal acts of control. Exp Clin Psychopharmacol 13: 15-24
Marczinski CA, Fillmore MT (2005b). Compensating for alcohol-induced impairment of control: effects on inhibition and activation of behavior. Psychopharmacology (Berl) 181: 337-346
Marinkovic K, Halgren E, Maltzman I (2004). Effects of alcohol on verbal processing: an event-related potential study. Alcohol Clin Exp Res 28: 415-423
Martin NG, Oakeshott JG, Gibson JB, Wilks AV, Starmer GA, Whitfield JB (1981). Prodromus to a twin study of sensitivity to intoxication and alcohol metabolism. Aust N Z J Med 11: 140-143
Martinez TT, Martinez RR (2003). Failure of standardized psychophysical tests for DWI to distinguish between blood alcohol levels of 0.000 and 0.080 to 0.125 g/dl. Proc West Pharmacol Soc 46: 170-173
Maylor EA, Rabbitt PM (1987a). Effect of alcohol on rate of forgetting. Psychopharmacology (Berl) 91: 230-235
Maylor EA, Rabbitt PM (1987b). Effects of alcohol and practice on choice reaction time. Percept Psychophys 42: 465-475
Maylor EA, Rabbitt PM (1988). Amount of practice and degree of attentional control have no influence on the adverse effect of alcohol in word categorization and visual search tasks. Percept Psychophys 44: 117-126
Maylor EA, Rabbitt PM, Connolly SA (1989). Rate of processing and judgment of response speed: comparing the effects of alcohol and practice. Percept Psychophys 45: 431-438
Maylor EA, Rabbitt PM, Kingstone A (1987a). Effects of alcohol on word categorization and recognition memory. Br J Psychol 78 (Pt 2): 233-239
Maylor EA, Rabbitt PM, Kingstone AF (1988). Effects of alcohol on lexical access. Psychopharmacology (Berl) 95: 119-123
Maylor EA, Rabbitt PM, Sahgal A, Wright C (1987b). Effects of alcohol on speed and accuracy in choice reaction time and visual search. Acta Psychol (Amst) 65: 147-163
McKee RH, Lammers JH, Hoogendijk EM, Emmen HH, Muijser H, Barsotti DA, Owen DE, Kulig BM (2006). Model studies for evaluating the acute neurobehavioral effects of complex hydrocarbon solvents I. Validation of methods with ethanol. Neurotoxicology 27: 1064-1079
McNair DM, Lorr M, Droppleman LF (1971). Manual for the Profile of Mood States. San Diego, CA: Educational and Industrial Testing Service.
McNamee JE, Piggins D, Tong J (1981). Confirmation of the influence of alcohol on heterophoria using a vision screener. Am J Optom Physiol Opt 58: 761-765
McNamee JE, Tong JE, Piggins DJ (1980). Effects of alcohol on velocity perception: I. Stimulus velocity and change in performance over time. Percept Mot Skills 51: 779-785
Millar K, Finnigan F, Hammersley RH (1999). Is residual impairment after alcohol an effect of repeated performance? Aviat Space Environ Med 70: 124-130
Mills KC, Bisgrove EZ (1983a). Body sway and divided attention performance under the influence of alcohol: dose-response differences between males and females. Alcohol Clin Exp Res 7: 393-397
Mills KC, Bisgrove EZ (1983b). Cognitive impairment and perceived risk from alcohol. Laboratory, self-report and field assessments. J Stud Alcohol 44: 26-46

Mills KC, Parkman KM, Spruill SE (1996). A PC-based software test for measuring alcohol and drug effects in human subjects. Alcohol Clin Exp Res 20: 1582-1591
Mintzer MZ, Griffiths RR (1999). Triazolam and zolpidem: effects on human memory and attentional processes. Psychopharmacology 144: 8-19
Moulton PL, PETros TV, Apostal KJ, Park RV, Ronning EA, King BM, Penland JG (2005). Alcohol-induced impairment and enhancement of memory: a test of the interference theory. Physiol Behav 85: 240-245
Mueller CW, Lisman SA, Spear NE (1983). Alcohol enhancement of human memory: tests of consolidation and interference hypotheses. Psychopharmacology (Berl) 80: 226-230
Mulvihill LE, Skilling TA, Vogel-Sprott M (1997). Alcohol and the ability to inhibit behavior in men and women. J Stud Alcohol 58: 600-605
Mundt JC, Perrine MW, Searles JS (1997). Individual differences in alcohol responsivity: physiological, psychomotor and subjective response domains. J Stud Alcohol 58: 130-140
Nagoshi CT, Noll RT, Wood MD (1992). Alcohol expectancies and behavioral and emotional responses to placebo versus alcohol administration. Alcohol Clin Exp Res 16: 255-260
Niaura RS, Nathan PE, Frankenstein W, Shapiro AP, Brick J (1987). Gender differences in acute psychomotor, cognitive, and pharmacokinetic response to alcohol. Addict Behav 12: 345-356
Nicholson ME, Wang M, Airhihenbuwa CO, Mahoney BS, Christina R, Maney D W (1992a). Variability in behavioral impairment involved in the rising and falling bac curve. J Stud Alcohol 53: 349-356
Nicholson ME, Wang MQ, Airhihenbuwa CO, Mahoney BS, Maney DW (1992b). Predicting alcohol impairment: perceived intoxication versus bac. Alcohol Clin Exp Res 16: 747-750
Nikulin VV, Nikulina AV, Yamashita H, Rossi EM, Kahkonen S (2005). Effects of alcohol on spontaneous neuronal oscillations: a combined magnetoencephalography and electroencephalography study. Prog Neuropsychopharmacol Biol Psychiatry 29: 687-693
Nyberg S, Wahlstrom G, Backstrom T, Poromaa IS (2004). No difference in responsiveness to a low dose of alcohol between healthy women and men. Pharmacol Biochem Behav 78: 603-610
O'Boyle DJ, Binns AS, Sumner JJ (1994). On the efficacy of alcohol placebos in inducing feelings of intoxication. Psychopharmacology (Berl) 115: 229-236
O'Malley SS, Maisto SA (1984). Factors affecting the perception of intoxication: dose, tolerance, and setting. Addict Behav 9: 111-120
Ortner CN, MacDonald TK, Olmstead MC (2003). Alcohol intoxication reduces impulsivity in the delay-discounting paradigm. Alcohol Alcohol 38: 151-156
Parker ES, Birnbaum IM, Weingartner H, Hartley JT, Stillman RC, Wyatt RJ (1980). Retrograde enhancement of human memory with alcohol. Psychopharmacology (Berl) 69: 219-222
Parker ES, Morihisa JM, Wyatt RJ, Schwartz BL, Weingartner H, Stillman RC (1981). The alcohol facilitation effect on memory: a dose-response study. Psychopharmacology (Berl) 74: 88-92
Patel SJ, Bollhoefer AD, Doty RL (2004). Influences of ethanol ingestion on olfactory function in humans. Psychopharmacology (Berl) 171: 429-434
Phillips JG, Ogeil RP (2007). Alcohol consumption and computer blackjack. Journal of General Psychology 134: 333-353
Pierucci-Lagha A, Covault J, Feinn R, Khisti RT, Morrow AL, Marx CE, Shampine LJ, Kranzler HR (2006). Subjective effects and changes in steroid hormone concentrations in humans following acute consumption of alcohol. Psychopharmacology (Berl) 186: 451-461
Pishkin V, Lawrence BE, Bourne LE, Jr. (1983). Cognitive and electrophysiologic parameters during ascending and descending limbs of the blood alcohol curve. Alcohol Clin Exp Res 7: 76-82
Post RB, Lott LA, Maddock RJ, Beede JI (1996). An effect of alcohol on the distribution of spatial attention. J Stud Alcohol 57: 260-266
Quintyn JC, Massy J, Quillard M, Brasseur G (1999). Effects of low alcohol consumption on visual evoked potential, visual field and visual contrast sensitivity. Acta Ophthalmol Scand 77: 23-26
Rammsayer T (1995). Extraversion and alcohol: Eysenck's drug postulate revisited. Neuropsychobiology 32: 197-207
Ray S, Bates ME (2006). Acute alcohol effects on repetition priming and word recognition memory with equivalent memory cues. Brain Cogn 60: 118-127
Ray S, Bates ME, Ely BM (2004). Alcohol's dissociation of implicit and explicit memory processes: implications of a parallel distributed processing model of semantic priming. Exp Clin Psychopharmacol 12: 118-125
Reynolds B, Richards JB, de Wit H (2006). Acute-alcohol effects on the Experiential Discounting Task (EDT) and a question-based measure of delay discounting. Pharmacol Biochem Behav 83: 194-202
Richards JB, Zhang L, Mitchell SH, de Wit H (1999). Delay or probability discounting in a model of impulsive behavior: effect of alcohol. J Exp Anal Behav 71: 121-143
Roehrs T, Zwyghuizen-Doorenbos A, Knox M, Moskowitz H, Roth T (1992). Sedating effects of ethanol and time of drinking. Alcohol Clin Exp Res 16: 553-557
Rohrbaugh JW, Stapleton JM, Parasuraman R, Frowein HW, Adinoff B, Varner JL, Zubovic EA, Lane EA, Eckardt MJ, Linnoila M (1988). Alcohol intoxication reduces visual sustained attention. Psychopharmacology (Berl) 96: 442-446
Rohrbaugh JW, Stapleton JM, Parasuraman R, Zubovic EA, Frowein HW, Varner JL, Adinoff B, Lane EA, Eckardt MJ, Linnoila M (1987). Dose-related effects of ethanol on visual sustained attention and event-related potentials. Alcohol 4: 293-300
Ross DF, Pihl RO (1988). Alcohol, self-focus and complex reaction-time performance. J Stud Alcohol 49: 115-125
Rupp TL, Acebo C, Carskadon MA (2007a). Evening alcohol suppresses salivary melatonin in young adults. Chronobiology International 24: 463-470
Rupp TL, Acebo C, Seifer R, Carskadon MA (2007b). Effects of a moderate evening alcohol dose. 11: Performance. Alcoholism-Clinical and Experimental Research 31: 1365-1371
Rupp TL, Acebo C, Van Reen E, Carskadon MA (2007c). Effects of a moderate evening alcohol dose. I: Sleepiness. Alcoholism-Clinical and Experimental Research 31: 1358-1364
Ryan C, Russo K, Greeley J (1996). Testing the global-slowing hypothesis: are alcohol's effects on human performance process-specific or task-general? Acta Psychol (Amst) 92: 59-78
Samson HH, Harris RA (1992). Neurobiology of alcohol-abuse. Trends in Pharmacological Sciences 13: 206-211
Sarkola T, Makisalo H, Fukunaga T, Eriksson CJ (1999). Acute effect of alcohol on estradiol, estrone, progesterone, prolactin, cortisol, and luteinizing hormone in premenopausal women. Alcohol Clin Exp Res 23: 976-982
Saults JS, Cowan N, Sher KJ, Moreno MV (2007). Differential effects of alcohol on working memory: distinguishing multiple processes. Exp Clin Psychopharmacol 15: 576-587
Schandler SL, Cohen MJ, Naliboff BD (1984). Alcohol-influenced changes in activation peaking during paired-associate verbal learning. J Stud Alcohol 45: 493-499
Schulte T, Muller-Oehring EM, Strasburger H, Warzel H, Sabel BA (2001). Acute effects of alcohol on divided and covert attention in men. Psychopharmacology (Berl) 154: 61-69
Schweizer TA, Jolicoeur P, Vogel-Sprott M, Dixon MJ (2004). Fast, but error-prone, responses during acute alcohol intoxication: effects of stimulus-response mapping complexity. Alcohol Clin Exp Res 28: 643-649
Schweizer TA, Vogel-Sprott M, Danckert J, Roy EA, Skakum A, Broderick CE (2006). Neuropsychological profile of acute alcohol intoxication during ascending and descending blood alcohol concentrations. Neuropsychopharmacology 31: 1301-1309
Schweizer TA, Vogel-Sprott M, Dixon MJ, Jolicoeur P (2005). The stage-specific effect of alcohol on human information processing. Psychopharmacology (Berl) 178: 52-57
Sher KJ (1985). Subjective effects of alcohol: the influence of setting and individual differences in alcohol expectancies. J Stud Alcohol 46: 137-146
Skalka HW, Helms H, Holman J (1986). Effects of ethyl alcohol on VECP. Doc Ophthalmol 62: 47-51
Soderlund H, Parker ES, Schwartz BL, Tulving E (2005). Memory encoding and retrieval on the ascending and descending limbs of the blood alcohol concentration curve. Psychopharmacology (Berl) 182: 305-317
Sommer W, Leuthold H, Hermanutz M (1993). Covert effects of alcohol revealed by event-related potentials. Percept Psychophys 54: 127-135
Spreen O, Stretton CD (1998). A compendium of neuropsychological tests; administration, norms and commentary. 2nd edition (ISBN 0-19-510019-0) New York: Oxford University Press, Inc.:
Stenberg G, Sano M, Rosen I, Ingvar DH (1994). eeg topography of acute ethanol effects in resting and activated normals. J Stud Alcohol 55: 645-656
Swartz BL (1992). Resistance of voice onset time variability to intoxication. Percept Mot Skills 75: 415-424
Teo RK, Ferguson DA (1986). The acute effects of ethanol on auditory event-related potentials. Psychopharmacology (Berl) 90: 179-184
Thomson JB, Newlin DB (1988). Effects of alcohol conditioning and expectancy on a visuo-motor integration task. Addict Behav 13: 73-77
Tiplady B, Baird R, Lutcke H, Drummond G, Wright P (2005). Effects of ethanol on kinaesthetic perception. J Psychopharmacol 19: 627-632
Tiplady B, Drummond GB, Cameron E, Gray E, Hendry J, Sinclair W, Wright P (2001). Ethanol, errors, and the speed-accuracy trade-off. Pharmacol Biochem Behav 69: 635-641
Tracy JI, Bates ME (1999). The selective effects of alcohol on automatic and effortful memory processes. Neuropsychology 13: 282-290
Tyson PD, Schirmuly M (1994). Memory enhancement after drinking ethanol: consolidation, interference, or response bias? Physiol Behav 56: 933-937
Tzambazis K, Stough C (2000). Alcohol impairs speed of information processing and simple and choice reaction time and differentially impairs higher-order cognitive abilities. Alcohol Alcohol 35: 197-201
Versavel M, Zuhlsdorf M, Unger S, Wensing G, Kuhlmann J (2005). Concentration-effect relationships of alcohol in a computerised psychometric test system. Arzneimittelforschung 55: 289-295

Vogel-Sprott M, Barrett P (1984). Age, drinking habits and the effects of alcohol. J Stud Alcohol 45: 517-521
Vogel-Sprott M, Fillmore MT (1993). Impairment and recovery under repeated doses of alcohol: effects of response-outcomes. Pharmacol Biochem Behav 45: 59-63
Vogel-Sprott M, Rawana E, Webster R (1984). Mental rehearsal of a task under ethanol facilitates tolerance. Pharmacol Biochem Behav 21: 329-331
Walsh JK, Humm T, Muehlbach MJ, Sugerman JL, Schweitzer PK (1991). Sedative effects of ethanol at night. J Stud Alcohol 52: 597-600
Wang MQ, Taylor-Nicholson ME, Airhihenbuwa CO, Mahoney BS, Fitzhugh EC, Christina R (1992). Psychomotor and visual performance under the time-course effect of alcohol. Percept Mot Skills 75: 1095-1106
Watten RG, Lie I (1996). Visual functions and acute ingestion of alcohol. Ophthalmic Physiol Opt 16: 460-466
Watten RG, Lie I (1997). The effects of alcohol on eye movements during reading. Alcohol Alcohol 32: 275-280
Watten RG, Magnussen S, Greenlee MW (1998). Spatial-frequency discrimination, brain lateralisation, and acute intake of alcohol. Perception 27: 729-736
Wegner AJ, Fahle M (1999a). Alcohol and visual performance. Prog Neuropsychopharmacol Biol Psychiatry 23: 465-482
Wegner AJ, Fahle M (1999b). Alcohol and visually guided saccades: gap effect and predictability of target location. Psychopharmacology (Berl) 146: 24-32
Weight FF, Aguayo LG, White G, Lovinger DM, Peoples RW (1992). Gaba-gated and glutamate-gated ion channels as molecular sites of alcohol and anesthetic action. Advances in Biochemical Psychopharmacology 47: 335-347
Weintraub AL, Goldman MS (1983). Alcohol and proactive interference: a test of response eccentricity theory of alcohol's psychological effects. Addict Behav 8: 151-166
Weissenborn R, Duka T (2000). State-dependent effects of alcohol on explicit memory: the role of semantic associations. Psychopharmacology (Berl) 149: 98-106
Weissenborn R, Duka T (2003). Acute alcohol effects on cognitive function in social drinkers: their relationship to drinking habits. Psychopharmacology (Berl) 165: 306-312
Wilkie H, Stewart SH (2005). Reinforcing mood effects of alcohol in coping and enhancement motivated drinkers. Alcohol Clin Exp Res 29: 829-836
Williams HL, Rundell OH (1984). Effect of alcohol on recall and recognition as functions of processing levels. J Stud Alcohol 45: 10-15
Young JA, Pihl RO (1982). Alcohol consumption and response in men social drinkers; the effects of causal attributions concerning relative response control. J Stud Alcohol 43: 334-351
Zack M, Vogel-Sprott M (1993). Response outcomes affect the retention of behavioral tolerance to alcohol: information and incentive. Psychopharmacology (Berl) 113: 269-273
Zeichner A, Allen JD, PETrie CD, Rasmussen PR, Giancola P (1993). Attention allocation: effects of alcohol and information salience on attentional processes in male social drinkers. Alcohol Clin Exp Res 17: 727-732
Zuurman L, Ippel AE, Moin E, Van Gerven JMA (2009). Biomarkers for the effects of cannabis and THC in healthy volunteers. Br J Clin Pharmacol 67(1): 5-21
Zuzewicz W (1981). Ethyl alcohol effect on the visual evoked potential. Acta Physiol Pol 32: 93-98
Zwyghuizen-Doorenbos A, Roehrs T, Timms V, Roth T (1990). Individual differences in the sedating effects of ethanol. Alcohol Clin Exp Res 14: 400-404



# CHAPTER 3

## *A comparative study of two methods for attaining constant alcohol levels*

*Br J Clin Pharmacol 2008, 66 (5), 674-681*

R.W.M. Zoethout, J.M.A. van Gerven, G.J.H. Dumont,
S. Paltansing, N.D. van Burgel, M. van der Linden, A. Dahan,
A.F. Cohen, R.C. Schoemaker

## ABSTRACT

*BACKGROUND* Alcohol effects or drug-alcohol interactions are preferably studied at constant blood levels. To achieve pseudo-steady state levels, various methods are used, which usually produce adequate averages but variable individual concentration profiles. In this study two modes of alcohol administration were compared: a 'two-step prekinetic procedure' and a 'clamping method'.

*METHODS* The two-step prekinetic procedure started with determination of individual pharmacokinetic parameters, during a prestudy occasion. Individual infusion regimens were calculated afterwards, based on a pseudo-steady state breath alcohol concentration (BrAC) of 0.65 $g{\cdot}L^{-1}$ and applied during a separate occasion. For the clamping procedure, a spreadsheet-based paradigm was developed using BrAC guided adjustments of infusion rates, to maintain stable BrAC levels of 0.6 $g{\cdot}L^{-1}$.

*RESULTS* The mean BrAC during clamping (0.61 $g{\cdot}L^{-1}$ (95% CI: 0.58 - 0.63 $g{\cdot}L^{-1}$)) did not differ from its intended level of 0.6 $g{\cdot}L^{-1}$ (1.0% on average). In contrast, the mean BrAC during the prekinetic procedure was significantly lower than the 0.65 $g{\cdot}L^{-1}$ set-point (0.59 $g{\cdot}L^{-1}$ (95% CI: 0.54 - 0.63 $g{\cdot}L^{-1}$)), and deviated from this target by 9.7% on average. The clamping method also showed less variation between subjects (CV: 6.2%), compared to the prekinetic procedure (CV: 14.6%).

*CONCLUSIONS* Although the two methods differ considerably in their approach, clamping of BrAC resulted in more accurate alcohol levels than infusion based on PK-modelling and does not require an extra prestudy occasion. The novel alcohol clamping paradigm can be of value in future studies of alcohol interactions or the pharmacodynamics of acute alcohol administration.




## INTRODUCTION

Alcohol, or ethanol is widely used in social settings and causes dose-dependent central nervous system (CNS) depression (Little, 1991). Its metabolising systems show a large ethnic (Eng *et al.*, 2007; Reed *et al.*, 1976) and interindividual variability, and there are many potential drug interactions. Alcohol intake can inhibit drug oxidation by competing with metabolising enzyme systems. On the other hand, chronic use of alcohol induces liver microsomal enzyme activity, which can lead to lower plasma levels of susceptible drugs when given concomitantly. Besides this pharmacokinetic interplay, alcohol consumption can also interact with drugs or substances on a pharmacodynamic level (Sellers and Holloway, 1978). Hence, research for possible interactions between alcohol and other pharmacological compounds is often necessary when developing a safe drug therapy. Interactions with alcohol are particularly important for psychoactive drugs, since such compounds are often metabolised by the liver and they may affect the CNS-effects of alcohol or vice versa. At the same time, alcohol abuse occurs with many psychiatric disorders (Kushner *et al.*, 2000; Soyka *et al.*, 1993) and alcohol consumption is an accepted and frequent habit in Western society. Therefore, alcohol interaction studies are performed in many early drug development programs of psychoactive agents.

In determining the effects of alcohol or assessing alcohol-drug interactions, it is helpful if alcohol plasma concentration can be maintained at pseudo-steady state levels. Most of the effects of alcohol are concentration- and time-dependent (Hiltunen *et al.*, 2000; Vogel-Sprott, 1979), and interpretation of the results can be complex if plasma levels change over time. Nonetheless, many studies of alcohol effects or drug interactions are not performed at pseudo-steady state levels, or under other conditions in which these complexities are regulated. In many cases, alcohol exposure is merely controlled by adaptation of the dose to weight, and sometimes to gender 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 efforts to maintain constant levels are rare (Wilkinson, 1980). A major reason for this lack of methodological stringency is the complexity and variability of alcohol's pharmacokinetic characteristics.

To achieve pseudo-steady state alcohol plasma concentrations, various methods have been reported in the literature. One method described by Hartmann *et al.* (Hartmann *et al.*, 1988), used a two-step infusion rate protocol with a prestudy day. The pharmacokinetic profiles for alcohol were established after intravenous administration of a fixed alcohol dose on a prestudy day, and the individual parameters were used to calculate the infusion regimens needed to achieve a certain plasma concentration profile. On the actual study day, plasma samples were taken for determination of alcohol concentration, during the first hour of infusion. The results were available within the next hour and were used to adapt the infusion rate. In practice however, Hartmann *et al.* showed that these adaptations decreased the stability of the plasma alcohol concentrations (Hartmann *et al.*, 1988). Therefore, the method was performed without these adjustments during several studies in our centre (van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1996). This technique produced a reliable average concentration profile with occasional over- or undershoots.

To avoid major fluctuations altogether and produce a more stable steady state serum level of alcohol with lower variability, it would be necessary to adapt the infusion rate to the plasma alcohol concentration instantaneously. O'Connor *et al.* (O'Connor *et al.*, 1998) developed an algorithm that uses breath alcohol concentration (BrAC) to guide infusion rate adjustments for a steady alcohol plasma concentration. Their initial method started with an oral loading dose, adapted to body weight. After one hour, an alcohol infusion was initiated and BrAC samples were taken with ten minutes intervals. An experienced technician adapted the infusion rate to the BrAC-changes. The authors were able to keep alcohol plasma concentrations within 10% of the desired steady state, thus reducing variance introduced by changing alcohol plasma levels. Ramchandani *et al.* (Ramchandani *et al.*, 1999), replaced the oral loading dose with a preprogrammed intravenous infusion rate profile. Using a physiology-based pharmacokinetic model, a steady BrAC within 5% window of the target concentration could be achieved.

Despite its apparent advantages, the alcohol BrAC clamping method has not gained wide application. This might be related to the perceived complexity of the procedure, which in addition to an intravenous route of administration seems to require specific expertise and frequent BrAC-measurements for adaptations of the infusion rate. In addition, although it seems clear that the alcohol clamp will improve the stability of alcohol levels, it has not yet been established how this compares to other procedures, such as two-step prekinetic methods. We describe a new method, based on O'Connor's principles in comparison to the adapted two-step infusion rate method, which had been used previously (van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1996).

## METHODS

All of the studies described below were approved by the Medical Ethics Review Board of Leiden University Medical Centre. Both oral and written informed consent were obtained from all participating subjects after protocol approval. All studies were performed in compliance with the law on clinical trials of the Netherlands (WMO).

### *'Two-step prekinetic procedure'*

The alcohol data obtained from eight healthy male and eight healthy female volunteers participating in a drug-alcohol interaction study (CHDR0111 – data on file) were used to evaluate the feasibility of the adapted two-step prekinetic procedure as described by Hartmann (Hartmann *et al.*, 1988). Here, we only report alcohol data from the treatment arm that consisted of alcohol administration alone. On the prestudy day, an infusion of 50 grams of alcohol was administered for one hour (500 mL of 100 $g \cdot L^{-1}$ alcohol solution

in 5% glucose). Breath alcohol concentrations were obtained at t = 0, 30, 55, 75, 90, 120, 180, 240, 300 and 360 minutes. A hand-held Alco-Sensor IV meter was used for BrAC assessments during the study. To avoid an 'overshoot' in the alcohol level, the infusion of alcohol was terminated whenever the level of 1.00 g·L$^{-1}$ was reached.

#### INDIVIDUAL INFUSION REGIMENS

For each subject, individual pharmacokinetic parameters were determined as follows. Previously, a nonlinear mixed effects population pharmacokinetic model had been built, based on the data from an earlier CHDR study with a similar design (van Steveninck *et al.*, 1996), using nonlinear mixed effect modelling with NONMEM version V software (Beal S.L. *et al.*, 2006). A two-compartment model was used with Michaelis-Menten elimination from the central compartment. V1, V2, $V_{max}$, $K_m$ and Q (inter-compartmental clearance) were estimated. Exponential models for inter-individial variabilty were used but inter-individual variability for $K_m$ was fixed to zero to avoid numerical problems. An additive residual error model was used to avoid overestimating the importance of low alcohol measurements. Parameters were calculated using first-order conditional estimation (FOCE). These population parameters were used as priors in a Bayesian non-linear regression analysis of the BrAC-levels of the prestudy day, to generate pharmacokinetic parameters. Next, individual infusion regimens were simulated using the Bayes estimates. The regimen that approached and stayed at 0.65 g·L$^{-1}$ was applied to the subsequent alcohol infusion occasions.

This infusion regimen consisted of a constant intravenous administration over one hour (loading phase), followed by a lower constant rate infusion over three hours (maintenance phase). Breath alcohol levels were obtained at t = 0, 60, 100, 150, 180, 210 and 300 minutes after the start of the loading phase. Contrary to Hartmann's original procedure, no further adjustments in infusion rate were made during the infusion period.



## *'Alcohol clamping procedure'*

The O'Connor method (O'Connor *et al.*, 1998) 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/min) rate, independent of concentration. Therefore, when the input (g/min) 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. The original BrAC clamping algorithm (O'Connor *et al.*, 1998) consisted of an oral loading dose and BrAC samples, with possible infusion rate adaptations every ten minutes. It was anticipated that an intravenous loading infusion would reduce variability and shorten the time required to reach pseudo-steady state. Additionally, 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 may interfere with the required measurements. Simulations were performed for a large range of scenarios using alcohol population parameters determined from previous CHDR studies (88128, CHDR0111, CHDR0210 – data on file). These parameters are shown in table 1. The same population model was used as for the two-step prekinetic procedure, but the increase in information from using data from two extra studies, resulted in the possibility to estimate correlations between population parameters and inter-individual variability for $K_m$ as well.

These simulations indicated that the original O'Connor (O'Connor *et al.*, 1998) 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

regime was empirically determined consisting of a time dependent reduction of the suggested O'Connor infusion rate. This regime is fully described in the Appendix and was implemented in a simple user-friendly Microsoft Excel® spreadsheet (available from the authors on request). During the procedure, online adjustments in infusion rates are made using the BrAC samples as a guideline to achieve a pseudo-steady state alcohol serum level of 0.6 $g \cdot L^{-1}$. This target level was expected to result in saturated alcohol elimination, since $K_m$ was estimated at 0.0452 $g \cdot L^{-1}$. At more than 10 times this concentration (during clamping at 0.6 $g \cdot L^{-1}$), elimination will be saturated, hence complying with the prerequisite for the clamping technique. This new infusion paradigm was tested for its feasibility in a subsequent study.

## *Clamping study*

Six healthy male and six healthy female volunteers were recruited for this alcohol clamping study, where alcohol was infused for five hours. BrAC samples were taken at baseline and every five minutes for the first 30 minutes after the start of the infusion, every ten minutes for the next 30 minutes and then every half hour until the end of the study. Again, the hand-held Alco-Sensor IV meter was used for BrAC sampling. Since product specifications for this apparatus state that sampling intervals shorter than five minutes cause fatigue of the BrAC sensor, two different measurement devices were alternated during the clamping study.

The clamping study was performed in a randomised, double-blind and placebo-controlled fashion. The intravenous alcohol infusion (alcohol 10% w/v solution in glucose 5% or placebo (glucose 5%)) took place for five hours in total. An infusion assistant, who was not a member of the study team, was made responsible for the BrAC measurements and the execution of the clamp, to maintain blinding during the study. During the whole study day, volunteers performed a battery of both cognitive and psychomotor CNS tests between sampling time points. The results of these pharmacodynamic assessments will be described elsewhere.



#### SPREADSHEET-BASED PROCEDURE

In advance of a study occasion, the study statistician prepared individual computer spreadsheets, according to a randomisation schedule. For alcohol occasions, this spreadsheet contained a blank 'measurement column', in which the measured BrAC-values were entered by the infusion assistant. Based on these results, the spreadsheet calculated the new infusion rate. For placebo occasions, the spreadsheet contained a separate column with 'sham' BrAC-values, determined from kinetic simulations. At each protocol time, the infusion assistant entered the corresponding 'sham' value into the measurement column. This value was used by the spreadsheet to calculate a new 'sham' infusion rate, which was subsequently applied. In this way, operations during the alcohol clamping and sham procedures were exactly similar for the subject.

## *Alco-Sensor IV Intoximeter*

Two Alco-Sensor IV Intoximeters (Honac, Apeldoorn, the Netherlands) were used for BrAC measurements during the studies. The Alco-Sensor utilizes an electrochemical sensor (fuel cell) to quantify alcohol in the provided sample. Prior to the study, the reliability of the BrAC devices was confirmed in a validation study. Twelve subsequent samples of a 1.08 $g \cdot L^{-1}$ calibration gas (taken at t = 0, 10, 20, 35, 55, 115, 175, 235, 295, 355, 415 and 475 min.) resulted in a mean value of 1.07 $g \cdot L^{-1}$ (95% CI: 1.06, 1.08 $g \cdot L^{-1}$) for the first apparatus and a mean value of 1.08 $g \cdot L^{-1}$ (95% CI: 1.07, 1.09 $g \cdot L^{-1}$) for the second apparatus. An important error in the measurement of alcohol levels during the clamping study could be exhaustion of the BrAC sensor. Since, exhaustion of the sensor, which was most likely during the ascending limb of the BrAC curve (where frequent BrAC measurements are required) could result in incorrect alcohol values, two BrAC devices were alternated. The instruments were calibrated prior to the start of the studies.

## *Subject characteristics*

Subjects participating in the two-step prekinetic study were on average 27 years of age (range 18 - 53 years old), had an average weight of 77 kg (range 53 - 95 kg) and an average height of 175 cm (range 155 - 187 cm). Subjects participating in the alcohol clamping study were on average 22 years old (range 18 - 39 years old), had an average weight of 74 kg (range 56 - 89 kg) and an average height of 173 cm (range 161 - 195 cm). All subjects participating in the previous alcohol studies (88128, CHDR0111, CHDR0210 – data on file) used to construct the pharmacokinetic model were on average 24 years of age (range 18 – 53 years old), had an average weight of 77 kg (range 53 - 97 kg) and an average height of 180 cm (range 155 – 194 cm). All subjects were occasional users of alcohol.

## *Safety*

To prevent local pain in the beginning of the alcohol infusion, an additional diluting glucose 5% infusion (100 $mL \cdot hr^{-1}$) was given to all participants, during the first ten minutes after the start of the alcohol infusion over the same infusion line. No further specific safety measurements were planned for both the alcohol clamping procedure and the two-step prekinetic procedure. During both studies, alcohol was infused to a level of 0.6 $g \cdot L^{-1}$, just above the legal limit of 0.5 $g \cdot L^{-1}$ for adult motor vehicle drivers in the Netherlands.

# RESULTS

## *Subjects*

During the prestudy day of the two-step prekinetic procedure, the infusion of one of the subjects was stopped 38 minutes after the start of the alcohol infusion, because BrAC exceeded 1.0 $g \cdot L^{-1}$ and concomitant nausea and inebriation occurred. The available data from this subject were used for the analyses, and for the determination of well-tolerated infusion regimens for the 'pseudo-steady state' part of the two-step procedure.

In the clamping study, one subject dropped out after the first occasion for personal reasons. This subject was replaced by another volunteer receiving the same order of alcohol/placebo administration. Another subject repeated his first occasion because he had hardly slept the night before the study occasion, despite instructions to maintain a regular diurnal rhythm during the study period. Both repeated occasions were used for the pharmacokinetic analysis. All other subjects completed the studies according to protocol.

## *Two-step prekinetic procedure*

The average BrAC-profiles over time for both steps of the two-step prekinetic procedure are presented in figure 1. A large variability in BrAC values was observed after the fixed intravenous alcohol dose in the prekinetic occasion. Individual peak BrAC levels ranged from 0.71 $g \cdot L^{-1}$ to 1.71 $g \cdot L^{-1}$. During the loading phase of the pseudo-steady state occasions, the maximum initial overshoot reached up to 0.93 $g \cdot L^{-1}$, which is an excursion of almost 50% above the target value. A slight, but clear declining trend in average BrAC level could be observed during the maintenance phase. During this period BrAC values ranged from 0.56 to 0.63 $g \cdot L^{-1}$. A 'plateau phase' could be observed between 100 and 210 minutes post-dose. During this part, BrAC values were 9.7% below the desired concentration on average.

## *Alcohol clamping procedure*

The average BrAC profiles of subjects participating in the alcohol clamping study are presented in figure 2. The 0.6 $g \cdot L^{-1}$ pseudo-steady state level was reached within 25 minutes after the start of the alcohol infusion. The intravenous loading regimen did not produce an overshoot as could be observed from the figure. A pseudo-steady state phase can be observed between 25 and 300 minutes. During this period, BrAC values ranged from

0.55 to 0.65 g·L$^{-1}$. On average, the clamping procedure slightly exceeded its target level by 1.0% during the plateau phase. The graph also shows that the stability of the clamp was not affected by the simultaneous performance of the pharmacodynamic test battery.

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 during the plateau phase were somewhat higher for males than for females (143.5 mL·hr$^{-1}$ (SD: 36.1) and 108.3 mL·hr$^{-1}$ (SD: 23.7) respectively), but the difference did not reach significance in this small group ($p$ = 0.08).

### *Comparison of the alcohol clamping procedure and the two-step prekinetic procedure*

To compare the two different infusion regimens the mean BrAC level during the relevant steady state interval and the coefficient of variation were calculated. During the two-step prekinetic procedure (target level 0.65 g·L$^{-1}$) a mean BrAC of 0.59 g·L$^{-1}$ (95% CI: 0.54 - 0.63 g·L$^{-1}$) was found. The mean BrAC of the novel alcohol clamping procedure (target level 0.60 g·L$^{-1}$) was 0.61 g·L$^{-1}$ (95% CI: 0.58 - 0.63 g·L$^{-1}$). The calculated coefficient of variation of the clamping method (6.2%) was more than twice as low as the two-step prekinetic procedure (14.6%). Thus, the novel clamping paradigm resulted in more accurate pseudo-steady state levels of alcohol compared to the mean BrAC during the two-step prekinetic procedure, with two-fold lower variability.

### *Adverse events*

No serious adverse reactions occurred during either study. The most frequently reported adverse events included inebriation, a painful arm at the start of the infusion, sleepiness and headache (see table 2). These symptoms were all transient and mild in severity.

## DISCUSSION

The two-step prekinetic procedure resulted in a substantial overshoot prior to the maintenance phase, and a declining plateau phase entirely located below the desired target level. Some fluctuations were expected since online corrections were not implemented in this method. Despite these inaccuracies, the adapted paradigm was able to maintain serum levels of alcohol that were on average relatively stable. The clamping method did not require prior determination of individual disposition parameters and was able to keep constant alcohol levels over an extended period of time. Online adjusted BrAC levels could easily be targeted to a preset target level and showed very little interindividual variability. The higher variability obtained with the two-step prekinetic procedure might in part be attributable to the interoccasion variability related with the study design of the two-step procedure. In this respect, the design of the alcohol clamp study appeared to be superior to the two-step design of the prekinetic procedure.

A less obvious, but potentially important advantage of the clamping method is that if a drug modifies alcohol kinetics, the BrAC guided method will instantly adapt. This will improve the safety and tolerability of drug-alcohol interaction studies. Using applied infusion rates and BrAC assessments, alcohol kinetics can still be determined; but a much simpler comparison of alcohol metabolism can be obtained from the total amounts of infused alcohol that were needed to maintain pseudo-steady state BrAC-levels.

Contrary to O'Connor's originally described procedure, the clamping study presented here used an intravenously administered loading dose. Although it was not investigated in this study, it is likely that intravenous loading will cause less variability than oral loading because between subject variability in absorption is removed. Two other practical advantages of the

new alcohol clamping method compared to the one originally proposed by O'Connor are its user-friendliness and the incorporation of wider inter-sampling periods. By converting the paradigm (see Appendix) electronically into an easy-to-handle spreadsheet, the procedure can be executed by anyone able to modify an infusion pump and use a breath alcohol meter, after a short introduction. Furthermore, reducing the number of BrAC samples increased the time between two sequential BrAC sampling moments. This allows for the performance of other measurements, which makes the clamp more suitable for pharmacodynamic alcohol effect or interaction studies. This study showed that a battery of pharmacodynamic CNS measurements could be integrated in the course of the clamp without affecting the stability of the target level.

Since the clamping method is based on empirical principles it is not necessarily state of the art. Systematic procedures like target-controlled infusions or physiology-based pharmacokinetic methods may result in more accurate alcohol concentrations over time. The procedure described by Ramchandani, based on precalculation of infusion rates, probably approaches this more closely (Ramchandani *et al.*, 1999). However, these state of the art approaches are rarely implemented in practice, because of the perceived complexity during execution. The clamping procedure presented here, is a simple to implement procedure that allows everyone to use it with good results.

Future studies should focus on further optimisation of the alcohol clamp. Both over- and undershoots that appear particularly during the transition from the ascending (loading) limb to the plateau (maintenance) phase of the BrAC curve can be minimised by individualisation of the initial infusion rates during the first ten minutes. The initial fixed infusion rates for the first ten minutes, could for example be replaced by infusion rates that are based on individual demographic characteristics (e.g. gender, age, height and weight). More research is needed to find out if the clamping method is also able to produce stable BrAC levels for longer periods than the five hours used in this study, or at different target BrAC levels.



In conclusion, the novel alcohol clamping paradigm was an accurate, user-friendly method, with low variability, able to maintain constant alcohol levels for hours. The current paradigm shows an opportunity to perform intensive pharmacodynamic or functional assessments during the execution of the clamp, which could be of great value for future studies of alcohol.

**FIGURE 1** RESULTS OF THE 'TWO-STEP PROCEDURE'

*Left: Average BrAC-profile of the participating subjects during the prekinetic occasion and their SD's. Minimum and maximum values are also presented. The straight vertical line marks the end of the one-hour infusion period. Right: Average BrAC-profile of the participating subjects during the pseudo-steady state experiment and their SD's. Minimum and maximum values are also presented. The area between the two straight vertical lines at t=0 and t=60 minutes marks the 'loading phase'. The area between the two straight vertical lines at t=60 and t=240 minutes marks the 'maintenance phase'.*

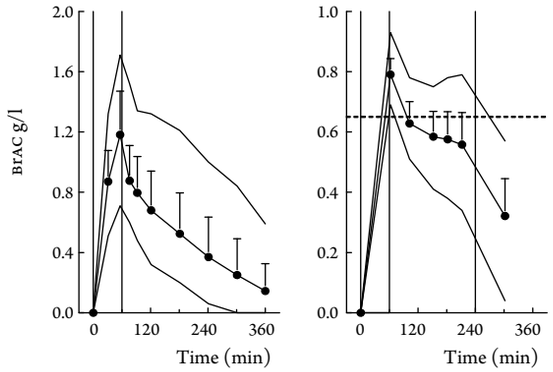



**FIGURE 2** AVERAGE BrAC-PROFILE OF THE PARTICIPATING VOLUNTEERS DURING ALCOHOL CLAMPING PROCEDURE WITH THEIR SD'S

*Minimum and maximum values are also presented. The vertical lines mark the start and the stop of the infusion.*

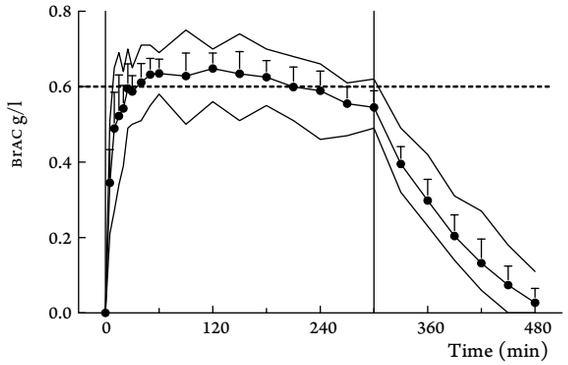





**TABLE 1** NONMEM ALCOHOL POPULATION PARAMETERS

*Mean: population average, IICV: inter-individual coeffcient of variation. These population parameters are based on 68 subjects and 6052 BrAC samples.*

| | **mean** | **IICV** | **between subject correlations** | | | |
|---|---|---|---|---|---|---|
| | | | $V_c$ | $V_p$ | $K_m$ | $V_{max}$ |
| $V_c$ (L) | 13.6 | 51% | | | | |
| $V_p$ (L) | 36.0 | 36% | -0.13 | | | |
| $K_m$ (g·L$^{-1}$) | 0.0452 | 46% | -0.56 | -0.49 | | |
| $V_{max}$ (g·L$^{-1}$·min$^{-1}$) | 0.0117 | 49% | -0.90 | 0.34 | 0.51 | |
| Q (L·min$^{-1}$) | 1.22 | 25% | -0.35 | 0.81 | -0.50 | 0.50 |
| SD residual error (g·L$^{-1}$) | 0.035 | | | | | |

**TABLE 2** DISTRIBUTION OF ADVERSE EVENTS DURING BOTH EVALUATED METHODS

*The numbers represent the occurrence of the symptoms as a percentage of the total number of adverse events per study.*

| | **'Clamping method'** | | **'Two-step prekinetic procedure'** | |
|---|---|---|---|---|
| | alcohol | placebo | alcohol | placebo |
| "inebriation" | 22% | 3% | 26% | 3% |
| "painful infusion" | 22% | 3% | 8% | 0% |
| "sleepiness" | 16% | 3% | 20% | 16% |
| "headache" | 8% | 3% | 8% | 9% |

Beal SL, Sheiner LB, and Boechmann AJ (Eds.) (2006). NONMEM Users Guides (1989-2006), Icon Development Solutions, Ellicott City, Maryland, USA.

Eng MY, Luczak SE and Wall TL (2007). ALDH2, ADH1B, and ADH1C genotypes in Asians: A literature review. Alcohol Research & Health *30*, 22-27.

Hartmann D, Fluckiger A, Ziegler WH, Timm U and Zell M (1988). Experimental model for the Investigation of kinetic and or dynamic interactions between drugs and ethanol in humans. Journal of Pharmaceutical Sciences *77*, 299-303.

Hiltunen AJ, Saxon L, Skagerberg S and Borg S (2000). Acute tolerance during intravenous infusion of alcohol: Comparison of performance during ascending and steady state concentrations - a pilot study. Alcohol *22*, 69-74.

Kushner MG, Abrams K and Borchardt C (2000). The relationship between anxiety disorders and alcohol use disorders: A review of major perspectives and findings. Clinical Psychology Review *20*, 149-171.

Little HJ (1991). Mechanisms that may underlie the behavioral effects of ethanol. Progress in Neurobiology *36*, 171-194.

O'Connor S, Morzorati S, Christian J and Li TK (1998). Clamping breath alcohol concentration reduces experimental variance: Application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcoholism-Clinical and Experimental Research *22*, 202-210.

Ramchandani VA, Bolane J, Li TK and O'Connor S (1999). A physiologically-based pharmacokinetic (PBPK) model for alcohol facilitates rapid BRAC clamping. Alcoholism-Clinical and Experimental Research *23*, 617-623.

Reed TE, Kalant H, Gibbins RJ, Kapur BM and Rankin JG (1976). Alcohol and acetaldehyde metabolism in Caucasians, Chinese and Amerinds. Canadian Medical Association Journal *115*, 851-855.

Sellers EM and Holloway MR (1978). Drug kinetics and alcohol ingestion. Clinical Pharmacokinetics *3*, 440-452.

Soyka M, Albus M, Kathmann N, Finelli A, Hofstetter S, Holzbach R, Immler B and Sand P (1993). Prevalence of alcohol and drug-abuse in schizophrenic inpatients. European Archives of Psychiatry and Clinical Neuroscience *242*, 362-372.

van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MSM, Kroon JM, Breimer DD and Cohen AF (1993). Pharmacokinetic and pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo-steady state. Psychopharmacology *110*, 471-478.

van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MSM, Breimer DD and Cohen AF (1996). Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Brit J Clin Pharmacol *41*, 565-573.

Vogel-Sprott MD (1979). Acute recovery and tolerance to low doses of alcohol: differences in cognitive and motor skill performance. Psychopharmacology (Berl) *61*, 287-291.

Wilkinson PK (1980). Pharmacokinetics of ethanol - Review. Alcoholism-Clinical and Experimental Research *4*, 6-21.





## ALCOHOL CLAMPING PARADIGM

Alcohol was infused for a total of five hours with a set point of 0.6 $g \cdot L^{-1}$.

BRAC measurement were obtained at 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420 and 480 minutes postdose.

Infusion rate between 0 and 5 minutes was fixed at 72 $g \cdot hr^{-1}$.

Infusion rate between 5 and 10 minutes was fixed at 60 $g \cdot hr^{-1}$.

Infusion rates were modified at 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240 and 270 minutes.

Regression equations of applied infusion rate vs. resulting change in alcohol concentration were calculated for all available pairs of data (i.e. starting with the measurement at 5 minutes) and updated with each new data point, up to 270 minutes.

If a negative infusion rate is suggested by the regression equation (e.g. to correct for an overshoot), then the infusion rate was set at 1.0 $g \cdot hr^{-1}$ (the lowest possible rate on the infusion apparatus).

At times (especially at early sampling points) it is possible for the regression equation to arrive at a negative slope. This means that the equation suggests that in order to increase the concentration, less alcohol needs to be infused. This anomaly was corrected by replacing the regression equation in this case by a population average equation, determined using a large number of simulated regression equations. The population average equation was: *new_rate = (required_rate_of_change + 0.013) / 0.06*.

In order to correct for the suggested infusion rates, they were reduced in a time dependent manner:

- at 10, 15, 20, 25 and 30 minutes the correction percentage was 75%,
- at 40 minutes the correction percentage was 70%,
- at 50 minutes the correction percentage was 60%,
- at 60 minutes the correction percentage was 50%,
- from 90 minutes up to 210 minutes the correction percentage was 45%,
- (a correction of 75% implies that the suggested rate was reduced by 25%).

If, after all these adjustments, rates were still too high, the following maximum time-dependent rates were applied:

- from 10 to 30 minutes (not incl.) the maximum allowed rate was 144 $g \cdot hr^{-1}$,
- from 30 to 60 minutes (not incl.) the maximum allowed rate was 72 $g \cdot hr^{-1}$,
- from 60 minutes onwards the maximum allowed rate was 36 $g \cdot hr^{-1}$.

The average total amount of infused alcohol is approximately 75 grams when a pseudo-steady state of 0.6 $g \cdot l^{-1}$ is achieved for five hours. This corresponds to the consumption of less than one bottle of wine, over that same time period.

# CHAPTER 4

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

*Br J Clin Pharmacol 2009, 68 (4), 524–534*

R.W.M. Zoethout, R.C. Schoemaker, L. Zuurman, J. van Pelt, A. Dahan,
A.F. Cohen, J.M.A. van Gerven

## 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-00-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.*

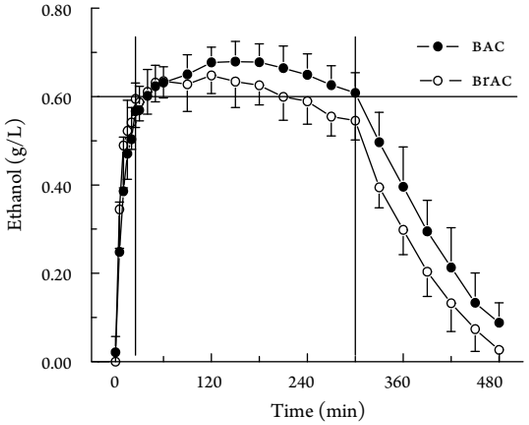



**FIGURE 2** LS MEANS GRAPH OF VAS ALERTNESS (MM): CHANGE FROM BASELINE WITH 95% CI ERROR BARS

*The plateau-phase is marked by the two vertical lines.*

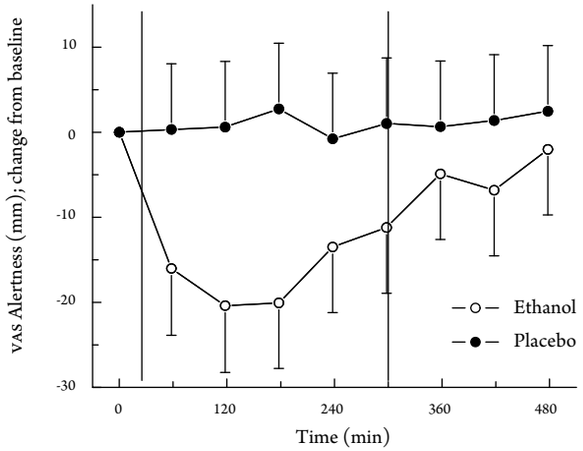





**FIGURE 3** LS MEANS GRAPH OF VAS ALCOHOL EFFECTS (MM): CHANGE FROM BASELINE WITH 95% CI ERROR BARS

*The plateau-phase is marked by the two vertical lines.*

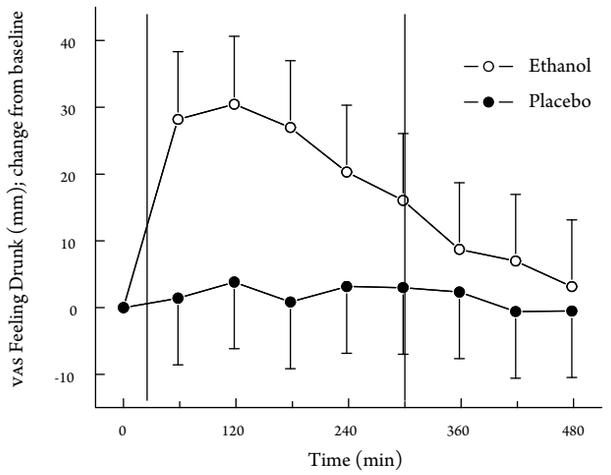



**FIGURE 4** LS MEANS GRAPH OF BODY SWAY (MM): % CHANGE FROM BASELINE WITH 95% CI ERROR BARS

*The plateau-phase is marked by the two vertical lines.*

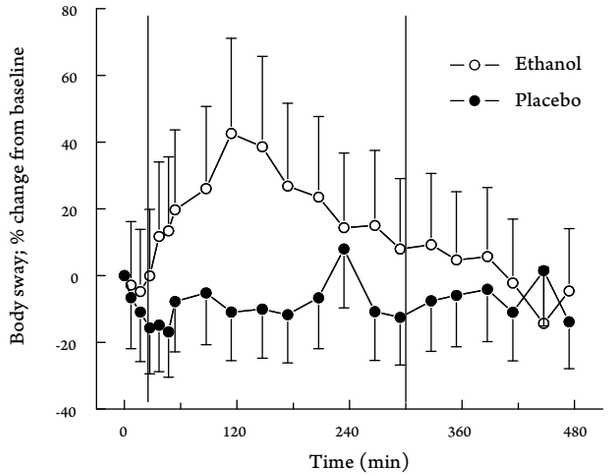


**FIGURE 5** LS MEANS GRAPH OF TRACKING (%): CHANGE FROM BASELINE WITH 95% CI ERROR BARS

*The plateau-phase is marked by the two vertical lines.*

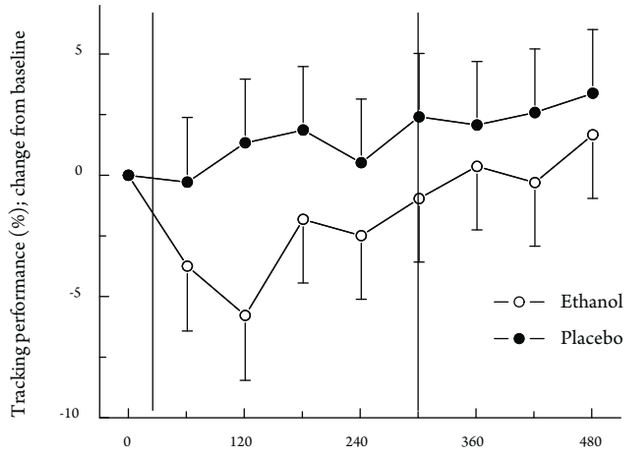



**FIGURE 6** LS MEANS GRAPH OF SMOOTH PURSUIT

*Change from baseline with 95% CI error bars. The plateau-phase is marked by the two vertical lines.*

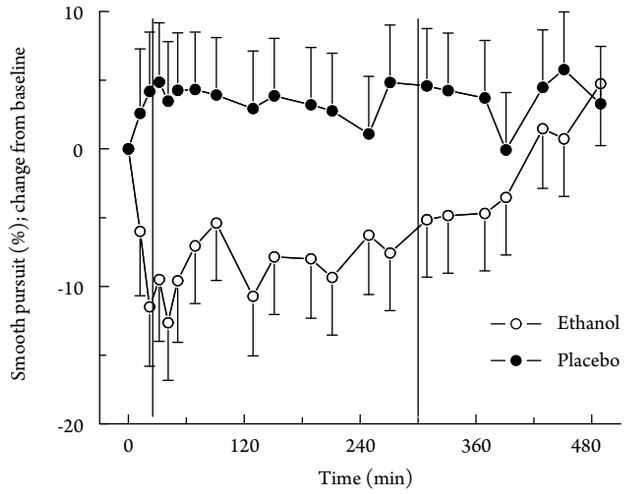





**FIGURE 7** EXPLORATORY PK/PD RELATIONSHIP BETWEEN AVERAGE SERUM ALCOHOL AND THE MEAN EFFECTS ON BODY SWAY

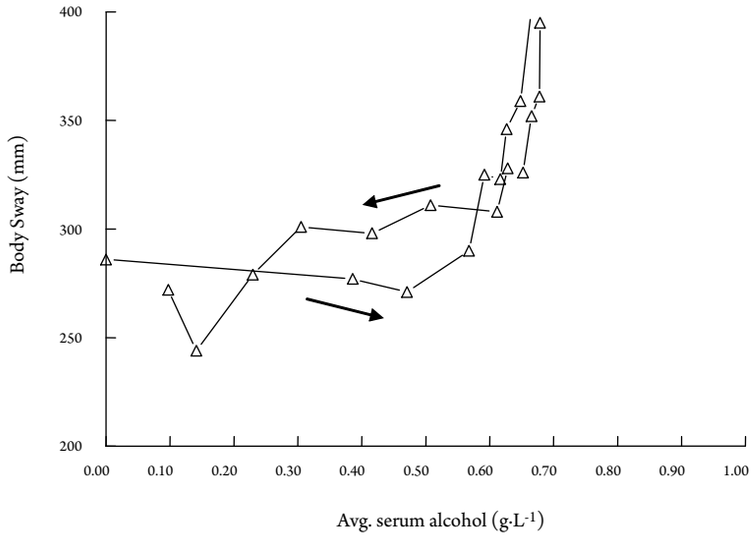



**FIGURE 8** EXPLORATORY PK/PD RELATIONSHIP BETWEEN AVERAGE SERUM ALCOHOL AND THE MEAN EFFECTS ON VAS ALERTNESS

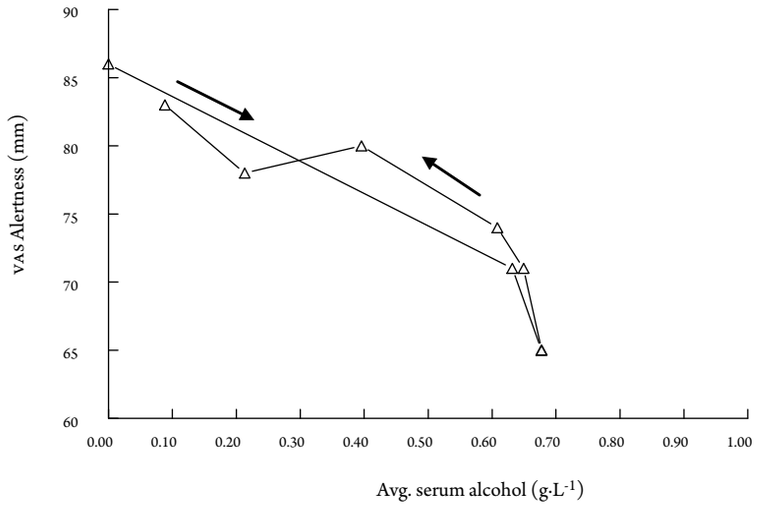


**FIGURE 9** EXPLORATORY PK/PD RELATIONSHIP BETWEEN AVERAGE SERUM ALCOHOL AND THE MEAN EFFECTS ON VAS ALCOHOL EFFECTS

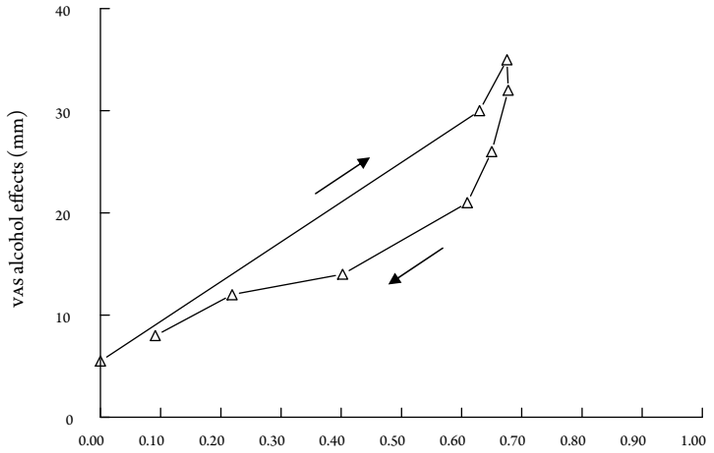



**FIGURE 10** EXPLORATORY PK/PD RELATIONSHIP BETWEEN AVERAGE SERUM ALCOHOL AND THE MEAN EFFECTS ON TRACKING PERFORMANCE

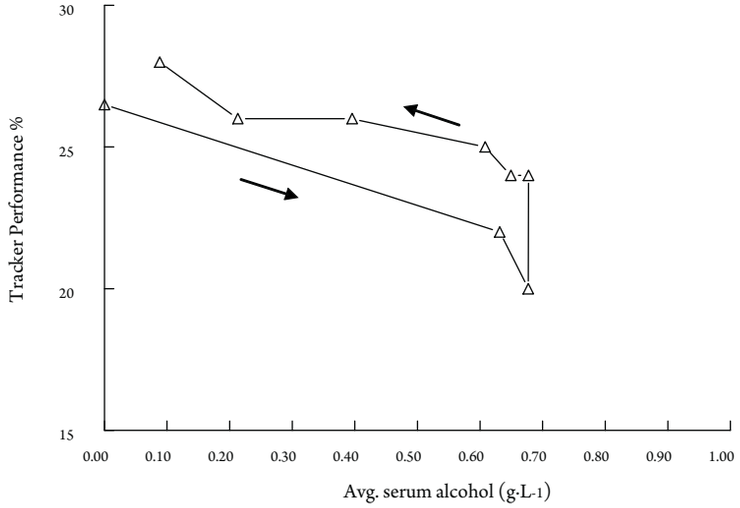





**TABLE 1** SUMMARY OF PHARMACODYNAMIC EFFECTS OF ALCOHOL COMPARED TO PLACEBO

| **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%) |

Baloh RW, Sills AW, Kumley WE and Honrubia V (1975). Quantitative measurement of saccade amplitude, duration, and velocity. Neurology 25, 1065-1070.

Bittencourt PRM, Wade P, Smith AT and Richens A (1983). Benzodiazepines impair smooth pursuit eye-movements. Br J Clin Pharmacol 15, 259-262.

Bond A and Lader M (1974). The use of analogue scales in rating subjective feelings. Br J Med Psychol 47, 211-218.

Borland RG and Nicholson AN (1984). Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol 18 Suppl 1, 69S-72S.

Cohen AF, Ashby L, Crowley D, Land G, Peck AW and Miller AA (1985). Lamotrigine (Bw430C), a potential anticonvulsant - effects on the central nervous-system in comparison with phenytoin and diazepam. Br J Clin Pharmacol 20, 619-629.

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 and van Gerven JM (2007). 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 21, 374-383.

de Visser SJ, van der Post J, Pieters MSM, Cohen AF and van Gerven JMA (2001). Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 51, 119-132.

de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF and van Gerven JM (2003). Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 55, 39-50.

Ehlers CL, Garcia-Andrade C, Wall TL, Cloutier D and Phillips E (1999). Electroencephalographic responses to alcohol challenge in Native American Mission Indians. Biol Psychiatry 45, 776-787.

Fox GR and Hayward JS (1987). Effect of Hypothermia on Breath-Alcohol Analysis. Journal of Forensic Sciences 32, 320-325.

Fox GR and Hayward JS (1989). Effect of Hyperthermia on Breath-Alcohol Analysis. Journal of Forensic Sciences 34, 836-841.

Heishman SJ, Arasteh K and Stitzer ML (1997). Comparative effects of alcohol and marijuana on mood, memory, and performance. Pharmacology Biochemistry and Behavior 58, 93-101.

Hiltunen AJ, Saxon L, Skagerberg S and Borg S (2000). Acute tolerance during intravenous infusion of alcohol: Comparison of performance during ascending and steady state concentrations - a pilot study. Alcohol 22, 69-74.

Jalava KM, Mattila MJ, Tarssanen M and Vanakoski J (1995). Lorazepam and diazepam differently impair divided attention. Pharmacol Biochem Behav. 51, 189-197.

Jones AW (1982). How Breathing Technique Can Influence the Results of Breath-Alcohol Analysis. Medicine Science and the Law 22, 275-280.

Jones AW (1997). Effect of high-fat, high-protein, and high-carbohydrate meals on the pharmacokinetics of a small dose of ethanol.

Jones AW and Andersson L (2003). Comparison of ethanol concentrations in venous blood and end-expired breath during a controlled drinking study. Forensic Science International 132, 18-25.

Kaplan HL, Sellers EM, Hamilton C, Naranjo CA and Dorian P (1985). Is there acute tolerance to alcohol at steady-state. Journal of Studies on Alcohol 46, 253-256.

Lehtinen I, Nyrke T, Lang AH, Pakkanen A and Keskinen E (1982). Quantitative effects of ethanol infusion on smooth pursuit eye-movements in man. Psychopharmacology 77, 74-80.

Magliozzi JR, Mungas D, Laubly JN and Blunden D (1989). Effect of Haloperidol on A Symbol Digit Substitution Task in Normal Adult Males. Neuropsychopharmacology 2, 29-37.

Martin CS and Moss HB (1993). Measurement of acute tolerance to alcohol in human subjects. Alcohol Clin Exp Res 17, 211-216.

Morzorati SL, Ramchandani VA, Flury L, Li TK and O'Connor S (2002). Self-reported subjective perception of intoxication reflects family history of alcoholism when breath alcohol levels are constant. Alcohol Clin Exp Res 26, 1299-1306.

Mulder JA and Neuteboom W (1987). The effects of hypo- and hyperventilation on breath alcohol measurements. Blutalkohol 24, 341-347.

Norris H (1971). Action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 10, 181-&.

Nutt DJ, Besson M, Wilson SJ, Dawson GR and Lingford-Hughes AR (2007). Blockade of alcohol's amnestic activity in humans by an alpha5 subtype benzodiazepine receptor inverse agonist. Neuropharmacology 53, 810-820.

O'Connor S, Morzorati S, Christian J and Li TK (1998). Clamping breath alcohol concentration reduces experimental variance: Application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcohol Clin Exp Res 22, 202-210.

Ramchandani VA, Bolane J, Li TK and O'Connor S (1999). A physiologically-based pharmacokinetic (PBPK) model for alcohol facilitates rapid BRAC clamping. Alcohol Clin Exp Res 23, 617-623.

Ramchandani VA, Flury L, Morzorati SL, Kareken D, Blekher T, Foroud T, Li TK and O'Connor S (2002). Recent drinking history: Association with family history of alcoholism and the acute response to alcohol during a 60 mg% clamp. Journal of Studies on Alcohol 63, 734-744.

Ramchandani VA, Kwo PY and Li TK (2001). Effect of food and food composition on alcohol elimination rates in healthy men and women. Journal of Clinical Pharmacology 41, 1345-1350.

Ramchandani VA, O'Connor S, Blekher T, Kareken D, Morzorati S, Nurnberger J and Li TK (1999). A preliminary study of acute responses to clamped alcohol concentration and family history of alcoholism. Alcohol Clin Exp Res 23, 1320-1330.

Santhakumar V, Wallner M and Otis TS (2007). Ethanol acts directly on extrasynaptic subtypes of GABA(A) receptors to increase tonic inhibition (vol 41, pg 211, 2007). Alcohol 41, 461.

Shamsi Z, Kimber S and Hindmarch I (2001). An investigation into the effects of cetirizine on cognitive function and psychomotor performance in healthy volunteers. European Journal of Clinical Pharmacology 56, 865-871.

van Steveninck AL (1993). Methods of assessment of central nervous system effects of drugs in man. Thesis/Dissertation, State University Leiden.

van Steveninck AL, Cohen AF and Ward TA (1989). A microcomputer based system for recording and analysis of smooth pursuit and saccadic eye movements. Br J Clin Pharmacol 27(5), 712P-713P.

van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MSM, Kroon JM, Breimer DD and Cohen A F (1993). Pharmacokinetic and pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo-steady state. Psychopharmacology 110, 471-478.

van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, Breimer DD and Cohen AF (1996). Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 41, 565-573.

van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD and Cohen AF (1991). 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. 50, 172-180.

van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM and Cohen AF (1999). The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J. Psychopharmacol. 13, 10-17.

Wilkinson PK (1980). Pharmacokinetics of ethanol - Review. Alcohol Clin Exp Res 4, 6-21.

Wright BM (1971). A simple mechanical ataxia-meter. J. Physiol 218, 27P-28P.

Zoethout RWM, van Gerven JMA, Dumont GJH, Paltansing S, Van Burgel ND, Van der Linden M, Dahan A, Cohen AF and Schoemaker RC (2008). A comparative study of two methods for attaining constant alcohol levels. Brit J Clin Pharmacol 66, 674-681.

# CHAPTER 5

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

*Manuscript submitted for publication (Alcohol)*

R.W.M. Zoethout, M.L. de Kam, A. Dahan, A.F. Cohen, J.M.A. van Gerven

## 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.*

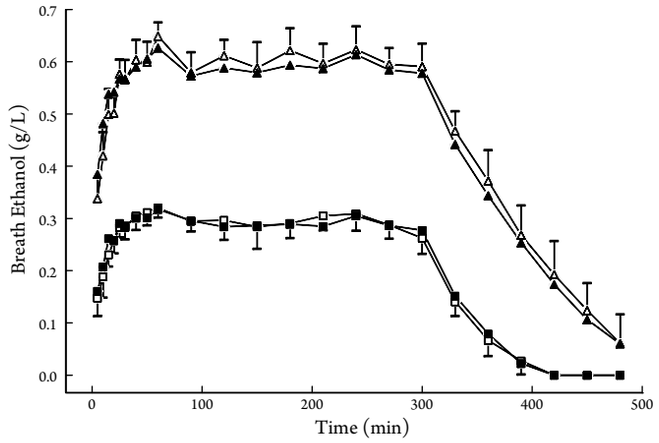



**FIGURE 2** MEAN INFUSION RATES PER SUBGROUP PER DOSE LEVEL

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

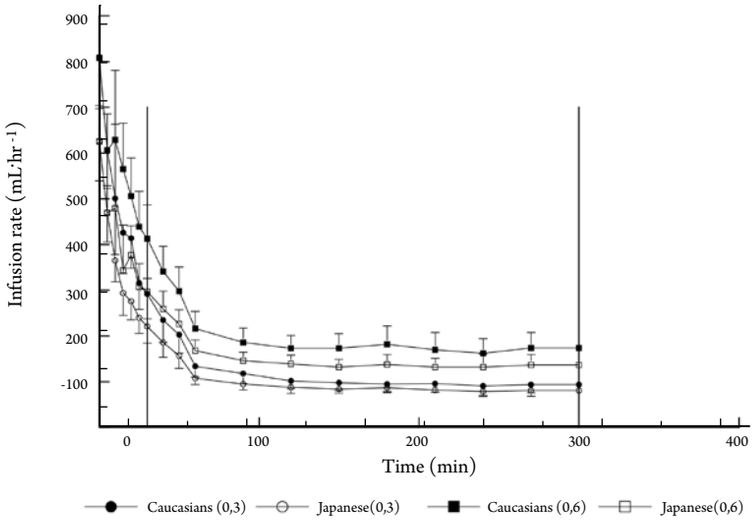





**FIGURE 3** LEAST SQUARE MEANS OF VAS ALCOHOL EFFECTS

*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.*

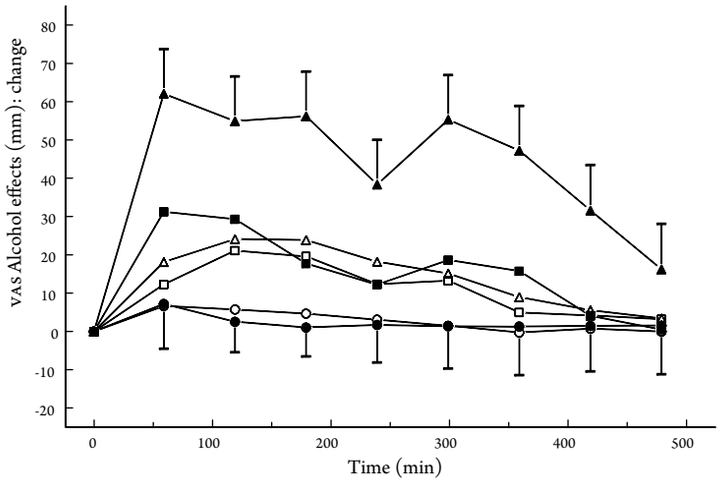



**FIGURE 4** LEAST SQUARE MEANS OF VAS ALERTNESS

*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.*

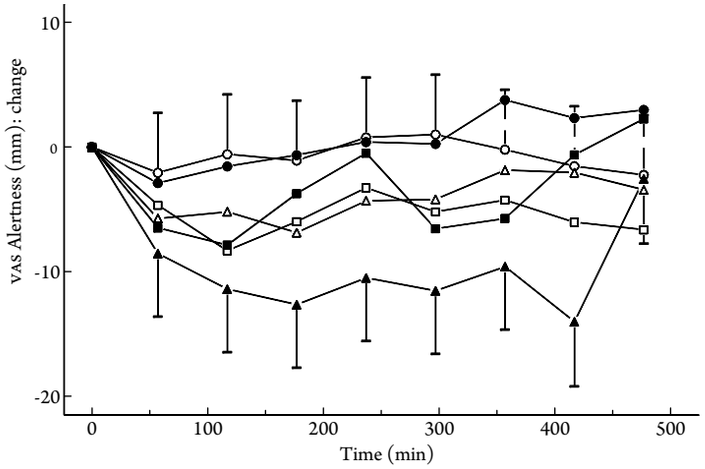


**FIGURE 5** LEAST SQUARE MEANS OF BODY SWAY

*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.*

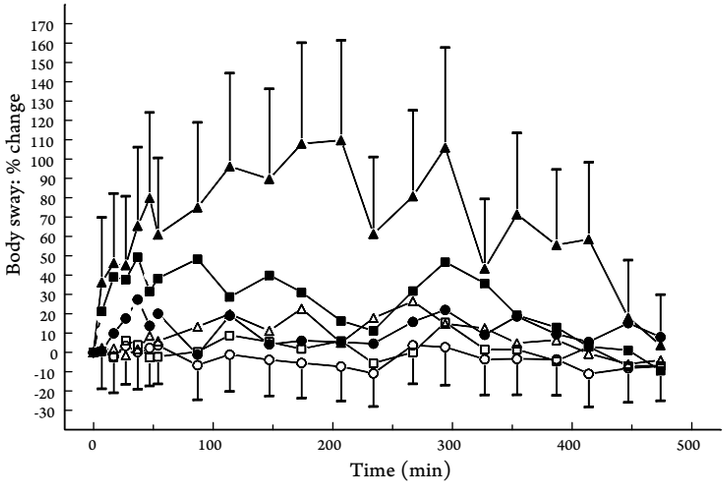



**FIGURE 6** LEAST SQUARE MEANS OF ADAPTIVE TRACKING

*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.*

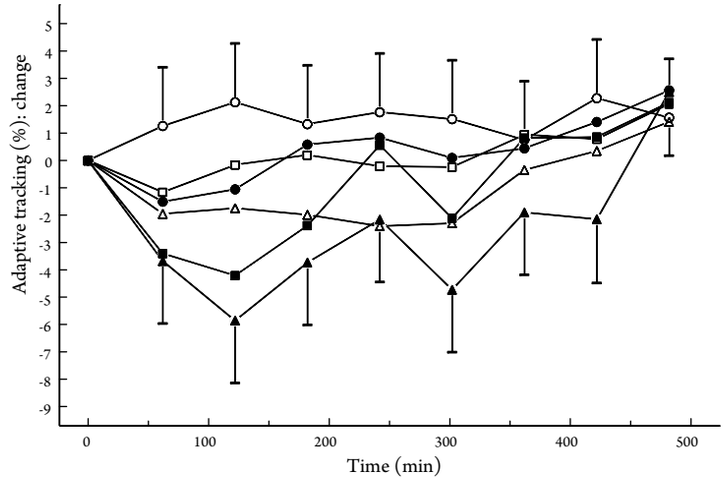





**FIGURE 7** LEAST SQUARE MEANS OF SACCADIC PEAK VELOCITY IN DEGREES PER SECOND

*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.*

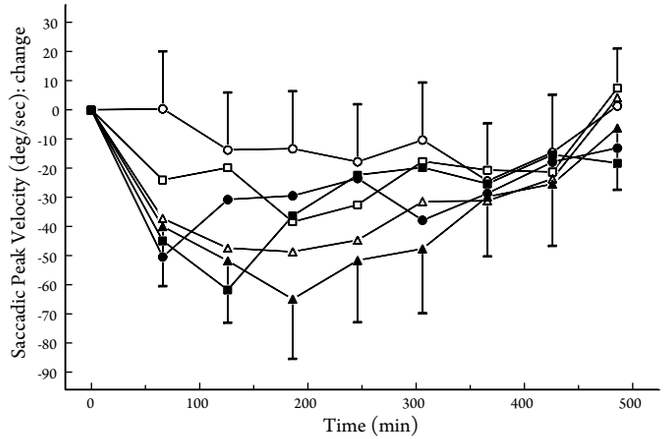



**FIGURE 8** LEAST SQUARE MEANS OF SMOOTH PURSUIT EYE MOVEMENTS

*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.*

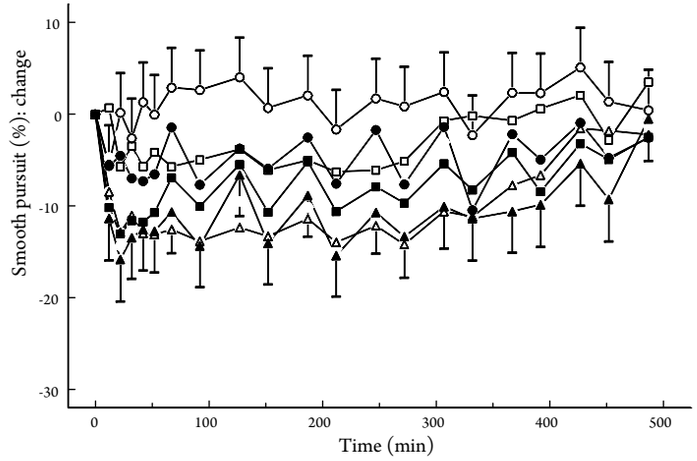


**TABLE 1** CONTRASTS BETWEEN TREATMENTS AND SUBGROUPS FOR EACH PHARMACODYNAMIC PARAMETER

*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%) |

Baloh RW, Sills AW, Kumley WE, Honrubia V (1975). Quantitative measurement of saccade amplitude, duration, and velocity. Neurology 25:1065-1070.

Bennett RH, Cherek DR, Spiga R (1993). Acute and chronic alcohol tolerance in humans - effects of dose and consecutive days of exposure. Alcohol Clin Exp Res 17:740-745.

Bittencourt PRM, Wade P, Smith AT, Richens A (1983). Benzodiazepines impair smooth pursuit eye-movements. Br J Clin Pharmacol 15:259-262.

Bond A, lader M (1974). The use of analogue scales in rating subjective feelings. pp 211-218.

Borland RG, Nicholson AN (1984). Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol 18 Suppl 1:69S-72S.

Chan AW (1986). Racial differences in alcohol sensitivity. Alcohol Alcohol 21:93-104.

Cohen AF, Ashby L, Crowley D, Land G, Peck AW, Miller AA (1985). Lamotrigine (Bw430C), A potential anticonvulsant - effects on the central nervous-system in comparison with phenytoin and diazepam. Br J Clin Pharmacol 20:619-629.

de Haas SL, de Visser SJ, van der Post JP, de Smet M, Schoemaker RC, Rijnbeek B, Cohen AF, Vega JM, Agrawal NGB, Goel TV, Simpson RC, Pearson LK, Li S, Hesney M, Murphy MG, van Gerven JMA (2006). Pharmacodynamic effects of TPA023, a GABA-A alpha(2,3) subtype selective agonist, compared with lorazepam and placebo in healthy volunteers. Br J Clin Pharmacol 61:627.

de Visser SJ, van der Post J, Pieters MSM, Cohen AF, van Gerven JMA (2001). Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br J Clin Pharmacol 51:119-132.

de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM (2003). Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 55:39-50.

de Visser SJ, van Gerven JMA, Schoemaker RC, Cohen AF (2001). 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 51:423-428.

Duranceaux NC, Schuckit MA, Luczak SE, Eng MY, Carr LG, Wall TL (2008). Ethnic differences in level of response to alcohol between Chinese Americans and Korean Americans. J Stud Alcohol Drugs 69:227-234.

Eriksson CJ (2001). The role of acetaldehyde in the actions of alcohol (update 2000). Alcohol Clin Exp Res 25:15S-32S.

Grant SA, Millar K, Kenny GNC (2001). Blood alcohol concentration and psychomotor effects (vol 85, pg 401, 2000). British Journal of Anaesthesia 86:302.

Hallynck TH, Soep HH, Thomis JA, Boelaert J, Daneels R, Dettli L (1981). Should Clearance be Normalized to Body-Surface Or to Lean Body-Mass. Brit J Clin Pharmacol 11:523-526.

Holdstock L, de Wit H (1999). Ethanol impairs saccadic and smooth pursuit eye movements without producing self-reports of sedation. Alcoholism-Clinical and Experimental Research 23:664-672.

Jones AW (1993). Pharmacokinetics of ethanol in saliva - comparison with blood and breath alcohol profiles, subjective feelings of intoxication, and diminished performance. Clinical Chemistry 39:1837-1844.

Lehtinen I, Nyrke T, Lang AH, Pakkanen A, Keskinen E (1982). Quantitative effects of ethanol infusion on smooth pursuit eye-movements in man. Psychopharmacology 77:74-80.

Lukas SE, Lex BW, Slater JP, Greenwald NE, Mendelson JH (1989). A microanalysis of ethanol-induced disruption of body sway and psychomotor performance in women. Psychopharmacology 98:169-175.

Martin NG, Oakeshott JG, Gibson JB, Wilks AV, Starmer GA, Whitfield JB (1981). Prodromus to a twin study of sensitivity to intoxication and alcohol metabolism. Australian and New Zealand Journal of Medicine 11:140-143.

Norris H (1971). Action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 10:181-&.

O'Connor S, Morzorati S, Christian J, Li TK (1998). Clamping breath alcohol concentration reduces experimental variance: application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcohol Clin Exp Res 22:202-210.

Santhakumar V, Wallner M, Otis TS (2007). Ethanol acts directly on extrasynaptic subtypes of GABA(A) receptors to increase tonic inhibition (vol 41, pg 211, 2007). Alcohol 41:461.

Shibuya A, Yasunami M, Yoshida A (1989). Genotypes of alcohol-dehydrogenase and aldehyde dehydrogenase loci in Japanese alcohol flushers and nonflushers. Human Genetics 82:14-16.

van der Post JP, de Visser SJ, Schoemaker RC, Cohen AF, van Gerven JMA (2004). Pharmacokinetic/pharmacodynamic assessment of tolerance to central nervous system effects of a 3 mg sustained release tablet of rilmenidine in hypertensive patients. Journal of Psychopharmacology 18:221-227.

van Gerven JMA, Uchida E, Uchida N, Pieters MSM, Meinders AJ, Schoemaker RC, Nanhekhan LV, Kroon JM, de Visser SJ, Altorf B, Yasuda K, Yasuhara H, Cohen AF (1998). Pharmacodynamics and pharmacokinetics of a single oral dose of nitrazepam in healthy male and female volunteers. An interethnic comparative study between Japanese and Caucasian volunteers. J Clin Pharmacol 38:1129-1136

van Steveninck AL, Cohen AF, Ward T (1989). A microcomputer based system for recording and analysis of smooth pursuit and saccadic eye movements. Br J Clin Pharmacol 27(5):712-713.

van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MSM, Kroon JM, Breimer DD, Cohen AF (1993). Pharmacokinetic and pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo-steady state. Psychopharmacology 110:471-478.

van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MSM, Breimer DD, Cohen AF (1996). Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 41:565-573.

van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF (1991). 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 50:172-180.

van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF (1999). The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 13:10-17.

Watson PE, Watson ID, Batt RD (1981). Prediction of blood-alcohol concentrations in human-subjects - updating the Widmark equation. Journal of Studies on Alcohol 42:547-556.

Wright BM (1971). A simple mechanical ataxiameter. J Physiol 218:27P-28P.

Zoethout RW, van Gerven JM, Dumont GJ, Paltansing S, Van Burgel ND, Van der Linden M, Dahan A, Cohen AF, Schoemaker RC (2008). A comparative study of two methods for attaining constant alcohol levels. Br J Clin Pharmacol 66:674-681.

Zoethout RW, Schoemaker RC, Zuurman L, van PH, Dahan A, Cohen AF, van Gerven JM (2009). Central nervous system effects of alcohol at a pseudo-steady-state concentration using alcohol clamping in healthy volunteers. Br J Clin Pharmacol 68:524-534.

Zoethout RWM, Delgado WL, Ippel AE, Dahan A, van Gerven JMA (2010). Functional biomarkers for the acute effects of alcohol on the central nervous system in healthy volunteers. Br J Clin Pharmacol 2011, 71(3), 331-350.

Zuurman L, Ippel AE, Moin E, van Gerven JM (2009). Biomarkers for the effects of cannabis and THC in healthy volunteers. Br J Clin Pharmacol 67:5-21.

# CHAPTER 6

## *Effects of morphine and alcohol on functional brain connectivity during 'resting state': a placebo-controlled crossover study in healthy young men*

*Hum Brain Mapp. 2012, 33(5), 1003-1018*

N. Khalili-Mahani, R.W.M. Zoethout, C.F. Beckmann, E. Baerends,
M.L. de Kam, R.P. Soeter, A. Dahan, M.A. van Buchem,
J.M.A. van Gerven, S.A.R.B. Rombouts

## ABSTRACT

A major challenge in central nervous system (CNS) drug research is to develop a generally applicable methodology for repeated measurements of drug effects on the entire CNS, without task-related interactions and *a priori* models. For this reason, data-driven resting-state fMRI methods are promising for pharmacological research. We aimed to investigate whether different psychoactive substances cause drug-specific effects in functional brain connectivity during resting-state. In this double blind placebo-controlled (double dummy) crossover study, seven resting-state fMRI scans were obtained in 12 healthy young men in three different drug sessions (placebo, morphine and alcohol; randomized). Drugs were administered intravenously based on validated pharmacokinetic protocols to minimize the inter- and intra-subject variance in plasma drug concentrations. Dual-regression was used to estimate whole-brain resting-state connectivity in relation to eight well-characterized resting-state networks, for each data set. A mixed effects analysis of drug by time interactions revealed dissociable changes in both pharmacodynamics and functional connectivity resulting from alcohol and morphine. Post hoc analysis of regions of interest revealed adaptive network interactions in relation to pharmacokinetic and pharmacodynamic curves. Our results illustrate the applicability of resting-state functional brain connectivity in CNS drug research.


## INTRODUCTION

One of the major challenges of central nervous system (CNS) drug discovery is to develop a generally applicable methodology to demonstrate the effects of a compound on the brain across the different phases of drug development. An optimal methodology should be minimally invasive, repeatable and able to identify overlapping and distinguishable effects of different CNS drugs with little reliance on *a priori* models for drug effects.

The advances in functional neuroimaging, especially positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), have opened important frontiers for CNS drug research. However, radiation dose restrictions in PET prevent repeated intra-subject measurements within short periods of time. This is a major limitation of PET because repeated measurements allow testing different drug compounds on the same individual and minimize the within-subject variances. More importantly, repeated neuroimaging acquisitions would allow the assessment of pharmacokinetic/pharmacodynamic (PK/PD) relationships, which form the scientific basis of modern drug development.

Compared to PET, blood-oxygen-level-dependent (BOLD) fMRI is non invasive, and benefits from high temporal and spatial resolution. Until recently, fMRI was applicable in task-related designs, where pharmachologically induced changes in regional BOLD signal in response to a hypothesized CNS function (e.g. pain alleviation) were investigated. For examples see (Honey and Bullmore, 2004). One of the major limitations of this approach was the difficulty in controlling for inter-subject variations in stimulus perception or performance in task-related experiments. In addition, fMRI tasks had to be chosen to activate regions of interest based on *a priori* hypotheses about the site of drug effect (Breiter *et al.*, 1997); or about the PK/PD profile (Stein *et al.*, 1998) – which in many cases are unknown.

A recent breakthrough in neuroimaging has resulted from discovering the complex (but consistent and reliable) functional architecture of brain activity from the BOLD fluctuations (Biswal *et al.*, 2010; Bullmore and Sporns, 2009; Smith *et al.*, 2009). Today, a growing body of evidence suggests that the 'resting-state' brain activity (i.e. spontaneous BOLD fluctuations in the absence of any specific stimuli) forms spatially correlated topographies, which represent functional connections that relate to, or even predict, emotional and cognitive behavioral outcomes (Fox *et al.*, 2007; Seeley *et al.* 2007) or clinical conditions (Greicius, 2008). These resting-state networks (RSNs) represent distinct functional systems (e.g. motor, vision, attention, etc), that are reliable and reproducible (Beckmann *et al.* 2005; Biswal *et al.*, 2010; Damoiseaux *et al.*, 2006; Zuo *et al.*, 2009). Hence, if different drugs produce specific and detectable changes in the functional topography of these networks, then RSNs may become a biomarker for CNS drug research.

In this study we investigate whether *different psychoactive drug compounds (alcohol and morphine) elicit distinguishable changes in functional topography of the resting-state brain networks*. The RSN connectivity is determined in terms of similarity of temporal fluctuations in the whole brain in relation to eight networks of interest (NOI). These NOIs represent the most reliably and reproducibly detectable RSNs of functional significance (e.g. visual, somatosensory, motor, attention, working memory) (Beckmann *et al.*, 2005; Damoiseaux *et al.*, 2006). Drugs are administered under PK-controlled infusion protocols. Seven resting-state fMRIs (RS-fMRI) and several PK and PD assessments are made at controlled intervals (figure 1). We report drug-specific changes in the profile of RSN connectivity, and show their temporal relation to the profiles of drug concentrations and pharmacodynamic effects in areas where effects of morphine and alcohol are expected.

# METHODS

## *Experimental Design*

A schematic diagram of the study design and the analyses is provided in figure 1. This study is randomized, double blind (i.e. both examiner and the participant are unaware of which drugs are given), and double dummy placebo-controlled (i.e. placebo was used for each treatment, consisting of infusion of the vehicle for treatment A in parallel to the administration of active treatment B or vice versa, and two vehicle infusions in the placebo session). Alcohol and morphine are two of the most commonly used substances in addiction and pain studies with similar analgesic and euphoric effects but they also result in different autonomic responses. In humans, alcohol exerts a wide range of psychoactive effects by interacting with GABAergic, dopaminergic, serotonergic and even opiate neurotransmitter systems (Koob *et al.*, 1998). In contrast, morphine targets specific receptors (μ-opioid), which in turn interact with many other neurotransmitter systems (Contet *et al.*, 2004). This within-subject design aimed to test whether drug-specific effects on the RSN connectivity would be detectable and whether their profile corresponded to the PK and PD profiles we measured. However, both alcohol and morphine have dose-dependent effects on the regional cerebral blood flow (Blaha *et al.*, 2003; Wagner *et al.*, 2001), and pharmacodynamic effects can significantly vary across individuals. Hence, we applied previously validated infusion regimens to achieve approximately steady state serum levels and controlled pharmacodynamic effects. The infusion protocols were based on validated PK models for each of these drugs (Sarton *et al.*, 2000; Zoethout *et al.*, 2009; Zoethout *et al.*, 2008). This allowed 1) minimizing the between- and within subject variations in plasma drug concentration and 2) maintaining each of these drugs' concentrations at a plateau level for 90 minutes (approximately 60 minutes after the onset of experiment).

## *Subjects*

Twelve healthy male subjects (age range 18-40; BMI 18-26 kg/m$^2$) were selected to participate in the neuroimaging study. (See supplemental material for exclusion criteria.) The study was approved by the Medical Ethics Review Board of Leiden University Medical Centre. Both oral and written informed consents were obtained from all participating subjects. All studies were performed in compliance with the law on clinical trials of the Netherlands (WMO). Each participant was scanned on three separate days, at least 7 days apart. Subjects fasted for at least four hours prior to the start of a study day. After arrival at the hospital a light standardized meal was provided. On each study day, negative scores on alcohol breath tests and a urinary drug screen for amphetamines, cocaine, morphine and Δ9-Tetrahydrocannabinol (THC) were required. Three intravenous cannulas (one for morphine or placebo administration, one for alcohol or placebo administration and one for blood sampling) were placed in veins of the arms. Before the start of drug or placebo administration, a baseline resting-state scan was made and two baseline measurements of each visual analogue scale (VAS) were taken. After the last scan, the intravenous cannulas were removed and a second meal was provided.

## *Morphine Infusion Protocols*

In order to reach stable serum levels of morphine (approximately 80 nmol/L) an initial bolus of 100 µg/kg/hour was infused during one minute; followed by a continuous infusion of 30 µg/kg/hour for 2.5 hours. Total volume of morphine infusion was approximately 14.5 mg. A prior morphine study using an identical infusion paradigm (Sarton *et al.*, 2000) showed that this infusion regimen could be safely applied, without the occurrence of major side effects. This dose was also associated with significant pharmacodynamic CNS effects. The total amount of morphine administered during one occasion using this dosage scheme, was approximately 14 mg for an average weighted male subject, infused over a time period of 2.5 hours. This is considered to be a safe and rational dose, since it is within the therapeutic range of morphine (i.e. 2.5 mg - 15 mg in 4-5 ml in 4-5 minutes intravenously, for acute pain). To determine the plasma concentration of morphine, venous blood was collected in 5 ml plain tubes (Becton and Dickinson). Blood samples were taken at 0, 15, 30, 50, 60, 90, 120, 150, 180, 210 and 270 minutes after the start of the placebo or drug administration. All samples were centrifuged for 10 minutes at 2000 G between 30 and 45 minutes after collection. Plasma samples were stored at -21° C. Plasma concentrations of morphine were determined using liquid chromatography with tandem mass spectrometry (Sarton *et al.*, 2000).

## *Alcohol Infusion Protocol*

Alcohol concentrations were controlled based on an intravenous alcohol clamping paradigm using ethanol 10% in glucose 5% (O'Connor *et al.*, 1998). We aimed to maintain alcohol levels at 600 mg/L, which barely exceeds the legal limits for driving in the Netherlands (approximately equivalent to two glasses of wine). The alcohol clamp has previously been well-tolerated at this serum level and produced statistically significant pharmacodynamic CNS effects (Zoethout *et al.*, 2009). Infusion rates required to maintain stable alcohol levels were computed by a non-blind staff member without any other involvement in the study, based on measurements of breath alcohol (BrAC) at 5-minute intervals between 0-30 minutes, at 10-minute intervals between minutes 30-60, and 30-minute intervals between minutes 60-300 after the start of the placebo or drug administration. The alcohol placebo condition consisted of a sham-procedure using a glucose 5% solution, including computer-driven adaptations of infusion rates and BrAC measurements.

## *Neuroimaging Acquisition and Processing Protocols*

A 3T Achieva scanner (Philips Medical System, Best, The Netherlands) was used for image acquisition. For each subject we obtained 21 resting-state T2*-weighted acquisitions (gradient echo EPI with parameters set to a TR = 2180 ms, TE = 30 ms, flip angle = 80; 64x64x38 isotropic resolution 3,44 mm, 220 frames, 8 minutes) and a T1-weighted high resolution scan for anatomical registration. During scanning, a pulse oximeter (INVIVO MRI 4500, Siemens Healthcare, Germany) was used to monitor heart rate and oxygen saturation. A flexible pressure belt was used to record the respiratory signals. These RS-FMRI data were preprocessed using the standard procedure including motion correction, brain extraction, Gaussian smoothing with a 5 mm FWHM, kernel, mean-based intensity normalization of all volumes by the same factor (i.e., 4D grand-mean), and high-pass temporal filtering (FWHM = 100 s). After preprocessing, the functional scans were affine-registered to an MNI152 standard space (Montreal Neurological Institute, Montreal, QC, Canada). In all stages Functional Magnetic Resonance Imaging of the Brain (FmriB) Software Library (fsl 4.0, Oxford, UK; www.fmrib.ox.ac.uk/fsl) was used.

## *Assessment of Drug Effects on RSN Connectivity*

We defined RSN connectivity in terms of the similarity of the BOLD fluctuations in each brain voxel in relation to characteristic fluctuation in eight predefined networks of interest (NOI). These networks are obtained from a weighted mask of networks that are most reliably (and reproducibly) identi-

fied from a model-free analysis of the spatio-temporal structure of the resting-state BOLD fluctuations (Beckmann *et al.*, 2005). These template NOIs include over 80% of the total brain volume and comprise: medial and lateral visual systems (NOIs 1 and 2, respectively), auditory and somatosensory system (NOI3), sensory motor system (NOI4), the default mode network (NOI5), executive salience network (NOI6), and visual-spatial and working memory networks (NOIs 7 and 8, which represent almost mirrored networks). Each NOI represents shared neurophysiological fluctuations of the anatomical locations it includes.

To measure connectivity, we used the dual-regression method (Beckmann *et al.*, 2009). Briefly, dual-regression is based on first extracting the temporal pattern of resting-state signal fluctuations within an RSN — for each resting fMRI dataset; and next regressing these 'fitted time courses' against fluctuations in the entire brain. Dual- regression analysis generates statistical maps of z-scores that represent connectivity to the given NOI. In other words, the higher the absolute value of the z-score, the stronger the connectivity to an NOI. These statistical maps can then be used in voxel-wise mixed model analyses of complex experimental designs to obtain a statistical representation of where the drug interactions with any particular network connectivity are significant. The statistical sensitivity of the dual-regression method has been successfully demonstrated in a study that reported distinct differences in brain connectivity in young carriers of the APOE4 gene (Filippini *et al.*, 2009).

Here, dual-regression resulted in 252 statistical parametric maps of whole brain connectivity to each of NOIs (12 subjects x 7 RSfMRIs x 3 treatments x 8NOIs). For each NOI, the respective RSN connectivity maps were entered in a mixed-effect generalized linear model (GLM) to identify which brain regions and which networks were most significantly affected by the drug x time interactions. We used fixed factors treatment and time, and random factor subject. Particularly, we tested the difference between morphine-placebo and alcohol-placebo, while accounting for the variance across time (6 post-injection time points vs. the first pre-infusion). The effects of morphine versus placebo; alcohol versus placebo, each of the time points 2 to 7 versus the pre-drug time point, and an intercept for each subject were modeled as covariates.

Pharmacologically induced variations in heart rate and respiration rate were expected. Previous studies have shown that both heart rate (Chang *et al.*, 2009; Shmueli *et al.*, 2007) and respiration (Birn *et al.*, 2008; Chang and Glover, 2009b; Wise *et al.*, 2004) fluctuations introduce variance in the resting-state BOLD signal. We controlled for global effects of respiration and heart rate by examining the linear association of average heart rate and average respiration rate with resting-state connectivity of each network. We also tested separate models, with and without respiration and heart rates as covariates to examine the robustness of detected effects to nuisance variables.

Permutation-based statistical inference (Nichols and Holmes, 2002) (5000 permutation tests) was used. Statistical significance was set at $p<0.05$, after cluster-based correction for family wise errors (based on the null distribution of the max cluster size across the image) was performed. In all stages FSL 4.0 was used.

## *Pharmacodynamic Assessment*

Computerised Visual Analogue Scales (VAS) were used to measure subjective CNS effects of drugs at baseline and repeated at 30, 60, 90, 120, 150, 180 and 210 minutes after infusion started. The assessments were performed outside the scanner. The VAS Bond and Lader (Bond and Lader, 1974) was used for the subjective assessment of the state of mind at that moment. Three factors corresponding to 'alertness', 'mood' and 'calmness' can be derived from the VAS Bond and Lader. The VAS Bond and Lader scores are expressed in millimeter (mm), in which 50 mm indicates a normal feeling.

An adapted version of the VAS Bowdle (Bowdle *et al.*, 1998) was used for subjective assessment of psychedelic effects. From the VAS Bowdle, three factors corresponding to 'internal perception' and 'external perception' (two modalities of psychedelic effects) and 'feeling high' can be derived. 'External perception' consist of the VAS scores changing of body parts, changes of surrounding, altered passing of time, difficulty controlling thought, changes in

color intensity and changes in sound intensity. It indicates a misperception of an external stimulus or a change in the awareness of the subject's surroundings. 'Internal perception' reflects inner feelings that do not correspond with the reality, e.g. hearing of unrealistic voices or sounds, unrealistic thoughts, paranoid feelings and anxious feelings. 'Feeling high' is a separate item of the VAS Bowdle. The minimum score for the VAS Bowdle (absence of psychedelic effects) is 0 mm. We also used VAS nausea (Mearadji *et al.*, 1998) and VAS alcohol effect (intoxication), each consisting of a single scale in which the extreme left side (0 mm) corresponds to 'not nauseous/drunk at all' and the extreme right side (100 mm) to 'maximum nauseous/drunk'.

Repeatedly measured pharmacodynamic and physiological data were compared with a mixed model analysis of variance with fixed factors treatment, period, time, and treatment by time and random factor subject, subject by treatment and subject by time and the average pre-value (average over all measurements at or before time=0) as covariate (SAS for windows V9.1.2 ; SAS Institute, Inc., Cary, NC, USA). Images were generated using Prism 5, GraphPad Software Inc., La Jolla, CA, USA).

# RESULTS

## *Controlled Pharmacokinetic Profiles*

To reduce inter- and intra-subject variability, we aimed to examine variations in resting-state connectivity under controlled pseudo-steady state plasma drug concentrations, using a target-controlled infusion regimen for morphine and an intravenous clamp for alcohol. Figure 2 illustrates the concentrations over time for each subject. Sixty minutes after the start of the infusion at the onset of RS-FMRI 2, the between-subject averaged (± SD) morphine level was 67.17± 10.22 *(nmol/L)*, which remained in the same range in the following RS-FMRIS 3, 4 and 5 (68.63 ± 8.0; 66.88 ± 5.8; 68.04 ± 8.8, respectively). Average alcohol concentrations were also stable, with low between-subject variability at RS-FMRI 2, 3, 4 and 5 (0.60 ± 0.05, 0.60 ± 0.056; 0.58 ± 0.069 and 0.63 ± 0.038 (*g*/L), respectively.)



## *Pharmacodynamic Effects of Morphine and Alcohol*

Figure 3 and table 1 summarize the pharmacodynamic effects. Morphine significantly reduced the respiration and heart rates and increased calmness and sensation of nausea compared to placebo. Alcohol significantly increased the heart rate and feelings of intoxication. Other effects did not reach statistical significance.

## *Effects of Physiological Factors on RSN connectivity*

Figure 4 shows overlaid F-stat maps for each network, at $p < 0.05$ (cluster corrected). Simple regression shows significant correlation between respiration rate and connectivity patterns of NO14, NO15, NO16 and NO17. In contrast, heart rate correlated with connectivity of several areas including the cerebellum, the brainstem and the amygdala in relation to NO13. Overlapping heart rate modulation of connectivity of a region close to major arteries (and extending to the amygdala) in relation to NO1 4, NO15, NO16 and NO18 was also present. However, when effects of treatment by time were modeled in the GLM, the patterns of correlation between physiological factors and connectivity were diminished. Including respiration and heart rate, the topography of morphine effects were changed, but alcohol effects were unchanged (figure 4). With the exception of the default mode network, it seems drug effects on resting-state connectivity were independent of global physiological variations of heart rate and respiratory depression (due to morphine).

## *Interactions of Morphine and Alcohol with RSN Connectivity*

Considering the location of regions where including physiological effects in the statistical model resulted in significant differences (white matter and a zone peripheral to the default mode network in relation to respiration), we expect these effect are non-neuronal and related to global cerebrovascular responses, potentially resulting from a hypercapnic condition due to respiratory

depression (Pattinson *et al.*, 2007), or physiological noise (Birn *et al.*, 2008). Therefore, for the purpose of this study, we have reported drug by time interactions while including these variables in the GLM, to avoid such confounds.

Figure 5 illustrates statistical maps (F-test; cluster p-values <0.05, corrected) of areas where connectivity in relation to a given NOI (in red) was significantly different due to morphine (green) and due to alcohol (blue) over time. Details in terms of cluster size, cluster p-value and the t-value of the peak of the cluster are provided in table 2.

MORPHINE Effects of morphine on RSN connectivity over time (compared to placebo) were significant and dissociable in different NOIs. The affected areas include prefrontal regions (subgenual ACC, medial prefrontal and basolateral prefrontal regions), posterior parietal areas (precuneus, posterior cingulate), medial temporal regions (amygdala and the hippocampal), primary sensory, primary motor, basal ganglia and cerebellum. The most extensive effects of morphine were observed in NOI4 (primary sensory motor network). Within the total areas affected (52.6 ml of brain volume) 39% of the effect was in the bilateral somatosensory areas (inside NOI4), but the rest of the effects were distal to NOI4 (26% in the limbic system and basal ganglia, 16% in the primary motor, 13% in the cerebellum and 10% in the visual area). The next most extensive effects were in the executive salience network, NOI6 (15.13 ml, 73% of which was prefrontal area that lies inside NOI6).

ALCOHOL Compared to morphine, effects of alcohol on RSN connectivity were more limited. The most extensive effects of alcohol were observed in NOI3 (15.15 ml of brain volume), where 62% of the change was in the posterior parietal cortex and the rest in the cerebellum and brainstem (which are outside NOI3). Small changes in connectivity of dorsocaudal ACC and precentral gyrus to NOI4 were present as well (4.52 ml) and in a small area (2.46 ml) inside NOI1.

OVERLAP In NOIs 1, 2, 3, 5, 6, and 7, the intersection of the significant clusters of each drug's effects did not reveal overlap, suggesting topographic differences in each drug effect on these functional networks. Only a small (42 voxels = 0.34 ml) overlap was observed in connectivity of the cerebellum to NOI4 and in connectivity of the superior frontal gyrus to NOI8 (52 voxels = 0.416 ml).

## *Post hoc Examination of Selected ROI Responses*

The purpose of the post-hoc analysis is to demonstrate the range and profile of changes in connectivity in some exemplar ROIs in relation to PK and PD profiles. These ROIs were chosen from NOIs that were most prominently affected by alcohol (NOI3) or morphine (NOI4).

In the sensorimotor network (NOI4), where the negative hippocampal connectivity emerged (figure 6-a), the hippocampal connectivity in relation to NOI4 was small in alcohol and placebo conditions (95% CI: -1.176 to -0.1104 and -1.230 to 0.09146; respectively). By contrast, morphine infusion increased the absolute value of hippocampal connectivity to NOI4 (95% CI: -4.309 to -3.043). We note that connectivity is defined in terms of linear fitting of spontaneous fluctuations at any given region to the 'specific' fluctuations within the entire network (i.e. the weighted average time course of all voxels). Therefore, the negative z-score suggests a distinct inverse relationship in terms of simple oscillations also a phase shift of the fluctuations of the hippocampus with respect to the sensory-motor network. By contrast, the connection of the central gyrus to NOI4, which was similar prior to both infusions, remained stable for alcohol and placebo (95% CI: 8.08 to10.45; and 8.34 to 10.33, respectively) but became stronger after morphine infusion (95% CI: 11.79 to 13.20) (figure 6-b).

Figure 6-c illustrates that alcohol significantly increased the connectivity within NOI1 within the first 90 minutes of infusion (95% CI: 0.36 to 2.22). By contrast, in the brainstem (figure 6-d) morphine and placebo showed an increase in negative connectivity to NOI3 (95% CI: -0.97 to -2.26 and -0.53 to -2.37; respectively), whereas this probably adaptive effect was not present with alcohol (95% CI: -0.07 to 0.87).

## DISCUSSION

Effects of morphine and alcohol on changes in the RSN connectivity were measured at several time points (together with the PD and PK profiles of each drug). We detected effects where the within-subject variation in pre-infusion connectivity in relation to a given NOI was minimal, whereas the difference in RSN connectivity (in relation to placebo) increased over the next six time-points—after plasma concentrations became stable and during the recovery phase. Drugs produced distinct CNS and clinical responses in terms of PD effects, physiological responses, and resting-state connectivity.

The question of the neurological substrates of resting-state BOLD fluctuations is still an open one. The BOLD signal originates from changes in cerebral blood flow, cerebral metabolism and oxygen extraction. At the present state of knowledge, interpretations of BOLD resting-state connectivity are tentative; although evidence for electrical (Britz *et al.*, 2010; Mantini *et al.*, 2007) and arterial perfusion (Chuang *et al.*, 2008) bases of functional connectivity in some of the putative resting-state networks is already available. Notwithstanding a definite biological interpretation of connectivity, we detected effects in most brain regions where changes in receptor binding, cerebral blood flow, or function in response to these specific substances are previously reported. For instance, PET studies with opiodergic radiotracers have shown that the ACC, opercular/insular cortex, thalamus, amygdala and putamen (the medial parts of the pain system) have the highest, and the primary somatosensory, sensorimotor areas (the lateral parts of the pain system) (Baumgartner *et al.*, 2006; Jones *et al.*, 1991; Zubieta *et al.*, 2001) and occipital areas (Sadzot *et al.*, 1991) to have the lowest binding potentials. As table 2 shows, we have detected predominant morphine effects both in areas that, according to PET studies, have low opioid binding potentials (such as primary sensorimotor connectivity to NOI4), and areas that have high opiodergic binding potentials (such as the ACC connectivity to NOI6). This observation precludes interpretation of the effects simply as an outcome of metabolic modulation at the site of receptor action. However, it is possible that the detected effects reflect interactions between these different regions in terms of functional adaptation. Future studies of combined RSfMRI and opiodergic PET studies can shed a light on this central question.

On the other hand, we have been able to demonstrate different connectivity effects resulting from morphine and alcohol, which have different sites of action. Unlike morphine, CNS effects of alcohol are primarily mediated via $GABA_A$ receptors, which are expressed across the brain, although different parts of the brain have higher affinity for different subunits (Kumar *et al.*, 2009). Presently, there are no radiotracer studies to have illustrated alcohol-induced variances in regional $GABA_A$ binding potentials in humans. The most direct *in vivo* evidence for the effect of alcohol on cerebral activation is observed in terms of reduced global metabolism (de Wit *et al.*, 1990; Volkow *et al.*, 2008; Volkow *et al.*, 2006; Wang *et al.*, 2000), with more relative decreases in the occipital cortex (Schreckenberger *et al.*, 2004; Volkow *et al.*, 2008; Wang *et al.*, 2000) and the cerebellum (Volkow *et al.*, 2008; Wang *et al.*, 2000), and relative increases in the brainstem, striatum and the ACC (Schreckenberger *et al.*, 2004). Also, a radiotracer study of a $GABA_A$ ligand ($[^{11}C]$,Ro 15-4513, which has preferred binding potential for ethanol (Hanchar *et al.*, 2006)) in monkeys indicates that the ACC, insula and the limbic system have the highest, and the occipital cortex and the cerebellum have the lowest binding potential for $[^{11}C]$,Ro 15-4513 (Maeda *et al.*, 2003). Here, we observed that alcohol affected the connectivity of the visual cortex (in relation to NOI1), the cerebellum (in relation to NOI3 and NOI4), the ACC (in relation to NOI3 and NOI4) and the brainstem (in relation to NOI3). Surprisingly, the most extensive effect of alcohol was in changing the connectivity of PCC (in relation to NOI3). It is known that alcohol effects can vary with dosage (Blaha *et al.*, 2003; de Wit *et al.*, 1990; Gordon *et al.*, 1995; Luksch *et al.*, 2009; Sano *et al.*, 1993), and they may depend on interindividual differences in regular alcohol consumption. The lesser extent of detected alcohol effects in our study can be hypothetically attributed to these sources of variance and future studies need to address the issue more closely.

With the exception of a small overlap in the cerebellum (in relation to NOI4) and the superior frontal gyrus (in relation to NOI8), drug effects on connectivity were distinct. Similarly, overlap in pharmacodynamic effects was limited. Alcohol significantly increased a feeling of drunkenness and morphine significantly increased nauseous sensation. The CNS effects expected to be common in both drugs such as calmness, alertness and mood were not significant for alcohol (although calmness was significantly increased by morphine). Of course, we cannot conclude that alcohol and morphine do not interact on common brain circuitry. In this experiment, the VAS scores indicate little overlap in terms of subjective changes in mood and feelings induced by alcohol and morphine. However, alcohol (ethanol) was administered at relatively low levels (0.6 g/L), whereas morphine was given in a therapeutically relevant dose (14.5 mg) with considerable functional impact. It is likely that higher doses of alcohol intoxication that result in stronger cognitive and affective responses, will elicit more pronounced changes in local functional connectivity. Presently, without extensive psychometric tests the behavioral correlates of the observed CNS effects cannot be discussed. More precise recordings of certain aspects of emotion and cognition would have enabled us to study the functional significance of RSN connectivity changes. In this experiment, we omitted cognitive testing between different resting-state measurements to avoid introducing performance-related confounds. In fact, our study underlines an important advantage of the resting-state pharma-fMRI (RS-PhfMRI). Significant changes were detected in regions that are expected to be associated with the CNS effects of these drugs; without *a priori* models and performance- or task-related confounds.

It is crucial to note the effects of global physiological variations due to morphine and alcohol. Previous studies have well documented that respiratory depression due to opiodergic drugs generates hypercapnic conditions that increases cerebral blood flow (MacIntosh *et al.*, 2008), and causes focal BOLD signal reduction in response to hypercapnic condition in the sensorimotor brain regions (Pattinson *et al.*, 2007). On the other hand, it has been shown that BOLD fluctuation correlating with respiration and heart rate can compromise delineation of the default mode network (Chang *et al.*, 2009; Chang and Glover, 2009b). Nevertheless, the impact of physiological noise on BOLD is nearly global and including the timecourse of physiological fluctuations do not drastically change the results of connectivity estimation (Birn *et al.*, 2006; Chang and Glover, 2009a). We observed a significant respiratory depression after morphine injection and an increase in heart rate due to alcohol. Our study does not address effect of physiological variables in dual regression outcome, but we have examined the effects of overall physiological variations (in terms of average respiration and average heart rates per each subjects per each session) on resting-state connectivity, and have shown that inclusion of physiological covariates in a repeated measures model does not change the main effects of the drug by time interaction. Interestingly, including respiration and heart rate in the model removes emerging connections from white matter to NOI4, and reduces the extent of morphine effect on connections to the NOI5, the default mode network, whose fluctuations seem to overlap in frequency with spontaneous BOLD changes in the default mode network (Birn *et al.*, 2006; Wise *et al.*, 2004). It should be noted that our framework is based on examining connectivity to eight NOI templates corresponding to the most consistently determined RSNs. These template networks are initially obtained from independent component analyses (CO). It has been suggested that the default-mode network obtained from ICA might not be fully respiration-proof (Birn *et al.*, 2008). It is therefore striking that modeling a global measure of respiration change at the group level analysis would demonstrate an effect expected from more careful analysis of the respiration spectra (Birn *et al.*, 2006; Wise *et al.*, 2004). We cannot preclude the possibility that variation in the temporal physiological profiles have a stronger explanatory power on effects of some of these networks, such as NOI4. Our aim in this study was to show that RS-fMRI is a sensitive and applicable method for detecting drug effects in the brain. Future studies that aim to validate RS-fMRI in relation to specific metabolic effects of especially opiodergic drugs should make precise recordings of variables such as end-tidal $CO_2$ or arterial $CO_2$ tension during the RS-fMRI session and incorporate them in such analyses.

It is worth mentioning that, without considering the time and the session effects in the model, we have detected significant associations between the heart rate and connectivity of several regions outside of NOI3 in relation to this network, in addition to changes in connectivity of a region near major arteries (circle of Willis) that also extends to the amygdala, in relation to NOI3, NOI4, NOI5, NOI6 and NOI8. By contrast, respiration changes affected connectivity to NOI4, NOI5, NOI6 and NOI7. An understanding of the neural or vascular substrates of physiological modulation is potentially crucial to interpretation of the neurological substrate of resting-state BOLD connectivity. Especially in pharmacological studies, the autonomic responses to the CNS drug are central to the research and these questions should be carefully considered in the study design. For instance, heart rate modulated the connectivity of amygdala to NOI4 (sensorimotor), NOI5 (default mode network), NOI6 (salience executive) and NOI8 (right dorsomedial visual stream). This observation is striking, since amygdalar association with visual-emotional (Critchley *et al.*, 2005; van Marle *et al.*, 2009) or executive-motor (Napadow *et al.*, 2008) modulation of the heart rate is previously reported. Regardless of neurobiological interpretations, our findings illustrate that different NOIs vary in degree of susceptibility to physiological modulation; and highlight a need for considering different statistical models to ensure important results are not obscured.

Our study overcomes some limitations in earlier pharmacological fMRI studies, and poses challenging questions. We have successfully detected effects reported in previous studies of morphine and alcohol—without subjectively delineating an ROI necessary for commonly used seed-based RS-fMRI analysis (Anand *et al.*, 2007; Hong *et al.*, 2009; Kelly *et al.*, 2009; Rack-Gomer *et al.*, 2009). It may be argued that predefined NOIs limit the scope of observations by restricting the criterion of connectivity to temporal similarity with a given NOI. However, these networks were chosen on the basis of previous studies that robustly reproduced them in entirely different population (Beckmann *et al.*, 2005; Damoiseaux *et al.*, 2006). By choosing these networks we avoided typical ambiguities associated with ICA approach



that depends on choosing the model order, or on subjective selection of similar or relevant components for further analysis. Nevertheless, we have detected significant changes in connectivity of regions such as the cerebellum and the hippocampus that are outside the reference NOIs. Therefore this method seems to be robust and less limiting than alternative ICA or ROI methods in detecting regions where the drug effects may not be expected. New approaches such as eigenvector centrality mapping (ECM) (Lohmann *et al.*, 2010) might bring us closer to the goal of total model-free analyses, but such methods are computationally more costly than dual-regression using pre-defined NOI templates, as we did. It would be interesting to compare results from ECM analysis and our proposed methodology.

An important advantage of resting-state connectivity, compared to other (more quantitative) neuroimaging methods is that it reveals the complexity of the temporal profile of drug effects in each region or interest. For instance, no significant connection is present between the hippocampus and the sensorimotor network (NOI4) under placebo or alcohol conditions, but during morphine infusion, a significant negative connectivity emerges. At the same time, morphine diminishes the connection of the supplementary motor area to NOI4 to zero. To date, the issue of negative connectivity remains a topic of debate (Murphy *et al.*, 2009). Several hypothetical explanations are possible: It may be argued that global vascular effects of morphine (especially due to respiratory depression) would give rise to the observed negative connectivity demonstrated in the hippocampus. However, these particular emerging negative correlations are anatomically confined to bilateral hippocampus, and although we cannot comment on the neurophysiological basis of the effect, they are unlikely to be non-specific artifacts. Another possibility is that reduction of physiological noise (e.g. due to respiratory depression due to morphine) would enhance negative correlations (Fox *et al.*, 2009). Alternatively, the negative connectivity in the hippocampus may correspond to a delayed, and perhaps adaptive function of this structure (Bast 2007; Khalili-Mahani *et al.*, 2010). We remind that these particular effects were not affected by inclusion of respiration and heart rate averages in the group-

level statistical model. Such inter-regional and inter-network heterogeneity of effects raise questions about compensatory and adaptive mechanisms that interact with the drug (which reflects interactions of different brain regions with each other); and underlines the added advantage of resting-state connectivity analysis compared to other neuroimaging modalities.

In conclusion, we have shown that drug-class specific effects on dynamics of brain function at resting-state can be detected even without *a priori* models. An important advantage of the resting-state Pharma-fMRI is that it is not confounded by differences in alertness or test strategies and other factors that influence the performance and outcomes of task-induced fMRI, and demonstrates effects even if no appropriate task is available. This study was designed to exhibit limited pharmacokinetic variability, and was hence unsuitable to investigate concentration-effect relationships, although the time profiles of the MRI-effects roughly followed those of the clinical responses. This study demonstrates that RS-fMRI could be useful for 'finger-printing' different drug actions within the same individual's brain. Notwithstanding the exploratory nature of the present study, and the inability to characterize the neurophysiological substrates of resting-state BOLD fluctuations, many of our results correspond to findings from previous quantitative PET studies. Because RS-fMRI is noninvasive, widely available and frequently repeatable, it may become an important biomarker for CNS-drug development, able to distinguish detailed drug-induced response patterns in healthy subjects and patients.





Anand A, Li Y, Wang Y, Gardner K, Lowe MJ. (2007). Reciprocal effects of antidepressant treatment on activity and connectivity of the mood regulating circuit: an FMRI study. J Neuropsychiatry Clin Neurosci 19(3):274-82.

Bast T (2007). Toward an integrative perspective on hippocampal function: from the rapid encoding of experience to adaptive behavior. Rev Neurosci 18(3-4):253-81.

Baumgartner U, Buchholz HG, Bellosevich A, Magerl W, Siessmeier T, Rolke R, Hohnemann S, Piel M, Rosch F, Wester HJ, Henriksen G, Stoeter P, Bartenstein P, Treede RD, Schreckenberger M. (2006). High opiate receptor binding potential in the human lateral pain system. Neuroimage 30(3):692-9.

Beckmann C, Mackay C, Filippini N, Smith S. (2009). Group comparison of resting-state FMRI data using multi-subject ICA and dual regression. NeuroImage 47(Supplement 1):1.

Beckmann CF, DeLuca M, Devlin JT, Smith SM. (2005). Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc Lond B Biol Sci 360(1457):1001-13.

Birn RM, Diamond JB, Smith MA, Bandettini PA. (2006). Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in FMRI. Neuroimage 31(4):1536-48.

Birn RM, Murphy K, Bandettini PA. (2008). The effect of respiration variations on independent component analysis results of resting state functional connectivity. Hum Brain Mapp 29(7):740-50.

Biswal BB, Mennes M, Zuo XN, Gohel S, Kelly C, Smith SM, Beckmann CF, Adelstein JS, Buckner RL, Colcombe S, Dogonowski AM, Ernst M, Fair D, Hampson M, Hoptman MJ, Hyde JS, Kiviniemi VJ, Kotter R, Li SJ, Lin CP, Lowe MJ, Mackay C, Madden DJ, Madsen KH, Margulies DS, Mayberg HS, McMahon K, Monk CS, Mostofsky SH, Nagel BJ, Pekar JJ, Peltier SJ, PETersen SE, Riedl V, Rombouts SA, Rypma B, Schlaggar BL, Schmidt S, Seidler RD, Siegle GJ, Sorg C, Teng GJ, Veijola J, Villringer A, Walter M, Wang L, Weng XC, Whitfield-Gabrieli S, Williamson P, Windischberger C, Zang YF, Zhang HY, Castellanos FX, Milham MP. (2010). Toward discovery science of human brain function. Proc Natl Acad Sci U S A 107(10):4734-9.

Blaha M, Aaslid R, Douville CM, Correra R, Newell DW. (2003). Cerebral blood flow and dynamic cerebral autoregulation during ethanol intoxication and hypercapnia. J Clin Neurosci 10(2):195-8.

Bond A, Lader M. (1974). Use of Analog Scales in Rating Subjective Feelings. British Journal of Medical Psychology 47(Sep):211-218.

Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. (1998). Psychedelic effects of ketamine in healthy volunteers - Relationship to steady-state plasma concentrations. Anesthesiology 88(1):82-88.

Breiter HC, Gollub RL, Weisskoff RM, Kennedy DN, Makris N, Berke JD, Goodman JM, Kantor HL, Gastfriend DR, Riorden JP, Mathew RT, Rosen BR, Hyman SE. (1997). Acute effects of cocaine on human brain activity and emotion. Neuron 19(3):591-611.

Britz J, Van De Ville D, Michel CM. (2010). BOLD correlates of EEG topography reveal rapid resting-state network dynamics. Neuroimage 52(4):1162-70.

Bullmore E, Sporns O. (2009). Complex brain networks: graph theoretical
analysis of structural and functional systems. Nat Rev Neurosci 10(3):186-98.

Chang C, Cunningham JP, Glover GH. (2009). Influence of heart rate on the BOLD signal: the cardiac response function. Neuroimage 44(3):857-69.

Chang C, Glover GH. (2009a). Effects of model-based physiological noise correction on default mode network anti-correlations and correlations. Neuroimage 47(4):1448-59.

Chang C, Glover GH. (2009b). Relationship between respiration, end-tidal CO2, and BOLD signals in resting-state fMRI. Neuroimage 47(4):1381-93.

Chuang KH, van Gelderen P, Merkle H, Bodurka J, Ikonomidou VN, Koretsky AP, Duyn JH, Talagala SL. (2008). Mapping resting-state functional connectivity using perfusion MRI. Neuroimage 40(4):1595-605.

Contet C, Kieffer BL, Befort K. (2004). Mu opioid receptor: a gateway to drug addiction. Curr Opin Neurobiol 14(3):370-8.

Critchley HD, Rotshtein P, Nagai Y, O'Doherty J, Mathias CJ, Dolan RJ. (2005). Activity in the human brain predicting differential heart rate responses to emotional facial expressions. Neuroimage 24(3):751-62.

Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF. (2006). Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci U S A 103(37):13848-53.

de Wit H, Metz J, Wagner N, Cooper M. (1990). Behavioral and subjective effects of ethanol: relationship to cerebral metabolism using PET. Alcohol Clin Exp Res 14(3):482-9.

Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, Beckmann CF, Mackay CE. (2009). Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci U S A 106(17):7209-14.

Fox MD, Snyder AZ, Vincent JL, Raichle ME. (2007). Intrinsic fluctuations within cortical systems account for intertrial variability in human behavior. Neuron 56(1):171-84.

Fox MD, Zhang D, Snyder AZ, Raichle ME. (2009). The global signal and observed anticorrelated resting state brain networks. J Neurophysiol 101(6):3270-83.
Gordon EL, Nguyen TS, Ngai AC, Winn HR. (1995). Differential effects of alcohols on intracerebral arterioles. Ethanol alone causes vasoconstriction. J Cereb Blood Flow Metab 15(3):532-8.
Greicius M. (2008). Resting-state functional connectivity in neuropsychiatric disorders. Curr Opin Neurol 21(4):424-30.
Hanchar HJ, Chutsrinopkun P, Meera P, Supavilai P, Sieghart W, Wallner M, Olsen RW. (2006). Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to alpha4/6beta3delta GABA A receptors. Proc Natl Acad Sci U S A 103(22):8546-51.
Honey G, Bullmore E. (2004). Human pharmacological MRI. Trends Pharmacol Sci 25(7):366-74.
Hong LE, Gu H, Yang Y, Ross TJ, Salmeron BJ, Buchholz B, Thaker GK, Stein EA. (2009). Association of nicotine addiction and nicotine's actions with separate cingulate cortex functional circuits. Arch Gen Psychiatry 66(4):431-41.
Jones AK, Qi LY, Fujirawa T, Luthra SK, Ashburner J, Bloomfield P, Cunningham VJ, Itoh M, Fukuda H, Jones T. (1991). In vivo distribution of opioid receptors in man in relation to the cortical projections of the medial and lateral pain systems measured with positron emission tomography. Neurosci Lett 126(1):25-8.
Kelly C, de Zubicaray G, Di Martino A, Copland DA, Reiss PT, Klein DF, Castellanos FX, Milham MP, McMahon K. (2009). L-dopa modulates functional connectivity in striatal cognitive and motor networks: a double-blind placebo-controlled study. J Neurosci 29(22):7364-78.
Khalili-Mahani N, Dedovic K, Engert V, Pruessner M, Pruessner JC. (2010). Hippocampal activation during a cognitive task is associated with subsequent neuroendocrine and cognitive responses to psychological stress. Hippocampus 20(2):323-34.
Koob GF, Roberts AJ, Schulteis G, Parsons LH, Heyser CJ, Hyytia P, Merlo-Pich E, Weiss F. (1998). Neurocircuitry targets in ethanol reward and dependence. Alcohol Clin Exp Res 22(1):3-9.
Kumar S, Porcu P, Werner DF, Matthews DB, Diaz-Granados JL, Helfand RS, Morrow AL. (2009). The role of GABA(A) receptors in the acute and chronic effects of ethanol: a decade of progress. Psychopharmacology (Berl) 205(4):529-64.
Lohmann G, Margulies DS, Horstmann A, Pleger B, Lepsien J, Goldhahn D, Schloegl H, Stumvoll M, Villringer A, Turner R. (2010). Eigenvector centrality mapping for analyzing connectivity patterns in FMRI data of the human brain. PLoS One 5(4):e10232.
Luksch A, Resch H, Weigert G, Sacu S, Schmetterer L, Garhofer G. (2009). Acute effects of intravenously administered ethanol on retinal vessel diameters and flicker induced vasodilatation in healthy volunteers. Microvasc Res 78(2):224-9.
MacIntosh BJ, Pattinson KT, Gallichan D, Ahmad I, Miller KL, Feinberg DA, Wise RG, Jezzard P. (2008). Measuring the effects of remifentanil on cerebral blood flow and arterial arrival time using 3D GRASE MRI with pulsed arterial spin labelling. J Cereb Blood Flow Metab 28(8):1514-22.
Maeda J, Suhara T, Kawabe K, Okauchi T, Obayashi S, Hojo J, Suzuki K. (2003). Visualization of alpha5 subunit of GABA A/benzodiazepine receptor by 11C Ro15-4513 using positron emission tomography. Synapse 47(3):200-8.
Mantini D, Perrucci MG, Del Gratta C, Romani GL, Corbetta M. (2007). Electrophysiological signatures of resting state networks in the human brain. Proc Natl Acad Sci U S A 104(32):13170-5.
Mearadji B, Straathof JWA, Biemond I, Lamers CBHW, Masclee AAM. (1998). Effects of somatostatin on proximal gastric motor function and visceral perception. Alimentary Pharmacology & Therapeutics 12(11):1163-1169.
Murphy K, Birn RM, Handwerker DA, Jones TB, Bandettini PA. (2009). The impact of global signal regression on resting state correlations: are anti-correlated networks introduced? Neuroimage 44(3):893-905.
Napadow V, Dhond R, Conti G, Makris N, Brown EN, Barbieri R. (2008). Brain correlates of autonomic modulation: combining heart rate variability with fMRI. Neuroimage 42(1):169-77.
Nichols TE, Holmes AP. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp 15(1):1-25.
O'Connor S, Morzorati S, Christian J, Li TK. (1998). Clamping breath alcohol concentration reduces experimental variance: application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcohol Clin Exp Res 22(1):202-10.
Pattinson KT, Rogers R, Mayhew SD, Tracey I, Wise RG. (2007). Pharmacological FMRI: measuring opioid effects on the BOLD response to hypercapnia. J Cereb Blood Flow Metab 27(2):414-23.
Rack-Gomer AL, Liau J, Liu TT. (2009). Caffeine reduces resting-state BOLD functional connectivity in the motor cortex. Neuroimage 46(1):56-63.
Sadzot B, Price JC, Mayberg HS, Douglass KH, Dannals RF, Lever JR, Ravert HT, Wilson AA, Wagner HN, Jr., Feldman MA, *et al.* (1991). Quantification of human opiate receptor concentration and affinity using high and low specific activity [11C]diprenorphine and positron emission tomography. J Cereb Blood Flow Metab 11(2):204-19.
Sano M, Wendt PE, Wirsen A, Stenberg G, Risberg J, Ingvar DH. (1993). Acute effects of alcohol on regional cerebral blood flow in man. J Stud Alcohol 54(3):369-76.
Sarton E, Olofsen E, Romberg R, den Hartigh J, Kest B, Nieuwenhuijs D, Burm A, Teppema L, Dahan A. (2000). Sex differences in morphine analgesia: an experimental study in healthy volunteers. Anesthesiology 93(5):1245-54; discussion 6A.
Schreckenberger M, Amberg R, Scheurich A, Lochmann M, Tichy W, Klega A, Siessmeier T, Grunder G, Buchholz HG, Landvogt C, Stauss J, Mann K, Bartenstein P, Urban R. (2004). Acute alcohol effects on neuronal and attentional processing: striatal reward system and inhibitory sensory interactions under acute ethanol challenge. Neuropsychopharmacology 29(8):1527-37.
Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, Reiss AL, Greicius MD. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci 27(9):2349-56.
Shmueli K, van Gelderen P, de Zwart JA, Horovitz SG, Fukunaga M, Jansma JM, Duyn JH. (2007). Low-frequency fluctuations in the cardiac rate as a source of variance in the resting-state fMRI BOLD signal. Neuroimage 38(2):306-20.
Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, Filippini N, Watkins KE, Toro R, Laird AR, Beckmann CF. (2009). Correspondence of the brain's functional architecture during activation and rest. Proc Natl Acad Sci U S A 106(31):13040-5.
Stein EA, Pankiewicz J, Harsch HH, Cho JK, Fuller SA, Hoffmann RG, Hawkins M, Rao SM, Bandettini PA, Bloom AS. (1998). Nicotine-induced limbic cortical activation in the human brain: a functional MRI study. Am J Psychiatry 155(8):1009-15.
van Marle HJ, Hermans EJ, Qin S, Fernandez G. (2009). From specificity to sensitivity: how acute stress affects amygdala processing of biologically salient stimuli. Biol Psychiatry 66(7):649-55.
Volkow ND, Ma Y, Zhu W, Fowler JS, Li J, Rao M, Mueller K, Pradhan K, Wong C, Wang GJ. (2008). Moderate doses of alcohol disrupt the functional organization of the human brain. Psychiatry Res 162(3):205-13.
Volkow ND, Wang GJ, Franceschi D, Fowler JS, Thanos PP, Maynard L, Gatley SJ, Wong C, Veech RL, Kunos G, Kai Li T. (2006). Low doses of alcohol substantially decrease glucose metabolism in the human brain. Neuroimage 29(1):295-301.
Wagner KJ, Willoch F, Kochs EF, Siessmeier T, Tolle TR, Schwaiger M, Bartenstein P. (2001). Dose-dependent regional cerebral blood flow changes during remifentanil infusion in humans: a positron emission tomography study. Anesthesiology 94(5):732-9.
Wang GJ, Volkow ND, Franceschi D, Fowler JS, Thanos PK, Scherbaum N, Pappas N, Wong CT, Hitzemann RJ, Felder CA. (2000). Regional brain metabolism during alcohol intoxication. Alcohol Clin Exp Res 24(6):822-9.
Wise RG, Ide K, Poulin MJ, Tracey I. (2004). Resting fluctuations in arterial carbon dioxide induce significant low frequency variations in BOLD signal. Neuroimage 21(4):1652-64.
Zoethout RW, Schoemaker RC, Zuurman L, van Pelt H, Dahan A, Cohen AF, van Gerven JM. (2009). Central nervous system effects of alcohol at a pseudo-steady-state concentration using alcohol clamping in healthy volunteers. Br J Clin Pharmacol 68(4):524-34.
Zoethout RW, van Gerven JM, Dumont GJ, Paltansing S, van Burgel ND, van der Linden M, Dahan A, Cohen AF, Schoemaker RC. (2008). A comparative study of two methods for attaining constant alcohol levels. Br J Clin Pharmacol 66(5):674-81.
Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA, Stohler CS. (2001). Regional mu opioid receptor regulation of sensory and affective dimensions of pain. Science 293(5528):311-5.
Zuo XN, Kelly C, Adelstein JS, Klein DF, Castellanos FX, Milham MP. (2009). Reliable intrinsic connectivity networks: Test-retest evaluation using ICA and dual regression approach. Neuroimage. 49:2163–2177.

**FIGURE 1** SCHEMATIC PRESENTATION OF STUDY DESIGN AND DATA COLLECTION *(full colour version inside cover)*

*Each subjects participated in three randomized sessions. Both examiners and the subject were blind to which drug they received. The expected pharmacokinetic profiles for alcohol and morphine are plotted (1). The placebo infusion was conducted concurrently with the drug infusion. After preprocessing (2) dual regression on all data sets to estimate whole-brain connectivity in relation to the eight template RSNs (3), followed by statistical testing (4).*

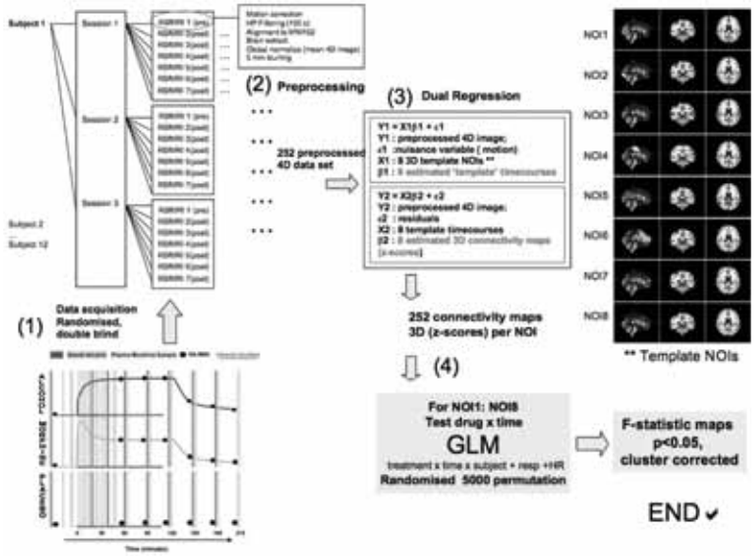



**FIGURE 2** PHARMACOKINETIC EFFECTS

*(a) Plasma morphine concentrations for each subject; (b) Breath alcohol readouts. The bars illustrate when the RS-fMRI acquisition took place.*

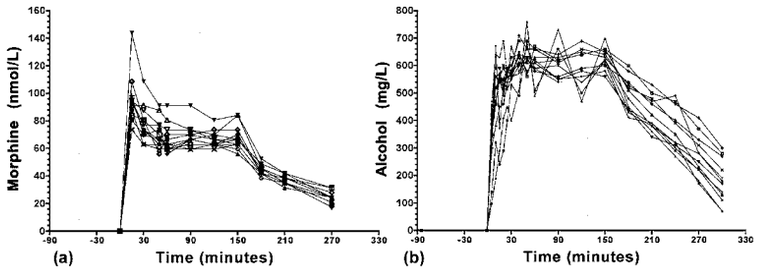



**FIGURE 3** PHARMACODYNAMIC EFFECTS OVER TIME (MEAN ± SEM)

*See table 1 for details of main effects.*

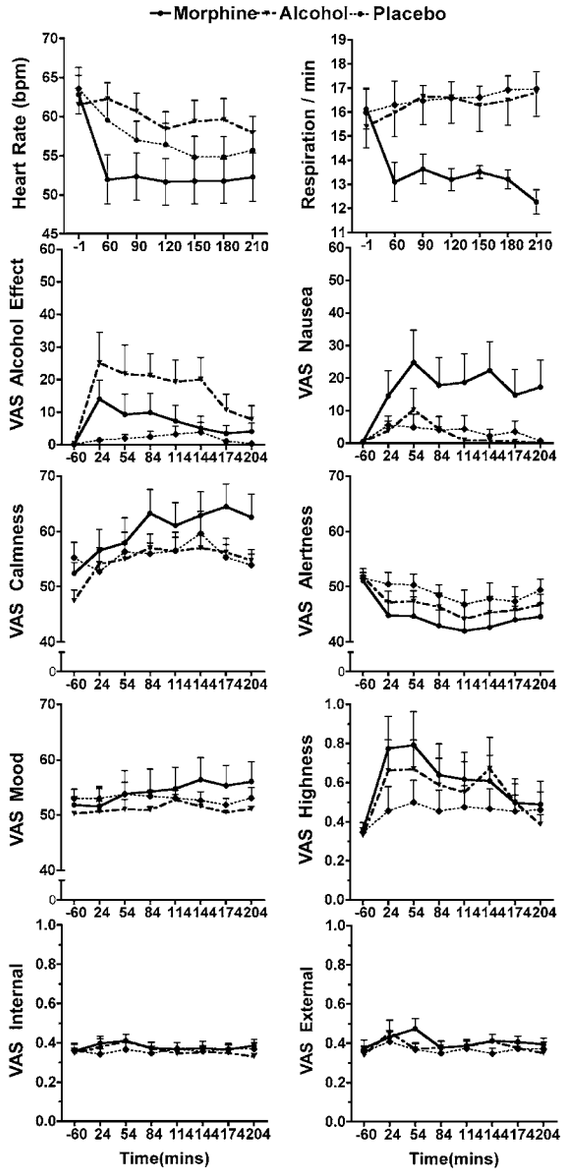


**FIGURE 4** OVERLAYING MAPS OF VOXEL-WISE REGRESSION OF RESPIRATION (RESP) AND HEART RATE (HR) WITH CONNECTIVITY TO THE 8 TEMPLATE NETWORKS *(full colour version inside cover) F-statistics; cluster corrected p<0.05 in GLM, including treatment by time interactions.*

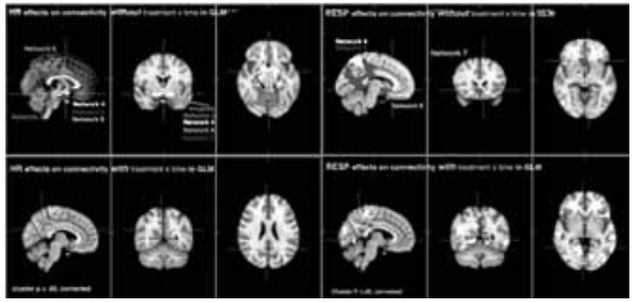


**FIGURE 5** OVERLAYING MAPS OF DRUG BY TIME INTERACTIONS WITH CONNECTIVITY TO THE 8 TEMPLATE NETWORKS *(full colour version inside cover) F-statistics voxel-wise p < 0.0001; cluster corrected p<0.05; average physiological rates included as covariates. The NOIs are represented in pale red, morphine effects in green and alcohol effects in blue. table 2 for the effects details. WM, working memory; DVS, dorsal visual stream.*

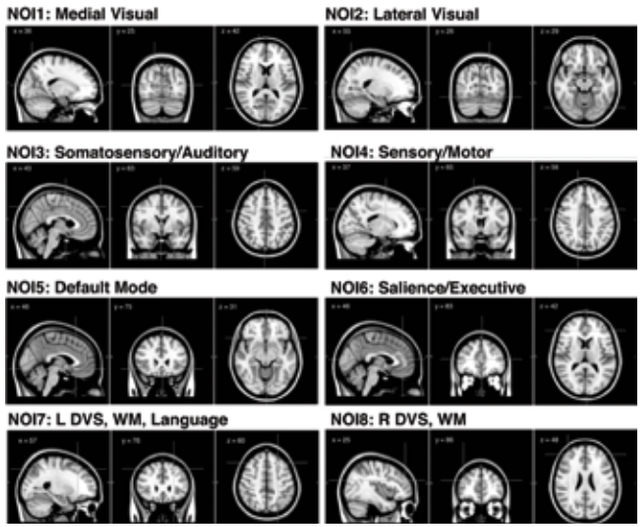





**FIGURE 6** PROFILE OF CHANGE IN RESTING-STATE CONNECTIVITY IN SELECT ROI'S *(full colour version inside cover)*
*Statistical parametric maps (t-statistics, cluster corrected p<0.05) reflect the amplitude of drug-induced changes in functional connectivity (drug versus placebo over time). Morphine leads to increased negative connectivity of the hippocampus (a), and positive connectivity of the central gyri (b) in relation to the sensory-motor network (NOI4). Alcohol enhances the intra-connectivity of the visual network (c) and disrupts adaptive connectivity of brainstem (d). In the ROI diagrams, red corresponds to placebo, green to morphine and blue to alcohol.*

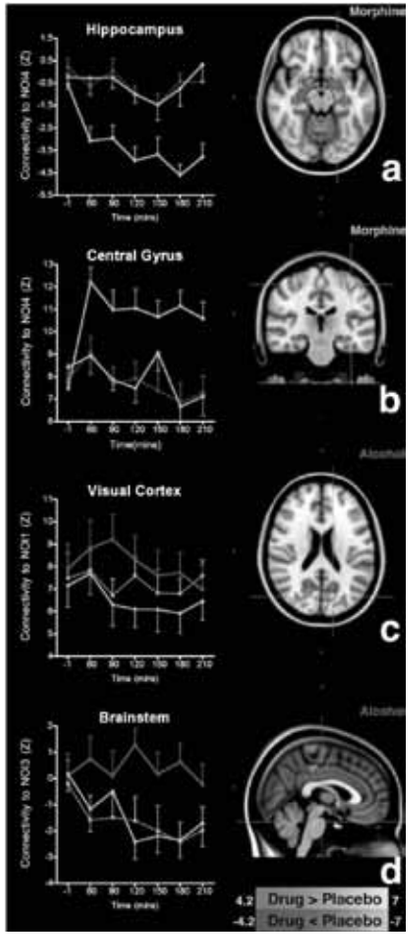


**TABLE 1** PHARMACODYNAMIC EFFECTS

| Pharmacodynamic Effects | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Least Square Means | | | | Contrasts | | |
| Parameter | Placebo | Alcohol | Morphine | Treatment P-value | Alcohol vs Placebo | Morphine vs Placebo | Morphine vs Alcohol |
| VAS Alcohol effects (mm) | 1.7 | 19.1 | 7.2 | 0.0096 | 17.4 (6.7, 28.2) p=0.0030 | 5.5 (-5.1, 16.2) p=0.2907 | -11.9 (-22.7, -1.1) p=0.0321 |
| VAS Alertness (mm) | 48.6 | 46.0 | 43.7 | 0.1843 | -2.6 (-8.0, 2.8) p=0.3236 | -5.0 (-10.3, 0.4) p=0.0696 | -2.3 (-7.7, 3.0) p=0.3742 |
| VAS Calmness (mm) | 53.4 | 58.2 | 60.6 | 0.0428 | 4.7 (-1.5, 11.0) p=0.1323 | 7.2 (1.6, 12.7) p=0.0136 | 2.5 (-3.3, 8.2) p=0.3855 |
| VAS Mood (mm) | 51.7 | 52.8 | 54.4 | 0.5171 | 1.1 (-4.1, 6.2) p=0.6623 | 2.8 (-2.2, 7.8) p=0.2618 | 1.7 (-3.4, 6.7) p=0.4982 |
| VAS Nausea (mm) | 3.7 | 3.0 | 18.6 | 0.0320 | -0.6 (-13.5, 12.2) p=0.9179 | 14.9 (2.1, 27.8) p=0.0247 | 15.6 (2.8, 28.4) p=0.0198 |
| VAS External log(mm) | 0.371 | 0.398 | 0.406 | 0.4601 | 0.027 (-.035, 0.089) p=0.3650 | 0.035 (-.026, 0.097) p=0.2413 | 0.008 (-.054, 0.071) p=0.7826 |
| VAS Internal log(mm) | 0.359 | 0.366 | 0.382 | 0.4592 | 0.007 (-.033, 0.046) p=0.7245 | 0.023 (-.017, 0.062) p=0.2359 | 0.016 (-.024, 0.056) p=0.3934 |
| VAS feeling high log(mm) | 0.467 | 0.575 | 0.635 | 0.5275 | 0.107 (-.202, 0.417) p=0.4766 | 0.168 (-.141, 0.476) p=0.2706 | 0.060 (-.251, 0.371) p=0.6908 |
| Heart Rate | 55 | 61 | 52 | 0.0004 | 5.2 (1.4, 9.0) p=0.0098 | -3.8 (-7.5, 0) p=0.0498 | -9.0 (-12.7, -5.2) p<0.0001 |
| Respiration | 16.6 | 16.6 | 13 | <0.0001 | 0.0 (-1.4 1.4) p = 0.9659 | -3.6 (-5, -2.2) p < 0.0001 | -3.6 (-5, -2.2) p<0.0001 |



**TABLE 2** DETAILS OF DRUG EFFECTS ON FUNCTIONAL CONNECTIVITY

*(cluster corrected threshold, p <0.05)*

| Network | Morphine | | | | | | Ethanol | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Location | #voxels | t | x | y | z | Location | #voxels | t | x | y | z |
| **NO11: Medial Visual Network** relaying visual input through thalamus to primary visual area. Includes, Calcarine, inferior precuneus, and primary visual cortex. | R cerebellum | 149 *** | 4.77 | 27 | 28 | 16 | R visual | 140 *** | 4.78 | 35 | 27 | 41 |
| | L cerebellum | 101 ** | 4.8 | 64 | 30 | 15 | L visual | 100 ** | 5.57 | 52 | 26 | 46 |
| | R hippocampus | 65 ** | 5.05 | 33 | 50 | 29 | L parietal lobule | 67 ** | -4.91 | 59 | 31 | 56 |
| | R superior frontal | 87 ** | 5.87 | 37 | 88 | 50 | | | | | | |
| | Precentral gyrus | 26* | 4.88 | 44 | 53 | 71 | | | | | | |
| **NO12: Lateral visual Network** visual spatial attention. | R occipital V5 | 91 ** | 5.52 | 23 | 26 | 40 | R cuneus | 56 ** | 4.9 | 22 | 33 | 21 |
| | L occipital V4 | 70 ** | 4.87 | 57 | 20 | 29 | Precuneus | 24* | 4.45 | 46 | 32 | 43 |
| | L posterior insula | 45* | -5.24 | 65 | 51 | 45 | | | | | | |
| | R superior frontal | 33* | -4.83 | 38 | 88 | 51 | | | | | | |
| **NO13: Superior Temporal Network** auditory, somatosensory and autonomic functions includes insular and dorsocaudal anterior cingualte cortices. | L precuneus | 348 *** | -5.78 | 45 | 29 | 53 | Bi posterior cingulated | 728 *** | 5.3 | 45 | 50 | 59 |
| | R paracingulate | 322 *** | -4.96 | 44 | 67 | 60 | R superior parietal lobule | 310 *** | 5.86 | 27 | 39 | 58 |
| | R cerebellum | 96 ** | -4.57 | 39 | 45 | 26 | Left cerebellum | 167 *** | 4.97 | 49 | 42 | 25 |
| | L cerebellum (tonsil) | 87 ** | -5.31 | 54 | 42 | 13 | Bi culmen | 153 *** | 5.73 | 45 | 37 | 15 |
| | L cerebellum (culmen) | 74 ** | -4.59 | 50 | 44 | 25 | Right cerebellum | 144 *** | 5.03 | 41 | 42 | 25 |
| | L amygdala | 63 ** | -4.6 | 54 | 62 | 25 | brainstem | 144 ** | 6.29 | 43 | 54 | 23 |
| | R amygdala | 40* | -4.28 | 35 | 64 | 25 | L supramarginal gyrus | 109 ** | 5.61 | 68 | 45 | 59 |
| | L frontal pole | 46* | -4.75 | 57 | 86 | 52 | L precuneus | 45* | 4.92 | 47 | 35 | 41 |
| | L occipital superior | 38* | -4.77 | 56 | 26 | 62 | R supramarginal gyrus | 38* | 5.44 | 18 | 45 | 59 |
| | R occipital superior | 44* | -4.37 | 37 | 26 | 58 | L orbitofrontal | 34* | 5.16 | 70 | 76 | 32 |
| | R postcentral | 53* | -4.58 | 18 | 59 | 57 | R anterior cingulate | 22* | 4.80 | 39 | 86 | 36 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **N014: Sensory Motor Network** includes pre- and postcentral somatosensory somatomotor areas | L primary somatosensory cortex | 1774 *** | 7.52 | 65 | 51 | 65 | R cerebellum & fusiform | 223 *** | -5.74 | 27 | 40 | 23 |
| | L hippocampus | 1679*** | -7.61 | 55 | 54 | 29 | L dorsocaudal anterior cingulate | 132 ** | -4.02 | 39 | 61 | 59 |
| | R hipocampus | | -6.06 | 32 | 56 | 29 | R inferior parietal lobule | 112 ** | -4.79 | 19 | 47 | 49 |
| | L putamen | | -4.3 | 58 | 58 | 41 | R precentral R | 98** | -5.3 | 49 | 65 | 56 |
| | L amygdala | | -6.27 | 37 | 61 | 27 | | | | | | |
| | R amygdala | | -6.02 | 52 | 61 | 28 | | | | | | |
| | L caudate | | -4.9 | 52 | 64 | 47 | | | | | | |
| | Bi primary motor | 1001 | 7.18 | 45 | 52 | 66 | | | | | | |
| | L cerebellum VI | **851** | -7.95 | 52 | 30 | 27 | | | | | | |
| | Vermis | | -5.37 | 45 | 29 | 28 | | | | | | |
| | L cerebellum VI | | -5.3 | 39 | 30 | 28 | | | | | | |
| | R primary somatosensory | 722 | 5.7 | 25 | 51 | 62 | | | | | | |
| | R putamen | **668** | -6.28 | 32 | 58 | 41 | | | | | | |
| | R caudate | | -5.53 | 39 | 64 | 46 | | | | | | |
| | L occipital fusiform | 494 | 6.11 | 54 | 19 | 31 | | | | | | |
| | R occipital fusiform | 314 | 6.01 | 33 | 23 | 30 | | | | | | |
| | L posterior insula | 122 | 5.39 | 63 | 52 | 45 | | | | | | |
| | R posterior insula | 84 | 5.12 | 23 | 56 | 44 | | | | | | |
| | thalamus | 87 | -4.64 | 45 | 52 | 41 | | | | | | |
| | Brainstem | 95 | 5.25 | 49 | 47 | 16 | | | | | | |
| | Paracingulate gyrus | 61 | 5.53 | 47 | 80 | 50 | | | | | | |
| **N015: Default Mode Network** Includes rostral medial prefrontal and posterior medial parietal precuneal and PCC areas | L subgen ACC | 40* | -5.27 | 47 | 76 | 31 | | | | | | |
| | L middle front | 40* | -4.81 | 61 | 76 | 59 | | | | | | |
| | Posterior cingulated | 37* | -4.53 | 45 | 40 | 48 | | | | | | |



| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **N016: Prefrontal Network** Includes medial and inferior prefrontal cortices. Regions in this network are implicated in executive control, attention and pain. Includes medial and inferior prefrontal cortices. Regions in this network are implicated in executive control, attention and pain. | Anterior cingulate | 490 *** | -6.47 | 45 | 85 | 41 | | | | | | |
| | L frontal pole | 392*** | -6.31 | 61 | 94 | 36 | | | | | | |
| | R frontal pole | 350*** | -6.65 | 30 | 89 | 50 | | | | | | |
| | R primary motor | 212 *** | -5.7 | 39 | 49 | 69 | | | | | | |
| | L insula | 115 ** | -6.13 | 63 | 71 | 33 | | | | | | |
| | Bi thalamus | 101** | -5.42 | 45 | 62 | 42 | | | | | | |
| | R lingual | 63** | -5.32 | 46 | 18 | 32 | | | | | | |
| | R inferior parietal lob | 50* | -4.82 | 21 | 39 | 51 | | | | | | |
| | L orbitofrontal | 42* | -5.43 | 54 | 71 | 27 | | | | | | |
| | L primary motor | 40* | -5.49 | 50 | 49 | 70 | | | | | | |
| | L lingual | 36* | -4.99 | 52 | 30 | 36 | | | | | | |
| **N017: Left Dorsal Visual Stream Network** Includes Broca rea 44, frontal pole, dorsolateral prefrontal cortex and parietal lobule. Working memory, visual spatial processing and language | L superior frontal | 31* | 4.83 | 46 | 91 | 45 | | | | | | |
| | R frontal pole | 81** | -5.56 | 25 | 86 | 48 | | | | | | |
| | R middle frontal | 55** | -4.8 | 32 | 73 | 63 | | | | | | |
| **N018: Right Dorsal Visual Stream Network** Includes homologous regions as N017, plus right paracingulate gyrus, left posterior cingulated and left medial prefrontal cortex. | L superior frontal | 270 *** | -5.16 | 56 | 75 | 58 | L superior frontal | 79 ** | -5.64 | 55 | 74 | 59 |
| | L basolateral PFC | 243 *** | -5.22 | 67 | 84 | 34 | | | | | | |
| | L superior parietal lobule | 160 *** | -5.36 | 62 | 35 | 60 | | | | | | |
| | Paracingulate, medial prefrontal | 144 *** | -5.29 | 47 | 80 | 56 | | | | | | |
| | R primary somatosensory | 63 ** | -4.83 | 21 | 46 | 63 | | | | | | |
| | L sup middle frontal | 52 ** | -5.5 | 28 | 67 | 63 | | | | | | |
| | R thalamus | 35* | -4.71 | 40 | 53 | 42 | | | | | | |

Cluster significance: * p < 0.05; ** p<0.01; *** p<0.001
Voxel dimension is 2mm x 2mm x 2mm (voxel volume 0.008ml)

# CHAPTER 7

## *The effects of TPA023, A $GABA_A$ α2,3 subtype-selective partial agonist, on essential tremor in comparison with alcohol*

*Journal of Psychopharmacology 2012, Feb; 26(2), 282-91*

R.W.M. Zoethout, S.L. de Haas, K. Van Dyck, M. De Smet,
L.B. Rosen, M.G. Murphy, K.M. Gottesdiener, R.C. Schoemaker,
A.F. Cohen, J.M.A. van Gerven *(first two authors contributed equally)*

## ABSTRACT

BACKGROUND Essential tremor (ET) is a relatively frequent neurological disorder that responds in some patients to $GABA_A$ agonists (like benzodiazepines). Partial subtype selective $GABA_A$-agonists may have an improved side effect profile compared to non-selective $GABA_A$ agonists. However, it is unknown which $GABA_A$ subtypes are involved in the therapeutic effects of benzodiazepines in ET.

METHODS The effects of 2 mg TPA023, a $GABA_A$ α2,3 selective partial agonist on ET were compared to the effects of a stable alcohol level (0.6 $g \cdot L^{-1}$) and placebo in nine ET-patients. Tremor evaluation included laboratory accelerometry and a performance-based scale. Additional measurements were performed to evaluate other effects on the central nervous system (CNS).

RESULTS Alcohol significantly diminished tremor symptoms in the postural and kinetic condition, as assessed by laboratory accelerometry, but the performance-based rating scale was unaffected. Tremor was also reduced after TPA023 treatment in the kinetic condition, albeit not significantly. Additionally, TPA023 decreased saccadic peak velocity, while alcohol decreased subjective feelings of alertness.

CONCLUSIONS This study showed that alcohol reduced maximum tremor power, as assessed by laboratory accelerometry, unlike TPA023, which decreased tremor symptoms to some extent, but not significantly. This study showed that treatment with an α2,3 subunit selective $GABA_A$ partial agonist was less effective compared to a stable level of alcohol in reducing ET-symptoms. These results provide no support for a therapeutic role of TPA023 in the suppression of ET-symptoms.


## INTRODUCTION

Essential tremor (ET) is one of the most common movement disorders (Pahwa and Lyons, 2003). The age- and gender-adjusted prevalence of ET is estimated to be 3 to 4 per 1000, with an annual incidence of 23.7 per 100,000. Approximately 4% of adults in the age group above 40 years are affected by ET (Zesiewicz *et al.*, 2010). ET has a 4-12 Hz frequency that predominantly affects the upper extremities, may also affect the head and voice, and rarely affects the legs. In contrast to resting tremor in Parkinson disease, essential tremor is characterised by postural and kinetic components (Elble, 2000). Although its cause is unknown the central oscillatory systems at the level of the inferior olivary nucleus of the medulla oblongata seem to play an important role in the pathophysiology of ET (Deuschl and Elble, 2000). Current therapy for ET includes beta blockers, phenobarbital (a primidone metabolite), benzodiazepines, and some antiepileptic agents. Treatment is symptomatic, effective in no more than roughly half of the patients, and often limited by side effects (Chen and Swope, 2003).

Preclinical and clinical studies suggest that a GABAergic pharmacologic agent could be effective in the treatment of ET (Louis, 1999). Double-blind studies have demonstrated efficacy of alprazolam versus placebo in treating ET (Gunal *et al.*, 2000; Huber and Paulson, 1988). Different $GABA_A$ receptor subtypes have been identified, and pre-clinically linked to well-known effects of $GABA_A$ agonists like sedation (α1) (Rudolph *et al.*, 1999), muscle relaxation (α2) (Rowlett *et al.*, 2005), anxiolysis (α2,3) (Atack *et al.*, 2005; Rudolph *et al.*, 2001) and memory impairment (α5) (Collinson *et al.*, 2002). Among these subtypes, the α2-receptor seems the most promising target for anti-tremor activity, although the exact mechanism of the therapeutic activity of GABA agonists in essential tremor is unknown. TPA023 is being developed as a $GABA_A$ α2,3 selective partial agonist. Based on the selective properties of this compound, it is believed to be a muscle relaxant (and anxiolytic) without causing sedation and instability. Healthy volunteer studies have shown the selective effect profile in comparison with lorazepam (de Haas *et al.*, 2007). Investigating the effects of this novel selective partial $GABA_A$ agonist on essential tremor might reveal new information about the pathophysiology of this disease, and possibly identify a new treatment modality.

Alcohol, which is an indirect agonist of the $GABA_A$-receptor (Santhakumar *et al.*, 2007) relieves tremor symptoms in an estimated 50 to 90% of ET patients (Rajput *et al.*, 1975; Deuschl, 1999) by reducing tremor

amplitude without affecting frequency (Koller and Biary, 1984). It most likely acts via a reduction of cerebellar over-activity, which results in reduced tremor amplitude, whereas the frequency is not altered (Koller and Biary, 1984; Koller *et al.*, 1994; Koller, 1991). In this study, alcohol was chosen as a positive control, using a novel clamping technique that was based on earlier publications (O'Connor *et al.*, 1998). Ideally, a novel therapy for ET would closely approach the tremor reducing effects of alcohol. Since the duration of action of TPA023 was estimated to be four hours, the clamping procedure took place during a similar period. To maximize the power of alcohol as a positive control, only patients who were familiar with the positive effects of alcohol on their symptoms were included in this project.

In this exploratory study, the novel α2,3-selective $GABA_A$ partial agonist TPA023 was compared with intravenous alcohol and placebo for their effects on tremor symptoms. A battery of Central Nervous System (CNS) function tests including body sway, eye movements, Visual Analogue Scales and adaptive tracking was also performed. The objectives were to distinguish between general CNS pharmacodynamic effects of the $GABA_A$ α2,3-selective drugs and tremor-specific effects in the accelerometry recordings, in a relatively small study.

## METHODS

### *Design*

This exploratory study was performed in a double-blind, double-dummy, randomized, placebo-controlled, 3-period, crossover fashion in nine patients diagnosed with essential tremor, with at least a five-day washout period.

### *Subjects*

Nine patients with essential tremor were recruited from the hospital database of the Leiden University Medical Centre, the Dutch patients' association and by advertising in local newspapers. Subjects were informed about the contents of the study during an information visit. When they had decided to participate, subjects visited the research unit for a medical screening. After signing informed consent, they were medically screened to evaluate eligibility for study participation. A neurologist (JvG) diagnosed essential tremor (ET) of the hands and forearms according to the diagnostic criteria for 'classic ET' defined by the consensus statement of the Movement Disorder Society (Deuschl *et al.*, 1998), adapted from previous criteria established by the Tremor Research Investigation Group (TRIG) (Deuschl *et al.*, 1995), as well as a more recent study indicating the importance of kinetic tremor (Brennan *et al.*, 2002). An isolated head tremor was not allowed.

Only subjects who stated a positive effect of alcohol on their tremor symptoms were included in the study. Subjects had to refrain from alcohol 48 hours prior to a treatment day and caffeine-containing products for at least 12 hours before treatment. Subjects were not allowed to use their own anti-tremor medications and had to abstain from grapefruit (juice) and St John's Wort for at least 2 weeks before the start until completion of the study, because these substances have stimulating and inhibiting effects on CYP3A4, respectively. They were not allowed to drink more than six units of caffeine-containing products or three units of alcohol per day or smoke more than five cigarettes per day during the total study period. On treatment days, the use of caffeine-containing products or smoking was not allowed. The study was approved by the Medical Ethics Review Board of Leiden University Medical Centre, and performed according to their standards.

### *Study treatments*

Each patient received a single oral dose of TPA023 2 mg or matching placebo. Also on each study day, an alcohol (10% in 5% glucose) or sham placebo (5% glucose) clamping procedure was performed. Only the person who was responsible for the clamping method was unblinded for alcohol infusion due to measurement of the breath alcohol concentrations (BrAC). This person was not involved in any other part of the study.

Alcohol was infused using a recently developed clamping method, in which the BrAC was used to guide intravenous dosing (Zoethout *et al.*, 2008). An alcohol level of 0.6 g·L$^{-1}$ was chosen, because this was expected to cause a significant tremor reduction in the majority of patients. Previous studies have shown that mean blood alcohol levels of 0.35 and 0.50 g·L$^{-1}$ had tremor diminishing effects, but this was after a single oral dose (Zeuner *et al.*, 2003). Levels of 0.6 g·L$^{-1}$ are routinely achieved during social drinking, without causing too many adverse effects.

In previous studies, the 0.6 g·L$^{-1}$ alcohol clamp was well-tolerated and produced statistically significant pharmacodynamic CNS effects (Zoethout *et al.*, 2009). It was estimated that the duration of action of TPA023 would be approximately four hours (de Haas *et al.*, 2007). To optimize the value of alcohol as a positive control, a clamping period of four hours was chosen for this study.

## *Safety*

Adverse events, ECG, blood pressure and heart rate measurements were assessed throughout the study. ECGs were assessed with a Cardiofax, equipped with ECAPS12 analysis program (Nihon Kohden, Japan). Blood pressure and heart rate were measured with an automated blood pressure monitor (MPV1072, Nihon Kohden, Japan), showing an average value for two sequential (duplicate) measurements at each time point. All safety measurements were made after sitting in a semi-recumbent position for at least 5 minutes.

## *Alcohol concentrations*

Breath alcohol (BrAC) samples were performed using the hand-held Alco-Sensor IV meter (Honac, Apeldoorn, The Netherlands). To reduce fatiguing of the Alco-Sensor meter, a minimum interval of approximately 10 minutes was maintained between BrAC samplings, by alternating two different measurement devices. A recent study using the described alcohol clamping technique shows similar findings for breath alcohol and blood alcohol levels (Zoethout *et al.*, 2008). Therefore, no pharmacokinetic blood samples were obtained for alcohol and only the breath alcohol samples were used for further pharmacokinetic analysis.

## *Pharmacodynamics*

TREMOR EVALUATION Tremor evaluations were performed at screening, predose (within 60 minutes prior to dosing) and 60, 150, 240, 330, 420 minutes postdose on each study day.

QUESTIONNAIRE/EVALUATION SCALE The Tremor Disability Questionnaire was only performed at screening and was executed to assess the level of disability due to ET according to the patients' opinion. This is a 36-item, 10 minute questionnaire that was designed in 1997 for the CADET study (Wendt *et al.*, 2000). It has shown substantial test-retest reliability and was validated against multiple other endpoints, including a neurologist's clinical ratings, the performance-based test of function, and quantitative computerized tremor analyses (Louis *et al.*, 2000). The total score of this questionnaire ranges from 0 (no disability) to 100 (completely disabled).

Additionally, a Performance-Based Tremor Evaluation (PBTE) was performed (Louis *et al.*, 1999). The test included the performance of 15 activities that were scored by a trained measurement assistant from 0 (no difficulty) to 4 (unable to perform), and the total score was converted to a percentage ranging from 0 (no disability) to 100 (maximally impaired) (Louis *et al.*, 1999).

LABORATORY TREMOGRAPHY Tremor was evaluated according the methodology of Gironell *et al.* (Gironell *et al.*, 1999) using three miniature linear piezo-electric accelerometers (Nihon Kohden, MT-3T), which were attached to the distal end of a clamp, above the fingertips of the dominant arm. The accelerometers were placed at right angles to one another to enable three-dimensional analysis of movement (Gironell *et al.*, 1999; Van Hilten *et al.*, 1991). An EMG recording of the flexor and extensor forearm muscles

was also obtained with silver-silver chloride electrodes applied 2cm apart at the belly of the muscles. The signals were amplified by use of a Grass 15LT (15A54/15A94), with a time constant of 1 second and a low pass filter at 100 Hz. For the fast Fourier analysis, data collection and analysis were performed using customized CED software (Cambridge Electronics Design, Cambridge, UK). The upper limb tremor was recorded in three positions, each held for a 60-second interval: (1) at rest, with the arm hanging relaxed along the body, (2) postural, with the arm held in an outstretched, horizontal, prone position and (3) kinetic, moving the hand from a set point to the nose (back and forth). Tremor was quantified by a power spectrum analysis to determine the dominant frequency peak (Hz) and the magnitude of the accelerometer signal (absolute power of the dominant frequency peak in μV).

#### CNS MEASUREMENTS

Pharmacodynamic measurements were performed predose (within 60 minutes prior to dosing) and 30, 120, 210, 300, 390 and 480 minutes postdose. Subjects underwent pharmacodynamic tests individually in a quiet room with ambient illumination. All subjects were thoroughly trained and familiarized with the tests within 14 days preceding study start to minimize learning effects before proceeding to the study.

SACCADIC EYE MOVEMENTS Saccadic eye movements were recorded using a micro-computer-based system for data recording (Cambridge Electronics Design, Cambridge, UK), Nihon Kohden equipment for stimulus display, signal collection and amplification (Nihon Kohden Corporation, Tokyo, Japan), and disposable surface electrodes (Medicotest N-00-S, Olstykke, Denmark) (van Steveninck *et al.*, 1989). Average values of latency (= reaction time), peak saccadic velocity and inaccuracy (difference between stimulus angle and corresponding saccade in %) were calculated for all artefact free saccades. Saccadic peak velocity (SPV) has been validated as the most sensitive measure for the sedative effects of benzodiazepines (van Steveninck *et al.*, 1991; van Steveninck *et al.*, 1992; van Steveninck *et al.*, 1999; de Visser *et al.*, 2003) Previous healthy volunteer studies showed that TPA023 caused SPV reductions, while alcohol did not (de Haas *et al.*, 2007; Zoethout *et al.*, 2009).

VISUAL ANALOGUE SCALES Visual analogue scales as originally described by Norris (Norris, 1971) were previously used to quantify subjective effects of benzodiazepines (van Steveninck *et al.*, 1991). From the set of sixteen scales three composite factors were derived as described by Bond and Lader (Bond and Lader, 1974), corresponding to alertness, mood and calmness. These factors were used to quantify subjective treatment effects. In contrast to TPA023, alcohol has previously shown to affect the VAS alertness scale (de Haas *et al.*, 2007; Zoethout *et al.*, 2009).

BODY SWAY Body sway was measured with an apparatus similar to the Wright ataxia meter (Wright, 1971), which integrates the amplitude of unidirectional body movement transferred through a string attached to the subject's waist. Two-minute measurements were made in the antero-posterior direction with eyes closed, with subjects standing comfortably on a firm surface with their feet slightly apart. In contrast to TPA023, alcohol has previously shown to increase postural instability (de Haas *et al.*, 2007; Zoethout *et al.*, 2009).

ADAPTIVE TRACKING The adaptive tracking test was first performed 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 were used for analysis. Adaptive tracking is a pursuit-tracking task. A circle moves randomly about a 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 test subject cannot maintain the dot inside the circle. Adaptive tracking was scored over a 3-minute period. Each test was preceded by a run-in period. The adaptive

tracking test has proved to be useful for measurement of CNS effects of alcohol (van Steveninck *et al.*, 1996), various psychoactive drugs (Gorman *et al.*, 1986), and sleep deprivation (van Steveninck *et al.*, 1999).

### *Statistical analyses*

The study was performed to explore the effects of TPA023 on ET compared to an alcohol infusion. Because of the exploratory character of the study, no formal power calculation could be performed.

Most PD parameters were analyzed by mixed model analyses of variance (using SAS PROC MIXED) with treatment, period, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline is defined as the average of the available values obtained prior to dosing. Treatment effects were reported as the contrasts specified below where the average of all post-dose measurements was calculated within the statistical model. Contrasts were reported along with 95% confidence intervals (95%CI) and analyses were two-sided with a significance level of 0.05.

Body sway and tremor parameters were analyzed after log-transformation due to skewed response distribution. All other parameters were analyzed untransformed. Log-transformed parameters were back-transformed after analysis where the results may be interpreted as percentage change. All calculations were performed using SAS V9.1.2 for Windows (SAS Institute, Inc., Cary, NC, USA).

## RESULTS

### *Subjects*

Seventeen patients were medically screened after giving written informed consent. Seven patients did not qualify for the study mainly because their tremor was too mild to be quantified reliably with the laboratory tremography. Nine patients (two female, seven male) fulfilled the study criteria and completed the study. The remaining patient was standby to replace any discontinuations, but never participated in the study. Apart from their tremor, they were judged to be in good health on the basis of medical history, physical examination and routine laboratory data. Subjects were on average 47 years of age (range 18-80), had an average weight of 77 kg (range 62-103 kg) and average height of 175 cm (range 159-189 cm).

The mean (range) dominant tremor frequency at screening was 7.7 (95%CI 5.4-9.4) Hz. Mean (standard deviation) scores on the Tremor Disability Questionnaire and Performance-Based Tremor Evaluation were 30 (18.2)% and 34 (30)% respectively. Two patients stopped their propranolol treatment two weeks before participation in this trial. The other patients did not use any treatment.

### *Clinical observations*

No serious adverse reactions occurred following any of the treatments. Frequently reported adverse events include headache, sleepiness, dizziness and a painful arm during infusion. Headache was the most common adverse event, occurring in five, one and two patient(s) after administration of alcohol, TPA023 and placebo, respectively. Dizziness and sleepiness occurred in four, two and one subject(s) after administration of alcohol, TPA023 and placebo, respectively. Five patients reported a painful arm just after the start of the alcohol infusion. All adverse events were single occasions and considered mild of intensity.

### *Pharmacodynamics*

#### TREMOR EVALUATION

LABORATORY TREMOGRAPHY During the postural condition of the measurement, alcohol infusion reduced maximum power of both the left-right (-31.4% (-45.9, -13.0%)) and backward-forward (-37.6% (-60.4, -1.7%))

tremor direction compared to placebo. For the kinetic condition, similar results were obtained (table 1). The maximum power of the left-right direction was reduced by 19.2% (-31.7, -4.3%) and that of the backward-forward direction by 29.5% (-44.8, -9.9%) after alcohol infusion compared to placebo. These changes resulted in a decrease in average power in both conditions (figure 2). In the resting condition, no effects were seen in any tremor direction for any treatment. TPA023 did not affect tremor symptoms as effectively as a stable level of alcohol.

PERFORMANCE-BASED TREMOR EVALUATION Mean total tremor score decreased shortly after alcohol infusion had begun (figure 3). However, mean scores during placebo and TPA023 treatment also decreased. No differences compared to placebo were present after alcohol (-2.2 (-6.3, 1.9)) and TPA023 (-1.0 (-5.1, 3.0)) treatment (table 1).

RELATIONSHIP BETWEEN LABORATORY TREMOGRAPHY AND PBTE Tremor amplitude and tremor rating scales are logarhithmically related and one can be estimated if the other parameter is known, using a set of formulas proposed by Elble (Elble *et al.*, 2006). We used these formulas to investigate to what extent the PBTE serves as a predictor for the effect on tremor amplitude. Based on the mean reduction in PBTE, which was measured after alcohol treatment (compared to placebo) a 15% decrease in tremor amplitude was expected, while a 20% reduction was observed (for the kinetic condition). Additionally, the mean reduction in PBTE scores, which was measured after TPA023 treatment in this study resulted in an estimated reduction in tremor amplitude of 7%, while a 6% reduction was observed in the kinetic condition.

#### CENTRAL NERVOUS SYSTEM TESTS

SACCADIC EYE MOVEMENTS TPA023 significantly decreased saccadic peak velocity (SPV) with 45.4 deg/sec (-65.6, -25.1deg/sec) compared to placebo. TPA023 also increased inaccuracy with 1.0% (0.3, 1.6) but not latency. Alcohol did not show significant effects on eye movements (table 2).



BODY SWAY Neither treatment significantly affected body sway compared to placebo (table 2).

ADAPTIVE TRACKING Adaptive tracking performance and SD of performance were not significantly affected compared to placebo (table 2).

VISUAL ANALOGUE SCALES Alcohol decreased the VAS alertness scale by 8.0 mm (-13.2, -2.7mm) compared to placebo. VAS mood and VAS calmness were not affected significantly by alcohol or TPA023 treatment (table 2).

### *Pharmacokinetics*

Mean breath alcohol concentrations increased in approximately 15 minutes to 0.58 g/L and stabilized for 4 hours, after which the infusion was stopped and concentrations returned to baseline (figure 1).

### *Post-hoc power calculation*

In this exploratory study, the potential tremor reducing effects of TPA023 were compared to the effects of alcohol that were identified in this study. A post-hoc power calculation revealed that a sample size of 139 ET-patients would have been necessary to have 80% power to detect the same average difference between TPA023 and placebo (i.e. 0.068 μV) that was observed in nine ET-patients during alcohol treatment.

## DISCUSSION

This placebo-controlled study explored the effects of a novel $GABA_A$ α2,3 selective partial agonist in patients with essential tremor. Results showed that only alcohol, which was used as a positive control, reduced tremor power, as assessed by the laboratory tremography.

Several studies have shown that alcohol is effective in reducing tremor amplitude in patients with essential tremor (Growdon *et al.*, 1975; Zeuner

*et al.*, 2003; Rajput *et al.*, 1975; Koller and Biary, 1984). It is thought that alcohol acts within the central nervous system and not by affecting peripheral tremorogenic mechanisms (Growdon *et al.*, 1975; Zeuner *et al.*, 2003). Previous studies showed tremor reductions after a single oral intake of alcohol or an infusion bolus, causing mean blood alcohol levels of 0.35-0.55 $g \cdot L^{-1}$ (Zeuner *et al.*, 2003). The current study was able to keep alcohol levels stable at 0.6 $g \cdot L^{-1}$ for approximately four hours. Tremor power was reduced as long as the alcohol levels were stable and returned to baseline after the infusion was stopped.

Allthough tremor reducing effects were observed after TPA023 treatment in the kinetic condition, these effects did not reach significance in this relatively small study. It is unlikely that this was due to doses that were too low. There were clear effects of TPA023 2 mg on saccadic eye movements in this study, and similar finding were obtained with the same dose in healthy volunteers (de Haas *et al.*, 2007). The dose was predicted to be therapeutically active for the treatment of anxiety. A positron emission tomography study (Atack *et al.*, 2010) demonstrated that the dose of TPA023 used in this study produces substantial and sustained occupancy (47 to 64% at ~2 hours and 34 to 59% at ~7 hours postdosing). Although the required receptor occupancy for the postulated GABAergic effect in ET is unknown, these levels are consistent with the occupancy associated with the minimum effective dose in animal models of anxiolysis (44 to 76%, 46% and 89%, for the elevated plus maze, fear-potentiated startle and conditioned suppression of drinking paradigms, respectively), indicating that they are likely to be centrally active (MSD, data on file). In addition, the preliminary results of a fear-potentiated startle paradigm in healthy subjects support the anxiolytic efficacy of single dose TPA023 2.0 mg (MSD, data on file). In the latter study, startle amplitude during threat conditions was significantly reduced when subjects received TPA023 2.0 mg compared to placebo ($p<0.0035$).

To maximize the power of alcohol in detecting a treatment effect, only patients who were familiar with the positive effects of alcohol on their symptoms were included in this project. Patients could also have been selected on their (prior) responses to benzodiazepines or barbiturates. However, the clinical acceptability of such treatments was expected to be determined not only by the effect on ET-symptoms but also by the individual tolerability to side effects. Moreover, benzodiazepine withdrawal can also induce tremor, which can be difficult to distinguish from recurrence of ET-symptoms. It was considered less problematic to use a positive alcohol response as a potential predictor of an effect of subtype-selective partial $GABA_A$-agonist. Many patients report a positive effect of alcohol, but still do not routinely use alcohol to suppress their tremor. The half-life of alcohol is shorter than for most tremor-reducing medications. This diminishes the chance of tolerance and withdrawal after stopping alcohol. Since this drug also acts as an allosteric $GABA_A$-agonist, subjective ET-suppression with alcohol was also expected to increase the chance of finding effects with a subtype-selective $GABA_A$-agonist.

For treatment of ET and anxiety, similar clinical doses of benzodiazepines are used (Chouinard *et al.*, 1982; Pahwa and Lyons, 2003). Early clinical studies also indicate that TPA023 has an anxiolytic effect (Atack *et al.*, 2006). Therefore, the lack of effects of TPA023, which has a selective activity at the $\alpha 2,3$ subunit of the $GABA_A$ receptor, suggests that effects of GABAergic treatments of tremor are probably not mediated via the $\alpha 2,3$ subunit. However, we cannot rule out the possibility that the $\alpha 2$ and/or $\alpha 3$ subtypes *are* involved, but that TPA023 does not have enough intrinsic efficacy to produce clinical efficacy. It is uncertain which other $GABA_A$-receptor subtypes are involved. The $\alpha 5$ subunit is mainly located in the hippocampus, and has been shown to be involved in memory processes (Wendt *et al.*, 2000). The $\alpha 1$ subunit is probably the best candidate, since it is the most widely distributed $GABA_A$-receptor subtype (Benke *et al.*, 2004). Recently, the $\alpha 1$ subunit knockout mouse has been introduced as an animal model for essential tremor as it exhibits postural and kinetic tremors that clearly reproduce the features of essential tremors (Jankovic and Noebels, 2005; Kralic *et al.*, 2005). This suggests that the expression of $\alpha 1$ subunits in the brains of patients with ET may be abnormal. A pilot study in ET patients,

however, could not demonstrate any relation between ET and variants in the gene coding for this α1 subtype receptor (Deng *et al.*, 2006). Another recent paper confirms this statement (Garcia-Martin *et al.*, 2011). Nevertheless, other defects in the α1 subunit may still play a role in the pathophysiology of ET. Since this receptor subtype is also involved in sedation, it may be difficult to find GABAergic tremor treatment that is completely devoid of this side effect, which is particularly cumbersome in elderly patients.

It appeared that tremor power was largely reduced in all treatment groups in the first hour after treatment had started, as shown in figure 2. After this initial decrease, tremor remained stable during placebo treatment. This suggests that tremor was enhanced at baseline, e.g. by stress associated with the start of the study day (Whitney, 2006), or that there is a substantial initial placebo effect in treating ET. The alcohol effects were still clearly present on top of these significant placebo effects. Nonetheless, a wash-in placebo infusion would be useful in future studies, to ensure a reduction to stable baseline tremor levels in this patient group.

Although clinical rating scales have proven their effectiveness in the assessment of ET severity (Bain, 2000; Bain, 1998), the performance-based tremor evaluation scale used in this study was not able to measure a statistically significant effect of alcohol on ET, compared to placebo. The scoring of the test was performed by four trained persons, which might have caused too much inter-rater variability to detect a significant effect. Interestingly, the relationship between PBTE and tremor amplitude in this relatively small study approximately complied with the logarhithmic criteria, which were proposed earlier (Elble *et al.*, 2006).

In healthy volunteers, alcohol causes readily detectable increases in body sway and decreases in adaptive tracking (Zoethout *et al.*, 2009), but this could not be found in our study. This could have been caused by two opposing effects of alcohol in this patient group. On the one hand, alcohol has positive effects on the tremor itself, counterbalancing the negative performance on this task caused by alcohol (Zoethout *et al.*, 2009). Similar factors could play a role in the lack of effects on body sway. Previous studies have shown that patients with essential tremor had higher baseline ataxia scores compared to healthy controls, which diminished after alcohol ingestion (Klebe *et al.*, 2005). As for general performance, the lack of a net effect of alcohol on body sway could be explained by a reduction of ataxia in ET, combined with alcohol-induced postural instability. This might be a reason for the difference of alcohol effects between healthy volunteers and this patient group.

The different effects of TPA023 in the current study and those observed in a previous study investigating the effects of TPA023 (de Haas *et al.*, 2007) are approximately similar. TPA023 accounted for significant decreases in SPV in both studies and VAS alertness and body sway were not affected by TPA023 treatment in both sessions. The only observed difference between the two studies is an increase in saccadic inaccuracy in the current study, which was not found in the previous study. The higher dose in the current study might be an explanation for this phenomenon.

This study has shown that treatment with an α2,3 subunit selective $GABA_A$ partial agonist was less effective compared to a stable level of alcohol in reducing ET-symptoms. This suggests that the α2,3 subunit of the $GABA_A$ receptor is probably of less importance in the pathophysiology of essential tremor or in the beneficial effects of non-selective benzodiazepines and barbiturates. Additionally, the study has shown that the alcohol clamp is a reliable method for studies in patients with essential tremor.

**FIGURE 1** AVERAGE GRAPH OF BREATH ALCOHOL CONCENTRATIONS (G/L) WITH SD ERROR BARS

*Maximum and minimum values are shown with thin lines.*

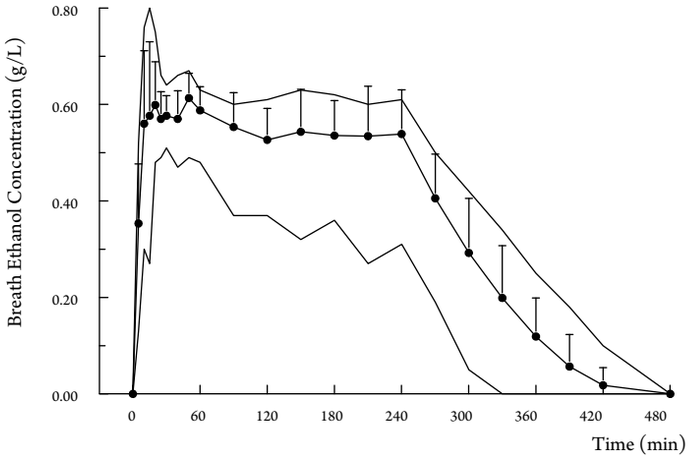



**FIGURE 2** GRAPHS SHOW CHANGES FROM BASELINE OF AVERAGE MAXIMUM POWER OF LABORATORY ACCELEROMETRY IN POSTURAL AND KINETIC CONDITION WITH 95%CI ERROR BARS

*Closed circle is TPA023; square is alcohol; open circle is placebo.*

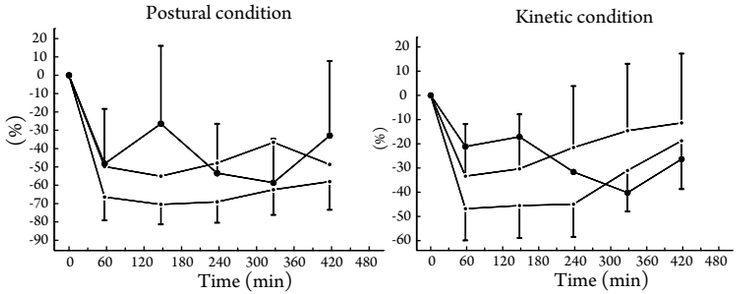



**FIGURE 3** GRAPH SHOWS CHANGE FROM BASELINE OF AVERAGE SCORE ON PERFORMANCE-BASED TREMOR EVALUATION SCALE WITH 95%CI ERROR BARS

*Closed circle is TPA023; square is alcohol; open circle is placebo.*

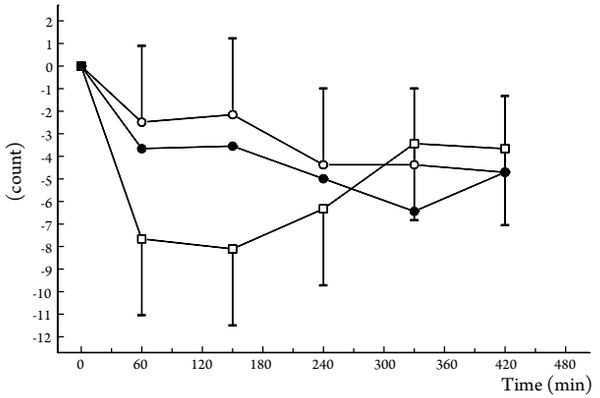


**TABLE 1** LEAST SQUARE MEANS AND TREATMENT DIFFERENCES RELATIVE TO BASELINE FOR DIFFERENT TREMOR VARIABLES

*ANOVA results are shown as contrasts (%), p-values and 95% CI.*

| **Laboratory accelerometry maximum power variables** | | **LS means** | | | **TPA023 - placebo** | | | | **Alcohol - lacebo** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TPA023 | Alcohol | Placebo | difference | P - value | 95%CI | | difference | P - value | 95%CI | |
| Rest | Up-Down (uV) | 0.192 | 0.149 | 0.151 | 26.8 | 0.19 | -12.2, | 83.1 | -1.3 | 0.94 | -31.7, | 42.7 |
| | Left-Right (uV) | 0.316 | 0.263 | 0.289 | 9.3 | 0.53 | -18.8, | 47.2 | -8.8 | 0.53 | -32.5, | 23.3 |
| | Back-Forward (uV) | 0.174 | 0.150 | 0.139 | 25.5 | 0.15 | -8.8, | 72.6 | 8.2 | 0.60 | -21.5, | 49.1 |
| | Average Maximum power (uV) | 0.233 | 0.191 | 0.195 | 19.0 | 0.26 | -13.4, | 63.6 | -2.3 | 0.88 | -29.0, | 34.5 |
| Postural | Up-Down (uV) | 0.623 | 0.390 | 0.588 | 6.0 | 0.77 | -31.2, | 63.3 | -33.6 | 0.060 | -56.8, | 2.1 |
| | Left-Right (uV) | 0.782 | 0.495 | 0.721 | 8.4 | 0.48 | -15.0, | 38.2 | -31.4 | 0.0049 | -45.9, | -13.0 |
| | Back-Forward (uV) | 0.482 | 0.317 | 0.508 | -5.1 | 0.80 | -39.7, | 49.4 | -37.6 | 0.043 | -60.4, | -1.7 |
| | Average Maximum power (uV) | 0.652 | 0.410 | 0.620 | 5.2 | 0.75 | -25.6, | 48.7 | -33.8 | 0.023 | -53.1, | -6.7 |
| Kinetic | Up-Down (uV) | 1.296 | 1.108 | 1.393 | -6.9 | 0.58 | -29.4, | 22.7 | -20.5 | 0.099 | -39.9, | 5.2 |
| | Left-Right (uV) | 2.719 | 2.344 | 2.899 | -6.2 | 0.41 | -20.5, | 10.6 | -19.2 | 0.018 | -31.7, | -4.3 |
| | Back-Forward (uV) | 1.112 | 0.866 | 1.228 | -9.5 | 0.40 | -29.6, | 16.5 | -29.5 | 0.0095 | -44.8, | -9.9 |
| | Average Maximum power (uV) | 1.741 | 1.486 | 1.862 | -6.5 | 0.48 | - 23.6, | 14.4 | -20.2 | 0.036 | -35.2, | -1.7 |
| **Performance Based Tremor Evaluation score** | | 15.1 | 13.9 | 16.1 | -1.0 | 0.59 | -5.1, | 3.0 | -2.2 | 0.26 | -6.3, | 1.9 |



**TABLE 2** LEAST SQUARE MEANS AND PHARMACODYNAMIC DIFFERENCES RELATIVE TO BASELINE FOR SACCADIC EYE MOVEMENTS, VISUAL ANALOGUE SCALES, BODY SWAY AND ADAPTIVE TRACKING

*ANOVA results are shown as contrasts, p-values and 95% CI.*

| **CNS Variable** | **LS means** | | | **TPA023 - placebo** | | | | **Alcohol - placebo** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **TPA023** | **Alcohol** | **Placebo** | **difference** | **P - value** | **95%CI** | | **difference** | **P – value** | **95%CI** | |
| Saccadic Peak Velocity (deg/sec) | 413.6 | 443.0 | 459.0 | -45.4 | 0.0002 | -65.6, | -25.1 | -16.0 | 0.095 | -35.3, | 3.2 |
| Latency (sec) | 0.233 | 0.228 | 0.224 | 0.009 | 0.14 | -0.003, | 0.021 | 0.004 | 0.47 | -0.008, | 0.016 |
| Inaccuracy (%) | 7.5 | 6.5 | 6.6 | 1.0 | 0.0080 | 0.3, | 1.6 | -0.1 | 0.80 | -0.8, | 0.6 |
| VAS Alertness (mm) | 61.6 | 56.2 | 64.2 | -2.6 | 0.33 | -8.1, | 2.9 | -8.0 | 0.0061 | -13.2, | -2.7 |
| VAS mood (mm) | 73.8 | 73.2 | 73.9 | -0.2 | 0.92 | -3.9, | 3.6 | -0.7 | 0.68 | -4.4, | 3.0 |
| VAS Calmness (mm) | 64.5 | 69.4 | 68.1 | -3.6 | 0.24 | -10.0, | 2.8 | 1.3 | 0.65 | -5.0, | 7.6 |
| Body Sway Eyes Closed (mm) | 426.7 | 465.9 | 413.7 | 3.1 | 0.64 | -10.6, | 19.0 | 12.6 | 0.11 | -3.1, | 30.9 |
| Adaptive tracking performance (%) | 13.60 | 14.01 | 15.03 | -1.43 | 0.065 | -2.96, | 0.10 | -1.02 | 0.17 | -2.54, | 0.50 |
| SD of adaptive tracking performance (%) | 2.47 | 2.61 | 2.53 | -0.06 | 0.71 | -0.37, | 0.26 | 0.09 | 0.54 | -0.22, | 0.40 |

Atack JR, Hutson PH, Collinson N, Marshall G, Bentley G, Moyes C, Cook SM, Collins I, Wafford K, McKernan RM, Dawson GR (2005). Anxiogenic properties of an inverse agonist selective for alpha3 subunit-containing GABA A receptors. Br J Pharmacol 144:357-366.

Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, Pike A, Sur C, Melillo D, Bristow L, Bromidge F, Ragan I, Kerby J, Street L, Carling R, Castro JL, Whiting P, Dawson GR, McKernan RM (2006). TPA023 (7-(1,1-dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine), an agonist selective for alpha2- and alpha3-containing GABA A receptors, is a nonsedating anxiolytic in rodents and primates. J Pharmacol Exp Ther 316:410-422.

Atack JR, Wong DF, Fryer TD, Ryan C, Sanabria S, Zhou Y, Dannals RF, Eng WS, Gibson RE, Burns HD, Vega JM, Vessy L, Scott-Stevens P, Beech JS, Baron JC, Sohal B, Schrag ML, Aigbirhio FI, McKernan RM, Hargreaves RJ (2010). Benzodiazepine binding site occupancy by the novel GABA A receptor subtype-selective drug 7-(1,1-dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)3(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine (TPA023) in rats, primates, and humans. J Pharmacol Exp Ther 332:17-25.

Bain PG (1998). Clinical measurement of tremor. Mov Disord 13 Suppl 3:77-80.

Bain PG (2000). Tremor assessment and quality of life measurements. Neurology 54:S26-S29.

Benke D, Fakitsas P, Roggenmoser C, Michel C, Rudolph U, Mohler H (2004). Analysis of the presence and abundance of GABA A receptors containing two different types of alpha subunits in murine brain using point-mutated alpha subunits. J Biol Chem 279:43654-43660.

Bond A, Lader M (1974). The use of analogue scales in rating subjective feelings. Br J Med Psychol 47:211-218.

Borland RG, Nicholson AN (1984). Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol 18 Suppl 1:69S-72S.

Brennan KC, Jurewicz EC, Ford B, Pullman SL, Louis ED (2002). Is essential tremor predominantly a kinetic or a postural tremor? A clinical and electrophysiological study. Mov Disord 17:313-316.

Chen JJ, Swope DM (2003). Essential tremor: diagnosis and treatment. Pharmacotherapy 23:1105-1122.

Chouinard G, Annable L, Fontaine R, Solyom L (1982). Alprazolam in the Treatment of Generalized Anxiety and Panic Disorders - A Double-Blind Placebo-Controlled Study. Psychopharmacology 77:229-233.

Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW (2002). Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABA A receptor. J Neurosci 22:5572-5580.

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 (2007). 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 21:374-383.

de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM (2003). Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 55:39-50.

Deng H, Xie WJ, Le WD, Huang MS, Jankovic J (2006). Genetic analysis of the GABRA1 gene in patients with essential tremor. Neurosci Lett 401:16-19.

Deuschl G (1999). Differential diagnosis of tremor. J Neural Transm Suppl 56:211-220.

Deuschl G, Bain P, Brin M (1998). Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. Mov Disord 13 Suppl 3:2-23.

Deuschl G, Elble RJ (2000). The pathophysiology of essential tremor. Neurology 54:S14-S20.

Deuschl G, Zimmerman R, Genger H, Lucking CH (1995). Physiologic classification of essential tremor. In: Handbook of tremor disorders pp 195-208. New York: Marcel Dekker.

Elble RJ (2000). Essential tremor frequency decreases with time. Neurology 55:1547-1551.

Elble RJ, Pullman SL, Matsumoto JY, Raethjen J, Deuschl G, Tintner R (2006). Tremor amplitude is logarithmically related to 4- and 5-point tremor rating scales. Brain 129:2660-2666.

Garcia-Martin E, Martinez C, Alonso-Navarro H, Benito-Leon J, Lorenzo-Betancor O, Pastor P, Puertas I, Rubio L, Lopez-Alburquerque T, Agundez JA, Jimenez-Jimenez FJ (2011). Gamma-aminobutyric acid GABRA4, GABRE, and GABRQ receptor polymorphisms and risk for essential tremor. Pharmacogenet Genomics 21(7):436-439.

Gironell A, Kulisevsky J, Barbanoj M, Lopez-Villegas D, Hernandez G, Pascual-Sedano B (1999). A randomized placebo-controlled comparative trial of gabapentin and propranolol in essential tremor. Arch Neurol 56:475-480.

Gorman WP, Cooper R, Pocock P, Campbell MJ (1986). A comparison of primidone, propranolol, and placebo in essential tremor, using quantitative analysis. J Neurol Neurosurg Psychiatry 49:64-68.

Growdon JH, Shahani BT, Young RR (1975). The effect of alcohol on essential tremor. Neurology 25:259-262.

Gunal DI, Afsar N, Bekiroglu N, Aktan S (2000). New alternative agents in essential tremor therapy: double-blind placebo-controlled study of alprazolam and acetazolamide. Neurol Sci 21:315-317.

Huber SJ, Paulson GW (1988). Efficacy of alprazolam for essential tremor. Neurology 38:241-243.

Jankovic J, Noebels JL (2005). Genetic mouse models of essential tremor: are they essential? J Clin Invest 115:584-586.

Klebe S, Stolze H, Grensing K, Volkmann J, Wenzelburger R, Deuschl G (2005). Influence of alcohol on gait in patients with essential tremor. Neurology 65:96-101.

Koller WC (1991). A new drug for treatment of essential tremor? Time will tell. Mayo Clin Proc 66:1085-1087.

Koller WC, Biary N (1984). Effect of alcohol on tremors: comparison with propranolol. Neurology 34:221-222.

Koller WC, Busenbark K, Miner K (1994). The relationship of essential tremor to other movement disorders: report on 678 patients. Essential Tremor Study Group. Ann Neurol 35:717-723.

Kralic JE, Criswell HE, Osterman JL, O'Buckley TK, Wilkie ME, Matthews DB, Hamre K, Breese GR, Homanics GE, Morrow AL (2005). Genetic essential tremor in gamma-aminobutyric acidA receptor alpha1 subunit knockout mice. J Clin Invest 115:774-779.

Louis ED (1999). A new twist for stopping the shakes? Revisiting GABAergic therapy for essential tremor. Arch Neurol 56:807-808.

Louis ED, Barnes LF, Wendt KJ, Albert SM, Pullman SL, Yu Q, Schneier FR (2000). Validity and test-retest reliability of a disability questionnaire for essential tremor. Mov Disord 15:516-523.

Louis ED, Wendt KJ, Albert SM, Pullman SL, Yu Q, Andrews H (1999). Validity of a performance-based test of function in essential tremor. Arch Neurol 56:841-846.

Norris H (1971). The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 10:181-191.

O'Connor S, Morzorati S, Christian J, Li TK (1998). Clamping breath alcohol concentration reduces experimental variance: Application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcoholism-Clinical and Experimental Research 22:202-210.

Pahwa R, Lyons KE (2003). Essential tremor: differential diagnosis and current therapy. Am J Med 115:134-142.

Rajput AH, Jamieson H, Hirsh S, Quraishi A (1975). Relative efficacy of alcohol and propranolol in action tremor. Can J Neurol Sci 2:31-35.

Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR (2005). Different GABA A receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proc Natl Acad Sci U S A 102:915-920.

Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Mohler H (1999). Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 401:796-800.

Rudolph U, Crestani F, Mohler H (2001). GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 22:188-194.

Santhakumar V, Wallner M, Otis TS (2007). Ethanol acts directly on extrasynaptic subtypes of GABA(A) receptors to increase tonic inhibition. Alcohol 41:211-221.

Van Hilten JJ, van Dijk JG, Dunnewold RJ, van der Velde EA, Kemp B, van Brummelen P, van der Krogt JA, Roos RA, Buruma OJ (1991). Diurnal variation of essential and physiological tremor. J Neurol Neurosurg Psychiatry 54:516-519.

van Steveninck AL, Cohen AF, Ward T (1989). A microcomputer based system for recording and analysis of smooth pursuit and saccadic eye movements. Br J Clin Pharmacol 27:712P-713P.

van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, Breimer DD, Cohen AF (1996). Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol 41:565-573.

van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF (1991). 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 50:172-180.

van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF (1999). The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol 13:10-17.

van Steveninck AL, Verver S, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF (1992). Effects of temazepam on saccadic eye movements: concentration-effect relationships in individual volunteers. Clin Pharmacol Ther 52:402-408.

Wendt KJ, Albert SM, Pullman SL, Schneier FR, Louis ED (2000). Columbia University Assessment of Disability in Essential Tremor (CADET): methodological issues in essential tremor research. Parkinsonism & Related Disorders 6:17-23.

Whitney CM (2006). Essential tremor. Neurologist 12:331-332.

Wright BM (1971). A simple mechanical ataxiameter. J Physiol 218:27P-28P.

Zesiewicz TA, Chari A, Jahan I, Miller AM, Sullivan KL (2010). Overview of essential tremor. Neuropsychiatr Dis Treat 6:401-408.

Zeuner KE, Molloy FM, Shoge RO, Goldstein SR, Wesley R, Hallett M (2003). Effect of ethanol on the central oscillator in essential tremor. Mov Disord 18:1280-1285.
Zoethout RW, Schoemaker RC, Zuurman L, van PH, Dahan A, Cohen AF, van Gerven JM (2009). Central nervous system effects of alcohol at a pseudo-steady-state concentration using alcohol clamping in healthy volunteers. Br J Clin Pharmacol 68:524-534.
Zoethout RW, van Gerven JM, Dumont GJ, Paltansing S, van Burgel ND, van der Linden M, Dahan A, Cohen AF, Schoemaker RC (2008). A comparative study of two methods for attaining constant alcohol levels. Br J Clin Pharmacol 66:674-681.



# CHAPTER 8

## *The effects of a novel histamine-3 receptor inverse agonist on essential tremor in comparison to stable levels of alcohol*

*Journal of Psychopharmacology 2012; 26(2), 292-302*

R.W.M. Zoethout, R. Iannone, B.R. Bloem, J. Palcza, G. Murphy, J. Chodakewitz, A. Buntinx, K. Gottesdiener, S. Marsilio, L. Rosen, K. Van Dyck, E.D. Louis, A.F. Cohen, R.C. Schoemaker, S. Tokita, N. Sato, K.S. Koblan, R.H. Hargreaves, J.J. Renger, J.M.A. van Gerven
*(first two authors contributed equally)*

## ABSTRACT

BACKGROUND Essential tremor (ET) is a common movement disorder. Animal studies show that histaminergic modulation might affect the pathological processes involved in the generation of ET. Histamine-3 receptor inverse agonists (H3RIA) have demonstrated attenuating effects on ET in the harmaline rat model.

METHODS In this double-blind, three-way cross-over, single-dose, double-dummy study the effects of 25 mg of a novel H3RIA (MK-0249) and a stable alcohol level (0.6 $g \cdot L^{-1}$) were compared to placebo, in 18 ET-patients. Tremor was evaluated using laboratory tremorography, portable tremorography and a clinical rating scale. The Leeds Sleep Evaluation Questionnaire (LSEQ) and a choice reaction time (CRT) test were performed to evaluate potential effects on sleep and attention, respectively.

RESULTS A steady state of alcohol significantly diminished tremor as assessed by laboratory tremorography, portable tremorography and clinical ratings compared to placebo. A high single MK-0249 dose was not effective in reducing tremor, but caused significant effects on the LSEQ and the CRT-test.

CONCLUSIONS These results suggest that treatment with a single dose of MK-0249 does not improve tremor in alcohol-responsive patients with ET, whereas stable levels of alcohol as a positive control reproduced the commonly reported tremor diminishing effects of alcohol.


## INTRODUCTION

Essential tremor (ET) is one of the most common neurological disorders among adults, and is the most common tremor disorder (Louis *et al.*, 1998; Louis, 2001; Louis, 2005). In contrast with the resting tremor in Parkinson's disease, ET is provoked by postural and kinetic movements and has a dominant frequency of 4-12 Hz (Pahwa and Lyons, 2003). The upper extremities are predominantly affected, but the head, the neck and the voice may also be involved, either separately or in combination. The prevalence of ET is estimated to be 4% within the general population (Pahwa and Lyons, 2003), and approximately 5% in the population above 65 years (Louis *et al.*, 1998).

Current pharmacological treatment, including beta blockers, benzodiazepines and primidone, act symptomatically and have variable effectiveness (Chen and Swope, 2003). Moreover, the occurrence of side effects, like sedation, weight gain and cognitive impairment, limit their use. Estimations indicate that alcohol is effective in approximately 70% of ET patients (Lou and Jankovic, 1991; Koller *et al.*, 1994). This finding is confirmed by controlled studies where alcohol was administered acutely (Growdon *et al.*, 1975; Koller and Biary, 1984). It most likely acts via a reduction of central over-activity, which results in reduced tremor amplitude, whereas the frequency remains unaffected (Koller and Biary, 1984; Koller, 1991). ET can also be treated surgically. Stereotactic thalamotomy and continuous deep-brain stimulation are promising techniques for patients with severe functional disability who are unresponsive to drug therapy (Koller, 1991; Louis, 2005). These two procedures are equally effective, but thalamic stimulation has fewer adverse effects and results in a greater improvement in function (Schuurman *et al.*, 2000).

Because little is known about the pathophysiology of ET, the search for novel pharmacological treatment options has been challenging. Recent studies indicate that ET might be attributed to a defect in the central oscillatory systems at the level of the inferior olivary nucleus of the medulla oblongata (Deuschl and Elble, 2000). Since histaminergic neurons project to the inferior olivary nucleus in the brainstem, the histaminergic system might play a role in the modulation of pathological processes involved in the generation of ET. In addition, animal models for ET show that modulating histaminergic tone in the central nervous systems (CNS) neurons might be a fruitful strategy in the development of future pharmacological treatment (Merck Research Laboratories (MRL) – data not shown).

The histaminergic system is widely disseminated in the CNS and regulates multiple functions including, arousal, satiety, attention and cognition. Histaminergic neurons originate in the tuberomammillary nucleus of the hypothalamus and project throughout the CNS, including the inferior olivary

nucleus in the brainstem. Currently, four types of histamine receptors have been identified. Postsynaptically localized histamine subtype-1 (H1) and histamine subtype-2 (H2) receptors mediate the preponderance of CNS effects, while the histamine subtype-3 (H3) receptors are localized on the presynaptic membrane as autoreceptors, and regulate the production and synaptic release of histamine as a part of a negative feedback mechanism. H3-receptors are mainly distributed in the CNS and function to modulate histaminergic effects in the brain, making them a potential target for pharmacological manipulation. H3-receptors signal constitutively, which serves to tonically suppress histamine production at baseline.

Recent preclinical findings indicate a potential role for the histaminergic system in the treatment of ET. Rats treated with the β-carboline harmaline, a toxin from the Syrian Rue plant, develop an 8-12 Hz postural and kinetic tremor that is clinically similar to ET (Martin *et al.*, 2005). The toxin also activates and synchronizes firing in the olivocerebellar system (Martin *et al.*, 2005). Harmaline has been used to develop a rat model of ET that responds in a dose dependent fashion to several of the currently available treatments for the disease, including propranolol, alcohol and benzodiazepines (Martin *et al.*, 2005). An increase in brain histamine induced by single doses of histamine modulating agents at the H3-receptor reduced tremor significantly in a dose-dependent fashion in the harmaline rat model of ET (MRL – data not published). Thus, histaminergic modulation might also contribute to the treatment of tremor seen in ET.

An increase in histamine concentration in the brain by pharmacological interference at the H3-receptor would avoid peripheral histamine effects. While a classical antagonist would only interfere with histamine-mediated negative feedback, H3-receptor inverse agonists have been demonstrated to decrease constitutive H3-receptor signalling, thus blocking tonic inhibition of histamine synthesis and release and further potentiating histaminergic effects (Arrang *et al.*, 2007).

The primary objective of this study was to evaluate the effects of a single dose of MK-0249, a recently developed H3-receptor inverse agonist (shown in figure 1), on average maximum tremor power in ET-patients. The chemical and biological characteristics of this class of H3-receptor inverse agonists have recently been published (Nagase *et al.*, 2008). Since most ET-patients experience attenuating effects of alcohol on their symptoms (Rajput *et al.*, 1975), the effects of MK-0249 on ET were compared to a steady state level of alcohol as a positive control, versus placebo. The study design, methodologies for measuring tremor and data analysis plan were based upon a prior, yet unpublished pilot study.

## METHODS

### *Design*

Eighteen patients diagnosed with ET, were included in the study. The effects of MK-0249 were investigated and compared to alcohol (as an active comparator) and placebo in a randomized, double-blind fashion. Since ethanol was delivered as an intravenous infusion and MK-0249 was administered orally, a double-dummy design was adopted to maintain the double-blind character of the study. On each study day, patients received one of three different treatment combinations: active ethanol with MK-0249 placebo, ethanol placebo (glucose 5% vehicle) with active MK-0249 and ethanol placebo with MK-0249 placebo. The washout period between treatments was at least seven days.

### *Patients*

Men and women of at least 18 years of age with documented ET for at least six months were recruited from the databases of the Leiden University Medical Centre, University Medical Centre St. Radboud in Nijmegen and from a number of local surrounding hospitals. Patients were informed about the contents of the study during an information visit. Once having agreed to participate and having signed informed consent, patients visited the research unit for a medical screening, at which time they were medically

screened to evaluate eligibility for study participation. A neurologist (J.G.) with experience in movement disorders clinically confirmed the diagnosis of ET of the hands and forearms according to the diagnostic criteria for 'classic ET' defined by the consensus statement of the Movement Disorder Society (Deuschl *et al.*, 1998). This statement was adapted from previous criteria established by the Tremor Research Investigation Group (TRIG) (Deuschl *et al.*, 1995) as well as from a more recent study indicating the importance of kinetic tremor (Brennan *et al.*, 2002). An isolated head tremor was not allowed. Patients were also selected for having symptoms that, by history, were relieved with alcohol consumption.

Only patients who stated a positive effect of alcohol on their tremor symptoms were included to maximize the power of alcohol as a positive control. Patients had to refrain from alcohol 48 hours prior to a treatment day and from caffeine-containing products for at least 12 hours before treatment. They were not allowed to use their own (anti-tremor) medications and had to abstain from grapefruit (juice) and St. John's Wort for at least 2 weeks before the start of the study until completion of the study. They were not allowed to drink more than six units of caffeine-containing products or three beverages of alcohol per day or smoke more than five cigarettes per day during the total study period. On treatment days, the use of caffeine-containing products or smoking was not allowed. The study was approved by the Medical Ethics Review Board of Leiden University Medical Centre, and performed according to their standards. The study was performed in compliance with the standards of Good Clinical Practice and the Declaration of Helsinki.

## *Study treatments*

On study days, the effects of a single oral dose of 25 mg MK-0249 or an intravenous alcohol infusion (10% w/v in 5% glucose) were examined. This MK-0249 dose was the highest single dose that was previously administered to healthy elderly male and female subjects. Elsewhere, single doses of MK-0249 up to 150 mg were safely administered to healthy young male volunteers. A yet unpublished positron emission tomography (PET) study in healthy young males suggests that the peak brain H3-receptor occupancy (after a single dose of 25 mg) would be approximately 90%, and be reached after approximately 6 hours. Given the long half life of this compound (approximately 14 hours), we would expect this level of receptor occupancy to be maintained during the entire observation period.

To diminish variability due to changing alcohol levels, alcohol was infused using a recently developed clamping method (Zoethout *et al.*, 2008). Based on a procedure for clamping breath alcohol concentrations (BrAC) previously described by O'Connor *et al.* (O'Connor *et al., 1998)* a spreadsheet-based alcohol clamping paradigm was developed. Changes in BrAC were used to adapt the intravenous infusion rate of alcohol (10% w/v in 5% glucose). This resulted in stable alcohol levels with minimal variability (Zoethout *et al.*, 2008). The alcohol clamp has previously been well-tolerated and produced statistically significant pharmacodynamic CNS effects (Zoethout *et al.*, 2009).

An alcohol level of 0.6 $g \cdot L^{-1}$ was chosen for this study, because this level is routinely achieved during social drinking, without causing too many adverse effects. Moreover, a prior study showed tremor reducing effects of alcohol at a mean level of 0.55 $g \cdot L^{-1}$, after a single oral dose (Zeuner *et al.*, 2003). Considering its pharmacokinetic profile, it was estimated that the action of MK-0249 would peak at about four hours and be sustained at high levels for at least seven hours. Consequently, to optimize the value of alcohol as a positive control, a level of 0.6 $g \cdot L^{-1}$ was maintained for seven hours . The research assistant who was responsible for performing breath samples was unblinded to the alcohol infusion, but this person was not involved in any other assessments, to preserve the double-blind character of the study.

This was a double-dummy study, so each active treatment was accompanied by administration of placebo for the alternative treatment. Thus, a matching placebo capsule for MK-0249 was given during the active alcohol occasion. On the day of administration of MK-0249 25 mg, a sham clamp was performed with glucose 5%, including preprogrammed pump rate changes and breath sampling (without revealing the results to the patient or the study team).

It has been suggested that tremor symptoms could be enhanced before dosing, e.g. by stress associated with the start of the study (Whitney, 2006) To by-pass this problem a 90-minute, a single-blind saline infusion (0.9%) preceded the actual alcohol/placebo infusion. After this 90 min 'run-in phase', MK-0249 (or placebo) was administered orally at t = 0 min and at the same time we started the alcohol (or placebo) clamping procedure. The run-in phase was single blind, and patients were told that the experiment started at t = - 90 min. The data collected between t = –90 min and t = 0 min were discarded.

## *Safety*

Adverse events, ECG, blood pressure and heart rate measurements were assessed throughout the study. During study days, a parallel glucose infusion (glucose 5%) was administered to all patients during the first ten minutes post-start, to minimize pain and discomfort in the infusion arm, caused by the alcohol infusion.

## *Drug concentrations*

Breath alcohol samples (BrAC) were performed using the hand-held Alco-Sensor IV meter (Honac, Apeldoorn, the Netherlands). The pharmacokinetic profile of MK-0249 had already been extensively investigated, and it was therefore decided not to analyze the pharmacokinetic samples of MK-0249 in this study.

## *Tremor evaluation*

Tremor evaluations were performed at screening, predose (within 60 minutes prior to dosing) and at 60, 150, 240, 330, 420 and 510 minutes postdose on each study day.



LABORATORY TREMOROGRAPHY Tremor evaluation was measured according to the methodology of Gironell *et al.* (Gironell *et al.*, 1999). Tremor was evaluated with three miniature linear piezo-electric accelerometers (Nihon Kohden, MT-3T), which were attached to the distal end of the clamp, above the fingertips of the dominant arm. The accelerometers were placed at right angles to one another to enable three-dimensional analysis of movement (Van Hilten *et al.*, 1991; Gironell *et al.*, 1999). The upper limb tremor was recorded in three positions, each held for a 60-second interval: (1) at rest, with the arm hanging relaxed along the body, (2) postural, with the arm held in an outstretched, horizontal, prone position and (3) kinetic, moving the hand from an outstretched position to the nose, as accurately as possible.

PORTABLE TREMOROGRAPHY Tremor was also evaluated using a portable accelerometer (Dynaport MiniMod3) that consisted of three linear accelerometers placed in a perpendicular array, a data logger and a power source. The portable accelerometer is the size of a match-box, and was affixed to the fingertips of the dominant hand. The portable tremorography assessment was performed immediately following each laboratory tremorography assessment on the same hand, in exactly the same way.

PERFORMANCE-BASED CLINICAL RATING SCALE (CRS) Clinical rating scales are often described as useful methods to assess tremor severity in ET patients (Bain *et al.*, 1993; Louis *et al.*, 2000). A performance-based clinical rating scale (Louis *et al.*, 1999) was used in this study to investigate the practical implications of alcohol and MK-0249 treatment on 15 simple, 'daily-life' activities (e.g. carry a tray with two filled glasses, thread a needle or pour liquid from a milk carton into a glass). Performance on the CRS was videotaped to permit post-hoc analysis by a blinded independent expert. Performance on each item was scored from 0 (no difficulty) to 4 (unable to perform). The total sum of activity scores was calculated.

## *Additional measurements*

CHOICE REACTION TIME (CRT) TEST To investigate the possible effects of MK-0249 (and alcohol) on psychomotor function, a CRT test was administered. Choice reaction time was chosen for its known sensitivity to the sedative effects of alcohol and other drugs (Grant *et al.*, 2000), as well as to stimulant effects of drugs like caffeine (Lieberman, 2007). During this test, either the word 'left' or the word 'right' was presented on a computer screen. All patients were instructed to press a corresponding button as quickly as possible. There were 32 trials for which each stimulus word was chosen randomly with equal probability. The duration of the interstimulus interval varied randomly. Both the amount of correct scores and the reaction time were assessed. CRT was tested at screening, predose (within 60 minutes prior to dosing) and at 60, 150, 240, 330, 420 and 510 minutes postdose on each study day.

LEEDS SLEEP EVALUATION QUESTIONNAIRE (LSEQ) Since MK-0249 is thought to increase CNS histamine levels, it is expected to be associated with alerting effects. Therefore the LSEQ was administered, to investigate the possible effects of MK-0249 on a wide scale of sleep parameters (Parrott and Hindmarch, 1980). The LSEQ is a standardized self-reporting instrument comprising ten 100 mm visual analogue scales that score the ease of getting to sleep, quality of sleep, ease of awakening from sleep and alertness and behavior following wakefulness (Parrott and Hindmarch, 1980). The LSEQ was administered at screening and pre dose on the morning of day 1 and at 24 hours post dose. To maximize compliance, patients were contacted by telephone the next morning, 24 hours after dosing, to check whether the LSEQ was completed.

## *Statistical analyses*

Changes in maximum kinetic tremor power (especially backward-forward movements), as assessed by laboratory accelerometry were of primary concern. Given their pharmacologic profiles, tremor measurements between 240 and 510 minutes post dose were specifically hypothesized to be affected to the largest extent by treatment. A mean treatment-induced reduction of 25% (or more) compared to average baseline tremor scores was considered clinically meaningful. With 18 patients, there was more than 99% power to detect such a difference between MK-0249 and placebo or between alcohol and placebo.

Because of their skewed response distribution all tremor parameters were analyzed after log-transformation. Log transformed changes from baseline for accelerometry tremor endpoints were analyzed with mixed effects models (using SAS PROC MIXED). This resulted in least square means estimates for each treatment that indicate the change from baseline on the log scale. Subsequently, mean treatment effects were calculated as the contrasts between these least square means for placebo and either MK-0249 or alcohol after back transformation from the log scale. The average of the measurements at 240, 330, 420 and 510 minutes was calculated within the statistical model. All results are presented on the original scale and reported as percent changes (all analyses were two-sided, with a significance level of 0.05). Between treatment comparisons were made for LSEQ, CRT and CRS on the original scale. All calculations were performed using SAS V9.1.2 for Windows (SAS Institute, Inc., Cary, NC, USA).

# RESULTS

## *Patients*

Nineteen ET-patients were found eligible for study participation. Apart from their tremor, they were judged to be in good health on the basis of medical history, physical examination and routine laboratory data. One patient dropped out after the first period because of the need to resume antidepressive medication. This patient was replaced by another patient, who received the same treatment randomization order. Eighteen patients (11 men, 7 women) completed the study per protocol. Patients were on average 47 years of age (range 19-81), had an average weight of 75 kg (range 61-98 kg) and an average height of 178 cm (range 161-191 cm).

## *Alcohol concentrations*

Figure 2 shows the mean graph of breath alcohol values for all 18 patients. The average target level of 0.60 $g \cdot L^{-1}$ was achieved within 30 minutes. The steady state level was maintained until approximately 7 hours post-dose, after which alcohol infusion was stopped. Thereafter, BrAC returned to baseline.

## *Tremor evaluation*

LABORATORY TREMOROGRAPHY Baseline tremor scores are presented in table 1. Treatment effects on maximum tremor power obtained by laboratory tremorography are summarized in table 2. MK-0249 treatment had no significant effect on the laboratory tremorography in any direction. For the kinetic condition, the mean maximum power of the backward-forward direction (primary endpoint) was reduced by 34.9% (95% CI: -52.7%, -17.3%) and that of the left-right direction was reduced by 42.0% (95% CI: -59.9%, -24.5%) after alcohol infusion compared to placebo. Also, mean average maximum power was reduced by 33.4% (95%CI: -49.8%, -17.2%) compared to placebo (figure 3). During the postural condition of the laboratory tremorography, alcohol infusion significantly reduced mean maximum power of both left-right (-38.9% (95% CI: -67.2%, -11.6%)) and backward-forward (-38.4% (95% CI: -66.1%, -11.9%)) tremor direction compared to placebo. The mean average maximum power was also significantly reduced after alcohol treatment (-37.0% (95% CI: -62.7%, -12.0%)) for the postural condition compared to placebo (figure 4). During the rest condition, alcohol infusion significantly reduced mean maximum power of both the up-down (-26.7% (95% CI: -53.8%, 0.0%)) and left-right condition (-46.1% (95% CI: -73.5%, -19.4%)) compared to placebo. Alcohol also reduced the mean average maximum power by 37.6% (95% CI: -62.5%, -13.4%) in the rest condition compared to placebo. Table 2 also shows additional contrasts (e.g. up-down for kinetic and postural and back-forward for rest) for which statistical significance was not noted.



PORTABLE TREMOROGRAPHY Treatment effects on tremor power variables obtained by portable accelerometry are summarized in table 3. No effects of MK-0249 were observed in the rest, postural or kinetic condition during portable accelerometry measurements. Alcohol decreased mean maximum power in the up-down (-25.7% (95% CI: -42.9%, -8.8%), left-right (-27.8% (95% CI: -43.2%, -12.6%) as well as in the backward-forward (-32.7% (95% CI: -55.5%, -10.3%) directions compared to placebo, during the kinetic assessment. Mean average maximum power was also significantly reduced by 27.9% (95% CI: -44.2%, -11.8%) compared to placebo (figure 5). During postural measurements, alcohol reduced mean maximum tremor power in both the left-right and the backward-forward direction compared to placebo by 28.4% (95% CI: -56.6%, -1.1%) and 35.9% (95% CI: -69.4%, -3.8%) respectively. Mean average maximum power was also significantly reduced (-29.0% (95% CI: -58.2%, -0.8%) during postural assessments compared to placebo (figure 6). No significant changes were observed during the rest condition for any treatment, compared to placebo.

PERFORMANCE-BASED CLINICAL RATING SCALE The videotaped clinical rating scales were analysed by a blinded independent expert (E.D.L.). While all assessment time points were videotaped, performance scores obtained at t = 240 minutes were selected (the expected $T_{max}$ for MK-0249) for comparison between treatments. This analysis showed a significant effect of alcohol. Alcohol scores were on average 17% lower (95% CI: 3, 28%) compared to placebo, when both treatments were corrected for baseline values. There was no apparent effect of MK-0249.

CLINICAL RATING SCALE VS. TREMOROGRAPHY
To estimate the predictive value of the experimental tremor registrations (tremorography) for clinical outcome, correlation coefficients of the clinical rating scale *vs.* tremorography were calculated at 240 minutes post dose. The Pearson correlation coefficient for the clinical rating scale *vs.* laboratory tremorography was 0.33 (p=0.018). The Pearson correlation coefficient for the clinical rating scale *vs.* portable tremorography was 0.38 (p=0.006).

## *Additional measurements*

CHOICE REACTION TIME (CRT) During the CRT test both reaction time (msec) and the number of correct responses were determined. Both alcohol and MK-0249 affected the reaction time significantly during this test. Alcohol infusion resulted in a mean increase of 43.2 msec (95% CI: 18.0, 68.5 msec) and MK-0249 resulted in a mean increase of 27.6 msec (95% CI: 1.8, 53.4 msec), as compared to placebo. The mean number of correct reactions after MK-0249 treatment (31.49) improved slightly but statistically significantly compared to placebo treatment (31.23) by 0.26 trials (95% CI: 0.00, 0.52; $p < 0.05$). There was no apparent effect of alcohol on the number of correct responses.

Leeds Sleep Evaluation Questionnaire (LSEQ)

There were no significant effects of alcohol on the LSEQ. On the night following administration, MK-0249 decreased the 'speed of getting to sleep' (i.e. getting to sleep more slowly than usual) by 12.4 mm (95% CI: -23.9, -0.9 mm) compared to baseline (pre-dose). MK-0249 also changed 'restlessness during sleep' (i.e. more restless than usual) and the 'periods of wakefulness' (i.e. more periods of wakefulness than usual) with 30.4 mm (95% CI: -38.9, -21.8 mm) and 30.2 mm (95% CI: -38.8, -21.6 mm) respectively. By comparison, in a study by Hindmarch *et al.* (Hindmarch *et al.*, 2000), 150 mg caffeine increased the time to sleep onset and was associated with deterioration in the perceived quality of sleep compared with placebo. Mean changes in the 100 mm scale were 22 and 16 mm for time to sleep onset and quality of sleep, respectively.

## *Adverse events*

All 19 subjects enrolled in the study were included in the assessment of safety and tolerability. Eighteen (18) of the 19 subjects enrolled, reported one or more clinical adverse experiences (AE). No laboratory adverse experiences were reported, no serious adverse experiences were reported, and no deaths occurred. If a subject reported the same AE more than once (per treatment period), this AE was counted only once. A total of 115 clinical adverse experiences were reported and 31 occurred while taking MK-0249. Of the 31 clinical adverse experiences reported while taking MK-0249, 25 were considered to be related to study drug. The most common drug-related adverse experiences during active MK-0249 treatment were perspiration (5 subjects), insomnia (4 subjects), and nausea (3 subjects). The most frequently reported adverse events after alcohol treatment were sleepiness (9 subjects), inebriation (7 subjects), a painful arm at the start of the infusion (7 subjects), dizziness (7 subjects) and headache (6 subjects). Headache was also reported by 6 subjects after placebo treatment. All symptoms were transient and mild in severity. One patient on placebo discontinued the study due to 'moderate lightheadedness', which was considered to be related to discontinuation of his own medication.

# DISCUSSION

A stable level of alcohol (0.6 $g \cdot L^{-1}$) for seven hours was associated with a reduction in average maximum tremor power by approximately 30%. The tremor diminishing effects of alcohol were not only observed using laboratory accelerometry, the pre-specified primary endpoint, but we also found that the portable tremorographer detected changes in tremor associated with alcohol treatment. Although it has only been tested under standard experimental conditions here, portable tremorography could be a promising technique for portable, standardized, ambulatory tremor registrations.

The present study confirms the commonly reported attenuating effects of alcohol on ET that have been demonstrated earlier, in both database studies (Lou and Jankovic, 1991; Koller *et al.*, 1994) and in controlled trials (Growdon *et al.*, 1975; Koller and Biary, 1984). However, in contrast to most controlled trials in which the effect of alcohol on ET is being studied, our results were obtained under tightly controlled, stable alcohol levels for a prolonged period of time. These fixed levels accounted for a stable condition that minimized the variability. The steady state level of alcohol clearly showed tremor relieving effects, confirming that the (partly experimental) methods and the design

of this study were sensitive enough to detect tremor reductions. The alcohol effects also set a benchmark for clinically significant tremor reductions, demonstrating that the effects of MK-0249 did not only fail to reach statistical significance, but also that they were much smaller than what is achieved with alcohol.

In contrast to resting tremor in Parkinson's disease, essential tremor is characterized by postural and kinetic components (Elble, 2000), which were both affected by alcohol treatment in this study. Tremor symptoms at rest do occur in some ET patients (Louis, 2006), and this finding may explain the effects of alcohol in the resting condition during laboratory accelerometry measurements. The effects of alcohol on ET were maintained for most of the infusion period. Although acute tolerance to the effects of alcohol is frequently described (Martin and Moss, 1993; Hiltunen *et al.*, 2000), no indications for acute changes in effects of alcohol on tremor power were observed in this study.

Despite the promising results during the preclinical phase, single doses of MK-0249 (25 mg) did not reduce maximum tremor power on any of the tremor measurements in this study of alcohol-responsive ET-patients. On average, most tremor measurements even seemed to deteriorate slightly with the H3-inverse agonist, although this was not statistically significant. The clear improvements with alcohol indicated that the power of the study and the sensitivity of the methods were sufficient to detect a clinically significant tremor reduction. Thus, a single dose of MK-0249 25 mg was not effective in reducing ET symptoms and does therefore not seem to have a beneficial effect on human ET. This was not due to a lack of brain penetration or CNS activity, since clear CNS effects were observed on the CRT and the LSEQ after MK-0249 administration. Moreover, a comparable single dose of MK-0249 was associated with alerting effects in a study of sleep-deprived healthy male volunteers (Iannone *et al.*, 2010). It cannot be excluded that a more sustained exposure of MK-0249 would be needed for amelioration of tremor than would have been achieved with a single dose.



Interestingly, an analysis of linkage in a two-generation pedigree of patients with Tourette's syndrome recently identified a rare functional mutation in the HDC gene encoding L-histidine decarboxylase, the rate-limiting enzyme in histamine biosynthesis. These findings pointed to a role for histaminergic neurotransmission in the mechanism and modulation of Tourette's syndrome and tics (Ercan-Sencicek *et al.*, 2010). This suggests that MK 0249 may be beneficial for patients with Tourette's syndrome and related features of compulsive behavior, but this hypothesis has not been tested.

Because neuronal histamine is one of the most important systems that stimulates and maintains wakefulness (Yanai and Tashiro, 2007), and because MK-0249 exerts its action through the histaminergic system, we expected that MK-0249 would be alerting. The significant effect on the LSEQ confirmed that MK-0249 had a pharmacodynamic effect in the CNS in this population of patients. This effect was in agreement with the reported sleep-related adverse events. An H3-inverse agonist was also expected to increase attentiveness. MK-0249 caused a marginal increase in reaction time as measured by the CRT-test, but was also associated with a statistically significant improvement in the number of correct scores. This could signify a shift in performance strategy from speed to accuracy. At the same time, it should be realized that the changes were very small in magnitude. These effects were however quite different from those of alcohol, which caused a significant delay in reaction time on the CRT test and only little effect on the LSEQ.

There was a weak positive correlation between the clinical rating scale and the tremorography data. Hence, experimental tremor registrations used in this study were only slightly predictive for clinical outcome as measured by the clinical rating scale. However, both clinical ratings and portable tremorography improved after alcohol administration, albeit with somewhat less sensitivity than laboratory tremorography, showing convergent evidence for the effects of alcohol on ET. These findings indicate the importance of performing clinical rating scales in addition to portable tremor registration methods, in studies focusing on tremor severity.

A prior tremor study at our centre (CHDR – data on file) showed large pharmacodynamic placebo effects during the first hour following the start of the study. Since tremor increases during anxiety or excitement (Whitney, 2006), this placebo effect was attributed to stress at the start of the experiment. It was therefore decided to precede the actual clamping phase by a single-blinded 90 min saline infusion period, to allow adaptation to the study circumstances before baseline values for each assessment were obtained. In contrast to the previous tremor study (CHDR – data on file), no placebo effect was observed in the present study. An 'adaptation period' therefore seems to be a useful procedure in studies focusing on ET measurements.

MK-0249 showed clear CNS-effects that were compatible with its pharmacological action as a H3RIA, but it did not reduce tremor in this study. In contrast to alcohol, most tremor measurements seemed to increase, although this never reached statistical significance. To the extent that the hypothesis could be tested with exposures from a single dose, these findings suggest that the histamine-3 receptor does not play an important role in the treatment of essential tremor. The harmaline rat model therefore does not seem a valuable predictor for the effects of histaminergic modulation on human ET. Moreover, H3-inverse agonism as a target for treatment of human ET seems of minor relevance. Clearly, more research is needed to investigate the role of histaminergic and other systems in the pathophysiology and possible treatment of this common movement disorder. This study shows that such studies are feasible and informative, using sensitive tremor detection methods in relatively small numbers of patients.



**FIGURE 1** CHEMICAL STRUCTURE OF MK-0249

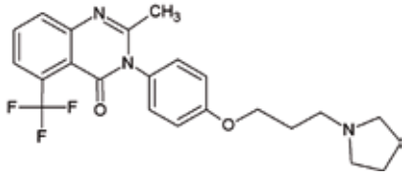



**FIGURE 2** AVERAGE GRAPH OF BREATH ALCOHOL CONCENTRATION (BrAC) WITH SD'S AS ERROR BARS

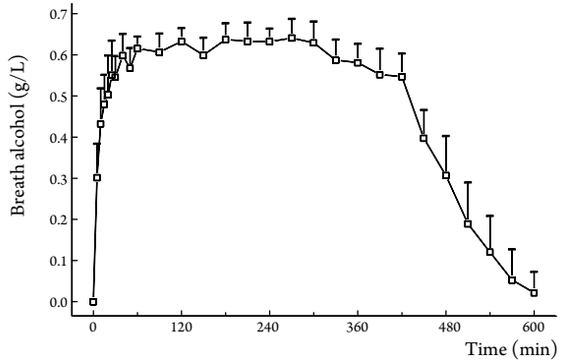



**FIGURE 3** LEAST SQUARE MEANS GRAPH OF LABORATORY ACCELEROMETRY IN THE KINETIC CONDITION

*Average max power (change from baseline), with 95% CI error bars for MK-0249 (up) and alcohol (down).*

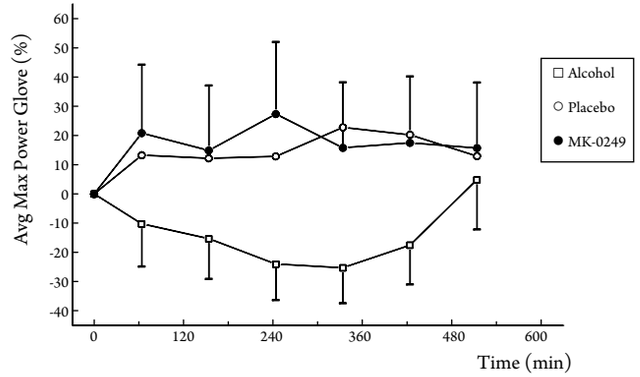


**FIGURE 4** LEAST SQUARE MEANS GRAPH OF LABORATORY ACCELEROMETRY IN THE POSTURAL CONDITION

*Average max power (change from baseline), with 95% CI error bars for MK-0249 (up) and alcohol (down).*

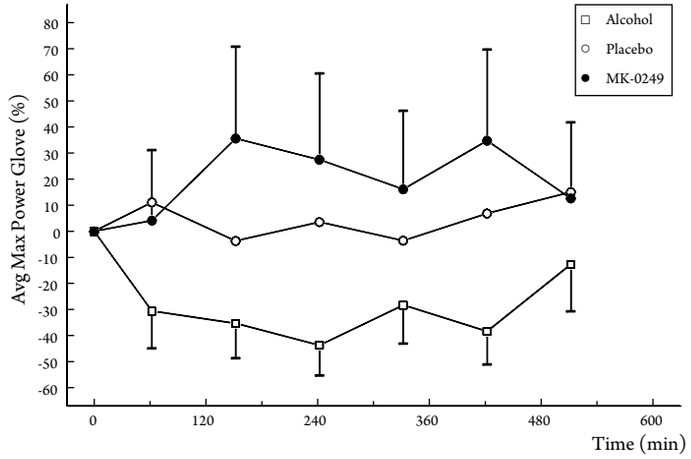



**FIGURE 5** LEAST SQUARE MEANS GRAPH OF PORTABLE ACCELEROMETRY IN THE KINETIC CONDITION

*Average maximum power (change from baseline), with 95% CI error bars for placebo (up) and alcohol (down).*

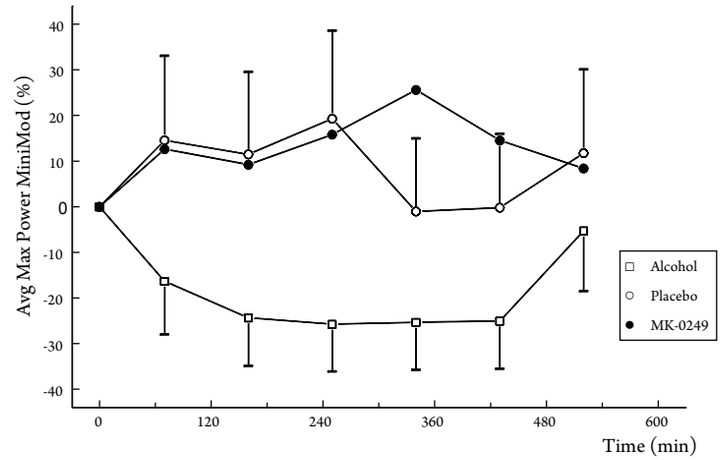





**FIGURE 6** LEAST SQUARE MEANS GRAPH OF PORTABLE ACCELEROMETRY IN THE POSTURAL CONDITION

*Average maximum power (change from baseline), with 95% CI error bars for MK-0249 (up) and alcohol (down).*

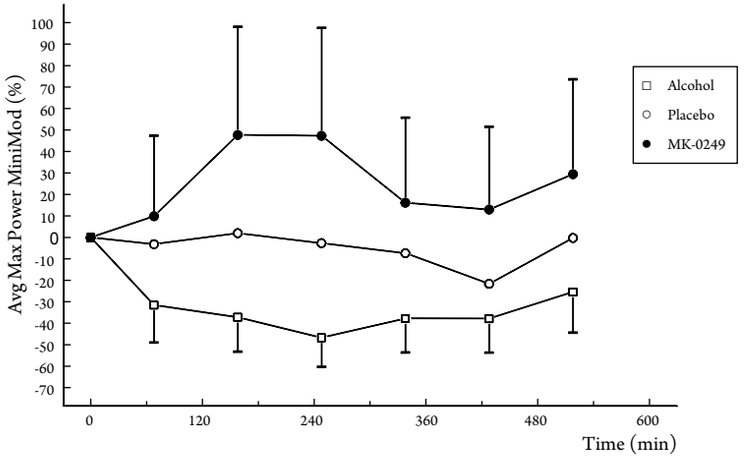



**TABLE 1** BASELINE TREMOR VALUES (IN μV) WITH STANDARD DEVIATIONS BETWEEN BRACKETS

| | | Placebo | MK-0249 | Alcohol |
|---|---|---|---|---|
| Kinetic | Back-Forward | 1.11 (0.73) | 1.21 (1.15) | 1.05 (0.77) |
| | Average | 1.88 (1.11) | 1.92 (1.32) | 1.89 (1.01) |
| | Up-Down | 1.81 (1.82) | 1.84 (2.23) | 1.72 (1.63) |
| | Left-Right | 2.70 (1.71) | 2.72 (1.90) | 2.91 (2.00) |
| Postural | Back-Forward | 0.89 (1.35) | 1.05 (2.13) | 1.08 (1.86) |
| | Average | 1.21 (1.37) | 1.16 (1.13) | 1.22 (0.98) |
| | Up-Down | 1.61 (2.31) | 1.35 (1.57) | 1.44 (1.53) |
| | Left-Right | 1.11 (0.83) | 1.07 (0.86) | 1.14 (0.63) |
| Rest | Back-forward | 0.15 (0.05) | 0.17 (0.10) | 0.20 (0.17) |
| | Average | 0.31 (0.19) | 0.46 (0.50) | 0.50 (0.38) |
| | Up-Down | 0.33 (0.34) | 0.50 (0.68) | 0.56 (0.77) |
| | Left-Right | 0.46 (0.34) | 0.70 (0.92) | 0.73 (0.58) |

**TABLE 2** LEAST SQUARE MEANS AND TREATMENT CONTRASTS OF TREMOR POWER AS ASSESSED BY LABORATORY TREMOROGRAPHY IN THREE DIFFERENT CONDITIONS.

| Laboratory accelerometry maximum power variables | | LS Means 4-8½h (change from baseline) | | | MK-0249 vs. Placebo | | | | Alcohol vs. Placebo | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Placebo | MK-0249 | Alcohol | difference | p-value | 95% CI | | difference | p-value | 95% CI | |
| Kinetic | Back-Forward (μV) | 10.4% | 20.1% | -24.4% | 9.7% | 0.3771 | -12.2% | 31.7% | -34.9% | 0.0003 | -52.7% | -17.3% |
| | Average (μV) | 17.1% | 19.0% | -16.3% | 1.9% | 0.8440 | -17.3% | 21.0% | -33.4% | 0.0002 | -49.8% | -17.2% |
| | Up-Down (μV) | 10.7% | 26.4% | -10.0% | 15.6% | 0.3527 | -18.1% | 49.7% | -20.8% | 0.1474 | -49.6% | 7.7% |
| | Left-Right (μV) | 23.6% | 19.6% | -18.5% | -4.0% | 0.7058 | -25.1% | 17.1% | -42.0% | <.0001 | -59.9% | -24.5% |
| Postural | Back-Forward (μV) | -1.5% | 27.9% | -39.9% | 29.4% | 0.1284 | -8.9% | 68.6% | -38.4% | 0.0056 | -66.1% | -11.9% |
| | Average (μV) | 5.2% | 22.4% | -31.7% | 17.2% | 0.3000 | -15.9% | 50.6% | -37.0% | 0.0047 | -62.7% | -12.0% |
| | Up-Down (μV) | -0.4% | 30.9% | -22.3% | 31.3% | 0.1200 | -8.5% | 72.1% | -21.9% | 0.1558 | -53.2% | 8.7% |
| | Left-Right (μV) | 8.5% | 16.6% | -30.4% | 8.0% | 0.6452 | -27.0% | 43.3% | -38.9% | 0.0063 | -67.2% | -11.6% |
| Rest | Back-Forward (μV) | 1.1% | 13.0% | -11.4% | 11.9% | 0.2048 | -6.7% | 30.6% | -12.6% | 0.1309 | -29.1% | 3.9% |
| | Average (μV) | 19.9% | 5.6% | -17.7% | -14.3% | 0.2905 | -41.6% | 12.8% | -37.6% | 0.0033 | -62.5% | -13.4% |
| | Up-Down (μV) | 14.0% | -5.6% | -12.7% | -19.6% | 0.1588 | -47.5% | 8.0% | -26.7% | 0.0499 | -53.8% | 0.0% |
| | Left-Right (μV) | 33.3% | 13.1% | -12.8% | -20.2% | 0.1791 | -50.4% | 9.7% | -46.1% | 0.0012 | -73.5% | -19.4% |

**TABLE 3** LEAST SQUARE MEANS AND TREATMENT CONTRASTS OF TREMOR POWER AS ASSESSED BY PORTABLE ACCELEROMETRY IN THREE DIFFERENT CONDITIONS

| Portable accelerometry maximum power variables | | LS Means 4-8½h (change from baseline) | | | MK-0249 vs. Placebo | | | | Alcohol vs. Placebo | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Placebo | MK-0249 | Alcohol | difference | p-value | 95% CI | | difference | p-value | 95% CI | |
| Kinetic | Back-Forward (mG) | 16.8% | 26.8% | -15.9% | 10.0% | 0.4640 | -17.4% | 37.5% | -32.7% | 0.0054 | -55.5% | -10.3% |
| | Average (mG) | 7.1% | 15.9% | -20.8% | 8.8% | 0.3620 | -10.6% | 28.3% | -27.9% | 0.0012 | -44.2% | -11.8% |
| | Up-Down (mG) | 1.4% | 10.6% | -24.3% | 9.2% | 0.3642 | -11.1% | 29.6% | -25.7% | 0.0039 | -42.9% | -8.8% |
| | Left-Right (mG) | 7.4% | 16.0% | -20.4% | 8.6% | 0.3474 | -9.7% | 27.0% | -27.8% | 0.0007 | -43.2% | -12.6% |
| Postural | Back-Forward (mG) | -0.4% | 27.9% | -36.3% | 28.3% | 0.2129 | -16.9% | 74.6% | -35.9% | 0.0294 | -69.4% | -3.8% |
| | Average (mG) | -8.3% | 25.8% | -37.3% | 34.2% | 0.0912 | -5.7% | 75.2% | -29.0% | 0.0443 | -58.2% | -0.8% |
| | Up-Down (mG) | -9.1% | 16.1% | -36.1% | 25.2% | 0.1833 | -12.5% | 63.8% | -27.0% | 0.0587 | -55.9% | 1.0% |
| | Left-Right (mG) | -9.6% | 27.0% | -37.9% | 36.6% | 0.0649 | -2.4% | 76.7% | -28.4% | 0.0415 | -56.6% | -1.1% |
| Rest | Back-Forward (mG) | 11.1% | 3.7% | -6.1% | -7.4% | 0.5310 | -31.2% | 16.3% | -17.2% | 0.1285 | -39.8% | 5.2% |
| | Average (mG) | -4.7% | 3.5% | -7.2% | 8.2% | 0.4163 | -12.0% | 28.5% | -2.5% | 0.7874 | -21.2% | 16.2% |
| | Up-Down (mG) | -13.8% | 0.6% | -9.4% | 14.4% | 0.1456 | -5.2% | 34.2% | 4.4% | 0.6257 | -13.8% | 22.7% |
| | Left-Right (mG) | -7.1% | 4.9% | -7.8% | 12.0% | 0.2939 | -10.8% | 34.9% | -0.7% | 0.9444 | -21.5% | 20.0% |





Arrang JM, Morisset S, Gbahou F (2007). Constitutive activity of the histamine H-3 receptor. Trends in Pharmacological Sciences 28: 350-357

Bain PG, Findley LJ, Atchison P, Behari M, Vidailhet M, Gresty M, Rothwell JC, Thompson PD, Marsden C D (1993). Assessing tremor severity. J Neurol Neurosurg Psychiatry 56: 868-873

Brennan KC, Jurewicz EC, Ford B, Pullman SL, Louis ED (2002). Is essential tremor predominantly a kinetic or a postural tremor? A clinical and electrophysiological study. Mov Disord 17: 313-316

Chen JJ, Swope DM (2003). Essential tremor: diagnosis and treatment. Pharmacotherapy 23: 1105-1122

Deuschl G, Bain P, Brin M (1998). Consensus statement of the Movement Disorder Society on Tremor. Ad Hoc Scientific Committee. Mov Disord 13 Suppl 3: 2-23

Deuschl G, Elble RJ (2000). The pathophysiology of essential tremor. Neurology 54: S14-S20

Deuschl G, Zimmerman R, Genger H, Lucking CH (1995). Physiologic classification of essential tremor. In: Handbook of tremor disorders New York: Marcel Dekker 195-208.

Elble RJ (2000). Essential tremor frequency decreases with time. Neurology 55: 1547-1551

Ercan-Sencicek AG, Stillman AA, Ghosh AK, Bilguvar K, O'Roak BJ, Mason CE, Abbott T, Gupta A, King RA, Pauls DL, Tischfield JA, Heiman GA, Singer HS, Gilbert DL, Hoekstra PJ, Morgan TM, Loring E, Yasuno K, Fernandez T, Sanders S, Louvi A, Cho JH, Mane S, Colangelo CM, Biederer T, Lifton RP, Gunel M, State MW (2010). L-histidine decarboxylase and Tourette's syndrome. N Engl J Med 362(20):1901-8

Gironell A, Kulisevsky J, Barbanoj M, Lopez-Villegas D, Hernandez G, Pascual-Sedano B (1999). A randomized placebo-controlled comparative trial of gabapentin and propranolol in essential tremor. Arch Neurol 56: 475-480

Grant SA, Millar K, Kenny GN (2000). Blood alcohol concentration and psychomotor effects. Br J Anaesth. 85: 401-406

Growdon JH, Shahani BT, Young RR (1975). The effect of alcohol on essential tremor. Neurology 25: 259-262

Hiltunen AJ, Saxon L, Skagerberg S, Borg S (2000). Acute tolerance during intravenous infusion of alcohol: Comparison of performance during ascending and steady state concentrations - a pilot study. Alcohol 22: 69-74

Hindmarch I, Rigney U, Stanley N, Quinlan P, Rycroft J, Lane J (2000). A naturalistic investigation of the effects of day-long consumption of tea, coffee and water on alertness, sleep onset and sleep quality. Psychopharmacology 149: 203-216

Iannone R, Palcza J, Renger JJ, Calder N, Cerchio K, Gottesdiener K, Hargreaves R, Dijk DJ, Boyle J, Murphy MG (2010). Acute alerting effects of a novel histamine subtype-3 receptor inverse agonist in healthy sleep-deprived male volunteers. Clin Pharmacol Ther. 88(6): 831-9

Koller WC (1991). A new drug for treatment of essential tremor? Time will tell. Mayo Clin Proc 66: 1085-1087

Koller WC, Biary N (1984). Effect of alcohol on tremors: comparison with propranolol. Neurology 34: 221-222

Koller WC, Busenbark K, Miner K (1994). The relationship of essential tremor to other movement disorders: report on 678 patients. Essential Tremor Study Group. Ann Neurol 35: 717-723

Lieberman HR (2007). Cognitive methods for assessing mental energy. Nutr Neurosci. Oct-Dec;10(5-6):229-42 (Review)

Lou JS, Jankovic J (1991). Essential tremor: clinical correlates in 350 patients. Neurology 41: 234-238

Louis ED (2001). Essential tremor. New England Journal of Medicine 345: 887-891

Louis ED (2005). Essential tremor. Lancet Neurology 4: 100-110

Louis ED (2006). Essential tremor. Clinics in Geriatric Medicine 22: 843-57

Louis ED, Barnes LF, Wendt KJ, Albert SM, Pullman SL, Yu Q, Schneier FR (2000). Validity and test-retest reliability of a disability questionnaire for essential tremor. Mov Disord 15: 516-523

Louis ED, Ottman R, Hauser WA (1998). How common is the most common adult movement disorder? estimates of the prevalence of essential tremor throughout the world. Mov Disord 13: 5-10

Louis ED, Wendt KJ, Albert SM, Pullman SL, Yu Q, Andrews H (1999). Validity of a performance-based test of function in essential tremor. Arch Neurol 56: 841-846

Martin CS, Moss HB (1993). Measurement of acute tolerance to alcohol in human subjects. Alcohol Clin Exp Res 17: 211-216

Martin FC, Le AT, Handforth A (2005). Harmaline-induced tremor as a potential preclinical screening method for essential tremor medications. Movement Disorders 20: 298-305

Nagase T, Mizutani T, Ishikawa S, Sekino E, Sasaki T, Fujimura T, Ito S, Mitobe Y, Miyamoto Y, Yoshimoto R, Tanaka T, Ishihara A, Takenaga N, Tokita S, Fukami T, Sato N (2008). Synthesis, structure-activity relationships, and biological profiles of a quinazolinone class of histamine H-3 receptor inverse agonists. Journal of Medicinal Chemistry 51: 4780-4789

O'Connor S, Morzorati S, Christian J, Li TK (1998). Clamping breath alcohol concentration reduces experimental variance: Application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcoholism-Clinical and Experimental Research 22: 202-210

Pahwa R, Lyons KE (2003). Essential tremor: differential diagnosis and current therapy. Am J Med 115: 134-142

Parrott AC, Hindmarch I (1980). The Leeds Sleep Evaluation Questionnaire in Psychopharmacological Investigations - A Review. Psychopharmacology 71: 173-179
Rajput AH, Jamieson H, Hirsh S, Quraishi A (1975). Relative efficacy of alcohol and propranolol in action tremor. Can J Neurol Sci 2: 31-35
Schuurman PR, Bosch DA, Bossuyt PMM, Bonsel GJ, van Someren EJW, De Bie RMA, Merkus MP, Speelman JD (2000). A comparison of continuous thalamic stimulation and thalamotomy for suppression of severe tremor. New England Journal of Medicine 342: 461-468
Van Hilten JJ, van Dijk JG, Dunnewold RJ, van der Velde EA, Kemp B, van Brummelen P, van der Krogt JA, Roos RA, Buruma OJ (1991). Diurnal variation of essential and physiological tremor. J Neurol Neurosurg Psychiatry 54: 516-519
Whitney C M (2006). Essential tremor. Neurologist 12: 331-332
Yanai K, Tashiro M (2007). The physiological and pathophysiological roles of neuronal histamine: An insight from human positron emission tomography studies. Pharmacology & Therapeutics 113: 1-15
Zeuner KE, Molloy FM, Shoge RO, Goldstein SR, Wesley R, Hallett M (2003). Effect of ethanol on the central oscillator in essential tremor. Mov Disord 18: 1280-1285
Zoethout RW, Schoemaker RC, Zuurman L, van Pelt H, Dahan A, Cohen AF, van Gerven JM (2009). Central nervous system effects of alcohol at a pseudo-steady-state concentration using alcohol clamping in healthy volunteers. Br J Clin Pharmacol 68: 524-534
Zoethout RW, van Gerven JM, Dumont GJ, Paltansing S, Van Burgel ND, Van der Linden M, Dahan A, Cohen AF, Schoemaker RC (2008). A comparative study of two methods for attaining constant alcohol levels. Br J Clin Pharmacol 66: 674-681



# CHAPTER 9

## *Pharmacokinetics and central nervous system effects of the novel dopamine D3 receptor antagonist GSK598809 and intravenous alcohol infusion at pseudo-steady state*

*Journal of Psychopharmacology 2012, Feb; 26(2), 303-14*

E.T. te Beek, R.W.M. Zoethout, M.S.G. Bani, A. Andorn, L. Iavarone, E.S. Klaassen, P. Fina and J.M.A. van Gerven

## ABSTRACT

GSK598809 is a novel selective dopamine $D_3$ receptor antagonist, currently in development for treatment of substance abuse and addiction. In a blinded, randomized, placebo-controlled study, effects of single oral doses of 175 mg GSK598809 were evaluated in healthy volunteers. Pharmacokinetics, central nervous system (CNS) effects and potential for interactions with alcohol were evaluated, using an alcohol infusion paradigm and analysis of eye movements, adaptive tracking, visual analogue scales, body sway, serum prolactin and verbal visual learning test. Adverse effects of GSK598809 included headache, dizziness and somnolence. Plasma concentration of GSK598809 was maximal 2-3 hours postdose and decreased with a half life of roughly 20 hours. CNS effects were limited to prolactin elevation and decreased adaptive tracking. Co-administration of GSK598809 and alcohol did not affect alcohol pharmacokinetics, but caused a 9% decrease of $C_{max}$ and a 15% increase of AUC of GSK598809. CNS effects of co-administration were mainly additive, except a small supra-additive increase in saccadic reaction time and decrease in delayed word recall. In conclusion, GSK598809 causes elevation of serum prolactin and a small decrease in adaptive tracking performance. After co-administration with alcohol, effects of GSK598809 are mainly additive and the combination is well tolerated in healthy volunteers.


## INTRODUCTION

A large body of evidence indicates that the mesolimbic dopaminergic pathway, which includes dopaminergic neurons in the ventral tegmental area projecting to the nucleus accumbens and other limbic forebrain structures, is one of the major neuronal circuits involved in the acute rewarding effects of drugs of abuse (Cami and Farre, 2003; Hyman and Malenka, 2001; Hyman, 2005; Koob and Nestler, 1997; Koob *et al.*, 1998). Although addictive drugs interact with many different neurotransmitter systems, most drugs ultimately cause an acute increase in synaptic dopamine in the nucleus accumbens and the mesolimbic dopaminergic system (Koob and Bloom, 1988; Nestler, 2005; Pierce and Kumaresan, 2006), as demonstrated by microdialysis studies in rats (Di Chiara and Imperato, 1988) and positron emission tomography (PET) studies in humans (Volkow *et al.*, 1999; Volkow *et al.*, 2003; Volkow *et al.*, 2004; Volkow *et al.*, 2007; Volkow *et al.*, 2009). Several important observations have suggested that dopamine $D_3$ receptors may play a significant role in the effects of drugs of abuse and the pathophysiology of drug addiction (Heidbreder *et al.*, 2005; Le Foll *et al.*, 2005). First, dopamine $D_3$ receptors are located primarily in mesolimbic regions such as nucleus accumbens and ventral striatum (Gurevich and Joyce, 1999; Herroelen *et al.*, 1994; Landwehrmeyer *et al.*, 1993; Murray *et al.*, 1994; Seeman *et al.*, 2006). Second, studies in animal models have demonstrated that dopamine $D_3$ receptor activation may be involved in the reinforcing effects and self-administration of cocaine (Caine and Koob, 1993). Third, long term drug exposure appears to cause upregulation of dopamine $D_3$ receptors as demonstrated in postmortem studies of cocaine overdose fatalities (Segal *et al.*, 1997; Staley and Mash, 1996). Accordingly, it has been suggested that dopamine $D_3$ antagonism may be an effective strategy in pharmacotherapy of addiction (Heidbreder *et al.*, 2005; Joyce and Millan, 2005; Levant, 1997).

GSK598809 is a novel, potent and selective dopamine $D_3$ receptor antagonist (Searle *et al.*, 2010), which is being developed as a novel treatment for substance dependence disorders. Functional assays showed that GSK598809 has greater than 100-fold selectivity for dopamine $D_3$ receptors over dopamine $D_2$, histamine $H_1$, muscarinic $M_1$, $M_2$, $M_3$, $M_4$, serotonin 5-$HT_{1A}$, 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors (data on file). Conditioned place preference (CPP) experiments in animal models indicated that GSK598809 significantly reduced nicotine- and cocaine-seeking behavior in a dose-dependent manner (data on file). In addition, GSK598809 significantly prevented relapse to nicotine-seeking behavior, although no effect was observed on reducing alcohol consumption in rats (data on file).

The present study was performed to evaluate the pharmacokinetics and central nervous system (CNS) effects of single oral doses of GSK598809 in

healthy volunteers. Special emphasis was given to evaluating possible interactions with alcohol, because the target population of patients will have alcohol dependence as primary disorder, or may abuse alcohol as comorbidity next to another substance abuse disorder. Pharmacokinetic interactions between alcohol and GSK598809 are theoretically possible, because a metabolite of GSK598809 shows *in vitro* to have a potential for inhibiting CYP2E1, which is one of the main enzymes involved in alcohol metabolism (Lieber, 1997). Also, pharmacodynamic interactions are theoretically possible as both compounds are centrally active and influence the dopamine system. However, apart from these theoretical possibilities, there are no reasons to assume *a priori* that any specific pharmacodynamic interaction will occur between GSK598809 and alcohol. Currently, no validated human pharmacodynamic markers for dopamine $D_3$ antagonism are available. For exploratory purposes, we used a battery of quantitative central nervous system tests, sensitive to various compounds, including alcohol (Zoethout *et al.*, 2009) and antipsychotic drugs (dopamine $D_2$ receptor antagonists) (de Visser *et al.*, 2001), was used to evaluate pharmacodynamic effects. An oral dose of 175 mg GSK598809 was chosen because positron emission tomography using [$^{11}$C]-(+)-PHNO in healthy volunteers has demonstrated that this dose can induce high occupancy (near 100%) of dopamine $D_3$ receptors in the substantia nigra (Searle *et al.*, 2010). Also, previous studies in healthy volunteers demonstrated that this dose is generally well tolerated (data on file).

## METHODS

### *Study design*

Twenty healthy volunteers, between 18 and 65 years of age and with a body mass index (BMI) between 18 and 30 kg/m$^2$, were planned to participate in a blinded, randomized, placebo-controlled, double-dummy, four-period crossover study. The study was approved by the medical ethics review board of the Leiden University Medical Center and registered at the NIH database of clinical trials (website http://clinicaltrials.gov) with identifier NCT00887367 and GSK ID number 106591. Prior to medical screening, all volunteers gave written informed consent. All volunteers underwent training sessions for the pharmacodynamic tests in order to minimize possible learning effects.

Volunteers were assigned to a randomized treatment sequence (see figure 1), consisting of one period of oral administration of 175 mg of GSK598809 combined with intravenous alcohol infusion (alcohol clamping, see below for further details), one period of oral administration of 175 mg of GSK598809 combined with intravenous placebo infusion, one period of oral placebo administration combined with intravenous alcohol infusion, and one period with oral placebo administration combined with intravenous placebo infusion. This study design enables analysis of the following comparisons (see figure 1):

1 Administration of 175 mg GSK598809 ($n$ = 20) versus placebo ($n$ = 20) Intravenous alcohol infusion ($n$ = 20) versus placebo ($n$ = 20)
2 Co-administration of 175 mg GSK598809 and intravenous alcohol infusion ($n$ = 20) versus placebo ($n$ = 20)
3 Co-administration of 175 mg GSK598809 and intravenous alcohol infusion ($n$ = 20) versus intravenous alcohol infusion alone ($n$ = 20)

GSK598809 or matching placebo was administered orally 30 minutes after the start of the ethanol (or placebo) infusion. The alcohol (or placebo) infusion continued for 5 hours in total to cover the main part of the plasma concentration curve of GSK598809. Each study period consisted of five study days. The randomized treatment was administered in the morning of the first study day, followed by pharmacokinetic and pharmacodynamic measurements at regular time points. All periods were separated by a washout time of at least five days.

Occasional (non-daily) smokers were eligible to participate in the study. Subjects were excluded from participation if they smoked on a daily basis. Also, subjects were excluded if they had an average daily intake of greater than 2 units (in case of females) or 3 units (in case of males) or an average weekly

alcohol intake of greater than 14 units (in case of females) or 21 units (in case of males). One unit is equivalent to a half-pint (220 mL) of beer or 1 (25 mL) measure of spirits or 1 glass (125 mL) of wine. Subjects were instructed to abstain from smoking and alcoholic drinks on the day preceding all study periods and all subsequent study days. In addition, use of illicit drugs was not permitted. In all study periods, breath alcohol measurements were performed to ascertain non-use of alcohol. Also, urine drug screening for cocaine, amphetamines, opiates (morphine), benzodiazepines, barbiturates and THC (Innovacon, Inc., San Diego, California, USA) was performed to ascertain non-use of illicit drugs.

## *Alcohol clamping*

The method for attaining constant alcohol levels has been described in detail elsewhere (Zoethout *et al.*, 2008; Zoethout *et al.*, 2009). In brief, alcohol (ethanol 10% w/v solution in 5% glucose) was infused intravenously over a period of five hours, guided by breath alcohol measurements to maintain a pseudo-steady state alcohol serum level of 0.6 g/L. This target level was chosen because this level produces significant central nervous system effects without causing too many inadvertent effects and is considered safe, since it is only just above the legal driving limit in the Netherlands (i.e. 0.5 g/L). Alcohol infusion started 30 minutes prior to administration of GSK598809. The infusion rate for the first ten minutes was determined using demographic data of the volunteer (weight, height, age and gender). Infusion rates were subsequently adjusted, guided by breath alcohol measurements at baseline and at every five minutes for the first 30 minutes after the start of the infusion, every 10 minutes for the next 30 minutes and then every half hour until the end, using two calibrated Alco-Sensor IV Intoximeters (Honac, Apeldoorn, the Netherlands), which were alternated to avoid fatigue of the sensors. To prevent local pain at the beginning of the alcohol infusion, an additional diluting glucose 5% infusion at 100 mL/h was given to all participants during the first 10 minutes after the start of the alcohol infusion over the same infusion line. Alcohol clamping was performed in a randomized, double-blind, placebo-controlled fashion by an infusion assistant, who was not a member of the study team. A sham procedure, consisting of saline infusion in a manner similar to the alcohol infusion, including repeated breath alcohol measurements and subsequent infusion rate adjustments, was used to maintain blinding of the subject and the rest of the team. The mock infusion rate adjustments were provided by the clamping program.

## *Safety monitoring*

Evaluation of adverse events, 12-lead electrocardiograms (ECG), blood pressure, heart rate, alcohol breath test, urinalysis and blood sampling for haematology and chemistry was performed at regular time points after each dose administration. Automated oscillometric blood pressures were measured using a Nihon-Kohden BSM-1101K monitor or a Colin Pressmate BP 8800. ECGs were obtained with Cardiofax V equipped with ECAPS12 analysis program (Nihon-Kohden, Tokyo, Japan). In addition, telemetry monitoring was started at the beginning of alcohol infusion and was continued for six hours. Volunteers were evaluated for akathisia and extrapyramidal symptoms, using the Barnes Akathisia Rating Scale (Barnes, 1989), Simpson-Angus Scale (Simpson and Angus, 1970) and Abnormal Involuntary Movement Scale (Munetz and Benjamin, 1988).

## *Pharmacokinetics*

Venous blood samples for GSK598809 concentration analysis were collected prior to dose administration and at 15 and 30 minutes and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 hours after dose administration. Concentration of GSK598809 in plasma samples was determined using protein precipitation followed by HPLC/MS analysis with a lower limit of quantification (LLQ) of 0.5 ng/mL. Pharmacokinetic parameters of GSK598809 include the maximum observed plasma concentration ($C_{max}$), time to reach maximum plasma concentration ($t_{max}$), area under the plasma concentration-time curve extrapolated to infinity ($AUC_{\infty}$) and terminal phase half life ($t_{½}$).

Venous blood samples for pharmacokinetic analysis of serum alcohol were taken prior to start of infusion and at 15, 30, 45, 60, 90, 150, 210, 270 and 390 minutes after start of alcohol infusion. Serum alcohol levels were measured with an enzymatic assay (Roche Diagnostics, Mannheim, Germany) using a Hitachi 911 (Boehringer Mannheim, Mannheim, Germany). In this enzymatic assay, alcohol and nicotinamide adenine dinucleotide ($NAD^+$) are converted to acetaldehyde and NADH by alcohol dehydrogenase (ADH). The NADH formed during the reaction, measured photometrically as a rate of change in absorbance, is directly proportional to the alcohol concentration.

## *Pharmacodynamic testing*

All pharmacodynamic measurements were performed as described previously (de Haas *et al.*, 2009; Liem-Moolenaar *et al.*, 2010a). Volunteers were tested individually in a quiet room with ambient illumination. Quantitative tests, sensitive to the effects of alcohol (Zoethout *et al.*, 2009) and single oral doses of antipsychotic drugs (dopamine $D_2$ receptor antagonists) (de Visser *et al.*, 2001) such as haloperidol (Liem-Moolenaar *et al.*, 2010a; Liem-Moolenaar *et al.*, 2010b) and risperidone (Liem-Moolenaar *et al.*, 2011) in healthy volunteers, included measurements of smooth pursuit and saccadic eye movements, adaptive tracking, body sway, visual analogue scales, the visual verbal learning test (VVLT) and serum prolactin levels. Previous studies using the alcohol clamping paradigm and this pharmacodynamic test battery (Zoethout *et al.*, 2009) demonstrated that smooth pursuit eye movements and body sway were the most sensitive pharmacodynamic parameters for the effects of alcohol. In order to obtain accurate time profiles of the effects of alcohol and GSK598809, smooth pursuit eye movements and body sway were recorded at a high frequency. The other pharmacodynamic tests could not be performed as frequently due to limitations in time and logistics.

ANALYSIS OF EYE MOVEMENTS To evaluate oculomotor performance and sedation, smooth pursuit and saccadic eye movements were recorded as described previously (Baloh *et al.*, 1975; Bittencourt *et al.*, 1983; van Steveninck *et al.*, 1991; van Steveninck *et al.*, 1999), using a microcomputer-based system for data recording and analysis (Cambridge Electronic Design Ltd., Cambridge, UK), Nihon-Kohden equipment for stimulus display, signal collection and amplification (Nihon-Kohden, Tokyo, Japan), and disposable surface electrodes (Medicotest N-00-S, Olstykke, Denmark). For smooth pursuit eye movements, a target light source moves sinusoidally over 20° eyeball rotation at frequencies ranging from 0.3 to 1.1 Hz. The time in which the eyes were in smooth pursuit was calculated for each frequency and expressed as the percentage of stimulus duration. The average percentage of smooth pursuit for all frequencies was used as parameter. Smooth pursuit eye movements were recorded prior to dose administration and at 10, 20, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420 and 450 minutes after dose administration. For saccadic eye movements, the target light source jumps from side to side. Peak velocity (degrees per second), reaction time and inaccuracy (%) was calculated of all artifact-free saccades. Saccadic eye movements were recorded prior to dose administration and at 30, 90, 150, 210, 270, 330, 390 and 450 minutes after dose administration.

ADAPTIVE TRACKING To evaluate visuo-motor coordination, the adaptive tracking task was performed as described previously (Borland and Nicholson, 1984; Gijsman *et al.*, 1998; van Steveninck *et al.*, 1991; van Steveninck *et al.*, 1993; van Steveninck *et al.*, 1999), using customized equipment and software developed by K.W. Hobbs (Hertfordshire, UK). Adaptive tracking is a pursuit tracking task in which a circle moves randomly over a computer screen and the volunteer must try to keep a dot inside the moving circle using a joystick. If this effort is successful, the speed of the moving circle is increased and if the effort is unsuccessful, the speed is reduced. The adaptive tracking task was performed prior to dose administration and at 30, 90, 150, 210, 270, 330, 390 and 450 minutes after dose administration and performance was scored over a fixed period of three minutes. Average performance and standard deviation of scores were used for analysis.

BODY SWAY Postural stability in the sagittal plane was measured with an apparatus similar to the Wright ataxiameter (Wright, 1971), using a string attached to the waist of the volunteer. Measurements were performed prior to dose administration and at 10, 20, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420 and 450 minutes after dose administration. Movements over a period of two minutes, while standing still with eyes closed, were integrated and expressed as mm sway.

VISUAL ANALOGUE SCALES Subjective effects were quantified prior to dose administration and at 30, 90, 150, 210, 270, 330, 390 and 450 minutes after dose administration using a Dutch translation of the visual analogue scales (VAS), originally described by Norris (Norris, 1971), to derive three composite factors corresponding to alertness, mood (contentedness) and calmness, as described by Bond & Lader (Bond and Lader, 1974). In addition, a visual analogue scale was used to quantify the subjective effects of alcohol.

VISUAL VERBAL LEARNING TEST The visual verbal learning test (VVLT) (Schmitt *et al.*, 2000) is an adapted version of the auditory verbal learning test (Rey, 1964) and was performed 150 minutes after dose administration. Three trials of 30 words are presented on a computer screen in the same sequence. The volunteer is requested to reproduce as many words as possible at the ending of each trial (immediate recall) and after 30 minutes (delayed recall). The number of correctly reproduced words is analyzed for each trial. Also, a recognition test is performed, consisting of 15 previously presented words and 15 new words, in which the volunteer has to indicate recognition of the word (delayed recognition) as quickly as possible. Response time and the number of correctly recognized words are analyzed.

SERUM PROLACTIN LEVELS Blood samples for measurement of prolactin levels were collected at baseline and at 60, 90, 120, 210, 390, 720 and 1320 minutes after study drug administration and serum was separated by centrifugation (2000 g at 4°C for 10 minutes). Prolactin levels were determined using an electrochemiluminescence immunoassay (ECLIA) on a Modular Analytics E170 (Elecsys module) immunoassay analyzer.

## *Statistical analysis*

Analysis of variance models were performed on the pharmacokinetic parameters, including the factors treatment and period as fixed effects and subject as random effect. AUC and $C_{max}$ pharmacokinetic parameters were log-transformed prior to analysis. Comparisons were expressed as ratios of the pharmacokinetic parameters after GSK598809 combined with ethanol relative to those after alcohol alone or relative to those after GSK598809 alone.

Pharmacodynamic data were compared using a mixed model analysis of variance with treatment, gender, period, time, and treatment by time as fixed factors, and with subject, subject by treatment and subject by time as random factors. VVLT data were compared using a mixed model analysis of variance with treatment, gender and period as fixed factors, and with subject as random factor. The parameters body sway, prolactin, saccadic eye movements and the delayed word variables were log-transformed prior to analysis to correct for the expected log-normal distribution of the data. The following contrasts were calculated (see figure 1): alcohol versus placebo, GSK598809 versus placebo, co-administration of GSK598809 and alcohol versus placebo, co-administration of GSK598809 and alcohol versus alcohol alone.

Supra-additive effects (defined as effects, resulting from co-administration of two independent agents, being greater than the sum of effects of each individual agent) were evaluated by analyzing the contrast of the effects of co-administration of GSK598809 and alcohol with subtraction of the effects of GSK598809 alone versus the effects of alcohol alone with subtraction of the effects of placebo.

After identifying gender effects on prolactin levels, analysis of prolactin data was repeated using a mixed model analysis of variance with treatment, gender, period, time, treatment by gender, treatment by time, gender by time and treatment by gender by time as fixed factors, and with subject, subject by

treatment and subject by time as random factors. Contrasts were calculated in original measurement unit with 95% confidence intervals and the associated *p*-value, except for the log-transformed parameters, which were calculated as a percentage relative to placebo or alcohol. All calculations were performed using SAS for Windows version 9.1.3 (SAS Institute Inc., Cary, North Carolina, USA).

# RESULTS

## *Subjects*

Twenty volunteers (10 males and 10 females) were included in the study. Volunteers had a mean age of 32.8 years (range 18-55), weight of 73.5 kg (range 54-108) and body mass index (BMI) of 23.6 kg/m² (range 18.5 - 29.8). One female volunteer tested positive for benzodiazepines on the drug screen in study period 2 and thereby violated the exclusion criteria. She was subsequently withdrawn from the study and not replaced. This volunteer was administered alcohol infusion and placebo capsules in the first study period.

## *Clinical observations*

All adverse events were transient and mild or moderate in severity and no serious adverse events occurred during the study. Overall, the most frequent adverse effect were headache, somnolence, feeling drunk, dizziness, fatigue, pain at infusion site, nausea and vomiting (see table 1). Somnolence and fatigue were reported more frequently after GSK598809 administration combined with alcohol ($n$ = 18), compared to alcohol alone ($n$ = 8), GSK598809 alone ($n$ = 4) or placebo ($n$ = 4). There were no consistent and clinically relevant changes on the Barnes Akathisia Rating Scale, Simpson-Angus Scale and Abnormal Involuntary Movement Scale. Mild short-lasting akathisia was reported spontaneously once after administration of GSK598809 combined with alcohol and once after GSK598809 alone, but these events were not verified objectively by the Barnes Akathisia Rating Scale, when this was performed as scheduled. There were no consistent and clinically relevant changes in vital signs, blood chemistry and haematology or any of the ECG intervals.

## *Pharmacokinetics of alcohol*

Following intravenous infusion, serum alcohol concentration increased rapidly and remained constant at the target level all over the time of infusion, after which serum concentrations declined (see figure 2).

## *Pharmacokinetics of GSK598809*

Pharmacokinetic parameters are presented in table 2. Oral administration of GSK598809 resulted in peak levels after roughly 2 to 3 hours (see figure 3) with an apparent bi-exponential decline and a half life of roughly 20 hours.

## *Pharmacokinetics of GSK598809 combined with alcohol*

No relevant effect of GSK598809 on ethanol pharmacokinetic parameters was observed. Regarding the effects of alcohol on GSK598809 pharmacokinetic parameters, an average 15% increase in $AUC_\infty$ of GSK598809 (ratio of LS geometric means 1.15; 90% confidence interval 1.02/1.30) and an average 9% decrease in $C_{max}$ of GSK598809 (ratio of LS geometric means 0.91; 90% confidence interval 0.83/1.00) was observed after administration of GSK598809 combined with alcohol compared to GSK598809 alone. Other parameters were roughly similar compared to GSK598809 alone (see table 2).

## *Pharmacodynamics of alcohol*

Following alcohol infusion, a statistically significant decrease in adaptive tracking and smooth pursuit eye movements and increase in body sway were

observed compared to placebo, but there were no effects on saccadic peak velocity, inaccuracy or reaction time (see table 3 and figures 4 to 7). Clear increases in the feeling of being drunk were noted. In addition, there was some decrease in alertness on the VAS Bond & Lader scales, compared with placebo (see table 4 and figure 8). Alcohol did not demonstrate any clear effect on VVLT performance (see table 5).

### *Pharmacodynamics of GSK598809*

Following administration of GSK598809, transient increases in serum prolactin were observed (see figure 9). Peak prolactin levels, which increased much more in females than in males ($p < 0.0001$), were reached roughly 3 hours after study drug administration and normalized within 12 hours. Administration of GSK598809 also caused a decrease in adaptive tracking performance, which was maximal between 2 and 6 hours after dose administration (see table 3 and figure 6). No statistically significant effects were observed on any of the other pharmacodynamic parameters (see tables 3 to 5 and figures 4, 5, 7 and 8).

### *Pharmacodynamics of GSK598809 combined with alcohol*

Co-administration of GSK598809 and alcohol resulted in additive effects on several pharmacodynamic parameters, compared to either treatment alone (see tables 3 to 5 and figures 4 to 8). While the effects of administration of GSK598809 alone or alcohol alone on saccadic eye movements did not reach statistical significance, co-administration of GSK598809 and alcohol resulted in a significant impairment. No significant supra-additive effects were found on any of the pharmacodynamic parameters, except a small increase in saccadic reaction time (see table 3) and a small decrease in delayed word recall on the VVLT (see table 5).



## DISCUSSION

The present study was performed to evaluate the pharmacokinetics and central nervous system (CNS) effects of single oral doses of 175 mg of the novel dopamine $D_3$ receptor antagonist GSK598809 in healthy volunteers and possible interactions with alcohol. Within the present group of healthy volunteers, single doses of GSK598809 were generally well tolerated. The most frequent adverse effects were mild headache, dizziness, somnolence, nausea and vomiting. GSK598809 did not induce any significant extrapyramidal symptoms. Mild short-lasting akathisia was reported spontaneously once after administration of GSK598809, although this was not verified objectively by the Barnes Akathisia Rating Scale, when this was performed according to protocol. Plasma concentration of GSK598809 increased rapidly after oral administration ($t_{max}$ of roughly 2 to 3 hours) and subsequently decreased in an apparent bi-exponential manner (terminal half life of roughly 20 hours). No effect of GSK598809 on the pharmacokinetics of alcohol was observed, but alcohol decreased $C_{max}$ and increased the AUC of GSK598809 to a limited extent, which is not considered to be of any clinical significance.

The CNS effects of GSK598809 alone were limited to an elevation of serum prolactin and a small decrease in adaptive tracking performance, with a time course that corresponds well with the observed pharmacokinetics. This study represents the first use of this pharmacodynamic test battery to evaluate the effects of a selective dopamine $D_3$ antagonist in healthy volunteers. As a result, no data of other dopamine $D_3$ receptor antagonists are available for comparison with the effects of GSK598809. Antipsychotic drugs (dopamine $D_2$ receptor antagonists) have been evaluated extensively with this pharmacodynamic test battery (de Visser *et al.*, 2001; Liem-Moolenaar *et al.*, 2010a; Liem-Moolenaar *et al.*, 2010b; Liem-Moolenaar *et al.*, 2011), but differences in tissue expression of $D_2$ and $D_3$ receptors and differences in receptor affinity profiles of the various drugs significantly limit the comparison of their effects to those of GSK598809.

Prolactin secretion by the lactotroph cells of the pituitary gland is under inhibitory control by dopamine, released predominantly from tuberoinfundibular dopaminergic neurons, acting on lactotrophic dopamine $D_2$ receptors (Ben-Jonathan and Hnasko, 2001; Freeman *et al.*, 2000). Pharmacological blockade of dopamine $D_2$ receptors removes this inhibitory influence and subsequently increases prolactin levels. However, the role of dopamine $D_3$ receptor antagonism in the control of prolactin secretion is unknown. An autoradiographic study has demonstrated presence of $D_3$ receptors in the pituitary gland (Herroelen *et al.*, 1994), but the density was quite low and any possible role for dopamine $D_3$ receptors in the pituitary gland in endocrine function remains unclear. Alternatively, dopamine $D_3$ antagonism may cause prolactin elevation by acting at the level of the hypothalamus. The periventricular and arcuate nuclei of the hypothalamus constitute the origin of the tuberoinfundibular dopaminergic pathway, which projects to the median eminence, where dopamine is released into the hypophyseal portal vessels (Albanese *et al.*, 1986; Ben-Jonathan and Hnasko, 2001; Moore and Bloom, 1978). Hypothalamic expression of dopamine $D_3$ receptors has not yet been examined in full detail, but one study found no detectable levels in the arcuate nucleus, whereas the periventricular nucleus was not investigated (Gurevich and Joyce, 1999). Therefore, any possible effect of dopamine $D_3$ antagonism on the hypothalamus, leading to prolactin elevation, also remains unclear. Another theoretical possibility is that GSK598809 could be acting on extra-dopaminergic mechanisms of prolactin control. However, a more likely explanation is that, despite a greater than 100-fold selectivity for $D_3$ receptors over $D_2$ receptors, GSK598809 at doses of 175 mg might cause enough $D_2$ receptor antagonism to modestly increase prolactin secretion.

The increases in serum prolactin following GSK598809 administration were much larger in female volunteers than in male volunteers (see figure 9). Similar gender differences in prolactin levels have been previously demonstrated after administration of typical antipsychotic drugs (Kuruvilla *et al.*, 1992; Meltzer and Fang, 1976; Meltzer *et al.*, 1983; Smith *et al.*, 2002; Wode-Helgodt *et al.*, 1977) and atypical antipsychotic drugs (Grunder *et al.*, 1999; Kinon *et al.*, 2003; Yasui-Furukori *et al.*, 2010), which have been attributed to an enhanced responsiveness of lactotrophs to prolactin-releasing stimuli by females, compared to males, due to the effects of estrogens (Ben-Jonathan and Hnasko, 2001; Buckman and Peake, 1973; Buckman *et al.*, 1976; PETty, 1999).

The other pharmacodynamic tests used in this study measure complex CNS functions. The neurophysiological and neurochemical mechanisms underlying these CNS functions have not yet been fully characterized, but are likely to involve multiple neurotransmitter receptor systems. The decrease in adaptive tracking performance after administration of GSK598809 indicates slight impairment in visuo-motor performance. Similar impairments in adaptive tracking performance have also been observed after administration of single doses of antipsychotic drugs (dopamine $D_2$ antagonists) such as haloperidol (Liem-Moolenaar *et al.*, 2010a) and risperidone (Liem-Moolenaar *et al.*, 2011) in healthy volunteers. However, unlike haloperidol and risperidone, GSK598809 did not affect smooth pursuit and saccadic eye movements, memory performance or any of the visual analogue scales. This clearly demonstrates the pharmacological distinctions between GSK598809 and antipsychotic drugs, but it is not necessarily an argument for $D_3$ receptor selectivity, since most antipsychotic drugs affect other neurotransmitter systems in addition to $D_2$ receptors. Recently, however, we examined the novel selective dopamine $D_2$ receptor antagonist JNJ-37822681 in healthy volunteers using similar pharmacodynamic tests (te Beek *et al*, 2011). Single oral doses of 15 mg JNJ-37822681 caused a reduction in adaptive tracking performance of about 2%, comparable to 175 mg doses of GSK598809. Also, this dose of JNJ-37822681 caused about 60% $D_2$ receptor occupancy and produced prolactin elevations of more than 700%, much larger than the 117% increase found with GSK598809 in this study. JNJ-37822681 also impaired saccadic and smooth pursuit eye movements, which were unaffected by GSK598809. Although these indirect comparisons have their limitations, they provide at least some support for the *in vivo* selectivity of GSK598809 for dopamine $D_3$ receptors.

In addition to obtaining a dopamine $D_3$ receptor-mediated profile of CNS effects, our study was specifically designed to evaluate potential pharmacokinetic and pharmacodynamic interactions between GSK598809 and alcohol. An intravenous alcohol clamping paradigm was used to achieve pseudo-steady state levels of alcohol, which produced clear and expected CNS effects, similar to previously reported results of this alcohol clamping paradigm (Zoethout *et al.*, 2008; Zoethout *et al.*, 2009). Co-administration of GSK598809 with intravenous alcohol levels at pseudo-steady state was generally well tolerated. However, somnolence and fatigue were reported more frequently, compared with the other treatments. Mild akathisia was reported spontaneously once, which was not verified objectively by the Barnes Akathisia Rating Scale, similar to the event after administration of GSK598809 alone. Co-administration of GSK598809 and alcohol generally produced additive CNS effects, without clear signs of supra-additive amplification of the effects of each treatment alone. Both GSK598809 and alcohol caused slight impairments of saccadic eye movements that failed to reach statistical significance by themselves, but the combination clearly differed from placebo. There was a small supra-additive increase in saccadic reaction time (see table 3) and there were also some indications that memory might be affected more by the combination than by each drug individually. These findings suggest that caution may be needed in the use of GSK598809 in individuals who consume alcohol moderately or excessively, although the effects will probably be dominated by alcohol.

In conclusion, the present study demonstrates elevation of serum prolactin and a small decrease in adaptive tracking performance after administration of the novel selective dopamine $D_3$ receptor antagonist GSK598809 within a small group of healthy volunteers. An interaction with intravenous alcohol infusion at pseudo-steady state was demonstrated, resulting in a decreased $C_{max}$ and increased AUC of GSK598809 and mainly additive effects on several CNS parameters. Although somnolence and fatigue were reported more frequently, the combination was generally well tolerated by healthy volunteers.



**FIGURE 1** STUDY DESIGN

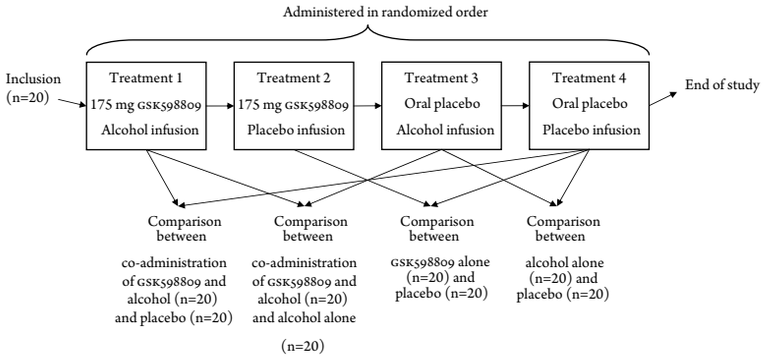



**FIGURE 2** SERUM ALCOHOL LEVELS AFTER INTRAVENOUS ALCOHOL INFUSION

*Starting at t = -0.5 hours and continuing until t = 4.5 hours, in combination with oral administration (at t = 0 hours) of GSK598809 (open circles) or placebo (closed circles). Means are presented with standard deviations as error bars.*

**FIGURE 3** PLASMA CONCENTRATIONS OF GSK598809 AFTER ORAL ADMINISTRATION

*At t = 0 hours, in combination with intravenous alcohol infusion (open circles) or placebo infusion (closed circles) starting at t = -0.5 hours and continuing until t = 4.5 hours. Means are presented with standard deviations as error bars.*

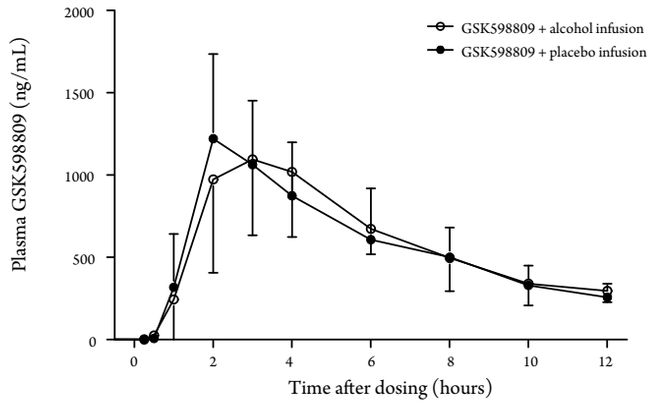



**FIGURE 4** TIME COURSE OF SMOOTH PURSUIT EYE MOVEMENTS

*Following administration of gsk598809 capsules combined with alcohol infusion. Least square means are presented with 95% confidence intervals as error bars.*

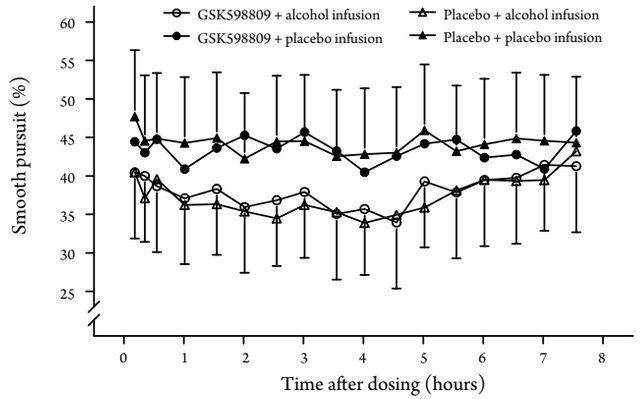





**FIGURE 5** TIME COURSE OF SACCADIC PEAK VELOCITY

*Following administration of GSK598809 capsules combined with alcohol infusion. Least square means are presented with 95% confidence intervals as error bars.*

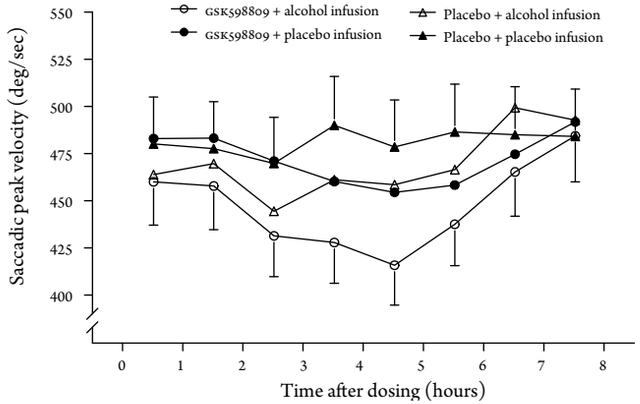



**FIGURE 6** TIME COURSE OF ADAPTIVE TRACKING PERFORMANCE

*Following administration of GSK598809 capsules combined with alcohol infusion. Least square means are presented with 95% confidence intervals as error bars.*

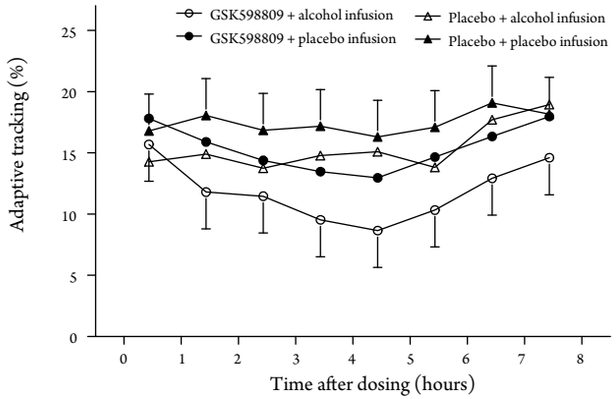


**FIGURE 7** TIME COURSE OF BODY SWAY

*Following administration of GSK598809 capsules combined with alcohol infusion. Least square means are presented with 95% confidence intervals as error bars.*

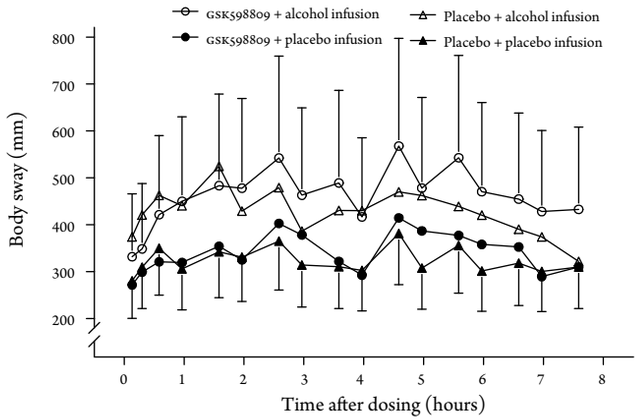



**FIGURE 8** TIME COURSE OF ALERTNESS (VISUAL ANALOGUE SCALES OF BOND & LADER)

*Following administration of GSK598809 capsules combined with alcohol infusion. Least square means are presented with 95% confidence intervals as error bars.*

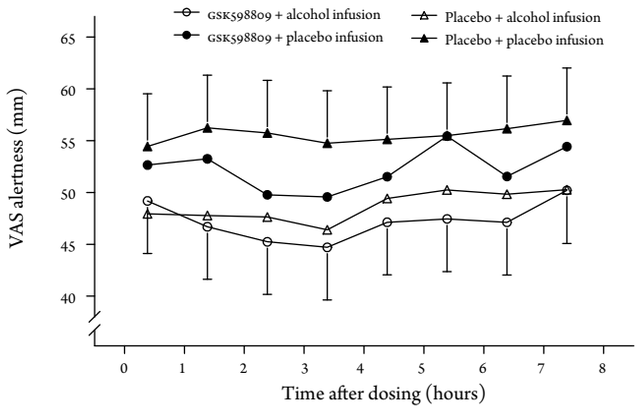





**FIGURE 9** TIME COURSE OF SERUM PROLACTIN IN FEMALE (LEFT PANEL) AND MALE SUBJECTS (RIGHT PANEL)

*Following administration of GSK598809 capsules combined with alcohol infusion. Means are presented with standard deviations as error bars.*

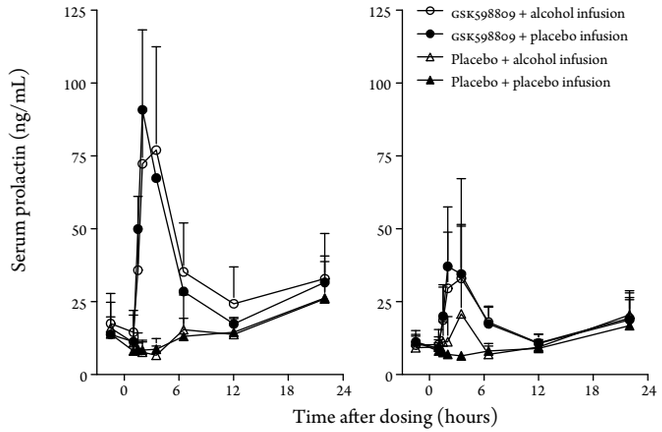


**TABLE 1** SUMMARY OF COMMON ADVERSE EVENTS, REPORTED BY TWO SUBJECTS OR MORE

*Incidence is based on the number of subjects, not the number of events.*

| **Adverse event** | **Placebo n = 19** | **Alcohol n = 20** | **GSK598809 n = 19** | **GSK598809 + Alcohol n = 19** |
|---|---|---|---|---|
| Headache | 6 (32%) | 9 (45%) | 5 (26%) | 7 (37%) |
| Somnolence | 1 (5%) | 4 (20%) | 3 (16%) | 11 (58%) |
| Dizziness | 1 (5%) | 6 (30%) | 6 (32%) | 5 (26%) |
| Akathisia | 0 | 0 | 1 (5%) | 1 (5%) |
| Feeling drunk | 0 | 7 (35%) | 0 | 6 (32%) |
| Fatigue | 3 (16%) | 4 (20%) | 1 (5%) | 7 (37%) |
| Infusion site pain | 0 | 3 (15%) | 0 | 2 (11%) |
| Catheter site related reaction | 0 | 1 (5%) | 1 (5%) | 0 |
| Nausea | 0 | 1 (5%) | 3 (16%) | 3 (16%) |
| Vomiting | 0 | 1 (5%) | 4 (21%) | 0 |
| Dry mouth | 0 | 0 | 0 | 2 (11%) |
| Upper respiratory tract infection | 0 | 2 (10%) | 0 | 0 |
| Dysmenorrhoea | 1 (5%) | 0 | 0 | 1 (5%) |
| Oropharyngeal discomfort | 1 (5%) | 0 | 0 | 1 (5%) |
| Skin reaction | 1 (5%) | 1 (5%) | 0 | 0 |

**TABLE 2** PHARMACOKINETIC PARAMETERS OF GSK598809 WITH AND WITHOUT CO-ADMINISTRATION OF ALCOHOL

*Data are presented as geometric means (with coefficient of variation), except $t_{max}$ which is presented as median (with range). Note: n = 19 for all calculated values, except t½ and $AUC_{\infty}$ of GSK598809 alone (n = 11) and combined with alcohol (n = 15), because these parameters could not be calculated reliably in 8 subjects and 4 subjects, respectively.*

| **Parameter** | **GSK598809** | **GSK598809 + alcohol** |
|---|---|---|
| $C_{max}$ (ng/mL) | 1320 (39) | 1190 (39) |
| $t_{max}$ (h) | 2.07 (2.0-6.05) | 3.03 (2.00-7.87) |
| $AUC_{0-t}$ (ng.h/mL) | 14000 (28) | 15700 (27) |
| $AUC_{\infty}$ (ng.h/mL) | 14000 (32) | 16600 (23) |
| Terminal half life (h) | 19.3 (33) | 21.6 (27) |



**TABLE 3** NEUROPHYSIOLOGICAL EFFECTS OF ADMINISTRATION OF ALCOHOL ALONE, GSK598809 ALONE AND CO-ADMINISTRATION OF GSK598009 AND ALCOHOL

*Treatment differences in least square means are shown with statistically significant results indicated in bold.*

| Parameter | Alcohol compared with placebo | GSK598809 compared with placebo | GSK598809 + alcohol compared with placebo | GSK598809 + alcohol compared with alcohol alone | Supra-additive effects |
|---|---|---|---|---|---|
| Prolactin (ng/mL) | | | | | |
| Contrast | 5.33% | **118.4%** | **113.1%** | **102.3%** | -7.35% |
| 95% CI | -6.17/18.25% | **4.49/145.1%** | **89.74/139.3%** | **80.26/127.1%** | -21.3/9.10% |
| *p*-value | 0.3711 | **≤0.0001** | **≤0.0001** | **≤0.0001** | 0.3525 |
| Saccadic peak velocity (deg/sec) | | | | | |
| Contrast | -2.53% | -1.98% | **-7.15%** | **-4.73%** | -2.81% |
| 95% CI | -5.01/0.01% | -4.45/0.57% | **-9.51/-4.72%** | **-7.18/-2.22%** | -6.30/0.80% |
| *p*-value | 0.0505 | 0.1239 | **≤0.0001** | **0.0005** | 0.1229 |
| Saccadic inaccuracy (%) | | | | | |
| Contrast | -0.23% | 3.96% | **10.17%** | **10.42%** | 6.21% |
| 95% CI | -8.67/9.00% | -4.82/13.56% | **0.79/20.42%** | **0.97/20.75%** | -6.34/20.45% |
| *p*-value | 0.9592 | 0.3805 | **0.0334** | **0.0306** | 0.3405 |
| Saccadic reaction time (sec) | | | | | |
| Contrast | 2.51% | 2.09% | **9.20%** | **6.53%** | **4.35%** |
| 95% CI | -0.43/5.54% | -0.83/5.09% | **6.05/12.44%** | **3.43/9.72%** | **0.11/8.76%** |
| *p*-value | 0.0937 | 0.1584 | **≤0.0001** | **≤0.0001** | **0.0443** |
| Smooth pursuit (%) | | | | | |
| Contrast | **-6.9** | -0.9 | **-6.1** | 0.8 | 1.7 |
| 95% CI | **-10.1/-3.7** | -4.1/2.3 | **-9.3/-2.9** | -2.4/4.0 | -2.9/6.2 |
| *p*-value | **≤0.0001** | 0.5930 | **0.0004** | 0.6210 | 0.4674 |
| Adaptive tracking (%) | | | | | |
| Contrast | **-2.0** | **-2.0** | **-5.6** | **-3.5** | -1.5 |
| 95% CI | **-3.7/-0.3** | **-3.7/-0.3** | **-7.3/-3.9** | **-5.2/-1.8** | -3.9/0.9 |
| *p*-value | **0.0207** | **0.0227** | **≤0.0001** | **0.0001** | 0.2099 |
| Body sway (mm) | | | | | |
| Contrast | **31.83%** | 4.80% | **41.31%** | 7.18% | 2.27% |
| 95% CI | **9.96/58.06%** | -12.6/25.70% | **17.69/69.67%** | -10.7/ 28.67% | -21.0/32.33% |
| *p*-value | **0.0035** | 0.6066 | **0.0004** | 0.4493 | 0.8618 |

**TABLE 4** VISUAL VERBAL LEARNING TEST (VVLT) RESULTS AFTER ADMINISTRATION OF ALCOHOL ALONE, GSK598809 ALONE AND CO-ADMINISTRATION OF GSK598009 AND ALCOHOL ON THE
*Treatment differences in least square means are shown with statistically significant results indicated in bold.*

| Parameter | Alcohol compared with placebo | GSK598809 compared with placebo | GSK598809 + alcohol compared with placebo | GSK598809 + alcohol compared with alcohol alone | Supra-additive effects |
|---|---|---|---|---|---|
| Immediate recall 1st trial | | | | | |
| Contrast | -0.3 | 0.3 | -0.8 | -0.5 | -0.8 |
| 95% CI | -1.6/1.1 | -1.1/1.7 | -2.1/0.6 | -1.8/0.8 | -2.7/1.1 |
| *p*-value | 0.6812 | 0.6725 | 0.2516 | 0.4554 | 0.4115 |
| Immediate recall 2nd trial | | | | | |
| Contrast | -1.3 | -0.1 | -1.3 | -0.0 | 0.1 |
| 95% CI | -2.8/0.3 | -1.7/1.5 | -2.9/0.2 | -1.6/1.5 | -2.1/2.3 |
| *p*-value | 0.1047 | 0.8822 | 0.0970 | 0.9640 | 0.9408 |
| Immediate recall 3rd trial | | | | | |
| Contrast | -1.1 | -1.0 | **-2.7** | -1.6 | -0.6 |
| 95% CI | -2.9/0.7 | -2.8/0.8 | **-4.5/-1.0** | -3.4/0.1 | -3.2/1.9 |
| *p*-value | 0.2210 | 0.2702 | **0.0034** | 0.0704 | 0.6206 |
| Delayed recall | | | | | |
| Contrast | -9.90% | 11.31% | **-25.5%** | -17.3% | **-25.7%** |
| 95% CI | -26.6/10.65% | -9.44/36.80% | **-39.4/-8.42%** | -32.7/1.56% | **-44.5/-0.60%** |
| *p*-value | 0.3135 | 0.3020 | **0.0061** | 0.0692 | **0.0456** |
| Word recognition (correct) | | | | | |
| Contrast | -0.82% | 2.24% | -8.62% | -7.87% | -9.88% |
| 95% CI | -12.1/11.91% | -9.49/15.48% | -19.1/3.22% | -18.3/3.95% | -24.1/6.97% |
| *p*-value | 0.8923 | 0.7169 | 0.1436 | 0.1790 | 0.2288 |
| Word recognition (incorrect) | | | | | |
| Contrast | 9.81% | 15.24% | 39.26% | 26.82% | 10.05% |
| 95% CI | -24.6/59.91% | -21.9/70.03% | -4.69/103.5% | -12.3/83.41% | -35.6/88.10% |
| *p*-value | 0.6193 | 0.4670 | 0.0855 | 0.2017 | 0.7212 |
| Reaction time (correct) | | | | | |
| Contrast | 1.97% | 1.72% | 0.54% | -1.40% | -3.07% |
| 95% CI | -4.70/9.11% | -4.98/8.90% | -6.08/7.64% | -7.85/5.50% | -11.9/6.70% |
| *p*-value | 0.5649 | 0.6172 | 0.8736 | 0.6775 | 0.5176 |



**TABLE 5** VISUAL ANALOGUE SCALES (VAS) RESULTS AFTER ADMINISTRATION OF ALCOHOL ALONE, GSK598809 ALONE AND CO-ADMINISTRATION OF GSK598009 AND ALCOHOL
*Treatment differences in least square means are shown with statistically significant results indicated in bold.*

| **Parameter** | **Alcohol compared with placebo** | **GSK598809 compared with placebo** | **GSK598809 + alcohol compared with placebo** | **GSK598809 + alcohol compared with alcohol alone** | **Supra-additive effects** |
|---|---|---|---|---|---|
| VAS alertness (mm) | | | | | |
| Contrast | **-6.9** | -3.3 | **-8.4** | -1.5 | 1.9 |
| 95% CI | **-11.4/-2.4** | -7.9/1.2 | **-12.9/-3.9** | -6.0/3.0 | -4.5/8.2 |
| *p*-value | **0.0031** | 0.1428 | **0.0005** | 0.5134 | 0.5574 |
| VAS calmness (mm) | | | | | |
| Contrast | -1.5 | -0.3 | -0.9 | 0.6 | 0.9 |
| 95% CI | -3.8/0.8 | -2.6/2.0 | -3.2/1.4 | -1.7/2.9 | -2.4/4.2 |
| *p*-value | 0.1911 | 0.7964 | 0.4288 | 0.6024 | 0.5820 |
| VAS mood (mm) | | | | | |
| Contrast | -2.0 | -0.9 | -0.9 | 1.1 | 2.0 |
| 95% CI | -5.0/0.9 | -3.9/2.1 | -3.9/2.1 | -1.9/4.1 | -2.2/6.2 |
| *p*-value | 0.1761 | 0.5537 | 0.5415 | 0.4542 | 0.3442 |
| VAS alcohol effects (mm) | | | | | |
| Contrast | **23.1** | 2.1 | **24.6** | 1.5 | -0.6 |
| 95% CI | **13.4/32.8** | -7.6/11.8 | **14.9/34.3** | -8.2/11.2 | -14.3/13.1 |
| *p*-value | **≤0.0001** | 0.6650 | **≤0.0001** | 0.7588 | 0.9280 |

Albanese A, Altavista MC and Rossi P (1986). Organization of central nervous system dopaminergic pathways. J Neural Transm. Suppl 22, 3-17.

Baloh RW, Sills AW, Kumley WE and Honrubia V (1975). Quantitative measurement of saccade amplitude, duration, and velocity. Neurology 25, 1065-1070.

Barnes TR (1989). A rating scale for drug-induced akathisia. Br. J. Psychiatry 154, 672-676.

Ben-Jonathan N and Hnasko R (2001). Dopamine as a prolactin (PRL) inhibitor. Endocr. Rev. 22, 724-763.

Bittencourt PR, Wade P, Smith AT and Richens A (1983). Benzodiazepines impair smooth pursuit eye movements. Br. J. Clin. Pharmacol. 15, 259-262.

Bond A and Lader M (1974). Use of analog scales in rating subjective feelings. British Journal of Medical Psychology 47, 211-218.

Borland RG and Nicholson AN (1984). Visual motor co-ordination and dynamic visual acuity. Br. J. Clin. Pharmacol. 18 Suppl 1, 69S-72S.

Buckman MT and Peake GT (1973). Estrogen potentiation of phenothiazine-induced prolactin secretion in man. J Clin Endocrinol Metab 37, 977-980.

Buckman MT, Peake GT and Srivastava LS (1976). Endogenous estrogen modulates phenothiazine stimulated prolactin secretion. J Clin Endocrinol Metab 43, 901-906.

Caine SB and Koob GF (1993). Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. Science 260, 1814-1816.

Cami J and Farre M (2003). Drug addiction. N. Engl. J. Med. 349, 975-986.

de Haas SL, Franson KL, Schmitt JA, Cohen AF, Fau JB, Dubruc C and van Gerven JM (2009). The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A alpha2,3 selective agonist, in comparison with lorazepam in healthy volunteers. J Psychopharmacol 23, 625-632.

de Visser SJ, van der Post J, Pieters MS, Cohen AF and van Gerven JM (2001). Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br. J. Clin. Pharmacol. 51, 119-132.

Di Chiara,G. and Imperato A (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U. S. A 85, 5274-5278.

Freeman ME, Kanyicska B, Lerant A and Nagy G (2000). Prolactin: structure, function, and regulation of secretion. Physiol Rev 80, 1523-1631.

Gijsman HJ, van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, Cohen AF and Van Kempen GM (1998). Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. J. Clin. Psychopharmacol. 18, 289-295.

Grunder G, Wetzel H, Schlosser R, Anghelescu I, Hillert A, Lange K, Hiemke C and Benkert O (1999). Neuroendocrine response to antipsychotics: effects of drug type and gender. Biol. Psychiatry 45, 89-97.

Gurevich EV and Joyce JN (1999). Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. Neuropsychopharmacology 20, 60-80.

Heidbreder CA, Gardner EL, Xi ZX, Thanos PK, Mugnaini M, Hagan JJ and Ashby CR Jr. (2005). The role of central dopamine D3 receptors in drug addiction: a review of pharmacological evidence. Brain Res. Brain Res. Rev. 49, 77-105.

Herroelen L, De Backer JP, Wilczak N, Flamez A, Vauquelin G and De Keyser J (1994). Autoradiographic distribution of D3-type dopamine receptors in human brain using [3H]7-hydroxy-N,N-di-n-propyl-2-aminotetralin. Brain Res. 648, 222-228.

Hyman SE (2005). Addiction: a disease of learning and memory. Am. J. Psychiatry 162, 1414-1422.

Hyman SE and Malenka RC (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. Nat. Rev. Neurosci. 2, 695-703.

Joyce JN and Millan MJ (2005). Dopamine D3 receptor antagonists as therapeutic agents. Drug Discov. Today 10, 917-925.

Kinon BJ, Gilmore JA, Liu H and Halbreich UM (2003). Hyperprolactinemia in response to antipsychotic drugs: characterization across comparative clinical trials. Psychoneuroendocrinology 28 Suppl 2, 69-82.

Koob GF and Bloom FE (1988). Cellular and molecular mechanisms of drug dependence. Science 242, 715-723.

Koob GF and Nestler EJ (1997). The neurobiology of drug addiction. J. Neuropsychiatry Clin. Neurosci. 9, 482-497.

Koob GF, Sanna PP and Bloom FE (1998). Neuroscience of addiction. Neuron 21, 467-476.

Kuruvilla A, Peedicayil J, Srikrishna G, Kuruvilla K and Kanagasabapathy AS (1992). A study of serum prolactin levels in schizophrenia: comparison of males and females. Clin Exp. Pharmacol. Physiol 19, 603-606.

Landwehrmeyer B, Mengod G and Palacios JM (1993). Dopamine D3 receptor mRNA and binding sites in human brain. Brain Res Mol. Brain Res 18, 187-192.

Le Foll B, Goldberg SR and Sokoloff P (2005). The dopamine D3 receptor and drug dependence: effects on reward or beyond? Neuropharmacology 49, 525-541.

Levant B (1997). The D3 dopamine receptor: neurobiology and potential clinical relevance. Pharmacol. Rev. 49, 231-252.

Lieber CS (1997). Cytochrome P-4502E1: its physiological and pathological role. Physiol Rev. 77, 517-544.

Liem-Moolenaar M, Gray FA, de Visser SJ, Franson KL, Schoemaker RC, Schmitt JA, Cohen AF and van Gerven JM (2010a). Psychomotor and cognitive effects of a single oral dose of talnetant (SB223412) in healthy volunteers compared with placebo or haloperidol. J Psychopharmacol 24, 73-82.

Liem-Moolenaar M, Rad M, Zamuner S, Cohen AF, Lemme F, Franson KL, van Gerven JMA and Merlo Pich E (2011). Central nervous system effects of the interaction between risperidone (single dose) and the 5HT6 antagonist SB742457 (repeated doses) in healthy males. Br J Clin Pharmacol.

Liem-Moolenaar M, te Beek ET, de Kam ML, Franson KL, Kahn RS, Hijman R, Touw D and van Gerven JM (2010b). Central nervous system effects of haloperidol on THC in healthy male volunteers. J Psychopharmacol 24, 1697-1708.

Meltzer HY, Busch DA and Fang VS (1983). Serum neuroleptic and prolactin levels in schizophrenic patients and clinical response. Psychiatry Res. 9, 271-283.

Meltzer HY and Fang VS (1976). The effect of neuroleptics on serum prolactin in schizophrenic patients. Arch. Gen. Psychiatry 33, 279-286.

Moore RY and Bloom FE (1978). Central catecholamine neuron systems: anatomy and physiology of the dopamine systems. Annu. Rev Neurosci. 1, 129-169.

Munetz MR and Benjamin S (1988). How to examine patients using the Abnormal Involuntary Movement Scale. Hosp. Community Psychiatry 39, 1172-1177.

Murray AM, Ryoo HL, Gurevich E and Joyce JN (1994). Localization of dopamine D3 receptors to mesolimbic and D2 receptors to mesostriatal regions of human forebrain. Proc. Natl. Acad. Sci. U. S. A 91, 11271-11275.

Nestler EJ (2005). Is there a common molecular pathway for addiction? Nat. Neurosci. 8, 1445-1449.

Norris H (1971). The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 10, 181-191.

PETty RG (1999). Prolactin and antipsychotic medications: mechanism of action. Schizophr. Res 35 Suppl, S67-S73.

Pierce RC and Kumaresan V (2006). The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci. Biobehav. Rev. 30, 215-238.

Rey A (1964). L'examen clinique en psychologie. (Paris: Presses Universitaires de France).

Schmitt JA, Jorissen BL, Sobczak S, van Boxtel MP, Hogervorst E, Deutz NE and Riedel WJ (2000). Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. J. Psychopharmacol. 14, 21-29.

Searle G, Beaver JD, Comley RA, Bani M, Tziortzi A, Slifstein M, Mugnaini M, Griffante C, Wilson AA, Merlo-Pich E, Houle S, Gunn R, Rabiner EA and Laruelle M (2010). Imaging dopamine D3 receptors in the human brain with positron emission tomography, [11C]PHNO, and a selective D3 receptor antagonist. Biol. Psychiatry 68, 392-399.

Seeman P, Wilson A, Gmeiner P and Kapur S (2006). Dopamine D2 and D3 receptors in human putamen, caudate nucleus, and globus pallidus. Synapse 60, 205-211.

Segal DM, Moraes CT and Mash DC (1997). Up-regulation of D3 dopamine receptor mRNA in the nucleus accumbens of human cocaine fatalities. Brain Res Mol. Brain Res 45, 335-339.

Simpson GM and Angus JW (1970). A rating scale for extrapyramidal side effects. Acta Psychiatr. Scand. Suppl 212, 11-19.

Smith S, Wheeler MJ, Murray R and O'Keane V (2002). The effects of antipsychotic-induced hyperprolactinaemia on the hypothalamic-pituitary-gonadal axis. J Clin Psychopharmacol 22, 109-114.

Staley JK and Mash DC (1996). Adaptive increase in D3 dopamine receptors in the brain reward circuits of human cocaine fatalities. J Neurosci. 16, 6100-6106.

te Beek ET, Moerland M, de Boer P, van Nueten L, de Kam ML, Burggraaf J, Cohen AF, van Gerven JM (2011). Pharmacokinetics and central nervous system effects of the novel dopamine D2 receptor antagonist JNJ-37822681. J Psychopharmacol Sep 2. (Epub ahead of print)

van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MS, Kroon JM, Breimer DD and Cohen AF (1993). Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. Psychopharmacology (Berl) 110, 471-478.

van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD and Cohen AF (1991). 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. 50, 172-180.

van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM and Cohen AF (1999). The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J. Psychopharmacol. 13, 10-17.

Volkow ND, Fowler JS and Wang GJ (1999). Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. J Psychopharmacol. 13, 337-345.

Volkow ND, Fowler JS and Wang GJ (2003). Positron emission tomography and single-photon emission computed tomography in substance abuse research. Semin. Nucl. Med. 33, 114-128.

Volkow ND, Fowler JS, Wang GJ, Baler R and Telang F (2009). Imaging dopamine's role in drug abuse and addiction. Neuropharmacology 56 Suppl 1, 3-8.
Volkow ND, Fowler JS, Wang GJ and Swanson JM (2004). Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. Mol. Psychiatry 9, 557-569.
Volkow ND, Fowler JS, Wang GJ, Swanson JM and Telang F (2007). Dopamine in drug abuse and addiction: results of imaging studies and treatment implications. Arch. Neurol. 64, 1575-1579.
Wode-Helgodt B, Eneroth P, Fyro B, Gullberg B and Sedvall G (1977). Effect of chlorpromazine treatment on prolactin levels in cerebrospinal fluid and plasma of psychotic patients. Acta Psychiatr. Scand. 56, 280-293.
Wright BM (1971). A simple mechanical ataxia-meter. J. Physiol 218, 27P-28P.
Yasui-Furukori N, Saito M, Nakagami T, Sugawara N, Sato Y, Tsuchimine S, Furukori H and Kaneko S (2010). Gender-specific prolactin response to antipsychotic treatments with risperidone and olanzapine and its relationship to drug concentrations in patients with acutely exacerbated schizophrenia. Prog. Neuropsychopharmacol Biol. Psychiatry 34, 537-540.
Zoethout RW, Schoemaker RC, Zuurman L, van Pelt H, Dahan A, Cohen AF and van Gerven JM (2009). Central nervous system effects of alcohol at a pseudo-steady-state concentration using alcohol clamping in healthy volunteers. Br. J Clin. Pharmacol. 68, 524-534.
Zoethout RW, van Gerven JM, Dumont GJ, Paltansing S, van Burgel ND, van der Linden M, Dahan A, Cohen AF and Schoemaker RC (2008). A comparative study of two methods for attaining constant alcohol levels. Br. J. Clin. Pharmacol. 66, 674-681.



# CHAPTER 10

## *General discussion*

# GENERAL DISCUSSION

Alcohol is one of the most widely used psychoactive substances in Western society. This makes it important to study effects of this compound, and its interactions with other medications or drugs. Alcohol has complex and variable pharmacokinetics, and some of the effects of alcohol are preferably studied at stable serum levels. The results of studies investigating alcohol clearance or the effects of alcohol on the central nervous system (CNS) or drug-alcohol interactions are often interpreted more easily when alcohol levels are kept within certain limits. Especially, because both its wide inter- and intra-individual variability are reduced when alcohol is 'clamped' to a pre-specified level. Methods to obtain stable serum concentrations are scarce, but efforts have been made before (Hartmann *et al.*, 1988; O'Connor *et al.*, 1998). However, these methods have not gained wide application. This might be related to the perceived complexity of the procedures, which seem to require specific expertise and frequent alcohol concentration measurements for adaptations of the alcohol infusion. The early drug development process would benefit from an accurate, user-friendly method, with low variability, which is able to maintain constant alcohol levels for prolonged periods of time. Such a method would greatly facilitate any (drug) research in which alcohol is involved. In this thesis the development and application of a new relatively straightforward alcohol clamping procedure is presented.

Reliable studies of the CNS-effects of alcohol do not only benefit from stable alcohol conditions, but also from sensitive tests that are able to reliably detect the acute effects of alcohol. Chapter 2 of this thesis contains a systematic review, which attempted to determine the sensitivity to alcohol for a large number of CNS-tests that are described in the literature. The results show that many different tests or biomarkers are currently used to study the various CNS-effects of alcohol, and that such studies would greatly benefit from a certain degree of standardization. Attention tasks, visuo-motor control tests and scales of subjective effects were identified as the most sensitive functional biomarkers for the acute CNS-effects of alcohol. The results of this review are helpful in selecting rational tests for studies investigating the acute CNS-effects of alcohol or for future alcohol-interaction studies.

In chapter 3 we introduced the alcohol clamp. Based on the results of O'Connor's 'Indiana alcohol clamp' (O'Connor *et al.*, 1998) we developed a new method to maintain stable alcohol serum levels for prolonged periods of time. A large data-set of population pharmacokinetics was used to reduce the amount of sampling moments that O'Connor's method requires throughout the clamping period. Besides, we introduced an intravenous loading dose instead of the oral dose originally described by O'Connor. These adaptations were incorporated into a new spreadsheet-based paradigm, to enhance its user-friendliness. The new clamping method was compared to a method described by Hartmann (Hartmann *et al.*, 1988), which is based on individual alcohol pharmacokinetics to predict individual infusion rates to achieve a desired target level on a subsequent study occasion. We showed that the novel alcohol clamping paradigm was more accurate and user-friendly, with low variability and the ability to maintain constant alcohol levels for hours. The paradigm provided an opportunity to perform intensive pharmacodynamic or functional assessments during the execution of the clamp, which was considered to be useful for future studies of alcohol.

The modified alcohol clamping procedure described in Chapter 3 is based on fewer actual alcohol samples than the Indiana alcohol clamp procedure (O'Connor *et al.*, 1998). This may reduce the variability of alcohol levels at a steady-state, but the O'Connor method does not leave any room for concomitant pharmacodynamic testing, due to its frequent sampling moments. The adapted alcohol clamp as described in this thesis was partly developed to overcome this problem. In chapter 4 we showed that an intensive battery of CNS-tests could be integrated in the course of the clamp without interfering with the sampling activities or with the stability of the clamp. Furthermore, we showed that attention, subjective effects and (visuo-) motor control tests were mainly affected by alcohol. Also, we found that some effects closely followed the relatively stable alcohol concentrations, whereas others fluctuated during the plateau-phase, which could be an

indication for time-related drug-effect changes such as acute tolerance. Based on chapter 3 and 4 we conclude that our 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.

During the alcohol clamp, the levels of ethanol that are infused in an attempt to maintain stable breath- and blood-levels provide detailed information about the individual disposition of the compound. Consequently, differences in the sensitivity to the effects of alcohol can be readily traced to pharmacokinetic and pharmacodynamic sources of variability. Japanese subjects are often more susceptible to the effects of alcohol, which is at least partly related to ethnic differences in alcohol metabolism but may also have pharmacodynamic causes. In chapter 5 we explored the pharmacokinetic and pharmacodynamic differences between a group of Caucasians and a group of Japanese healthy male volunteers at two different clamp levels at two different occasions. We found that significantly lower amounts of alcohol were needed for the Japanese group to maintain similar stable concentrations than for the Caucasians. However, these differences disappeared when values were corrected for lean body mass. Despite similar alcohol levels, several pharmacodynamic differences between the groups were observed, primarily on body sway and on the visual analogue scale (VAS) for subjective alcohol effects, and mainly at the highest dose level. We concluded that the alcohol clamp is a useful method to compare differences in alcohol metabolism between groups and that 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.

The selection of CNS-tests within the CNS-battery we introduced in chapter 4 and 5 was based on a thorough literature review (chapter 2). Together, these tests cover a broad range of neuropsychological functioning. However, the results are only indirect read-outs of the acute effects of alcohol on the CNS. Ideally, a generally applicable methodology for repeated measurements of direct drug effects on the entire CNS, without task-related interactions and



*a priori* models, would constitute a major improvement in CNS drug development. Resting-state functional magnetic resonance imaging (RS-fMRI) could satisfy many of these requirements, and was hence hypothesized to be a promising technique for pharmacological research. The validity and sensitivity of this new method as an instrument in future CNS-drug development programs was explored in a proof-of-concept study, during which we used the alcohol clamp at a low level of 0.6 g/L, to calibrate the new technique under steady-state conditions (chapter 6). Besides alcohol, a morphine infusion was used on a different occasion to explore the specificity of the effects of CNS-depressants on RS-fMRI. Our results revealed dissociable changes in both pharmacodynamics and functional connectivity resulting from alcohol and morphine. *Post hoc* analysis of regions of interest revealed adaptive network interactions in relation to pharmacokinetic and pharmacodynamic curves for both alcohol and morphine. This study suggests that resting-state functional brain connectivity could play a role as a drug-class specific and –sensitive method in CNS drug research.

In the previous chapter, alcohol was used as a positive control for a new method to test CNS-active drugs, because it has a wide range of CNS-effects within clearly defined socially and clinically acceptable blood levels. Alcohol also has a limited number of established therapeutic effects, for instance on essential tremor and methanol intoxication, which makes it a useful positive control for novel therapies in these conditions. In chapter 7 and 8 we investigated the effects of a GABAa α2,3 subtype selective partial agonist (TPA023) and a histamine-3 inverse agonist (MK-0249) on essential tremor symptoms. In both studies we added an alcohol arm to the study design as a positive control. The alcohol clamp was used to obtain steady state breath alcohol levels. Although some CNS-effects were observed for both new compounds, no significant tremor reducing effects were reported. In contrast, the stable alcohol levels did result in significant reductions in tremor symptoms, and the alcohol clamp successfully fulfilled its role as a positive control in both studies. The alcohol clamp procedure seems a valid method to use in future studies investigating the effects of new therapeutic options in ET

research. Additionally, the alcohol clamp can be used during the validation process of new methods, which quantify essential tremor or assess tremor severity and its responsiveness to therapeutic interventions.

In this thesis a novel alcohol clamping method was introduced, which was developed to maintain stable serum levels of alcohol for prolonged periods of time by continuous intravenous administration, during which a BrAC input and feedback system is used, based on population pharmacokinetics. The procedure provides an environment in which the acute (CNS-)effects of stable alcohol levels can accurately be investigated, including the metabolic disposition of ethanol, the effects on a wide range of CNS-functions, and the changes in sensitivity across time. One drawback of the clamping paradigm is that alcohol levels do not show enough variation during the steady state to explore PK/PD relationships. However, prolongation of the clamp-run phase or attainment of multiple consecutive steady-state levels will provide the opportunity for future studies to investigate such relations in larger detail.

Our alcohol clamp might serve as an interesting tool in the early drug development process. We showed that alcohol clamp could be used a benchmark in proof-of-concept studies (chapter 6) and as a robust positive control during ET-studies (chapter 7 and 8). The alcohol clamp may also play a future role in the validation process of new tremor registration devices, which assist in the search for new pharmacological treatment strategies for tremor disorders. The clear legal limits of acceptable alcohol levels and its broad spectrum of CNS-effects also creates a useful framework for the clamp, to serve as a functional benchmark for the effects of new drugs, to get a feel for the safety and acceptability of inevitable CNS-effects in work and traffic and other domains of daily life.

For safety reasons, drug-alcohol interaction studies are incorporated into the course of the development program of most new CNS-drugs. Both pharmacokinetic and pharmacodynamic interactions should be explored to generate recommendations and/or restrictions for its future users. We believe that the alcohol clamp might be a helpful tool for drug-alcohol interaction studies, since steady state alcohol level will allow the measurement of multiple pharmacodynamic endpoints with reduced variability. The methods described in this thesis have already been implemented in successful drug-alcohol interaction studies (chapter 9). This thesis has examined several other examples where the alcohol clamp has been a useful research instrument to the advancement of alcohol research.



## REFERENCES

Hartmann D, Fluckiger A, Ziegler WH, Timm U, Zell M (1988). Experimental model for the Investigation of kinetic and or dynamic interactions between drugs and ethanol in humans. Journal of Pharmaceutical Sciences 77:299-303.

O'Connor S, Morzorati S, Christian J, Li TK (1998). Clamping breath alcohol concentration reduces experimental variance: application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcohol Clin Exp Res. 22(1):202-10.

# *Samenvatting (Dutch summary)*

Alcohol is een veel gebruikt genotmiddel in de westerse wereld. Vanwege dit frequente gebruik is het belangrijk om interacties met geneesmiddelen die gelijktijdig worden ingenomen goed in kaart te brengen. De verwerking van alcohol door het lichaam is echter een complex proces en stabiele alcohol concentraties zouden theoretisch het bestuderen van dit proces kunnen vereenvoudigen. Bovendien zijn de resultaten van studies, waarbij er gekeken wordt naar de interactie tussen alcohol en een bepaald geneesmiddel ook vaak makkelijker te interpreteren als de alcohol concentratie een vast gegeven is. Dit is hoofdzakelijk het gevolg van een afname van de spreiding, die optreedt op het moment dat het alcohol niveau op een vast niveau wordt gehouden, ofwel op een vast niveau wordt "geclampt".
In de literatuur worden er wel enkele methoden beschreven (Hartmann *et al.*, 1988; O'Connor *et al.*, 1998) om voorspelbare alcohol spiegels te bereiken, maar deze worden niet heel veel toegepast door onderzoekers. Dit heeft mogelijk te maken met de complexiteit van deze procedures, waarbij intensieve metingen en specifieke expertise vaak een vereiste zijn om een stabiel alcohol niveau te bereiken. Een nauwkeurige, maar vooral gebruiksvriendelijke methode met geringe spreiding, waarbij alcohol voor langere tijd op een stabiel level in het bloed kan worden gehouden, zou goed van pas kunnen komen tijdens het ontwikkelingproces van nieuwe geneesmiddelen. Op deze manier kunnen interacties tussen alcohol en een bepaald nieuw geneesmiddel eenvoudiger en zorgvuldiger worden bestudeerd en kunnen er meer gedetailleerde uitspraken worden gedaan over de veiligheid van het gebruik van een toekomstig geneesmiddel in combinatie met alcohol. In dit proefschrift wordt de ontwikkeling en de toepasbaarheid van een nieuwe procedure beschreven (de alcohol clamp), die onderzoekers in staat moet stellen om voor langere periodes en op eenvoudige wijze, stabiele alcohol concentraties in het bloed te bewerkstelligen.

Naast stabiele alcohol concentraties is het bij studies waarbij acute alcohol effecten op het centrale zenuwstelsel (CZS) worden onderzocht ook belangrijk om *sensitieve* testen (ook wel sensitieve 'biomarkers' genoemd) te gebruiken om deze effecten te meten. In hoofdstuk 2 wordt aan de hand van wat er te vinden is in de literatuur een overzicht gepresenteerd van de gevoeligheid van dit soort biomarkers. De resultaten benadrukken de enorme diversiteit aan testen. Een vorm van standaardisatie van deze grote hoeveelheid biomarkers zou toekomstig onderzoek kunnen faciliteren. Aandachtstaken, visueel motorische testen en testen waarbij subjectieve alcohol effecten worden gemeten, bleken de meest sensitieve biomarkers om acute alcohol effecten te meten. De resultaten van dit overzichtsartikel zouden kunnen worden gebruikt bij het maken van een rationele keuze bij toekomstig alcohol onderzoek en in het bijzonder bij toekomstig onderzoek naar de interacties tussen alcohol en geneesmiddelen.

In hoofdstuk 3 wordt de nieuwe alcohol clamp gepresenteerd: een methode om stabiele alcohol concentraties in het bloed te creëren, gebaseerd op de eerder gepubliceerde *Indiana alcohol clamp*, beschreven door O'Connor (O'Connor *et al.*, 1998). Eerder verzamelde data van een grote populatie proefpersonen werd gebruikt om het optimale sample schema van de nieuwe clamp te voorspellen, om zo het aantal momenten te beperken waarop de infuussnelheid van de clamp moet worden aangepast aan de gemeten alcohol concentraties. Verder werd de orale oplaaddosis in het begin van de clamp vervangen door een oplaaddosis via het infuus.

Het nieuwe paradigma werd verwerkt in een spreadsheet programma om de uitvoering van de clamp gebruiksvriendelijker te maken. In ditzelfde hoofdstuk wordt de nieuwe alcohol clamp vergeleken met een andere methode beschreven door Hartmann (Hartmann *et al.*, 1988). Bij de Hartmann procedure wordt er een gewenst alcohol niveau bereikt op basis van gegevens die verzameld zijn tijdens een eerdere alcohol toediening. In vergelijking met de Hartmann methode is de alcohol clamp nauwkeuriger met een lagere spreiding. De vernieuwde alcohol clamp methode is gebruiksvriendelijk en alcohol concentraties in het bloed kunnen urenlang stabiel worden gehouden, er is sprake van een zogenaamde steady-state. Deze eigenschap zou van pas kunnen komen bij toekomstige alcohol studies.

Hoewel de oorspronkelijke Indiana clamp zeer nauwkeurig is en consistente resultaten laat zien, is het door de intensieve metingen die vereist zijn voor het in stand houden van de steady-state concentratie bijna onmogelijk om tegelijkertijd testen uit te voeren die alcohol effecten meten. Door op basis van populatie gegevens individuele voorspellingen te maken van het alcohol concentratie verloop in de tijd blijkt het mogelijk om de frequentie van de metingen te reduceren zonder dat de stabiliteit van de steady-state hier belangrijk door verandert (hoofdstuk 3). Op deze manier kunnen acute alcohol effecten worden gemeten onder stabiele alcohol condities. In hoofdstuk 4 wordt aangetoond dat er tijdens de clamping procedure een flink aantal CZS-testen kan worden verricht, zonder dat dit interfereert met de alcohol metingen en zonder dat de stabiliteit van het alcohol niveau wordt aangetast. Daarnaast wordt er in dit hoofdstuk aangetoond dat met name aandachtstaken, testen waarbij subjectieve effecten worden gemeten en (visueel) motorische testen door het stabiele alcohol niveau worden beïnvloed. Sommige effecten blijven heel stabiel tijdens de alcohol infusie, terwijl andere effecten juist veel meer variëren over de tijd. Dit laatste zou ten dele verklaard kunnen worden door het optreden van acute tolerantie. In hoofdstuk 3 en 4 laten we zien dat alcohol spiegels met behulp van de alcohol clamp niet alleen zeer nauwkeurig binnen bepaalde grenzen kunnen worden gehouden, maar dat er tegelijkertijd voldoende metingen kunnen worden verricht om nauwkeurig het tijdsverloop van de effecten van alcohol te meten, zonder dat deze belangrijk interfereren met de stabiliteit van het alcohol niveau. Deze combinatie maakt de alcohol clamp tot een unieke methode om de complexe acute effecten van alcohol op het CZS te bestuderen.

De hoeveelheid alcohol die nodig is om een bepaald alcohol niveau in stand te houden kan dienen als eenvoudige maat voor de verwerkingscapaciteit van alcohol van een individu, dit wordt ook wel de klaring genoemd. Bij een stabiele bloedspiegel geldt immers dat wat erin gaat (de infuussnelheid), overeenkomt met wat eruit gaat (de klaringssnelheid). Verschillen in klaring tussen individuen kunnen zo op simpele wijze met elkaar worden vergeleken. De alcohol clamp corrigeert automatisch voor verschillen in klaring en op deze manier kunnen verschillen in alcohol effecten tussen groepen individuen ook eerlijker met elkaar worden vergeleken. Japanners en Kaukasiërs verschillen in hun efficiëntie waarmee ze alcohol klaren en in de manier waarop ze op alcohol reageren. Andere testen (zoals bijvoorbeeld oogbewegingen en tracking) lieten daarentegen geen verschillen zien. In hoofdstuk 5 worden klaring en de effecten van twee verschillende alcohol niveaus bij een groep Japanners en een groep Kaukasiërs met elkaar vergeleken. Er blijkt significant minder alcohol nodig te zijn bij de groep Japanners voor het onderhouden van een bepaald stabiel alcohol niveau. Dit verschil verdwijnt echter als er gecorrigeerd wordt voor het vetpercentage van de vrijwilligers. Ondanks de vergelijkbare alcohol concentraties worden er verschillende effecten waargenomen tussen de groepen, met name tijdens de body sway en bij de visual analogue scale (VAS), een maat voor subjectieve alcohol effecten. In hoofdstuk 5 laten we zien dat de alcohol clamp een bruikbare methode is om verschillen in alcohol metabolisme aan te tonen tussen bepaalde ethniciteiten. Verder laten we zien dat bij een zelfde alcohol niveau de Japanse groep op sommige testen verschilt van de Kaukasische groep, terwijl er bij andere testen geen verschil wordt gevonden.

De CZS-testen waarmee we alcohol effecten gemeten hebben in hoofdstuk 4 en 5 zijn zorgvuldig geselecteerd op hun sensitiviteit en gebaseerd op de resultaten van een uitgebreid literatuur onderzoek (hoofdstuk 2). Ondanks het feit dat deze verzameling testen ons in staat stelt om over een groot spectrum uitspraken te doen over de acute effecten van alcohol op het neuropsychologisch functioneren, blijven het *indirecte* metingen van de effecten van alcohol. Idealiter, zouden alcohol (of geneesmiddelen) effecten op het CZS als geheel *direct* gemeten moeten kunnen worden zonder de tussenkomst van een test of taak. Dit zou een grote vooruitgang betekenen in het ontwikkelingsproces van geneesmiddelen die werken op het CZS.

Resting-state functional magnetic imaging (RS-fMRI) lijkt een veelbelovende techniek die voldoet aan bovenstaande criteria. Bij RS-fMRI wordt er zonder tussenkomst van een bepaalde taak (in een resting-state) *direct* naar bepaalde functionele effecten (bijvoorbeeld de invloed van een genees-

middel) op het brein gekeken met behulp van magnetic resonance imaging (MRI). In hoofdstuk 6 hebben we in een pilot-study onderzocht of RS-fMRI ons in staat stelt om de directe effecten van een stabiele alcohol concentratie en een stabiele morfine concentratie op het CZS als geheel te meten. Zowel alcohol als morfine laten in deze studie meetbare veranderingen zien van zowel de indirecte metingen (conventionele CZS-metingen) als de directe metingen (RS-fMRI) uitgedrukt in functionele connectiviteit. Daarnaast blijken er interacties tussen bepaalde netwerken mee te veranderen met zowel de alcohol/morfine concentratie als met de effecten gemeten met de conventionele testen. Omdat we met RS-fMRI blijkbaar geneesmiddelen van elkaar kunnen onderscheiden, lijkt het een veelbelovende methode. Daarnaast zijn er ook aanwijzingen dat het RS-fMRI beloop de plasmaspiegels volgt. Deze eigenschappen zouden een aanwinst kunnen zijn voor het ontwikkelingsproces van nieuwe geneesmiddelen.

Alcohol heeft ook een aantal therapeutische effecten, bijvoorbeeld bij de behandeling van een methanol intoxicatie en bij het reduceren van tremor symptomen bij essentiële tremor (ET). De alcohol clamp zou daarom kunnen dienen als een positieve controle bij studies naar nieuwe behandelingen van dit soort aandoeningen.

In hoofdstuk 7 en 8 hebben we de effecten onderzocht van twee nieuwe geneesmiddelen (respectievelijk TPA023 en MK-0249) op essentiële tremor, een veelvoorkomende bewegingsstoornis, waarbij met name de armen en handen trillen bij bewegen. In beide studies hebben we de alcohol clamp toegevoegd om de beoogde tremor reducerende effecten van de twee nieuwe medicijnen te vergelijken met die van een stabiel alcohol niveau. Er worden CZS-effecten gemeten bij beide geneesmiddelen, maar tremor reducerende effecten blijven uit. De alcohol clamp laat daarentegen wel een verbetering van de tremor symptomen zien in beide studies en de clamp lijkt haar rol als positieve controle bij ET-studies adequaat te vervullen. De alcohol clamp zou bij toekomstig onderzoek naar nieuwe therapeutische opties voor ET een belangrijke bijdrage kunnen leveren. Daarnaast zou de clamp een rol kunnen vervullen bij het validatie proces van nieuwe methoden die ontwikkeld worden voor het kwantificeren van tremor symptomen.



In dit proefschrift is de alcohol clamp geïntroduceerd: een methode waarbij alcohol in het bloed op een stabiel niveau kan worden "geclampt" voor langere perioden door gebruik te maken van een feedback system dat gebaseerd is op de hoeveelheid alcohol in de uitademingslucht. De clamp biedt ruimte om tijdens de uitvoering ervan testen of taken uit te voeren, waarmee de effecten van alcohol tegelijkertijd kunnen worden gemeten. Door het principe van de clamp kunnen er globale uitspraken worden gedaan over de efficiëntie van het alcoholmetabolisme van een individu en over veranderingen van alcohol effecten in de tijd (bijv. tolerantie). Een nadeel van de alcohol clamp is dat de beoogde alcohol concentratie te weinig variabiliteit bezit om modellen te maken die de relatie tussen alcohol concentratie en effect kunnen beschrijven (PK/PD studies). In dit proefschrift worden de alcohol concentraties geclampt op verschillende niveaus, maar steeds op andere studiedagen. Om concentratie-effect relaties betrouwbaar te onderzoeken, zouden binnen één "clamp-run" meerdere opeenvolgende stabiele alcohol levels aangelegd moeten worden om vervolgens de bijbehorende effecten te meten. Dit valt echter buiten de scope van dit proefschrift.

Omdat de clamp vanwege haar brede effectprofiel en haar stabiliteit een functioneel kader biedt voor proof-of-concept studies (hoofdstuk 6) en functioneert als robuuste positieve controle bij studies naar nieuwe therapeutische opties voor ET (hoofdstuk 7 en 8) is het een interessante methode die gebruikt kan worden tijdens het proces van de ontwikkeling van bepaalde nieuwe geneesmiddelen. De alcohol clamp zou tevens een rol kunnen gaan spelen bij de ontwikkeling en validatie van nieuwe tremor registratie apparatuur. Deze apparatuur kan dan uiteindelijk weer gebruikt worden bij de ontwikkeling van nieuwe farmacologische behandelingen van ET. Daarnaast kunnen we met behulp van de clamp en dankzij het brede spectrum aan effecten van alcohol en de wettelijk vastgestelde concentratie limieten (met de daarbij behorende bekende effecten) een *nauwkeurig* functioneel kader bieden voor acceptabele en ongewenste CNS-effecten. Op deze manier zou een nieuw geneesmiddel getoetst kunnen worden aan dit kader om zo een concreet idee te krijgen van de veiligheid en verdraagzaamheid van vaak onvermijdelijke bijwerkingen van een nieuw CNS-middel. Aan de hand hiervan kunnen er

ten tijde van de registratie van een bepaald medicament beslissingen worden genomen en medico-legale regels worden opgesteld met betrekking tot het toekomstig gebruik tijdens verkeersdeelname, tijdens werkgerelateerde activiteiten of gewoon tijdens gebruik in het dagelijks leven.

Om veiligheidsredenen worden er tegenwoordig steeds vaker studies uitgevoerd tijdens de ontwikkeling van een nieuw geneesmiddel die de interactie tussen alcohol en het nieuwe geneesmiddel bestuderen. Deze interacties moeten nauwkeurig in kaart worden gebracht voor het vormen van aanbevelingen en/of restricties voor haar toekomstige gebruikers ten aanzien van het gebruik in combinatie met alcohol. Wij denken dat de alcohol clamp een adequate en nauwkeurige methode is voor het uiteindelijk genereren van zulke aanbevelingen, vanwege de stabiliteit van de clamp en de mogelijkheid om herhaaldelijk (en onder stabiele concentraties) de effecten van het stabiele alcohol niveau te meten. De alcohol clamp is hierbij superieur aan methoden die momenteel worden toegepast, omdat deze vaak gehinderd worden door zowel hun complexiteit als hun variabiliteit. Wij zien de alcohol clamp als een state-of-the-art techniek, die in de toekomst door de autoriteiten als vereiste zou moeten worden gezien bij het uitvoeren van alcohol interactie studies. Een voorbeeld van het gebruik van de clamp tijdens een interactie studie wordt uitgebreid beschreven in hoofdstuk 9. Dit proefschrift is een uiteenzetting van de toepasbaarheid van de alcohol clamp als research instrument bij alcohol- en geneesmiddelenonderzoek.

REFERENCES

Hartmann D, Fluckiger A, Ziegler WH, Timm U, Zell M. Experimental model for the Investigation of kinetic and or dynamic interactions between drugs and ethanol in humans. Journal of Pharmaceutical Sciences 1988; 77:299-303.

O'Connor S, Morzorati S, Christian J, Li TK (1998). Clamping breath alcohol concentration reduces experimental variance: application to the study of acute tolerance to alcohol and alcohol elimination rate. Alcohol Clin Exp Res. 22(1):202-10



## *Curriculum Vitae*

Remco Zoethout was born in Hoorn, on the 29th of June 1979. He graduated from Grammar School (Tabor College, locatie Oscar Romero, Hoorn) in 1997. Subsequently he started Medical School at the University of Amsterdam (Academical Medical Center). After his graduation in December 2004, he started working as a resident (ANIOS) at the Flevoziekenhuis on the Emergency Department. In January 2006 he started working as a research physician at the Center for Human Drug Research until January 2009. The research described in this thesis was performed in this period. In January 2009 he started as a resident (AIOS) at the Anesthesiology department in the Leiden University Medical Center. He will finish his residency in July 2013.

te Beek ET, **Zoethout RW**, Bani MS, Andorn A, Iavarone L, Klaassen ES, Fina P, van Gerven JM. Pharmacokinetics and central nervous system effects of the novel dopamine D3 receptor antagonist GSK598809 and intravenous alcohol infusion at pseudo-steady state. J Psychopharmacol. 2012 Feb;26(2):303-14. Epub 2012 Jan 4.

de Haas SL, **Zoethout RW**, Van Dyck K, De Smet M, Rosen LB, Murphy MG, Gottesdiener KM, Schoemaker RC, Cohen AF, van Gerven JM. The effects of TPA023, a GABAAα2,3 subtype-selective partial agonist, on essential tremor in comparison to alcohol. J Psychopharmacol. 2012 Feb;26(2):282-91. Epub 2011 Sep 2.

Khalili-Mahani N, **Zoethout RW**, Beckmann CF, Baerends E, de Kam ML, Soeter RP, Dahan A, van Buchem MA, van Gerven JM, Rombouts SA. Effects of morphine and alcohol on functional brain connectivity during "resting state": a placebo-controlled crossover study in healthy young men. Hum Brain Mapp. 2012 May;33(5):1003-18. Epub 2011 Mar 9.

**Zoethout RW**, Iannone R, Bloem BR, Palcza J, Murphy G, Chodakewitz J, Buntinx A, Gottesdiener K, Marsilio S, Rosen L, van Dyck K, Louis ED, Cohen AF, Schoemaker RC, Tokita S, Sato N, Koblan KS, Hargreaves RH, Renger J, van Gerven JM. The effects of a novel histamine-3 receptor inverse agonist on essential tremor in comparison to stable levels of alcohol. J Psychopharmacol. 2012 Feb;26(2):292-302. Epub 2011 Feb 18.

**Zoethout RW**, Delgado WL, Ippel AE, Dahan A, van Gerven JM. Functional biomarkers for the acute effects of alcohol on the central nervous system in healthy volunteers. Br J Clin Pharmacol. 2011 Mar;71(3):331-50. Review.

Khalili-Mahani N, van Osch MJ, Baerends E, Soeter RP, de Kam M, **Zoethout RW**, Dahan A, van Buchem MA, van Gerven JM, Rombouts SA. Pseudocontinuous arterial spin labeling reveals dissociable effects of morphine and alcohol on regional cerebral blood flow. J Cereb Blood Flow Metab. 2011 May;31(5):1321-33. Epub 2011 Jan 19.

Liem-Moolenaar M, **Zoethout RW**, de Boer P, Schmidt M, de Kam ML, Cohen AF, Franson KL, van Gerven JM. The effects of the glycine reuptake inhibitor R213129 on the central nervous system and on scopolamine-induced impairments in psychomotor and cognitive function in healthy subjects. J Psychopharmacol. 2010 Nov;24(11):1671-9. Epub 2010 Feb 8.

**Zoethout RW**, Schoemaker RC, Zuurman L, van Pelt H, Dahan A, Cohen AF, van Gerven JM. Central nervous system effects of alcohol at a pseudo-steady-state concentration using alcohol clamping in healthy volunteers. Br J Clin Pharmacol. 2009 Oct;68(4):524-34.

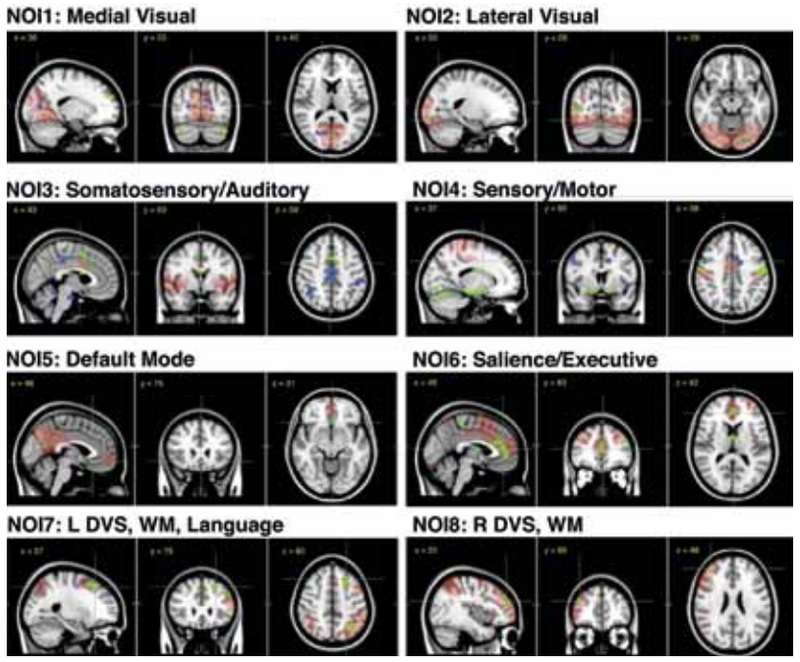
Liem-Moolenaar M, **Zoethout RW**, de Boer P, Schmidt M, de Kam ML, Cohen AF, Franson KL, van Gerven JM. The effects of a glycine reuptake inhibitor R231857 on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. J Psychopharmacol. 2010 Nov;24(11):1681-7. Epub 2009 Jul 31.

**Zoethout RW**, van Gerven JM, Dumont GJ, Paltansing S, van Burgel ND, van der Linden M, Dahan A, Cohen AF, Schoemaker RC. A comparative study of two methods for attaining constant alcohol levels. Br J Clin Pharmacol. 2008 Nov;66(5):674-81.



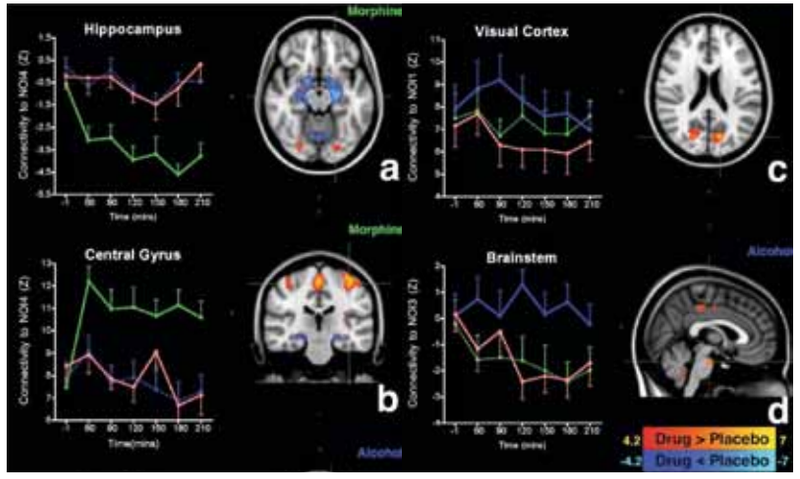
**CHAPTER 6 * FIGURE 5**

OVERLAYING MAPS OF DRUG BY TIME INTERACTIONS WITH CONNECTIVITY TO THE 8 TEMPLATE NETWORKS




**CHAPTER 6 * FIGURE 6**

PROFILE OF CHANGE IN RESTING-STATE CONNECTIVITY IN SELECT ROI'S



# Treatment in Early Drug or Alcohol Dependent Adolescents

Remco Zoethout