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DRUID
Driving under the Influence of Drugs, Alcohol and Medicines

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Effects of stimulant drugs on actual and simulated driving

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Effects of stimulant drugs on actual and simulated driving

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Executive summary

Background and rationale

The present deliverable focuses on two major stimulant drugs of abuse: i.e. 3,4-methyldioxymethamphetamine (MDMA) and amphetamine. Recent population surveys indicate that lifetime prevalence of amphetamine and MDMA use varies between nations, from nearly zero to 12% of all adults. The prevalence of amphetamine and MDMA consumption is however most common among young adults (15-34 yrs), particularly in recreational settings were young people congregate such as dance events and music festivals. Both drugs became increasingly popular during the 1990's. Population surveys now point to an overall stabilization, or even moderate decrease, in the popularity of both drugs (EMCCDA 2009). A summary of last year lifetime prevalence of the use of amphetamines and MDMA among youngsters is given in Figure 1.

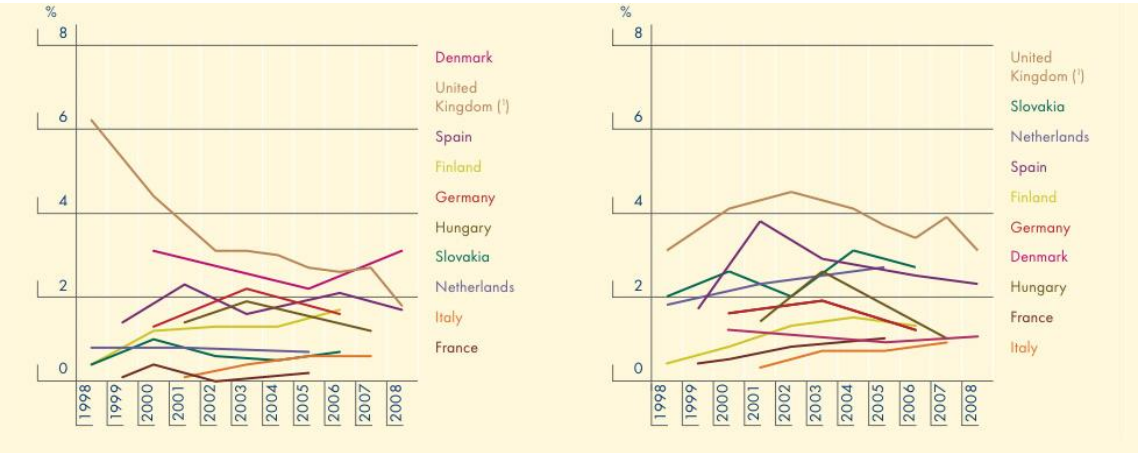


Figure 1. *Trends in last year prevalence of use of amphetamines (left) and MDMA (right) among young adults, aged 15-34 yr (ref, EMCCDA, 20009)*

Epidemiological data on the use of MDMA, amphetamines and other psychostimulants in traffic are sparse, but seem to point out that driving home after a party under the influence of so-called 'party drugs' or 'dance drugs' is on the rise (Koesters et al 2002; Morgan 2000; Ojaniemi et al 2009; Walsh et al 2004). When interviewed at the location of a dance party in the Netherlands, 6% of those who indicated they would be driving home afterwards had used MDMA and was still under the influence (Spruit 2001). A recent report of the National Highway and Traffic Safety Association (NHTSA) reported that 10.5% of drivers during night-time were under the influence of illicit drugs (NHTSA 2009). The use of MDMA and amphetamines has been implicated in road-accidents and both drugs have been reported in the plasma of drivers held responsible for the crashes (Henry et al 1992; Verschraagen et al 2007). Moreover, anecdotal reports on drivers under the influence of

amphetamines and MDMA describe increased risk taking, speeding inadequate attention as typical drug induced behaviours (Schifano 1995). These findings clearly stress the need to further investigate the putative impairing influence of amphetamines and MDMA on driving performance or driving-related psychomotor performance in placebo controlled, experimental studies.

A small number of experimental studies have assessed the effects of stimulant drugs on psychomotor functions or skills related to driving in experimental, placebo-controlled studies employing users of MDMA or amphetamines as participants. These studies generally showed that psychostimulants boost the confidence of users and temporally enhance feelings of well being and euphoria. Under experimental conditions, small doses of amphetamines (5-20 mg) have been shown to increase arousal and improve task performance in fatigued, as well as non-fatigued, subjects. The effects include improvements in reaction speed, vigilance, accuracy in a spatial delay response task, verbal reasoning, coordination and cognitive processing speed (Fleming et al 1995; Koelega 1993; Newhouse et al 1989; Silber et al 2006). However, impairments of overall driving ability after single doses of dexamphetamine have been reported as well. Silber et al (Silber et al 2005) assessed the acute effects of dexamphetamine on simulated driving performance of 24 volunteers in a double blind, placebo controlled , cross-over design. Mean dexamphetamine blood concentrations were 83 ng/ml and 98 ng/ml at 120 min and 170 min post drug, respectively. Results indicated a decrease in overall simulated driving ability following dexamphetamine administration during the day-time but not the night-time scenario tasks. Contributing to this performance reduction, "incorrect signaling", "failing to stop at a red traffic light" and "slow reaction times" were typical behaviors reported after dexamphetamine.

Several acute studies have shown that MDMA acts as a psychomotor stimulant that increases arousal, mood en psychomotor function. Lamers et al (Lamers et al 2003) demonstrated that a single dose of MDMA improved simple reaction time task performance and tracking performance under single and dual task conditions. Ramaekers and Kuypers (Ramaekers and Kuypers 2006) demonstrated that MDMA improved impulse control in a stop signal task. In addition, two actual driving studies showed improvement after a single dose of MDMA of lane tracking as indicated by a reduction in road tracking error (Kuypers et al 2006; Ramaekers et al 2006). When combined with alcohol however, MDMA's stimulant effects were not strong enough to fully overcome alcohol induced impairment of psychomotor function and actual and simulated driving performance (Brookhuis et al 2004; Dumont et al 2008; Hernandez-Lopez et al 2002; Kuypers et al 2006; Ramaekers and Kuypers 2006)

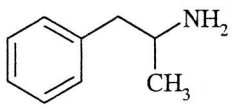
Experimental studies thus generally show that stimulants can improve arousal, energy and performance. These finding have supported general claims that stimulant drugs do not pose a safety hazard in traffic, or might even enhance traffic safety. Yet, it should be remembered that amphetamines and MDMA may also affect cognitive functions in ways that might be detrimental for traffic safety. For example, MDMA has been shown to decrease spatial and working memory performance and movement perception during intoxication (Kuypers and Ramaekers 2005; Lamers et al 2003; Ramaekers et al 2009) which may influence judgment and decision making during the driving tasks. Moreover, stimulant effects have usually been established in laboratory settings, during the

daytime, and after single doses, whereas stimulants a normally used during the night-time, in multiple doses and in combination with other drugs in recreational settings. The predictive validity of such experimental studies thus is very limited as long as real-life confounders are not taken into account. The experimental studies described in the present deliverable were designed to assess the contributory roles of two confounding variables on the effects of stimulant drugs on driving performance: i.e. sleep deprivation and concomitant alcohol use; and to determine driving impairment as a function of drug concentration in blood. In short, the main objectives of the study where:

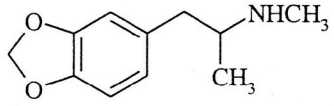
- To provide limits of impairment for MDMA and amphetamine
- To assess potential drug-alcohol interactions
- To assess drug-sleep deprivation interactions
- To provide a reference database for converting stimulant drugs concentrations from whole blood to plasma.

3,4-methylmethamphetamine (MDMA)

MDMA is a synthetic drug which is popular among clubbers who love it for its euphoric and energizing effects. MDMA is structurally related to amphetamines but differs in one important aspect (see Figure 2). It has a methylenedioxy group attached to the aromatic ring of the amphetamine molecule (i.e., it is "ring-substituted"). In this respect, it resembles the structure of the hallucinogenic material mescaline. As a result, the pharmacological effects of MDMA are a blend of those of the amphetamines and mescaline. MDMA is believed to the increase the net release of monoamines (serotonin, dopamine and noradrenaline) from their respective axon terminals. The release of dopamine and noradrenaline is mainly responsible for the physical effects that MDMA shares with amphetamine. The release of serotonin and (in)direct stimulation of post-synaptic serotonergic receptors are believed to be responsible for the mental effects observed during MDMA intoxication. The most frequent effects after MDMA/ecstasy administration are euphoria, well-being, happiness, stimulation, increased energy, extroversion, feeling close to others, increased empathy, increased sociability, enhanced mood, mild perceptual disturbances, changed perception of colors and sounds, somatic symptoms related to its cardiovascular and autonomic effects (blood pressure and heart rate increase, mydriasis), and moderate derealization but not hallucinations. Pharmacokinetic data demonstrate that that peak plasma concentrations of MDMA and it's metabolite MDA are achieved at approximately 2 hours after intake. Physiological and mental effects of MDMA can last up to 6 hrs after intake (de la Torre et al 2004).



amphetamine



3,4-methylenedioxymethamphetamine (MDMA)

Figure 2. *Chemical structures of amphetamine and MDMA.*

Amphetamine

Amphetamine is a synthetic drug with strong stimulant effects. In medicine, amphetamine derivatives such as dexamphetamine and methylphenidate are used for treatment of attention deficit disorders and narcolepsy. Within the armed forces, it is also frequently prescribed as an anti-fatigue pill for pilots or other individuals in situations requiring vigilance and alertness. Amphetamine is also used illegally to take advantage of these properties. In traffic, truck drivers have been known to use amphetamine to combat sleep during long haul rides. Amphetamine is traditionally comprised of 50% levo- and 50% dextro-amphetamine. Dextroamphetamine (d-amphetamine or dexamphetamine) is the most potent of both isomers and is the most predominant form of the drug used today. Amphetamine exerts its effects primarily on the dopamine system. It increases the release of dopamine form synaptic vesicles and blocks the reuptake of dopamine after release in the synaptic cleft. The net effect is greater availability of dopamine levels throughout the brain. Acute administration of amphetamine in humans leads to a number well described behavioural reactions such as: increased arousal, reduced fatigue and feeling of exhilaration. Sleep is delayed and performance is simple tasks is improved. Physiologically, amphetamine acts as a sympathomimetic and elevates blood pressure, heart rate and respiration rate. Amphetamine effects are usually experienced within 15-30 minutes. Plasma levels peak much more rapidly following smoking than following oral administration (i.e. a few minutes vs 2 hrs respectively). It's elimination half-life ranges from 7-30 hrs (Feldman 1997).

Design, dosing and study procedures

The following experimental studies were designed to assess the effects of dexamphetamine and MDMA on actual or simulated driving performance:

1) The effects of singles doses of MDMA (25, 50 and 100mg) on actual driving performance before and after a night of sleep deprivation (UMaas, Maastricht University)
2) The effects of a single dose of MDMA (100 mg) with and without alcohol (0.5.g/kg) on simulated driving performance (RUGPsy, University of Groningen)
3) The effects of singles doses of dexamphetamine (10 and 40 mg) on simulated driving performance before and after a night of sleep deprivation (VTI)
4) The effects of a single dose of dexamphetamine (10mg) with and without alcohol (0.8 g/kg) on simulated driving performance (TNO)

What all studies have in common is their use of placebo controlled, double-blind, within-subjects study designs. The studies employed representative subject samples, i.e. recreational users of MDMA and amphetamines, who went through strict medical screening and selection procedures. The studies furthermore employed cross-over designs which are generally preferred for their efficiency while providing maximal statistical power with relatively small sample sizes, and they proceeded from conventional laboratory testing of psychomotor skills and cognition to sophisticated driving simulators (i.e. VTI, TNO and University of Groningen) and actual on-the-road driving tests (Maastricht

University) for establishing the driving hazard potential of the respective drugs. Driving tests were conducted at Tmax, when drug concentrations were maximal and, in case of sleep deprivation, also in morning after a night of sleep loss. More details on study designs, screening, subject characteristics and in- and exclusion criteria can be found in the separate study reports that are included as separate chapters in the present deliverable. All studies adhered to the following common set of pre-defined instructions with respect to study procedures:

- Number of subjects: the minimum number of subjects was 16. The choice for a subjects' sample-size was always corroborated by a statistical power analysis.
- Subject selection: studies with MDMA and dexamphetamine only included recreational users of these drugs. Drug-naïve, healthy volunteers were excluded.
- Drug screens: subjects were always tested for drugs in urine prior to administration of drugs or medicines. Urine was checked for 5 major drugs: i.e. THC, benzodiazepines, opiates, stimulants and cocaine. Subjects that tested positive for drugs were dismissed (sent home) and asked to return to the lab at another date and drug negative.
- Alcohol screens: subjects were always tested for alcohol (by breathalysing) prior to drug or medicine administration. Subjects that tested positive for alcohol were dismissed (sent home) and asked to return to the lab at another date and alcohol negative.
- Driving experience: subjects needed to have a driver's license. In case of the stimulant studies there will be no demand regarding driving experience as the target population is expected to be very young.
- Blood alcohol concentration (BAC): all partners employed a standard BAC unit: i.e. mg/mL
- Training sessions: all subjects received training sessions of actual driving tests, simulator driving tests and/or laboratory performance tests in order minimize learning effects. Training was performed in all subjects to achieve a stable performance level prior to study entrance.
- Subjective measures: all partners included a set of subjective measures on drug effects (e.g. alertness, mental effort etc).
- Ethics: all partners obtained study approval from their local (and national) ethics review boards and conducted their study according the declaration of Helsinki and Good clinical practice.

Standard driving parameters

All partners adhered to a standard set of driving parameters to increase comparability between studies. These driving parameters basically covered 3 core levels of driving behaviours:

- Automated behaviours – Well-learned (over-learned) skills
- Controlled behaviours – Controlled manoeuvres in traffic
- Executive, strategic behaviours - Interactive functions with ongoing traffic, planning, risk taking

All partners agreed on a **minimum** of 3 driving scenarios to be included in each and every study. These scenarios represent the behavioural levels above, and constituted the primary driving measures

over all studies.

Road tracking scenario (automated behaviours)
The road tracking scenario was based on the Road Tracking Tests that has been used in the Netherland in over 100 studies for measuring drug effects on driving (O'Hanlon et al 1982). Participants are required to drive a 100km course maintaining a constant speed of 95 km/h and a steady lateral position in traffic lanes. The primary driving measure is the standard deviation of lateral position or SDLP. SDLP is an index of road tracking error or weaving, swerving and overcorrecting. SDLP is measured using an electro-optical device mounted on the rear of the vehicle which continuously records lateral position relative to the traffic lane. An increase in SDLP, measured in centimeters, indicates driver impairment, as the driver's ability to hold the car in a steady lateral position diminishes.

Car-Following scenario (controlled behaviours)
The Car Following task was developed to measure attention and perception performance, as errors in these areas often lead to accident causation. In this task participants are required to match the speed of a lead vehicle and to maintain a constant distance from the vehicle as it executes a series of deceleration and acceleration manoeuvres. The primary dependant variable is reaction time to lead vehicle's speed decelerations. This test assesses a driver's ability to adapt to manoeuvres of other motorists (Brookhuis and de Waard 1993; Ramaekers and O'Hanlon 1994).

Risk taking scenario (strategic behaviours)
Risk taking scenarios were only embedded in studies using a driving simulator. Standard parameters that were used by respective partners were gap acceptance, number of crashes, number of red light crossings and number of crashes during sudden event scenarios.

In addition, all partners including a number of laboratory tests measuring skills related to driving. These test included tracking tasks, attention tasks, reaction tasks and cognitive tasks. Performance parameters associated with these laboratory tests were considered secondary driving parameters.

Definition of clinically relevant drug effects

All partners employed alcohol effects on driving parameters as a standard reference to quantify impairment for any other drug. Any drug induced performance change > performance change induced by BAC 0.5 mg/mL was qualified as a clinical relevant drug effect. Drug effects equal to those produced by a BAC of 0.5 mg/ml were also considered to define the "threshold" of impairment for an individual drug. Drug effects were tested for comparability to BAC 0.5 mg/ml effects by means of equivalence testing (see section statistics). All partners conducted a placebo-controlled alcohol study in order to calibrate their primary driving parameters for the effects of BAC 0.5 mg/ml.

Standard toxicological analyses

All partners collected whole blood, serum and blood spots for determining concentrations of dexamphetamine and MDMA during driving tasks. Blood samples were analysed by Prof dr Gisela Skopp at the University of Heidelberg. Analytes were amphetamine, MDMA und MDA. Analysis was performed on whole blood, plasma and dried blood spots (DBS) by LC/MS/MS following evaluation of the analytical method according to international guidelines. B/p ratios were derived from *in vitro* partition experiments (different hematocrit values) and from corresponding blood and plasma samples (*ex vivo).* Bland Altman analysis was used to test agreement of concentrations determined from whole blood and corresponding DBS. Toxicological analysis served two objectives: 1) determination of drug concentration in whole blood and corresponding plasma samples to estimate *ex vivo* blood to plasma (b/p) ratios, and 2) comparison of drug levels in whole blood and corresponding DBS.

Standard statistical methods

All primary driving parameters were analysed according to the following predefined statistical procedures.

Superiority testing and equivalence testing

The general statistical analyses consisted of 2 steps: 1) Assessment of overall treatment effect by means of superiority testing (e.g. ANOVA for within group comparisons). Superiority testing basically indicates whether drug effects differ from placebo. 2) Equivalence testing of drug effects was based on difference scores from placebo (within group) relative to the alcohol criterion (i.e. equivalence to a BAC of 0.5 mg/ml). Basically, equivalence testing assessed whether the alcohol criterion values falls within the 95% CI for the drug effect. If yes, than the drug effect was considered equivalent to a BAC of 0.05 mg/ml (and thus clinically relevant for traffic safety). If the 95% CI was below the alcohol criterion value than a drug effect was considered not relevant

Concentration effect relations.

Concentration effect relations were conducted for those studies that administered multiple doses of a MDMA (Maastricht University) and dexamphetamine (VTI).

Data sets were analyzed according to a 2 step procedure. Data collected during different doses of a drug were converted into difference scores from placebo for analyses of the association between drug concentration and performance (i.e.: difference score = performance during drug treatments - performance during placebo treatment). A linear regression analysis was conducted to establish linear relationships between changes (from placebo) in task performance during drug treatment and log-transformed drug concentrations in serum. The total number of data points included in these equations was defined by the number of subjects x maximal number test repetitions x the number of drug doses.

Second, individual drug concentrations in serum prior to performance assessments in each of the drug dose conditions was divided over a number mutually exclusive categories covering the full range of drug concentrations in a particular study. Corresponding change scores of task performance

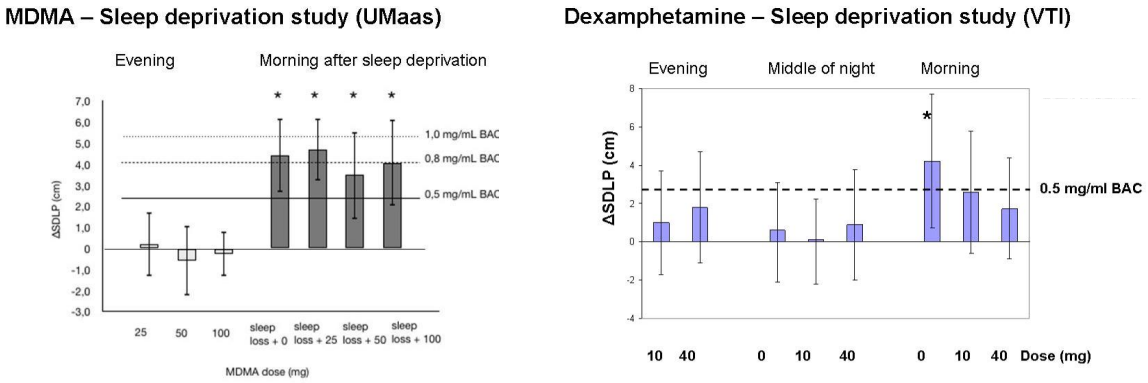
were then classified either as showing “impairment” or “no impairment” for all individual cases within each of these categories. Impairment was defined as a positive change score from placebo in case of SDLP (road tracking) and RT to speed decelerations (Car Following). Binomial tests were applied to measure whether the proportion of observations showing impairment or no impairment significantly differed from the hypothesized proportion. It was hypothesized that in case of no effect of a drug on task performance the proportion of observations showing impairment or no impairment will be equal; i.e. 50 percent.

Main results

The present section summarizes the main results from each of the 4 experimental studies, as well the additional toxicology analyses conducted at the University of Heidelberg. For full reports of the studies are reported in Chapters 1-2 (Maastricht University), Chapter 3 (University of Groningen), Chapter 4 (VTI), Chapter 5 (TNO) and Chapter 6 (University of Heidelberg).

Sleep deprivation studies

Studies assessing the interaction between stimulant drugs and sleep deprivation generally reported similar results. Both studies reported grossly impairing effects of sleep deprivation on road tracking performance (i.e. SDLP) and secondary measures of psychomotor function and cognition. The effects of sleep deprivation did not significantly change in the presence of MDMA and dexamphetamine, independent of dose or concentration, as indicated by the absence of a significant statistical interaction between the factors Stimulant (MDMA or Dexamaphetamine) and Sleep deprivation. Impairments of road tracking performance were always bigger than those observed after a BAC of 0.5 mg/ml and was considered clinically relevant. In the MDMA study, road tracking impairments observed after sleep deprivation with and without MDMA even were comparable to BAC > 0.8 mg/ml. In total 26 (20.3%) on-the-road driving tests were prematurely terminated due to extreme fatigue of which 23 (88.5%) in the morning and 3 (11.5%) in the evening. Dexamphetamine appeared to decrease the impairing effect of sleep deprivation on road tracking (see Fig 1) even the interaction between dexamphetaine and sleep deprivation was not of statistical significance. Equivelance tests demonstrated a large variability in road tracking performance as evidenced by wide 95%CIs. The upper limit of the 95%CI exceeded the alcohol criterion value associated with a BAC of 0.5 mg/ml but the lower limit also exceeded zero. The fact that the 95%CI includes both the alcohol criterion value as well as the placebo reference values basically means that the evaluation of the clinical relevance of the combined effect of dexamphetamine and sleep deprivation remains undecided or ambiguous: i.e. it is predicted that some individuals will show impairment whereas others may not.



A B

Fig 1a) Mean (95% CI) SDLP difference from placebo after single doses of MDMA during the road tracking test in the evening and in the morning after a night of sleep loss. (= equivalence to BAC 0.5 shown, upper bound of the 95% CI is above the non-inferiority margin or alcohol criterion of 2.4 cm)*
Fig 1 b) Mean (95% CI) SDLP difference from placebo after single doses of DEX during the road tracking test in the evening, middle of the night and in the morning after a night of sleep loss. (= equivalence to BAC 0.5 not shown, upper bound of the 95% CI is above the non-inferiority margin or alcohol criterion of 2.5 cm).*

MDMA and dexamphetamine alone (prior to sleep deprivation) generally did not affect primary and secondary driving measures. However, there were some signs of stimulatory effects as well. MDMA improved rapid information processing in a secondary laboratory task, and dexamphetamine improved RT to speed decelerations in the Car Following scenario and RT to crossing cars in a risk taking scenario. Subjective measures also indicated that MDMA and dexamphetamine produced stimulant effects (i.e. increased arousal, decreased sleepiness). These stimulatoy effects were most prominent in the evening before sleep deprivations but gradually dissolved over time as a function of hours without sleep. In the moring, subject ratings of arousal and sleepiness were always worse as compared to the prior evening. A summary of results from the sleep deprivation studies is given in Table 1.

Table 1. *Summary of MDMA and dexamphetamine effects on primary and secondary driving parameters (improvement, neutral or impairment), as well as subjective measures of arousal or sleep. Green color codings indicate neutral effects or "stimulating effects"; red color codings indicate "impairing" effects; orange color coding indicate impairments associated with a wide 95%CI. The latter indicates a large variety in response, some subjects are as impaired as under alcohol, others perform as under placebo.*

	MDMA – sleep deprivation study (Maastricht University)			**Dexamphetamine study – sleep deprivation study (VTI)**		
	MDMA	*Sleep deprivation*	*MDMA + Sleep deprivation*	*Dexamphetamine*	*Sleep deprivation*	*Dexamphetamine + Sleep deprivation*
Road tracking	No effect	Increased SDLP; Impairment > BAC 0.8 mg/ml	Increased SDLP; Impairment > BAC 0.8 mg/ml	No effect	Increased SDLP Impairment > BAC 0.5 mg/ml SDLP	Increased SDLP Relevance of impairment undecided (95%CI drug effect includes BAC 0.5 as well as 0)
Car Following	No effect	No effect	No effect	Dose related improvement of phase delay	Impairment of phase delay	Impairment of phase delay
Risk Taking	Not assessed	Not assessed	Not assessed	Improvement RT to crossing cars	Improvement RT to crossing cars	Improvement RT to crossing cars
Laboratory measures of skills related to driving	Neutral on most measures, Improvement on rapid information processing	Impairment of attention and impulse control	Impairment of attention and impulse control	Not assessed	Not assessed	Not assessed
Subjective measures	Increased arousal	Decreased arousal	Decreased arousal	Decreased sleepiness	Increased sleepiness	Increased sleepiness

Alcohol interaction studies

Single doses of alcohol (doses ranging 0.5 - 0.8 g/kg) produced impairments in a number of driving performance domains. Alcohol increased road tracking error in the MDMA-alcohol interaction study when BACs were about 0.5 mg/ml. Alcohol increased risk taking behaviors in the dexamphetamine-alcohol interaction study and also produced impairments psychomotor functions, such as road tracking, attention and RT when BACs where about 0.8 mg/ml. Alcohol induced impairment were not affected by the co-adminstration of dexamphetamine 20mg as evinced by the absence of a dexamphetamine x alcohol interaction in tests of superiority. The interaction between MDMA and

alcohol however did reach statistical significance in the road tracking task. It indicated that the stimulant effect MDMA alone (i.e. MDMA 100 mg improved road tracking) was no longer present when combined with alcohol. Equivalence testing demonstrated that the combined effects of stimulants and alcohol produced a large variation in driving performance as measured in the road tracking task. The upper limit of the 95%CI associated with stimulant/alcohol combinations exceeded the alcohol criterion value but the lower limit exceeded zero as well. Mean road tracking performances under stimulant, alcohol and their combinations is shown in Fig 2

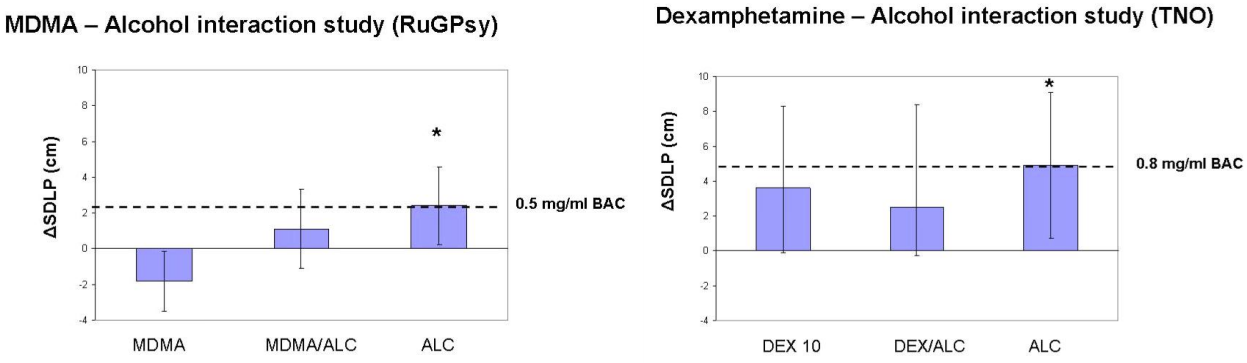


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Fig 2a) Mean (95% CI) SDLP difference from placebo after single doses of MDMA, ALC and MDMA+ALC during the road tracking test. (= non-inferiority not shown, upper bound of the 95% CI is above the non-inferiority margin of 2.4 cm)*
Fig 2b) Mean (95% CI) SDLP difference from placebo after single doses of DEX, ALC and DEX+ALC during the road tracking test.

The fact the the 95%CI includes both the alcohol criterion value as well as the placebo reference values basically means that the evaluation of the clinical relevance of the combined effects of dexamphetamine and alcohol or MDMA and alcohol remains undecided or ambiguous: i.e. it is predicted that some individuals will show impairment whereas others may not.

When given alone, MDMA and dexamphetamine only produced neutral or stimulatory effect. The stimulatory effects were most promintent in the road tarcking task and on subjective measures, MDMA 100mg improved road tracking performance as evinced by a reduction in SDLP or “weaving”. In addition, both MDMA and dexamphetamine produced subjective feelings of decreased sleepiness. For dexamphetamine, decreased sleepiness was even reported when given in combination with alcohol. A summary of results from the drug-alcohol interaction studies is given in Table 2.

Table 2. *Summary of MDMA and dexamphetamine effects on primary and secondary driving parameters (improvement, no effect or impairment) as well as subjective measures of arousal and sleep, alone and in combination with alcohol. Green color codings indicate neutral effects or "stimulating effects"; red color codings indicate "impairing" effects; orange color coding indicate impairments associated with a wide 95%CI. The latter indicates a large variety in response; some subjects are as impaired as under alcohol, others perform as under placebo.*

	MDMA-alcohol study (University of Groningen)			**Dexamphetamine – alcohol study (TNO)**		
	MDMA	*Alcohol*	*MDMA + Alcohol*	*Dexamphetamine*	*Alcohol*	*Dexamphetamine + Alcohol*
Road tracking	Decrease SDLP	Increase SDLP	Increase SDLP Relevance of impairment undecided (95%CI drug effect includes BAC 0.5 as well as 0)	No effects	Increased SDLP	Increased SDLP Relevance of impairment undecided (95%CI drug effect includes BAC 0.8 as well as 0)
Following	No effect	No effect	No effect	No effect	No effect	No effect
Risk Taking	No effect	No effect	No effect	No effect	Shorter gap acceptance; increased red light crossings and number of crashes	Shorter gap acceptance; increased red light crossings and number of crashes Relevance of impairment undecided (95%CI drug effect includes BAC 0.8 as well as 0)
Laboratory measures of skills related to driving	Not assessed	Not assessed	Not assessed	No effect	Impairment of attention, tracking and RT	Impairment of attention, tracking and RT
Subjective measures	Decreased sleepiness	Increased sleepiness	Increased sleepiness	Decreased sleepiness	No effect	Decreased sleepiness

Toxicology

Mean blood concentrations of MDMA, MDA and amphetamines were within the expected normal range in all experimental studies. MDMA concentrations ranged from not detectable to 310 or 236

ng/mL in blood and plasma respectively. Whenever MDMA could be determined in the plasma specimen, it was also present in the blood, and vice versa. MDA concentrations were up to 10.4 ng/mL in blood and 16.3 ng/mL in plasma, respectively. For most samples, MDA concentration were below the LOQ. At 11.5 hours post drug, during the morning driving sessions, mean MDMA concentrations ranged between 14.2 and 84.3 (6.7) ng/mL depending on the evening dose. Amphetamine concentrations ranged between 10 and 124 ng/mL during performance testing in studies conducted by TNO and VTI. There were no significant correlations between drug concentration and driving performance during MDMA and dexamphetamine treatments. In general, both MDMA and dexampetamine, when given alone did not affect driving performance independent of dose or concentration.

B/p ratios of amphetamine, MDMA and MDA were dependent on the hematocrit value, but not on the concentration (≤ 500 ng/mL). B/p ratios of MDMA and MDA averaged 1.16 +/- 0.13 and 1.27+/- 0.20, respectively. Mean ratios of amphetamine were 0.89 +/- 0.10 and 0.91 +/- 0.12. Bland Altman analysis revealed that < 5% of the concentration differences (DBS-blood) were not within the limits of "agreement" for both MDMA and amphetamine. Mean differences were 1.4 ng/mL for MDMA and -0.63 ng/mL / 1.03 ng/mL for amphetamine using DBS instead of whole blood for analysis which is considered acceptable.

Discussion

Effects of MDMA and dexamphetamine

The effects of MDMA and dexamphetamine on measures of simulated and actual driving were neutral for most of the driving measures. A small number of primary (road tracking and car following) and secondary driving parameters even demonstrated performance improvement during intoxication with either drug. Such stimulatory effects were also supported by subjective data that indicated that MDMA and dexamphetamine increased arousal and decreased sleepiness. These findings are in line with previous driving studies that demonstrated that stimulant drugs such as MDMA and methylphenidate can improve road tracking performance as measured in on-the-road driving tests (Ramaekers et al, Kuypers et al) after single dose administrations. The stimulatory effects of MDMA and dexamphetamine on human performance have been widely acknowledged, and as such are no real surprise. It should be remember however that previous research had also demonstrated that stimulant drugs such as MDMA, can improve certain aspects of performance while impairing other performance domains at the same time. For example, stimulants have repeatedly been shown to improve neuropsychological skills such as tracking, impulse control and reaction time, while impairing cognitive functions such as working memory and movement perception (Kuypers and Ramaekers 2005; Lamers et al 2003; Silber et al 2006; Silber et al 2005). Thus, the finding that MDMA and dexamphetamine can improve performance in particular driving domains, does not automatically mean that these drugs do never have detrimental effects in other domains, relevant to driving as well.

Effects of alcohol alone and combination with MDMA and dexamphetamine

Alcohol was administered in two simulated driving studies in order to assess the potential interaction between alcohol – MDMA and alcohol – dexamphetamine. Alcohol significantly impaired road tracking performance in the study by TNO. Co-adminstration of dexamphetamine did dod significantly change the imapring effect of alcohol als evinced the lack of statistical interaction between dexamphetamine and alcohol. Equivalence testing demonstrated that the 95%CI of the change in road tracking performance (i.e SDLP) after combined use of dexamphetamine and alcohol included both the alcohol criterion as wel the placebo reference (zero). The latter basically means that the evaluation of the clinical relevance of the combined effects of dexamphetamine and alcohol are undecided or ambiguous: i.e. it is predicted that some individuals will show impairment whereas others may not.

Risk scenario's and secondary driving measures employed by TNO were very sensitive to the effects of alcohol alone and to alcohol – dexamphetamine combined. These measures demonstrated that single doses of alcohol (0.8 g/kg body weight) increased risk taking behaviors (i.e. shorter gap acceptance, increase of red light crossings and number of crashes) and impaired tracking, attention and reaction time during a 3 hr period after drinking when BACs declined from 0.9 mg/ml to 0.2 mg/ml. Moreover, these alcohol impairments were not affected by the co-administration of dexamphetamine 20mg, indicating that the stimulatory effects of dexamphetamine were not sufficient to overcome the impairing effects of alcohol on skills related to driving.

Alcohol effects were most prominent in the road tracking scenario in the MDMA-alcohol interaction study conducted by University of Groningen. As expected, alcohol significantly increased SDLP or the amount of "weaving" during highway driving, suggesting a decrement of road tracking control. This finding nicely replicates earlier demonstration of alcohol induced impairment of road tracking performance in actual, on-the-road driving test scenarios (Kuypers et al 2006; Louwerens et al 1987; Ramaekers et al 2000). Average BACs during the simulated driving tests were around 0.45-0.50 mg/ml during treatments with alcohol. The stimulatory effects of MDMA (100mg) were sufficient to counteract some of the impairing effects of this low dose of alcohol on SDLP as indicated by a significant MDMA x Alcohol interaction. However, equivalence tests again demonstrated that change in SDLP after the combination of MDMA and alcohol included both the alcohol criterion as well as the placebo reference. In other words, due to large variation in subject sensitivity to combination of MDMA and alcohol, some subject will show impairment whereas others will not.

These findings are in line with previous research that has also indicated that stimulatory effects of MDMA are not sufficient to fully overcome alcohol induced impairments of driving performance. For example, Kuypers et al (Kuypers et al 2006) demonstrated that alcohol (BAC around 0.5 mg/ml) induced impairment of SDLP, as measured in an on-the-road tracking scenario were not or only partly mitigated by concomitant administration of 75 and 100mg respectively. Also, MDMA did not interact with the impairing effect of low doses of alcohol on a range of psychomotor an cognitive functions in a number of psychomotor studies performance (Brookhuis et al 2004; Dumont et al 2008; Kuypers et al 2006; Ramaekers and Kuypers 2006)

Hernandez-Lopez (Hernandez-Lopez et al 2002) reported that MDMA reversed the subjective sedation induced by alcohol but did not change the impairing effect of alcohol on measures of psychomotor function such as simple reaction time and digit symbol substitution. Collectively, these

studies seem to indicate that the stimulatory effects of dexamphetamine and MDMA are mild and generally not sufficient to overcome alcohol induced impairments in every performance domain. The lack of mitigating effects of MDMA and dexamphetamine on alcohol induced performance impairment may be of particular importance in terms of road safety issues. Many of the amphetamine and MDMA-impaired driving cases that have been reported in the scientific literature consist of drivers who have taken multiple drugs and/or alcohol (Logan 1996; Logan and Couper 2001; Verschraagen et al 2007). The present data indicates that the CNS stimulating effects of MDMA and amphetamine do not suffice to overcome alcohol induced impairment of motor control which is one of the most common causal factors in vehicle crashes.

Effects of sleep deprivation with or without MDMA or dexamphetamine

The sleep deprivation studies demonstrated that sleep loss produced severe impairment in actual and simulated driving performance as expressed by a significant rise in SDLP in the road tracking scenario. In the on-the-road driving study, a large number of driving tests during were prematurely terminated during the early morning sessions, due to excessive fatigue.

On average, SDLP increased with 4.2 cm in the morning after sleep deprivation, relative to SDLP before sleep deprivation. This increment is about 1.5-2 times greater than found in two recent driving under the influence of alcohol studies with blood alcohol concentrations between 0.29-0.5 mg/mL (Kuypers et al 2006; Ramaekers et al 2000). From a previous alcohol study that was conducted in order to calibrate SDLP for the dose-related effects of alcohol (Louwerens et al 1987) it can be concluded that a mean increase in SDLP of 4.2 cm is equivalent to a blood alcohol concentration of approximately 0.8 mg/mL. Equivalence testing even demonstrated that the upper limit of the 95% CI associated with the mean change in SDLP after sleep deprivation widely exceeded the criterion level of 1.0 mg/mL BAC. Together this indicates that sleep deprivation caused severe driving impairment comparable to driving under the influence of high to very high BAC.

These findings were corroborated by results from secondary driving measures as measured in laboratory tests. Critical tracking performance significantly decreased over the night, as a function of hours of sleep loss. Similar findings have previously been reported by Dawson and Reid (Dawson and Reid 1997). They measured tracking performance as a function of hours of sleep loss and BAC intoxication. According to their model, tracking performance of subjects after 17-24 hours of wakefulness is equivalent to that observed at BAC between 0.5-1.0 mg/mL. This again indicates that one night of sleep deprivation causes serious impairment of driving skills. In terms of BAC equivalents, these impairments exceeded those observed at legal BAC limits of 0.5 mg/mL and 0.8 mg/mL that are currently in place for driving under influence of alcohol in most of Europe and the US.

It is also apparent from the present studies that the stimulant effects of MDMA and dexamphetamine, if any, could not compensate for the impairing effect of sleep loss on simulated and actual driving performance. None of the primary driving measures demonstrated any significant MDMA x sleep loss interaction. The effects of sleep deprivation on driving were highly prominent during MDMA and dexamphetamine treatments and did not change as a function of dose and concentration. These data indicate that stimulatory effects of MDMA and dexamphetamine on driving

and psychomotor performance are not sufficient to overcome impairments caused by sleep deprivation.

Concentration effect relations of MDMA and dexamphetamine

Two studies were specifically designed to assess driving performance across a wide range of doses and concentrations. The MDMA-sleep deprivation study included 3 doses of MDMA (25, 50 and 100 mg) whereas the dexamphetamine-sleep deprivation study included a low dose (10mg) and a high dose (40mg) of dexamphetamine. It was apparent in both studies that neither MDMA nor dexamphetamine produced any dose or concentration related effects on driving. Also, the inability of both stimulants to compensate for the impairing effects of alcohol and sleep deprivation was not affected by dose or concentration.

It should however be noted that doses administered in the present study may have been relatively low for (some) recreational drug users. Dexamphetamine doses (10-40mg) were well with the accepted therapeutic window when used for medical purposes. Likewise, MDMA doses were close to the amount of MDMA that is generally present in a single ecstasy tablet (i.e. 50-100mg). However, it is very likely that a significant proportion of recreational drug users will take much higher doses of dexamphetamine and MDMA in real life situations. High dose effects of stimulant on driving performance can not be readily assessed in experimental, placebo controlled studies due to obvious medical and ethical constraints. It has become evident however, that MDMA and amphetamine concentrations that are observed in actual DUI cases can be 10-fold higher than during controlled administration in experimental studies. A recent study analyzing drug concentrations in port-mortem cases and DUI cases in the Netherlands in 1999-2004 may serve to illustrate this point (Verschraagen et al 2007). Amphetamine-based drugs were present in 70 post-mortem cases and 467 DUI cases. The most detected amphetamine-based drug was MDMA, followed by amphetamine. Median blood concentrations of MDMA in post-mortem and DUI cases were 1600 and 330 ng/mL respectively. MDMA blood concentrations in the MDMA related deaths (n=20) and in the DUI cases (n=360) varied up to 3700 and 4000 ng/mL, respectively. The median concentrations of amphetamine in the amphetamine related deaths (n=13) and the DUI cases (n=208) were 280 and 220 ng/mL, respectively. Amphetamine blood concentrations up to 6000 and 2300 ng/mL were seen in the drug related deaths and DUI cases, respectively. The most frequently encountered amphetamine-based drugs in the investigated deaths were MDMA and amphetamine. The majority of MDMA- and amphetamine-caused deaths, i.e. 90% of these deaths, occurred with blood concentrations above 1500 and 800 ng/mL, respectively. Clearly, these that data show that amphetamine concentrations in DUI cases can be much higher than amphetamine concentrations that are achieved in controlled studies. Of course, high concentrations of amphetamines in DUI cases do not necessarily mean that amphemine use is an underlying cause of accidents. In fact, the majority of DUI hospital cases that are positive for amphetamines are also positive for alcohol and other drugs (see also DRUID deliverable 2.2.5).

Toxicology

Toxicological analysis of MDMA and amphetamine concentrations in whole blood, plasma and DBS revealed that B/p ratios of amphetamine, MDMA and MDA were dependent on the hematocrit value, but not on the concentration (≤ 500 ng/mL). Bland Altman analysis revealed that < 5% of the concentration differences (DBS-blood) were not within the limits of "agreement" for both MDMA and amphetamine. Mean differences were 1.4 ng/mL for MDMA and -0.63 ng/mL / 1.03 ng/mL for amphetamine using DBS instead of whole blood for analysis which is considered acceptable. Dividing the concentration of MDMA or amphetamine in blood by 0.78 or 1.11, respectively, may give a reasonably good estimate of the coexisting concentration in plasma. For law enforcement purposes, it is recommended to consider inherent biological variations in the b/p relationship. There is sound evidence that the DBS assay has potential as a precise and inexpensive option for the determination of amphetamine and amphetamine derivates in small blood samples.

MDMA, dexamphetamine and driving safety

Several experimental studies have indicated that therapeutic doses of stimulant drugs produce neutral or even stimulating effects on a range of psychomotor functions. However, a few studies also indicated that therapeutic doses of stimulants can still be potentially dangerous for driving because of detrimental effects there are apparent on movement estimation and working memory (Kuypers and Ramaekers 2005; Lamers et al 2003; Silber et al 2005) .

There was no clear relation between drug concentrations and driving impairment for MDMA and dexamphetamine. Instead, impairing effect of therapeutic doses of stimulants became prominent in specific situations or settings: i.e. in combination with alcohol or after a night of sleep loss. Combinations of stimulants with alcohol and/or sleep deprivation produced severe impairments over a large range of performance domains. The pharmacological effects of stimulant and drug use settings seem very much intertwined and are likely to play a crucial role when evaluating driving under the influence (DUI) offenders. Some will take the present data as an argument to show that the primary reason for impairment observed in DUI cases with stimulants will be sleep deprivation or concomitant use of alcohol and drugs. Others can argue that the use of stimulants may affect a person's ability to subjectively evaluate or recognize their state of impairment. Stimulants increase subjective feelings of arousal, energy and mood. Such feelings affect the subjective judgment of stimulant users on whether or not it is safe to drive home after spending a night at a rave party. During intoxication with a stimulant, they may not be able to subjectively experience the debilitating effects of sleep loss or concomitant alcohol use to the same degree as stimulant free drivers, because they feel more energetic (see for example the VTI study). As a consequence, they may decide to drive because they subjectively feel alert, thereby neglecting the objectively impairing effects of other co-factors such as sleep deprivation or alcohol use.

In the context of a pan-European initiative to combat driving under the influence of drugs it is advised to distinguish between 1) potential medicinal use of amphetamines (therapeutic doses) and 2)

drug abuse of stimulants (polypharmacy, combat of sleep). Stimulants are generally safe for driving when taken alone at regular doses (e.g. as in medicinal use), but stimulant effects are less safe when taken in combination with sleep loss or alcohol intoxication as is often the case in drug abusers. In such cases it will be very difficult to separate stimulant effects from those of drug use setting. Consequently, drivers should receive specific warnings on driving impairment arising from the use of stimulants during sleep loss or alcohol intoxication.

Conclusions

- Most experimental studies indicate that effects of stimulant drugs on driving performance are generally mild and safe. Therapeutic doses of stimulant drugs produce neutral or even stimulating effects on a range of psychomotor functions and driving skills. A few studies reported that stimulants may also produce detrimental effects on specific cognitive functions such as memory, spatial orientation, time estimation of moving objects and increase risk taking behaviours, particularly in the case of MDMA. However, the relevance of frequently observed MDMA induced impairments of working memory for traffic safety seems minimal.
- Stimulant effects of MDMA and amphetamine are not sufficient to overcome or compensate for driving impairments produced by concomitant alcohol use or sleep deprivation.
- There was no clear relation between drug concentrations and driving performance for MDMA and dexamphetamine in doses up to 100 mg and 40 mg respectively.
- The pharmacological effects of stimulants and the effects of drug use setting (e.g. polydrug use, concomitant alcohol use and sleep deprivation) are intertwined and significantly contribute to driver impairment.
- In the context of a pan-European initiative to combat driving under the influence of drugs it is advised to distinguish between 1) potential medicinal use of amphetamines (therapeutic doses) and 2) drug abuse of stimulants (polypharmacy, sleep loss). Stimulants are generally safe for driving when taken alone at regular doses (e.g. as in medicinal use), but stimulant effects are less safe in combination with sleep loss or alcohol intoxication as is often the case in drug abusers. In such cases it will be very difficult to separate stimulant effects from those of drug use setting. Consequently, drivers should receive specific warnings on driving impairment arising from the use of stimulants during sleep loss or alcohol intoxication.
- There is evidence that the DBS assay has potential as a precise and inexpensive option for the determination of amphetamine and amphetamine derivates in small blood samples.

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Chapter 1

MDMA (ecstasy) effects on actual driving performance before and after sleep deprivation, as function of dose and concentration in blood and oral fluid.

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Abstract

Experimental research has shown that MDMA can improve some psychomotor driving skills when administered during the day. In real life however, MDMA is taken during the night, and driving may likely occur early in the morning after a night of “raving” and sleep loss. The present study was designed to assess the effects of MDMA on road tracking and car following performance in on-the-road driving tests in normal traffic. Sixteen recreational MDMA users participated in a randomized double-blind placebo-controlled 4-way cross-over design. They received single, evening doses of 0, 25, 50 and 100 mg MDMA on separate occasions. Actual driving tests were conducted in the evening when MDMA serum concentrations were maximal and in the morning after a night of sleep loss. The primary measure of driving, i.e. standard deviation of lateral position (SDLP, a measure of road tracking control) was significantly increased during driving tests in the morning in all treatment conditions, irrespective of MDMA dose and concentration. The increments in SDLP were of high clinical relevance and comparable to those observed for alcohol at BAC concentrations > 0.8 mg/mL. These impairments were primarily caused by sleep loss. In general, MDMA did not affect driving performance nor did it change the impairing effects of sleep loss. It is concluded that MDMA can not compensate for the impairing effects of sleep loss and that drivers who are under the influence of MDMA and sleep deprived are unfit to drive.

Keywords
Driving under the influence of drugs; DUID; MDMA; ecstasy; sleep deprivation

Introduction

3,4-methylenedioxymethamphetamine (MDMA) is the main psychoactive constituent of ecstasy. Ecstasy has stimulant and hallucinogenic effects, and has often been described as an entactogenic drug, because of its characteristic that it makes people feel close to each other. Ecstasy is a popular drug: in 2007 9.5 million European adults had ever used ecstasy, which is 2.8% of the general population (European Monitoring Centre for Drugs and Drug Addiction, 2008). In the US a trend of increasing ecstasy use among adults in 2006-2007 is noticeable, which remained stable in 2008 (Substance Abuse and Mental Health Services Administration, 2009). In Europe as well as in the US ecstasy is one of the most commonly used illicit drugs after cannabis.

The widespread use of ecstasy, might have implications for traffic safety. Epidemiological studies show an increased risk of accidents while driving under the influence of drugs and/or alcohol (European Monitoring Centre for Drugs and Drug Addiction, 2008). Data on illicit substances such as cannabis, amphetamines (including MDMA) and other stimulants in traffic seem to point out that driving home after a party under the influence of so called 'party drugs' is increasing (Morgan, 2000; Ojaniemi, Lintonen, Impinen, Lillsunde, & Ostamo, 2009; Walsh, de Gier, Christopherson, & Verstraete, 2004). A recent report of the National Highway and Traffic Safety Association (NHTSA) reported that 10.5% of drivers during nighttime were under the influence of illicit drugs (Lacey, et al., 2009).

Driving is a complex task that requires several cognitive functions. There is ample evidence that long term use as well as acute use of MDMA detrimentally affects cognition. Multiple reviews have indicated that chronic MDMA use impairs cognitive performance on tasks measuring working and episodic memory, attention, frontal-executive functions, impulsiveness and psychomotor speed (e.g. Kalechstein, De La Garza, Mahoney, Fantegrossi, & Newton, 2007; Morgan, 2000; Parrott, 2006; Zakzanis, Campbell, & Jovanovski, 2007). Acute studies demonstrated attentional and memory deficits during MDMA intoxication. For example, Kuypers and Ramaekers (2005, 2007) reported impairments in verbal and spatial memory performance after a single dose of 75 mg MDMA. Dumont et al. (2008) also described acute impairment in memory as well as in attention after a single dose of 100 mg MDMA. In contrast, psychomotor performance improved after a single acute dose of MDMA (Lamers, et al., 2003), showing that MDMA also possesses stimulating properties.

A number of studies have assessed the effects of MDMA on driving performance in actual driving studies and driving simulator studies. Studies that assessed on-the-road driving performance showed that acute administration of regular recreational doses of 75 or 100 mg MDMA improved road tracking performance (Kuypers, Samyn, & Ramaekers, 2006; Ramaekers, Kuypers, & Samyn, 2006). However, MDMA also impaired other aspects of driving such as car-following performance (Ramaekers, et al., 2006). Subjects overreacted to speed decelerations of a leading car as indicated by a significant ‘overshoot’ in their adaptive response. Another on-the-road driving study indicated that the stimulating effects of MDMA on driving performance were only mild, and not sufficient to counteract the impairing effect of alcohol when used in combination (Kuypers, et al., 2006). The latter finding was also reported by Brookhuis et al. (2004) who assessed simulated driving performance of

rave party visitors before and after the party in a quasi controlled study. All of the participants used multiple drugs, including MDMA and alcohol. Drug users clearly had higher accidents rates and displayed more risk taking behaviors early in the morning, when compared to non-drug using controls. However it was difficult to determine whether these impairments in the drug using group resulted from MDMA use, polydrug use or sleep deprivation.

The present study was designed to assess effects of MDMA and sleep deprivation on actual driving performance, separately and in combination. In addition, the present study assessed the association between MDMA concentration in blood and oral fluid. In order to cover a wide range of concentrations three doses of MDMA (25, 50 and 100 mg) were included in the design. Driving performance was measured in the evening after administration of placebo or MDMA and in the morning after a night of sleep loss. It was expected that MDMA would produce stimulant effects in the evening, but impairment in the morning after a night of sleep loss.

Method

Subjects

Eight males and eight females participated in this study (N=16). Their mean (SE) age was 22.0 (0.41) years and their mean (SE) lifetime MDMA use was 27.0 (8.4) times. Participants were recruited by advertisements at Maastricht University and were paid upon completion of the study. Before enrollment all subjects were screened by means of a telephone interview to determine whether they qualified for the study. The inclusion criteria were: experience with MDMA, i.e. at least 1 time in the last year; free from psychotropic medication; good physical health as determined by a medical examination; absence of any major medical, endocrine and neurological condition; normal weight, i.e. BMI between 18 and 28; valid driving license and written informed consent. The exclusion criteria were history of drug abuse or addiction; pregnancy or lactation; cardiovascular abnormalities on electrocardiogram; excessive drinking, i.e. more than 20 alcoholic consumptions a week; hypertension, i.e. systolic blood pressure over 170 mmHg or diastolic blood pressure over 100 mmHg; history of or current psychiatric disorder. If subjects met the inclusion criteria, they received a medical history and a drug questionnaire to get a more precise view on their health and drug use. Finally, participants underwent a medical examination and took part in a training session.

The study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008). Approval for the study was obtained from the Medical Ethics committee of the Academic Hospital of Maastricht and Maastricht University. A permit for obtaining, storing and administering MDMA was obtained from the Dutch drug enforcement administration.

Study design

The study was conducted according to a double blind, placebo-controlled, randomized, 4-way cross-over design. Treatments consisted of single doses of placebo, 25, 50 and 100 mg MDMA. Treatment orders were balanced over subjects and treatment periods. Placebo and MDMA were administered orally in identically appearing formulations. MDMA was dissolved in 25 mL bitter orange peel syrup

and placebo consisted of only the bitter orange peel syrup. The syrup was mixed with 200 mL juice before it was given to the participants. The wash-out period between treatments was at least one week.

Procedure

Subjects were asked to refrain from any drugs one week before the medical examination until two weeks after study completion. Subjects were not allowed to drink alcohol and caffeine or smoke tobacco during a 24 hour period prior to testing. Subjects were always tested for alcohol and drugs in breath and urine upon arrival (4:30 pm) at the laboratory on test days. In case of a positive result, subjects were send home and asked to come back on another day. This happened in case of one subject. After repeated violation this subject was excluded and replaced. At 5:00 pm participants received a light, standard dinner and at 5:15 pm MDMA or placebo was administered. Driving performance was assessed in the evening and in the morning after a night of sleep loss and psychomotor performance in the evening, in the middle of the night and in the morning. Subjective questionnaire were administered throughout the night. The timeline for performance testing, questionnaires and blood draws is displayed in Figure 1. An additional blood sample was drawn one week after each testing day to monitor renal and liver function. A test day ended at 9:00 am the next morning at which time participants were driven home.

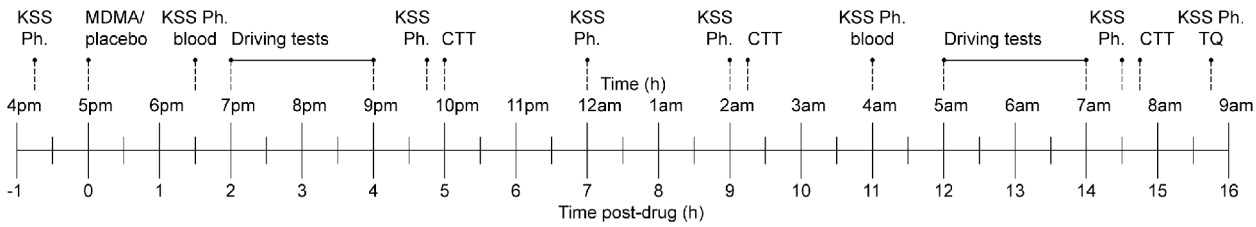

Fig. 1 Timeline for blood samples, questionnaires and performance tests relative to drug administration. KSS: Karolinska sleepiness scale; Ph: physiological measures; CTT: crtitcal tracking task; TQ: treatment questionnaire.

Actual driving tests

The road tracking test (O'Hanlon, 1984) consists of driving in a specially instrumented car with a constant speed of 95 km/h and as straight as possible on the right lane of primary highway during a 1 hour test ride. A video-camera mounted on the rear end of the car registers its lateral position relative to the road delineation. The images are recorded onto a hard drive in the car with a frequency of 4 Hz and are transformed into a file containing the measures of the lateral position. An off line editing routine is applied for removal of all data segments that reveal signal loss, disturbance or occurrence of passing maneuvers. The edited dataset is then used to calculate means and variances for lateral position. The primary dependent measures of this test is the standard deviation of lateral position (SDLP; i.e. a measure of weaving). Speed and standard deviation of speed (SDSP) are recorded as secondary control measures. The highway driving test has been calibrated in a manner allowing expression of any sedative drug effect in terms of the BAC required to achieve the equivalent level of driving

impairment (Louwerens, Gloerich, de Vries, Brookhuis, & O'Hanlon, 1987). The alcohol calibration curve demonstrates that drinkers' mean SDLP rises exponentially with BAC. Results from the alcohol calibration study can be used for describing drugs' effects on SDLP in terms of respective BAC equivalencies. The change in SDLP at a BAC of 0.5 mg/ml (i.e. 2.4 cm) has been used as a criterion level to quantify drug effects. Any drug induced changes in SDLP that exceed this criterion value are defined as clinically relevant impairing drug effect in the present study.

The car-following test (Karel A. Brookhuis, de Waard, & Mulder, 1994; Ramaekers, Muntjewerff, & O'Hanlon, 1995) consists of two cars driving in tandem on a secondary road. The leading vehicle is operated by a study staff member, the following vehicle is operated by the subject who is accompanied by a driving instructor. The test begins with the two vehicles traveling in tandem at speeds of 70 km/h on a secondary highway. Subjects attempt to drive 15-30 m behind the preceding vehicle and to maintain that headway as it executes a series of deceleration manoeuvres. During the test, the speed of the leading car is automatically controlled by a modified cruise-control system. At the beginning it is set to maintain a constant speed of 70 km/h, and by activating a microprocessor, the investigator can start sinusoidal speed changes reaching an amplitude of -10% and returning to the starting level within 50 sec. The maneuver is repeated 6-10 times. Speed signals collected during speed maneuvers enter a power spectral analysis for yielding phase-delay between the vehicle's velocities at the maneuver cycle frequency (0.02) Hz. Phase delay converted to a measure of Time to Speed Adaptation (TSA, in ms) is the primary measure. Gain and coherence are secondary control measures. Gain is the amplification factor between both speed signals collected from the leading and following vehicle and indicates the magnitude of overshoot in reaction. Coherence is a measure to control for correspondence between both speed signals. Test duration is 25 minutes.

Critical tracking task

The critical tracking task measures the participant's ability to control a displayed error signal in a 1st-order compensatory tracking task. Error appears as horizontal deviation of the cursor from midpoint on a horizontal, linear scale. Compensatory joy-stick movements null the error by returning the cursor to the midpoint. The frequency of cursor deviations, and therefore its velocity, increases as a stochastic, linear function of time. The participant is required to make compensatory movements with a progressively higher frequency until the participant loses control. The frequency at which control loss occurs is λ_c (the critical frequency). The reciprocal of this frequency is theoretically the perceptual/motor delay lag for humans operating in a closed-loop system. The participant performs this test in five trials and the mean λ_c is recorded as the final score (Jex, McDonnell, & Phatak, 1966).

Karolinska sleepiness scale

The Karolinska sleepiness scale is a subjective rating scale with scores that range from 1 "extremely alert" to 9 "very sleepy, great effort to keep alert, fighting sleep" (Åkerstedt & Gillberg, 1990). Participant are instructed to report their experienced sleepiness during the preceding 10 minutes. Reyner (1998) modified the original scale by adding verbal descriptions to intermediate

steps, which do not have any descriptions in the original version.

Pharmacokinetic assessment

Blood samples (10 mL) and oral fluid (1-2 mL) samples were collected throughout a testing day/night i.e. at 1.5 and 11 hours post-drug. Blood sample was centrifuged immediately and the resulting serum was frozen at -20°C until analyses for pharmacokinetic assessments. MDMA concentrations and its main metabolite MDA were determined in the corresponding serum samples using solid phase extraction and gas chromatography with mass spectrometric detection with a limit of quantification of 16.8 ng/mL. Oral fluid was collected with the Orasure Intercept® device for a quantitative analysis of MDMA concentrations by GC-MS.

Statistical analyses

All statistical analyses were conducted by means of SPSS 16.0 for Mac. Statistical analyses consisted of 2 steps: 1) Assessment for overall treatment effects by means of superiority testing; 2) Equivalence or non-inferiority testing of drug effects based on difference scores from placebo (within group) relative to the pre-established alcohol criterion, and 3) determination of concentration effect relations. Steps 2 and 3 were only conducted in case of treatment effects, and only for the primary measures of driving performance.

During step one all data entered the general linear model (GLM) repeated measures ANOVA procedures with MDMA (4 levels) and hours of sleep loss (2 levels for driving tests, 3 levels for cognitive test and 8 levels for subjective test) as main within subject factors. If the sphericity assumption was violated or not applicable, the Greenhouse-Geisser correction was used. In case of an overall effect of MDMA, separate drug-placebo contrast analyses were conducted for each MDMA dose.

Step two assessed whether a pre-established alcohol criterion falls within the 95% confidence interval (CI) of the drug effect. If yes, than the drug effect was considered to be equivalent or bigger than a BAC of 0.5 mg/mL, and thus relevant for traffic safety. If the 95% CI was lower than the alcohol criterion value, than a drug effect was considered of no clinical relevance.

In step 3, concentration-effect relations were determined according to the following procedure. Data collected during different doses of a drug were converted to change scores from placebo for analyses of the association between drug concentration and performance. A linear regression analysis was conducted to establish linear relationships between changes (from placebo) in task performance during drug treatment and log-transformed drug concentrations in serum. The total number of data points included in these equations was defined by the number of subjects x maximal number test repetitions x the number of drug doses. Individual drug concentrations in serum prior to performance assessments in each of the drug dose conditions were divided over three mutually exclusive categories covering the full range of drug concentrations. The concentration ranges in serum were 0-50, 50-100 and >100 ng/mL during evening sessions and 0-25, 25-50 and >50 ng/mL during morning sessions. The concentration ranges in oral fluid were 0-250, 250-1000 and >1000 ng/mL during evening sessions and 0-100, 100-500 and >500 ng/mL during morning sessions

Corresponding change scores of task performance were then classified either as showing "impairment" or "no impairment" for all individual cases within each of these categories. Impairment was defined as a positive change score from placebo. Binomial tests were applied to measure whether the proportion of observations showing impairment or no impairment significantly differed from the hypothesized proportion. It was hypothesized that in case of no effect of a drug on task performance the proportion of observations showing impairment or no impairment would be equal, i.e. 50%.

Results

Dropouts and missing data

Two participants dropped out. One was positive for THC in urine. The other indicated that the sleep loss interfered too much with his daily life. Both drop outs were replaced. Data was missing in the car following test on some occasions. These missing values were replaced by the overall group mean for the respective treatments .

Table 1: Mean (SE) of the driving and psychomotor tests and subjective measure for the treatment conditions and measuring times. Significance indicated by p-value.

						ANOVA		
Test	Test repetition	Placebo	25 mg MDMA	50 mg MDMA	100 mg MDMA	Sleep deprivation	MDMA	MDMA x sleep deprivation
Road tracking								
SDLP (mm)	1	18.2 (0.7)	18.4 (0.8)	18.1 (1.0)	18.0 (0.6)	0.000	-	-
	2	22.7 (0.7)	22.9 (0.6)	21.7 (0.9)	22.3 (1.1)			
SD Speed (km/h)	1	1.70 (0.13)	1.74 (0.14)	1.88 (0.17)	2.14 (0.14)	0.000	-	0.029
	2	2.65 (0.21)	2.62 (0.19)	2.66 (0.18)	2.50 (0.18)			
Critical tracking task								
λ_c (rad/s)	1	3.77 (0.13)	3.83 (0.16)	3.70 (0.15)	3.78 (0.19)	0.000	-	-
	2	3.63 (0.16)	3.56 (0.18)	3.73 (0.16)	3.77 (0.16)			
	3	3.09 (0.19)	2.87 (0.22)	3.09 (0.19)	3.36 (0.17)			
Karolinska sleepiness scale								
score	1	2.94 (0.21)	2.94 (0.27)	2.88 (0.36)	2.94 (0.25)	0.000	-	-
	2	3.13 (0.32)	2.69 (0.25)	2.19 (0.21)	1.81 (0.25)			
	3	3.62 (0.32)	3.69 (0.29)	3.62 (0.38)	3.62 (0.41)			
	4	4.44 (0.35)	4.62 (0.38)	4.25 (0.41)	5.13 (0.40)			
	5	5.31 (0.40)	5.81 (0.45)	5.44 (0.35)	6.12 (0.42)			
	6	5.88 (0.39)	6.50 (0.45)	6.19 (0.38)	5.81 (0.44)			
	7	7.38 (0.30)	7.69 (0.37)	7.50 (0.32)	7.56 (0.33)			
	8	7.38 (0.44)	7.88 (0.36)	7.62 (0.40)	7.62 (0.42)			

Driving tests prematurely terminated

In total 26 (20.3%) highway driving tests were prematurely terminated of which 23 (88.5%) in the morning and 3 (11.5%) in the evening. In 19 of the 26 cases (73.1%) the test was terminated by the driving instructor because the subject was too sleepy to perform the test adequately and 7 (26.9%) by the subject because they felt unable to perform the test. Terminations occurred during placebo (6x), MDMA 25 mg (5x), MDMA 50 mg (8x) and MDMA 100 mg (7x).

The car following test was prematurely terminated in 2 cases (1.6%), once in the placebo condition and once in the 25 mg MDMA condition. Both tests were terminated in the morning and by the instructor, because of sleepiness of the subject. In case of a premature stop, available data till time of test termination were used for statistical analysis.

Driving tests

Mean (SE) performances on the primary (SDLP and Time to speed adaptation) and secondary measures of the highway driving and car-following test are shown in Table 1.

MDMA did not affect any of the driving measures. Sleep loss significantly affected SDLP ($F_{1,15}$=41.341, p=0.000) but none of the other measures. SDLP was markedly higher in the morning as compared to the evening. The interaction between MDMA and sleep loss only reached significance for the secondary driving parameter SD Speed ($F_{3,45}$=3.297, p=0.029). SD speed was higher in the morning as compared to the evening, particularly after treatment with MDMA 100 mg (p=0.022). In general, variations in mean SD Speed were very small and ranged between 1-2.5 km/h.

Equivalence testing demonstrated that increments in SDLP in the morning in all treatment conditions were equivalent to a BAC of 0.8 mg/mL when compared to placebo performance in the evening. The upper limits of the 95% CI of changes induced by sleep loss clearly exceeded the pre-established inferiority margin of 2.4 cm. Mean change SDLP and 95% CI in every treatment condition are shown in Figure 2.

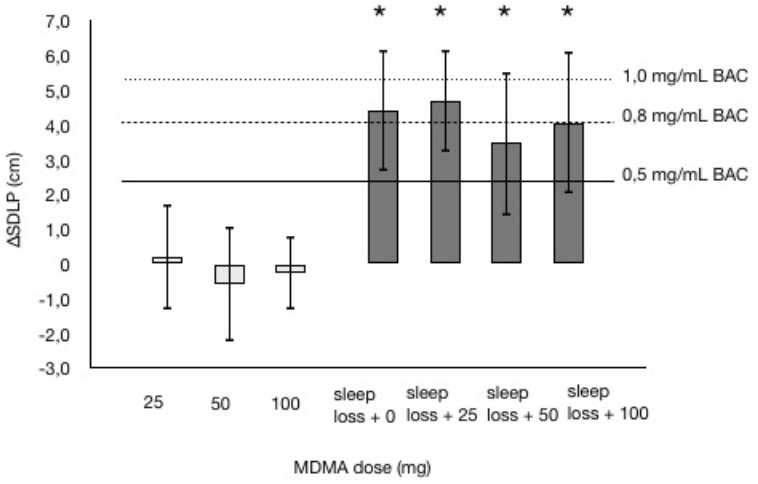

Fig. 2 Mean (95% CI) SDLP difference from placebo after single doses of MDMA during the road tracking test in the evening and in the morning after a night of sleep loss. (* = non-inferiority not shown, upper bound of the 95% CI is above the non-inferiority margin of 2.4 cm)

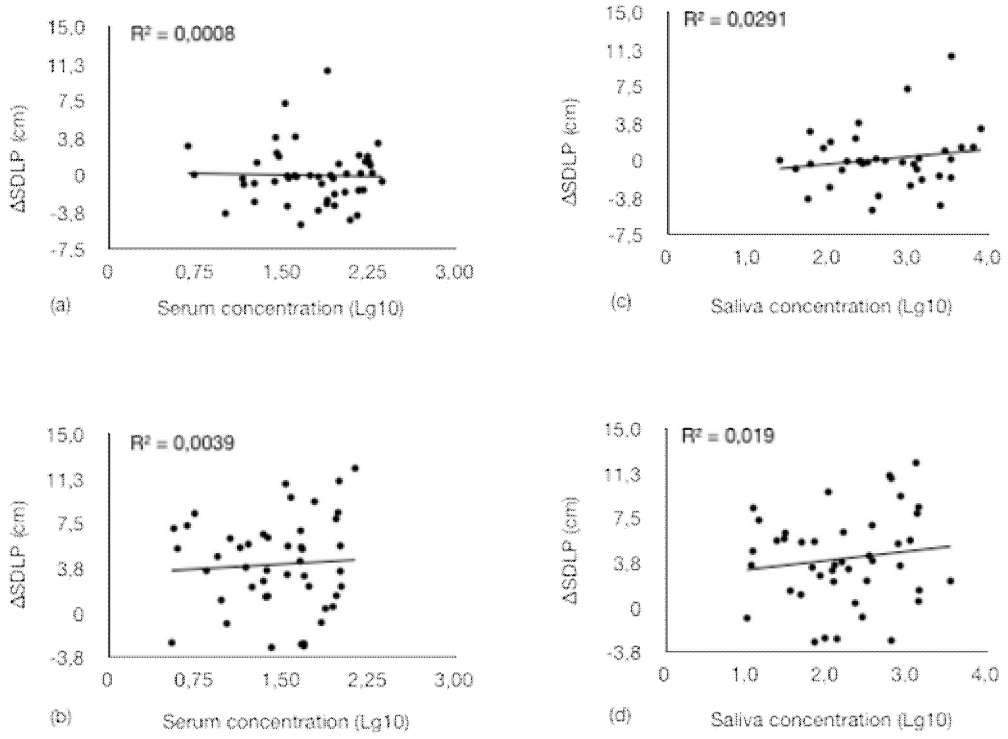

Fig. 3 Correlations between change SDLP and log converted MDMA concentrations in serum (left panels, a-b) and saliva (right panels, c-d) during driving tests in the evening (upper panels, a and c) and in the morning (lower panels, b and d)

Regression analysis of MDMA levels in serum and SDLP change scores showed a general lack of correlation between the measures. Scatter plots showing the linear relationship between MDMA levels in serum and oral fluid and changes in SDLP are shown in Figure 3.

Binomial tests showed a significant increase in the proportion of observations showing impairment in the highway driving test when conducted in the morning for serum MDMA concentrations between 0-25 ng/mL and >50 ng/mL (p<.05). In oral fluid all concentration ranges were associated with impairment (p<.05). Impairments were only apparent when compared to placebo SDLP during evening sessions. Distributions of observations showing 'impairment' and 'no impairment' in the highway driving test as a function of MDMA in serum and oral fluid are shown in Figure 4.

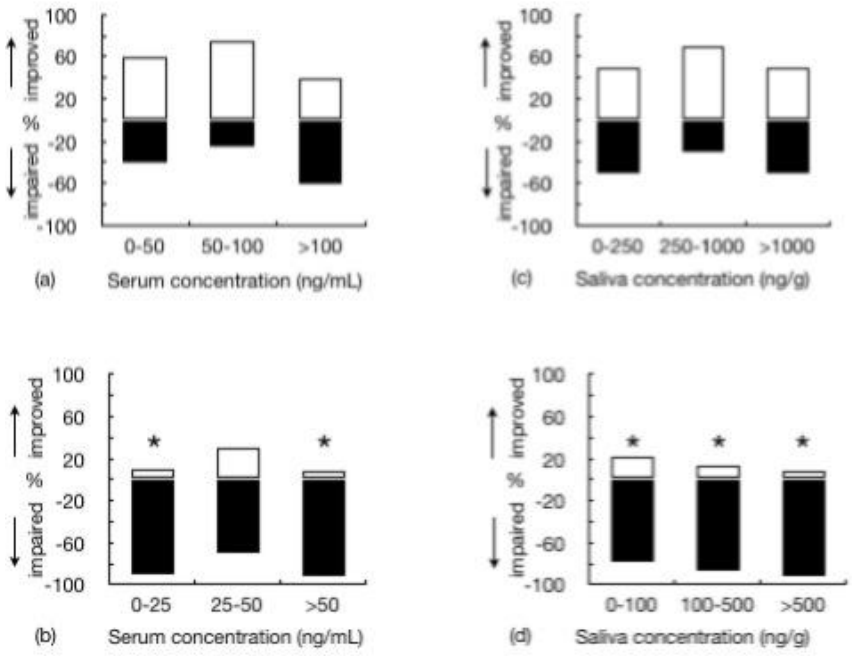

Fig. 4 Percentage of observations showing MDMA induced impairment or MDMA induced improvement of SDLP in the road tracking task, as a function of MDMA concentrations in serum (left panels, a-b) and saliva (right panels, c-d) during driving tests in the evening (upper panels, a and c) and in the morning after a night of sleep loss (lower panels, b and d). * $p<0.05$.

Critical tracking task

Mean λ_c (critical frequency) of the critical tracking task was significantly affected by sleep deprivation ($F_{2,30}$=34.516, p=0.000). Mean critical frequency generally decreased during the night, indicating diminished psychomotor control. Critical tracking was not affected by MDMA or MDMA x sleep loss.

Subjective measures

The Karolinska sleepiness scale showed a significant effect of sleep loss ($F_{2.972,44.576}$=108.717, p=0.000). The Karolinska sleepiness scale also demonstrated a significant dose-related MDMA effect at T_{max} ($F_{3,45}$=6.006, p=0.002). Contrasts indicated that the 100 mg as well as 50 mg MDMA conditions differed significantly from placebo (p=0.009 and p=0.023 respectively).

Pharmacokinetic assessment

PK analysis in serum revealed mean (SE) MDMA concentrations of 25.8 (3.3), 63.9 (6.4) and 157.2 (9.5) ng/mL at 1.5 hours after administration of a 25, 50 and 100 mg dose respectively. At 11.5 hours post drug mean MDMA concentrations were 14.2 (2.7), 34.0 (3.9) and 84.3 (6.7) ng/mL respectively. MDA concentrations were 3.5 (0.1), 3.9 (0.4) and 5.8 (0.2) ng/mL 1.5 hours post drug and 2.9 (0.5), 5.8 (0.2) and 9.7 (0.6) ng/mL after 11 hours for 25, 50 and 100 mg MDMA respectively.

Mean (SE) MDMA concentrations in oral fluid were 208.2 (72.4), 833.0 (270.9) and 3417.8

(694.2) ng/g and MDA concentrations were 3.2 (1.5), 13.9 (4.2) and 56.4 (13.5) for 25, 50 and 100 mg MDMA respectively at 1.5 hour post drug. The concentrations at 11 hours after drug intake were respectively 57.0 (16.3), 292.6 (79.0) and 925.3 (224.7) for MDMA and 3.6 (1.4), 18.9 (4.4) and 56.8 (12.8) for MDA.

Discussion

The present study demonstrated that sleep deprivation produced severe impairment in actual driving performance as expressed by a significant rise in SDLP and a large number prematurely terminated driving tests during early morning sessions. In general, MDMA did not affect actual driving performance and did not interact with the effects of sleep deprivation.

On average, SDLP increased with 4.2 cm in the morning after sleep deprivation, relative to SDLP before sleep deprivation. This increment is about 1.5-2 times greater than found in two recent driving under the influence of alcohol studies with blood alcohol concentrations between 0.29-0.5 mg/mL (Kuypers, et al., 2006; Ramaekers, Robbe, & O'Hanlon, 2000). From a previous alcohol study that was conducted in order to calibrate SDLP for the dose-related effects of alcohol (Louwerens, et al., 1987) it can be concluded that a mean increase in SDLP of 4.2 cm is equivalent to a blood alcohol concentration of approximately 0.8 mg/mL. Equivalence testing even demonstrated that the upper limit of the 95% CI associated with the mean change in SDLP after sleep deprivation widely exceeded the criterion level of 1.0 mg/mL BAC. Together this indicates that sleep deprivation caused severe driving impairment comparable to driving under the influence of high to very high BAC.

These findings were corroborated by results from the critical tracking task. Critical tracking performance significantly decreased over the night, as a function of hours of sleep loss. Similar findings have previously been reported by Dawson and Reid (1997). They measured tracking performance as a function of hours of sleep loss and BAC intoxication. According to their model, tracking performance of subjects after 17-24 hours of wakefulness is equivalent to that observed at BAC between 0.5-1.0 mg/mL. This again indicates that a night of sleep deprivation causes serious impairment of driving skills. In terms of BAC equivalents, these impairments exceeded those observed at legal BAC limits of 0.5 mg/mL and 0.8 mg/mL that are currently in place for driving under influence of alcohol in most of Europe and the US.

The car following test was the only driving measure that did not show an effect of sleep loss. This diverging effect between the two driving tests, i.e., road tracking and car following, could be explained by a study of Harrison and Horne (2000). They conducted a study in which they showed that dull and monotonous tasks are more sensitive to sleep deprivation than more complex, rule based tasks, because the latter ones generate more interest and effort to compensate for the effects of sleep deprivation. The highway driving test is monotone and subjects reported that it was more boring than the car-following test, which is more complex. This is corroborated by the fact that 20% of road-tracking and 2% of car-following tests were prematurely terminated of which the majority in the morning, i.e., after sleep deprivation. Therefore, this divergence between the two driving tests are in line with the results of Harrison and Horne (2000), in that the more monotone road-tracking test is

more sensitive to sleep deprivation than the car-following test.

In general, MDMA did not produce any significant effects on driving parameters independent of dose or concentration. Stimulating effects of MDMA on actual driving parameters or psychomotor measures that have been demonstrated before (e.g. Dumont, et al., 2008; Kuypers, et al., 2006; Kuypers, Wingen, Samyn, Limbert, & Ramaekers, 2007; Lamers, et al., 2003; Ramaekers, et al., 2006). could not be clearly replicated in the present study. This discrepancy could be due to the fact that the present study also included lower doses of MDMA, i.e. 25 and 50 mg that may be less likely to produce stimulatory effects than MDMA between 75 and 125 mg that have been tested in the studies mentioned above. However, even in the present study some measures tended to show stimulatory effects of MDMA. For example, SD speed was significantly affected by an interaction between sleep loss and MDMA. Overall, SD speed increased after a night of sleep loss, but this increase was somewhat less after 100 mg MDMA. Also, binomial tests of SDLP change scores in the evening demonstrated that the majority of observations indicated a reduction in SDLP, particularly at lower MDMA concentrations. However, this effect failed to reach statistical significance.

It is also apparent from the present study that the stimulant effects of MDMA, if any, could not compensate for the impairing effect of sleep loss on driving performance. None of the primary driving measures demonstrated any significant MDMA x sleep loss interaction. The effects of sleep deprivation on driving were highly prominent during MDMA treatments and did not change as a function of dose and concentration. These findings are in line with those in a previous study that assessed the stimulant effects of MDMA on SDLP during alcohol intoxication (Kuypers, et al., 2006). That study demonstrated that stimulatory effects of MDMA effects did not compensate for driving impairment caused by alcohol. Together, these data indicate that the effects of MDMA on psychomotor functions are neutral or mildly stimulating, but that these effects are not sufficient to overcome impairments caused by other factors such as sleep deprivation or alcohol intoxication.

The latter notion is of crucial importance when evaluating driving under the influence (DUI) offenders involving the use of MDMA. Subjects in the present study were significantly impaired during MDMA treatments when deprived of sleep for one night. However, the prime factor causing these impairments was sleep deprivation rather than the use of MDMA itself. When applied in courts, one could rightfully pose the argument that such drivers should not be prosecuted for DUI since MDMA did not contribute to the impairments of the driver. There are a number of counter arguments that should be taken into consideration when evaluating MDMA cases in traffic. First, it has been demonstrated in previous studies that single doses of MDMA increase subjective feelings of arousal and mood (Bosker, Kuypers, Conen, & Ramaekers, 2010; Kuypers, Wingen, & Ramaekers, 2008) and the current study showed a decrease in sleepiness from baseline at T_{max} in the MDMA conditions. Such feelings may affect the subjective judgment of MDMA users on whether or not it is safe to drive home after spending a night at a rave party. During MDMA intoxication, they may not be able to subjectively experience the debilitating effects of sleep loss to the same degree as drug free drivers, because they feel energetic. As a consequence, they may decide to drive because they feel alert, thereby neglecting the impairing effects of other impairing factors such as sleep deprivation or even alcohol use. Secondly, several studies have also demonstrated that cognitive functions such as working memory,

spatial memory and timing of moving objects (Kuypers & Ramaekers, 2005; Lamers, et al., 2003; Ramaekers, Kuypers, Wingen, Heinecke, & Formisano, 2009) are impaired during MDMA intoxication. Such cognitive impairments may also affect a person's ability to reflect on their fitness to drive as well driving ability in general.

It is concluded from this study that drivers who are under the influence of MDMA and are sleep deprived are unfit to drive. The impairing effects of sleep deprivation during MDMA intoxication occurred independent of MDMA dose and concentration.

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Chapter 2

Dose related effects of MDMA on psychomotor function and mood before, during and after a night of sleep loss

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Abstract

3,4-methylenedioxymethamphetamine (MDMA) is known to improve psychomotor function and mood when measured during daytime. However, MDMA users tend to take this drug at dance parties while staying awake for prolonged periods of time. This study was designed to assess dose related, residual effects of MDMA on psychomotor function and mood after a night without sleep. Sixteen recreational MDMA users received single doses of 25, 50 and 100 mg MDMA in a randomized, double-blind, placebo-controlled cross-over study. Results showed that sleep loss significantly impaired psychomotor function. MDMA generally did not affect performance, but did improve rapid information processing at the highest dose in the morning after administration. In the evening, MDMA also increased subjective ratings of positive mood at every dose, and subjective arousal at the highest dose. These subjective effects were no longer present after a night of sleep loss. It is concluded that sleep deprivation impairs psychomotor function and that stimulatory effects of MDMA are not sufficient to compensate for this impairment.

Keywords

MDMA; ecstasy; psychomotor; cognition; mood; sleep deprivation

Introduction

3,4-methylenedioxymethamphetamine (MDMA) is the main psychoactive substance of the party drug ecstasy. Ecstasy is a popular drug: about 0.4% of the Dutch general population (40.000 people) has been described as current users in 2005 (National Drug Monitor 2008) and 9.5 million European adults indicated to have ever used ecstasy, which is 2.8% of the general population (European Monitoring Centre for Drugs and Drug Addiction 2008). Ecstasy has been described as an entactogen, because of its subjective effect of feeling close to and connected with others. Other drug effects are enhanced visual and auditory perception: colors appear brighter and sound more intense. This is why people generally take ecstasy at dance parties where rhythmic music is being played in combination with colorful lights. Also, the stimulant effects enable people to dance for longer periods of time.

An extensive body of literature showed that ecstasy may produce detrimental effects on cognitive functions. For example, Morgan et al. (2006) found that ecstasy users were impaired on several measures of impulsivity compared to groups of polydrug users and drug-naïve controls. Two meta-analytic reviews demonstrated that ecstasy users performed significantly worse on cognitive tasks measuring learning/memory, attention, executive function and psychomotor performance compared to non-using control subjects, although effect sizes were medium to small (Kalechstein et al. 2007; Zakzanis et al. 2007). However, a large number of studies have also failed to exclusively link MDMA use to long term cognitive impairments. Roiser et al. (2007) reported that performance of current ecstasy users, former-ecstasy users, polydrug users and drug-naïve controls on tests measuring memory, executive function and impulsivity did not differ between groups. Clark et al. (2009) reported no performance difference between ecstasy users and controls on a task that measured impulsivity. Hoshi et al. (2007) and Hanson et al. (2008) concluded that the subtle impairments of memory and impulse control in MDMA users were due to polydrug use rather than ecstasy use alone. Likewise, Lamers et al. (2006) and Jager et al. (2008) concluded that impairments in cognitive and psychomotor function of MDMA users may be attributed to other drugs than MDMA, such as cannabis and amphetamine.

These conflicting findings of MDMA impairing performance in some, but not all studies have been attributed to methodological problems inherent in cross-sectional designs in abstinent drug users. For example, the fact that most ecstasy users also take other drugs is an important confounder. Thus, the results of the aforementioned studies could be due to the use of other drugs than MDMA. Another related problem is causality: the nature of cross-sectional designs does not allow inferences on whether the effects are caused by ecstasy or another factor, e.g. premorbid factors that might make people more vulnerable to use illicit drugs (Morgan, 2000).

One way to overcome these problems is to study acute effects of MDMA on cognitive performance in placebo controlled experimental designs. In such designs, changes in performance can be exclusively linked to the experimental drug. Experimental studies have previously shown that single doses of MDMA impair memory function (Dumont et al. 2008; Kuypers and Ramaekers 2005; 2007; Ramaekers et al. 2009) and attention (Dumont et al. 2008) and improve performance on tasks measuring impulse control (Ramaekers and Kuypers 2006) and psychomotor function (Lamers et al.

2003) when given during the day. The magnitude of impairment on memory and attention increased when MDMA was given during the night to subjects who stayed awake throughout the night. Moreover, stimulating effects of nocturnal doses of MDMA on psychomotor function, vigilance and sleepiness were very mild or no longer present after a night of sleep loss (Kuypers et al. 2008; Kuypers et al. 2007). In the latter studies repeated doses of 75 mg MDMA and 50 mg MDMA were administered to subjects, which resulted in psychomotor impairment additive to impairment produced by sleep loss. However MDMA also produced some mild stimulatory effects, it reduced subjective feelings of sleepiness and mildly increased vigilance performance in the morning.

At present it is not clear at which MDMA dose or serum concentration residual impairments start to emerge. Residual effects are limited to the time window in which the drug is still present in blood, but at low concentrations. The objectives of the present study were to establish the residual effects of MDMA on cognitive performance early in the morning as a function of MDMA dose. Subjects were treated on 4 separate occasions with evening doses of 0, 25, 50 and 100 mg. Because identical MDMA doses can lead to differing MDMA blood concentrations, it is important to study MDMA effects or it's interaction with sleep deprivation as a function of MDMA concentrations rather than dose. We decided to include a broad range of (low) nocturnal doses in order to be able to measure residual MDMA effects at very low concentrations in the morning. This wide range of residual blood concentrations will help determine the MDMA concentration threshold at which residual performance effects will still be present. Performance was tested in the evening, in the middle of the night and in the morning after a night of sleep loss.

Subjects and Methods

Subjects

Eight males and eight females participated in this study. Their mean (SE) age was 22.0 (0.41) years and their mean (SE) lifetime MDMA use was 27.0 (8.4) times. Subjects were recruited by advertisements at Maastricht University and were paid upon completion of the study. Before enrollment all subjects were screened by means of a telephone interview to determine whether they qualified for the study. The inclusion criteria were: experience with MDMA, i.e. at least 1 time in the last year; free from psychotropic medication; good physical health as determined by a medical examination; absence of any major medical, endocrine and neurological condition; normal weight, i.e. BMI between 18 and 28 and written informed consent. The exclusion criteria were history of drug abuse or addiction as assessed by means of a medical questionnaire by the physician at the medical checkup; pregnancy or lactation; cardiovascular abnormalities on electrocardiogram; excessive drinking, i.e. more than 20 alcoholic consumptions a week; hypertension, i.e. systolic blood pressure over 170 mmHg or diastolic blood pressure over 100 mmHg; history of or current psychiatric disorder. If subjects met the inclusion criteria, they received a medical history and a drug questionnaire to get a more precise view on their health and drug use. Finally, subjects underwent a medical examination and took part in a training session to get familiar with the driving and psychomotor tests.

This study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008). Approval for the study

was obtained from the Medical Ethics committee of the Academic Hospital of Maastricht and Maastricht University. A permit for obtaining, storing and administering MDMA was obtained from the Dutch drug enforcement administration.

Study design

The study was conducted according to a double blind, placebo-controlled, randomized, 4-way cross-over design. Treatments consisted of single doses of placebo, 25, 50 and 100 mg MDMA. Treatment orders were balanced over subjects and treatment periods. Placebo and MDMA were administered orally in identically appearing formulations. MDMA was dissolved in 25 mL bitter orange peel syrup and placebo consisted of only the bitter orange peel syrup. The syrup was mixed with 200 mL juice before it was given to the subjects. The wash-out period between treatments was at least one week.

Procedure

Subjects were asked to refrain from any drugs one week before the medical examination until two weeks after study completion. Subjects were not allowed to drink alcohol and caffeine or smoke tobacco during a 24 hour period prior to testing. Subjects were always tested for alcohol and drugs, i.e. THC, opiates, amphetamine/ecstasy, benzodiazepines, cocaine and methamphetamine/ecstasy, respectively in breath and urine upon arrival (4:30 pm) at the laboratory on test days. At 5:00 pm subjects received a light, standard dinner and at 5:15 pm MDMA or placebo was administered. Performance was assessed in the evening, the middle of the night and in the morning after a night of sleep loss. The timeline for performance testing, questionnaires and blood draws are displayed in figure 1. Subjects watched television or movies, played games, read a book or magazine or used the internet in between testing episodes during a test night. An additional blood sample was drawn one week after each testing day to monitor renal and liver function. A test day ended at 9:00 am the next morning at which time subjects were driven home.

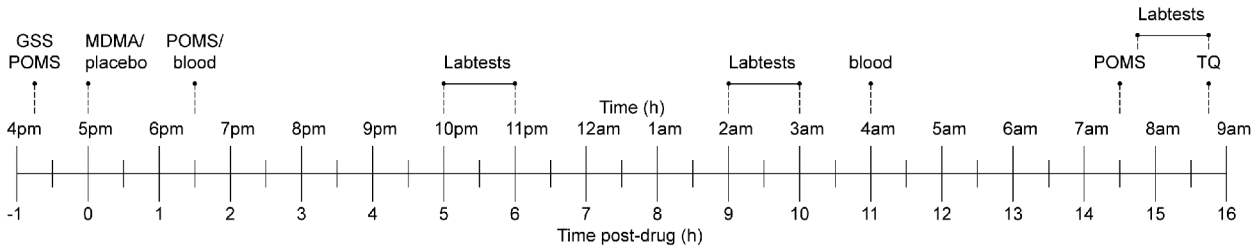

Fig. 1 Timeline for blood samples, questionnaires, and laboratory tests relative to drug administration. *GSS* Groninger sleep scale, *POMS* profile of mood states, *TQ* treatment questionnaire

Psychomotor assessment

The stop signal task required subjects to make quick key responses to visually presented go signals and to inhibit their response when a visual stop signal was suddenly presented. The go signals were four 1.5 cm letters (A, B, C, and D) presented one at a time in the center of a computer screen.

Subjects were required to respond to each letter as quickly as possible by pressing one of two response buttons. One button was pressed to indicate that A or C appeared and the other to indicate B or D. Letters were displayed for 500 ms and the computer screen was blank for 1.5 s inter-stimulus interval. This provided a period of 2 s in which the subject could respond to a letter. A single test consisted of 176 trials in which each of the 4 letter-stimuli was presented equally often. A stop-signal occurred in 48 trials during a test. The stop-signal consisted of a visual cue, i.e. *, that appeared in one of the four corners of the screen. Stop signals were presented 12 times at each of the four delays after the onset of a letter: 50, 150, 250 and 350 ms. Trials always began with a 500 ms preparation interval in which a fixation point appeared in the center of the screen. The task lasted about 10 minutes. Dependent variables were the proportion of commission errors on stop trials and the reaction times (RT) on go as well as stop trials, i.e. stop RT. Stop RT represents the estimated mean time required to inhibit a response and is a measure of impulsivity (Fillmore et al. 2002; Ramaekers and Kuypers 2006).

The rapid information processing task is a self-paced interactive working memory task that assessed subjects' information processing capacity. A pseudo-random sequence of 250 digits (1-8) was presented in the center of the screen. The digits were presented one at a time for 67 ms with an initial inter-stimulus-interval (ISI) of 600 ms. Subjects had to press a button when they saw the third digit of a 3-digit sequence (triad) that was comprised of even (e.g. 6, 2, 4) or odd (e.g. 5, 1, 7) digits. The entire 250-digit sequence contained 11 even-digit triads and 10 odd-digit triads. Each correct response speeded up the presentation rate by decreasing the ISI with 33 ms and each false response slowed it down with 33 ms. Task duration was 5 minutes; the 250-digit sequence was presented in a repeated loop. The initial presentation rate of the test was 90 digits per minute and the dependent variable was the average number of digits per minute presented in the test (Fillmore et al. 2005).

The divided attention task assessed the ability to divide attention between two tasks performed simultaneously. The primary task required the use of a joystick to continuously null the horizontal movement of a cursor from the center of a display. The cursor traveled in both directions with irregular velocity, but on average 50% of what the subject could just control. The dependent measures of this subtask were control losses and tracking error, which was measured by the absolute distance (mm) between the cursor's position and the center. The secondary task involved monitoring 24 single-digit numbers (0-9) that were arranged in the four corners of the display. The numbers changed asynchronously every 5 seconds. The requirement was to react as rapidly as possible by lifting the foot from a pedal every time a target, i.e. the number 2, appeared. Average reaction time to targets was recorded as the dependent measure (Moskowitz 1973).

The psychomotor vigilance task assessed the reaction time in response to a visual stimulus. The visual stimulus was a counter in the center of a computer screen that ran in one minute from 0 to 60 with a fixed inter-stimulus interval of 1 ms. The counter started at random intervals between 2 and 10 seconds and the subject had to react to the onset of the counter as quickly as possible by pressing a response button. Duration of the task was 10 minutes. This task has often been used to assess the impact of sleep loss on performance (Loh et al. 2004).

Subjective measures

The Groninger sleep scale assessed sleep quality and quantity (hours of sleep). It consisted of fifteen dichotomous questions about sleep complaints and an open question concerning the duration of sleep (Mulder-Hajonides van der Meulen et al. 1980). The quality score ranged from 0 (best quality of sleep) to 15 (worst quality of sleep).

The profile of mood states is a self-assessment mood questionnaire with 72 five-point Likert scale items, representing eight mood states, i.e. anxiety, depression, anger, vigor, fatigue, confusion, friendliness and elation. Three composite scales were derived, i.e. arousal ((anxiety + vigor) – (fatigue + confusion)), positive mood (elation – depression) and a total score ((anxiety + depression + anger + fatigue) – vigor), which is a measure of malaise. The subject had to indicate to what extent these items were representing his/her mood (de Wit et al. 2002).

Pharmacokinetic assessment

A blood sample (8 mL) was collected two times throughout a testing day/night i.e. at 1.5 and 11 hours post-drug. MDMA and MDA concentrations were determined afterwards in serum. The blood sample was centrifuged immediately and the resulting serum was frozen at -20°C until analyses for pharmacokinetic assessments.

Statistical analyses

All statistical analyses were conducted by means of SPSS 16.0 for Mac. All data were entered in the general linear model (GLM) repeated measures ANOVA procedures with MDMA (4 levels) and hours of Sleep loss (3 levels) as main within subject factors. If the sphericity assumption was violated, the Greenhouse-Geisser correction was used. In case of an overall effect of MDMA or an interaction effect between MDMA and Sleep loss, separate drug-placebo contrast analyses were conducted for each MDMA dose. In case of the profile of mood states, difference scores between post-drug and the pre-drug score entered the statistical analysis.

Results

Psychomotor assessment

Mean (SE) performance scores obtained from psychomotor tests are displayed in Table 1 along with p-values associated with GLM ANOVA.

MDMA did not affect performance in any of the psychomotor tasks. Sleep loss impaired performance on a range of tasks. It increased RT ($F_{2,30}$=6.415, p=0.005) and Stop RT ($F_{2,30}$=3.685, p=0.037) in the stop signal task, processing speed in the rapid information processing task ($F_{2,30}$=7.896, p=0.002), and RT in the psychomotor vigilance task ($F_{2,30}$=16.598, p=0.000). Sleep loss also increased control losses ($F_{1.345,20.171}$=14.272, p=0.001), tracking error ($F_{2,30}$=16.449, p=0.000) and RT ($F_{2,30}$35.538, p=0.000) in the divided attention task.

The interaction between MDMA and Sleep loss was significant for processing speed

($F_{6,90}$=2.616, p=0.022) in the rapid information processing task. Simple drug-placebo contrasts showed that this interaction effect was attributable to MDMA 100 mg (p=0.001). Performance during the placebo condition worsened during the night, while in the 100 mg MDMA condition subjects' performance remained stable throughout the night and slightly improved in the morning (see Figure 2).

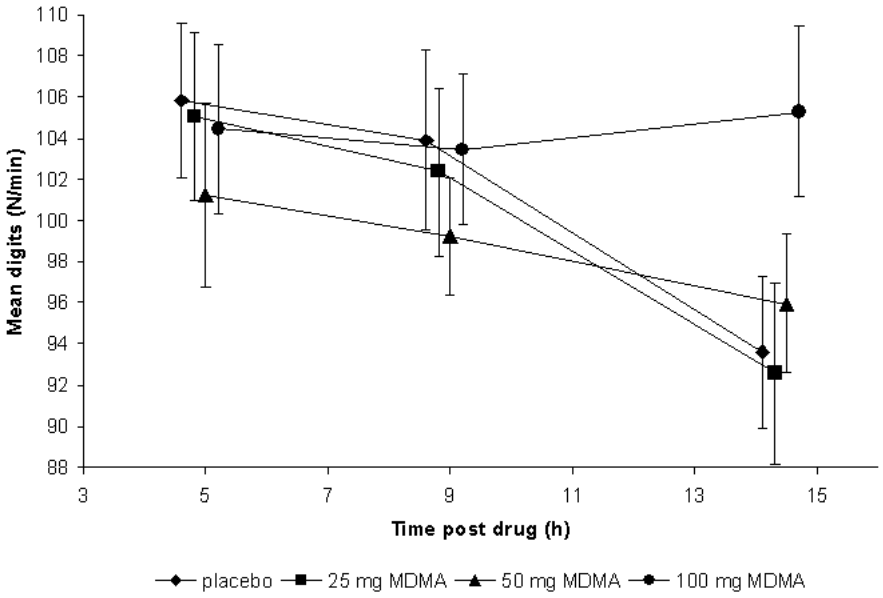

Fig. 2 Mean (SE) performance on the rapid information processing task in every treatment condition as a function of time after drug administration

Subjective measures

There were no significant differences in sleep quality and quantity between any of the MDMA conditions as measured by Groninger sleep scale. Subjects' mean (SE) quality of sleep and hours of sleep were 2.22 (0.36) and 8.5 (0.24) respectively.

ANOVA of the profile of mood states showed a significant interaction between MDMA and Sleep loss on the arousal ($F_{3,45}$=4.294, p=0.010) and positive mood scale ($F_{3,45}$=9.526, p=0.000). Simple drug-placebo contrasts indicated that MDMA 100 mg increased arousal (p=0.012) in the evening but not in the morning, as compared to placebo. Drug-placebo contrasts also showed that all MDMA doses (p<0.046), increased positive mood in the evening after drug intake but not in the morning. Mean (SE) arousal and positive mood ratings during treatment are given in Figure 3 and 4 respectively.

Table 1 Mean (SE) of the cognitive and psychomotor tests for the treatment conditions and measuring times

Test	Measure	Placebo	25 mg MDMA	50 mg MDMA	100 mg MDMA	ANOVA		
						Sleep deprivation	MDMA	MDMA × sleep deprivation
Stop signal task								
Commission errors (N)	1	4.8 (0.7)	4.5 (0.6)	5.1 (0.7)	5.3 (0.8)	–	–	–
	2	5.5 (0.8)	3.0 (0.6)	4.5 (0.7)	4.8 (0.7)			
	3	5.6 (0.7)	5.6 (0.6)	4.9 (0.6)	5.1 (0.7)			
RT go (ms)	1	543 (36)	540 (23)	528 (25)	523 (23)	0.005	–	–
	2	551 (35)	595 (30)	552 (27)	542 (28)			
	3	555 (34)	585 (29)	562 (26)	578 (30)			
Stop RT (ms)	1	276 (20)	271 (11)	292 (23)	315 (33)	0.037	–	–
	2	327 (22)	290 (11)	279 (18)	299 (22)			
	3	309 (25)	297 (16)	320 (33)	334 (23)			
Rapid information processing								
Processing speed (N/min)	1	529.0 (18.9)	525.2 (20.3)	506.0 (22.2)	522.1 (20.6)	0.002	–	0.022
	2	519.4 (20.3)	511.8 (20.3)	496.1 (14.2)	517.3 (18.2)			
	3	468.0 (18.3)	462.9 (22.1)	479.9 (17.1)	526.4 (20.6)			
Divided attention task								
Control loss (N)	1	1.9 (0.7)	1.8 (0.5	2.9 (1.3)	1.6 (0.5)	0.001	–	–
	2	12.6 (8.9)	14.4 (6.2)	5.6 (1.8)	6.1 (2.9)			
	3	48.4 (12.3)	33.4 (7.2)	47.9 (14.3)	24.7 (5.7)			
Tracking error (mm)	1	15.2 (1.1)	15.7 (1.2)	15.2 (1.0)	14.1 (1.2)	0.000	–	–
	2	15.9 (1.3)	17.3 (1.1)	16.1 (1.2)	15.5 (1.3)			
	3	19.3 (0.9)	19.7 (0.9)	18.0 (0.8)	18.5 (1.0)			
RT (ms)	1	1,822 (63)	1,853 (79)	1,885 (74)	1,920 (70)	0.000	–	–
	2	2,046 (89)	2,013 (79)	2,017 (89)	1,973 (74)			
	3	2,180 (65)	2,189 (66)	2,246 (69)	2,285 (73)			
Psychomotor vigilance task								
RT (ms)	1	289 (8)	286 (8)	295 (15)	301 (13)	0.000	–	–
	2	384 (47)	493 (108)	456 (76)	349 (26)			
	3	464 (29)	463 (45)	544 (49)	515 (38)			

Significance indicated by *p* value

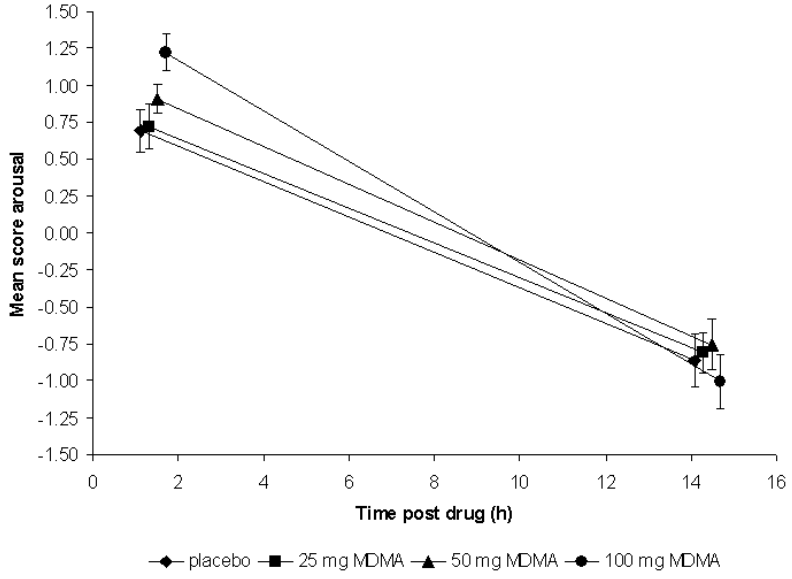

Fig. 3 Mean (SE) score on profile of mood states arousal scale in every treatment condition as a function of time after drug administration

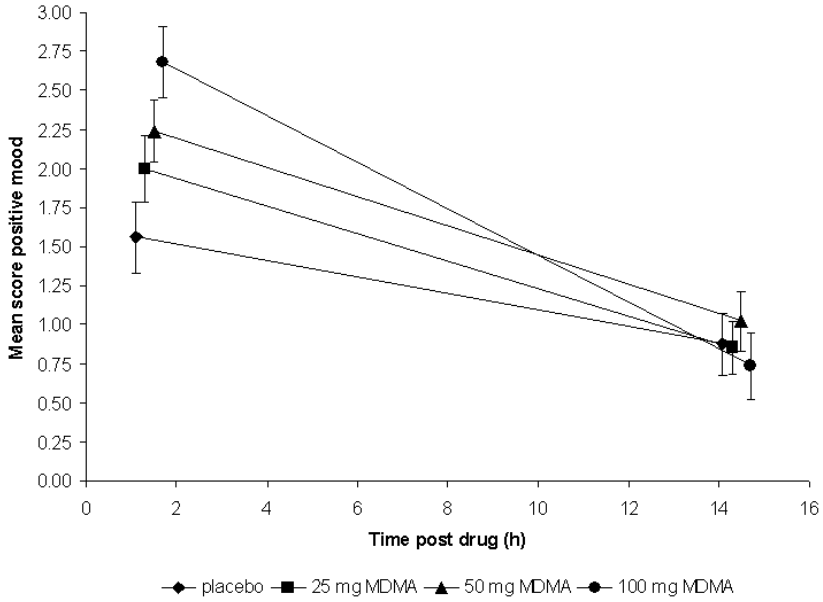

Fig. 4 Mean (SE) score on profile of mood states positive mood scale in every treatment condition as a function of time after drug administration

Pharmacokinetic assessment

PK analysis in serum revealed mean (SE) MDMA concentrations of 25.8 (3.3), 63.9 (6.4) and 157.2 (9.5) ng/mL at 1.5 hrs after administration of a 25, 50 and 100 mg dose respectively. At 11 hours post drug these concentrations were 14.2 (2.7), 34.0 (3.9) and 84.3 (6.7) ng/mL respectively. MDA concentrations were 3.5 (0.1), 3.9 (0.4) and 5.8 (0.2) ng/mL 1.5 hours post drug and 2.9 (0.5), 5.8 (0.2) and 9.7 (0.6) ng/mL after 11 hours for 25, 50 and 100 mg MDMA respectively.

Discussion

The present study showed significant impairing effects of sleep loss on various psychomotor measures. Performance deteriorated without exception on all tasks as the hours of sleep loss increased. RT in the stop-signal, divided attention and psychomotor vigilance task as well as processing speed in the rapid information processing task slowed down over time. Tracking error increased in the divided attention task, which indicates that subjects were unable to allocate sufficient cognitive resources to perform the primary tracking task at a normal, placebo level, despite a general slowing of RT in the secondary task. The number of control losses increased dramatically in the morning after sleep deprivation. Together this shows that sleep deprivation produced gross impairment of cognitive and psychomotor functions. Similar findings have previously been reported by Kuypers et al. (Kuypers et al. 2008; Kuypers et al. 2007) and Dawson and Reid (1997). The latter even demonstrated that performance decrements for each hour of wakefulness between 10 and 26 hours were equivalent to a performance decrement observed with a 0.004% rise in blood alcohol concentration. According to their model, performance of subjects in the present study after 17-24 hours of sustained wakefulness would have decreased to a level equivalent to the performance impairment observed at a blood alcohol concentration of roughly 0.05-0.10%.

This study showed no main effects of MDMA on performance, neither in the evening after drug administration nor throughout the night. This contrasts somewhat with findings of previous studies who reported neutral or even stimulating effects on psychomotor function after single doses of MDMA, particularly around T_{max} (Kuypers et al. 2007; Lamers et al. 2003; Ramaekers and Kuypers 2006; Ramaekers et al. 2006). It should be noted however that the MDMA doses/concentrations in the present study were relatively low compared to previous studies. This was particularly true for MDMA concentrations early in the morning. In the present study, the mean MDMA concentration in the 100 mg MDMA condition was 84.3 ng/mL at 11.5 hrs post dosing. This concentration was about 2.5 times lower than the concentration reported in a previous study assessing the effect of repeated doses of MDMA on psychomotor function after a night of sleep loss (Kuypers et al. 2008; Kuypers et al. 2007).

The only significant interaction between MDMA and sleep loss was caused by the highest dose of MDMA in the present study. Relative to placebo, performance in the rapid information processing task slightly improved 14-15 hrs after MDMA 100mg, after a full night of sleep loss. This interaction confirms some of the mild stimulatory characteristics that have been reported before when measuring MDMA effects at T_{max} (Kuypers et al. 2006; Ramaekers et al. 2006). In general, acute drug studies have shown that MDMA effects subside over time and normalize after about 6 hours after administration when taken during the day (Hernández-López et al, 2002; Dumont et al, 2009).

However MDMA effects on cognition may last longer or become more noticeable when taken during the night or after a night without sleep, because they may add to or even interact with the detrimental effects of sleep loss on cognition. Kuypers et al. (2007) demonstrated that nocturnal doses of MDMA significant increased vigilance and decreased sleepiness in the morning after a night of sleep loss. These results confirm the residual, mild, stimulatory effects of MDMA on psychomotor function that were measured in the present study after a night of sleep loss. These data seem to indicate that mild stimulatory effects of MDMA may compensate somewhat for the impairing effects of sleep loss on performance. However this effect of MDMA was very mild and only apparent in a single performance task. Overall, MDMA did not compensate for the detrimental effects of sleep loss on performance. This conclusion is in line with work of others who also showed that stimulant effects of MDMA are generally mild and not sufficient to counteract the impairing effects of other sources such as alcohol (Dumont et al. 2008; Kuypers et al. 2006).

The profile of mood states rating scales confirmed that subjects actively experienced an MDMA effect even after the lowest dose of 25 mg. Ratings of positive mood were significantly elevated after all MDMA doses between 1-2 hours after dosing. Likewise, feelings of arousal also increased at Tmax, albeit only after the highest dose. These positive feelings however did not last through the night. When rated in the morning mood and arousal were considerable lower as compared to the evening before, due to sleep loss, and not different from placebo. It again shows that residual concentrations of MDMA or stimulatory effects of MDMA on mood and arousal cannot compensate for decrements in subjective mood and arousal as a result of fatigue.

In summary, it can be concluded that sleep deprivation has a major impairing effect on cognitive and psychomotor performance. Low doses of MDMA generally failed to affect performance, but produced some stimulatory effect on rapid information processing at the highest dose. MDMA increased ratings of positive mood and alertness. However, the stimulatory effects of MDMA were only mild and never sufficient to overcome decrements in performance, mood or arousal due to sleep loss.

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Chapter 3 Effects of alcohol (BAC 0.5‰) and ecstasy (MDMA 100mg) on (simulated) driving performance, driving related performance and traffic safety

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Abstract

Drug use and particularly multiple drug use, including drug-alcohol combinations, among drivers constitute an important risk factor for traffic accidents. Since drug- and medicine use is proportionally increasing over the years, special efforts have to be directed towards gaining better knowledge of the various aspects of this problem and developing appropriate solutions. The objective of the EU-project DRUID (Driving under the Influence of Drugs, Alcohol and Medicines) is to give scientific support to the EU transport policy (White Paper, 2001) by establishing guidelines and measures to combat impaired driving.

To establish thresholds for the extent of driver impairment as a consequence of MDMA or combined MDMA and alcohol use, a study was carried out to assess the effects of single dose of (±) 3, 4-Methylenedioxymethamphetamine (100 mg MDMA, "ecstasy") and a single dose of alcohol (0.5 ‰), and a combination of the two, on simulated driving performance. A group of 20 relatively experienced, healthy MDMA-users were administered MDMA (0 and 100 mg) and alcohol (0 and 0.5 ‰) according to a 4-way, double-blind, placebo controlled, repeated measures, cross-over design. The data of 19 participants were compared to performance on the influence of alcohol alone as derived from a separate study. Data for this comparison were previously gathered in an alcohol calibration experiment in which a group of 17 experienced drivers were administered 0.3 ‰, 0.5 ‰ and 0.8 ‰ of alcohol according to the same design.

The effect of alcohol (alone) on weaving was very similar to the effect of alcohol in the special alcohol calibration study as well as in many on the road studies so far. The increase in SDLP at a BAC of 0.5 ‰ was about 2.5 cm, which is almost identical to all other findings. MDMA diminished that effect slightly, but a significant effect on SDLP persisted. The participants in this study were hardly, but if so, positively affected by MDMA (alone), as expected, at least with respect to weaving. In almost all other respects the expectations for what MDMA would do were not met, at least not with respect to average values. However, variance in several parameters increased when driving under influence. Specifically regarding speed, which is definitely related to accident likelihood, the variance was similarly increased when under influence of alcohol and alcohol combined with MDMA. Additionally, a subset of the participants predicted their driving in the combined condition to be worst, while afterwards they rated their performance to be not so bad. Both in the MDMA and in the combined condition the predicted ratings differed significantly from the experienced ratings. In general, it is concluded that alcohol impairs primary functions such as weaving. MDMA produced neutral or stimulatory effect when given alone, but did not fully compensate for the effects of alcohol when given in combination.

Introduction

Traffic safety is compromised whenever people participate in traffic under the influence of psychoactive substances, i.e. alcohol and prescription- and non-prescription drugs (Ramaekers, 1998). According to Christophersen and Morland (1997) the incidence of drivers who drive under the influence of psychoactive drugs in actual traffic is considerable (5-17%). Since drug- and medicine use is proportionally increasing over the years, special efforts have to be directed towards gaining better knowledge of the various aspects of this problem before developing appropriate solutions. This is the objective of the EU-project DRUID (Driving under the Influence of Drugs, Alcohol and Medicines).

DRUID aims to give scientific support to the EU transport policy, which states that by the year 2025 the number of persons killed or severely injured in road accidents should be reduced with 75%. The long term vision of the EU is to render road transport as safe as all other modes of transportation (White Paper, 2001). DRUID aims to contribute to this policy by establishing guidelines and developing measures to combat impaired driving under influence of drugs, alcohol and medicines. In the framework of DRUID, a series of experiments will be carried out in driving simulators and on the road studies, to assess the effects of alcohol, prescription and non-prescription drugs on driving performance.

Several fatal and non-fatal injurious road-accidents have been reported in which ecstasy was found in the blood of drivers, or those held responsible for the crashes (Henry et al. 1992). This is not surprising considering that ecstasy is the second most popular drug in Europe after cannabis (Report European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2009). The EMCDDA estimates that the life time prevalence (ever used) of ecstasy use for adults (16-64 years) in Europe ranges between 0.3 and 7.5 %. Current ecstasy use (used last year) is estimated to range between 1 and 3.5 %.

Ecstasy refers to a synthetic substance that is chemically related to amphetamines but differs to some extent in the effects. Whereas amphetamines are mainly known for their energizing effects, ecstasy is commonly appreciated for its entacogenic properties. Concretely this means that besides increased energy, an intensification of impressions and euphoria is experienced (e.g. contact with other people, music, light) after ingesting ecstasy (Baylen & Rosenberg, 2006). The best-known member of the ecstasy group of drugs is 3,4-methylenedioxy-methamphetamine (MDMA), but other analogues are also sometimes found in ecstasy tablets (MDA, MDEA). The drug is commonly used in social scenes such as dance events and is frequently combined with other drugs such as cannabis and amphetamines and (most popular), alcohol (Brookhuis et al., 2004, Nabben et al., 2007).

In the framework of the DRUID project the influence of ecstasy and combined ecstasy and alcohol use on simulated driving performance was investigated to determine thresholds for driver impairment as a consequence of MDMA or combined MDMA and alcohol use. In this report the results are published. Recently, a group at the University of Maastricht has also investigated the effects of MDMA on driving performance, alone and in combination with alcohol in controlled experimental designs. In a series of studies the effects of single dose (75 and 100 mg) and repeated (75 + 50 mg) doses of MDMA alone, or in combination with alcohol on cognitive and psychomotor performance and on the road driving performance was investigated (Lamers et al., 2003; Ramaekers & Kuypers, 2006)(

Kuypers & Ramaekers, 2005; Kuypers & Ramaekers, 2006; Kuypers, Samyn, & Ramaekers, 2006; Kuypers, Wingen, Limbert, Samyn, & Ramaekers, 2007; Ramaekers, Kuypers, & Samyn, 2006). The main conclusions from psychomotor tasks and on the road driving performance tasks were that: single doses of MDMA (75, 100mg) (1) improved or left psychomotor task performance unaffected (2) caused improvement in automated (control level) driving behaviour but impairment in 'controlled' (manoeuvring level) driving behaviour (3) did not exert stimulant effects in combination with alcohol. The drawback of these studies is that in an on the road study it is difficult to investigate risk taking behaviour, because of the obvious reason that an accident-prone situation in an experiment on the road can not be (ethically) acceptable. Accident-prone situations are therefore to be avoided at all costs. Since the aim of DRUID is to define risk thresholds the intention of the present study is to investigate the risks involved in driving under the influence of MDMA and alcohol in a controlled environment, i.e. a driving simulator.

Brookhuis et al. (2004) used the driving simulator to test recreational MDMA users who were going to a rave (dance party). The participants drove in the simulator before going to the rave, shortly after the use of their own, self-bought and self-administered MDMA. After the rave, they were tested again while they were under the influence of MDMA and various other substances at their choice. The participants were also tested in sober condition on a different day at a comparable time. Results indicated that automated (control level) driving behaviour was only moderately and non-significantly affected, but there where strong indications that participants were willing to adopt higher levels of risk when they were under the influence of MDMA. Furthermore, the combination of MDMA with other drugs were detrimental for driving performance on all levels and was described as being extremely dangerous. Because of the quasi-experimental design, i.e. no control over the active substances, however, it is hard to draw straightforward conclusions. To determine the earlier mentioned thresholds for driver impairment as a consequence of MDMA or combined MDMA and alcohol use a controlled experimental design is needed.

The aim of the present study is therefore to investigate the risks involved in driving under the influence of MDMA and alcohol in a controlled environment, i.e. a driving simulator in a controlled design. The practical outcome of the study is to generate recommendations for the definition of risk thresholds for driving under the influence of MDMA alone and combined with alcohol, in the framework of the EU project DRUID. To this end a set of scenarios was developed. In these scenario's driving performance is assessed on the three levels of driving as described by Michon, (1985). That is, the strategic level (general plans), manoeuvre level (controlled action patterns) and control level (automatic action patterns) (Michon, 1985). To determine the earlier mentioned thresholds for driver impairment as a consequence of MDMA or combined MDMA and alcohol use, an "alcohol calibration" study was performed earlier (Veldstra, Brookhuis & De Waard, 2008). In this calibration study the influence of three levels of alcohol (0.3 ‰, 0.5‰, and 0.8 ‰) against a placebo-alcohol condition was tested on a set of measures within specifically developed scenarios, similar to the ones used in the present study.

Method

Participants

Originally twenty volunteers participated in this study. Because one participant did not comply with the rules of participation, nineteen healthy volunteers (eleven male, nine female) aged between 21 and 40 years (mean (sd) 30.8 (5.65)), who were recruited by flyers distributed through and at the University of Groningen, were used in further analyses. On the basis of post-experimental substance analysis, one participant was removed for reasons of non-compliance. They all were experienced drivers who held their drivers licence between 3 to 20 years (mean (sd) 8.8 (5.7)) and had all used MDMA before participating in the study (lifetime use varied between 10 and about 100). Moreover, they had experience with alcohol use, but were not abusers (participants on average drank 7.8 (sd= 5.8) beverages per week). All participants were screened on their medical history and medically examined by the medical supervisor. Vital signs were checked and standard blood chemistry was examined. Inclusion criteria were: experience with the use of MDMA (at least 5 times in the past 12 months); experience with the use of alcohol (> 2 consumptions a week); free from psychotropic medication; good physical health as determined by examination and laboratory analysis; absence of any major medical, endocrine and neurological condition; normal weight, body mass index (weight/length^2 between 18 and 28 kg/m^2); valid driving license (minimum 3 years, driving experience 5000 km per year) and a written informed consent. Exclusion criteria were: history of drug abuse or addiction as determined by examination; excessive drinking (> 20 alcoholic consumptions a week); heavy smoking (during the laboratory visits smoking is restricted to breaks only); current or history of psychiatric disorder; history of malignant hyperthermia /serotonin syndrome; cardiovascular abnormalities as assessed by standard ECG; hypertension (diastolic> 100; systolic> 170); pregnancy, lactation or wishing to become pregnant in the period of the study; participation in any clinical trial including blood sampling and/or administration of substances up to 6 weeks before day 1 of this study; susceptibility to simulator sickness (participants were pre-tested).

This study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (Latest revision, Seoul 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO). This implies that all participants participated voluntarily and were fully informed of all procedures, possible adverse reactions to drug treatments, legal rights and responsibilities, expected benefits of a general scientific nature, and their right to voluntary termination without penalty or censure. All participants gave their informed consent, in writing. Approval for the studies was obtained from the Medical Ethics Committee (MEtC) of the Academic Medical Centre of Groningen (UMCG). A permit for obtaining, storing and administering MDMA was obtained from the Dutch drug enforcement administration (CCMO).

Design and treatment

The study design was a double-blind, placebo-controlled, 4-way cross-over design with treatment orders counter-balanced according to a Latin square. Testing took place on one day per condition plus an extra day for practising the tasks and to familiarize themselves with the driving simulator. Consequently, the participants visited the facilities five times, with a washout period of one week in

between. On the testing days the participants randomly received a single dose of 100 mg MDMA or placebo and a single dose of alcohol (0.5 g/kg) or placebo. Placebo, alcohol and MDMA where administered orally in identically appearing formulations. MDMA was administered in a capsule; placebo was given as a capsule as well. The alcohol containing beverage that was used is Vodka (40 %) mixed with orange juice up to 250 ml; placebo was orange juice topped with a tiny bit of vaporised alcohol. The amount of administered alcohol was dependent on the weight, length and sex of the participants and was calculated using the Widmark procedure (Widmark, 1932).

The alcohol level was kept constant during tests by analyzing alcohol in breath every 15 minutes and administering a drink every time, with extra alcohol in case of insufficient BAC. Driving tests were conducted between 1.5 and 2.5 hours post drug (30 minutes after the first alcohol administration 10 minutes after subsequent administrations) and cognitive tests were done between 3 and 4 hours post drug (20 minutes after subsequent alcohol administrations).

See the appendix for a schedule on treatment and procedure.

Procedure

Prior to participation, participants underwent a physical examination. When there was no medical objection for participation, participants were invited to come for a training day to check for simulator sickness and practice all driving and cognitive tasks.

Participants were asked to refrain from any drugs starting one week before the screening and during the whole study period. Drug screens in urine and alcohol screened in breath were conducted before the start of every testing day. Female participants were also screened for pregnancy. Participants were allowed to take part on the testing day only if they had passed the screening. Furthermore, participants were asked to refrain from alcohol and caffeine containing beverages on the day prior to the testing day and to make sure they were well-rested for testing days.

On testing days the experimenters made sure that the participants were transported from and to their home in a safe manner to prevent them from driving while under the influence. The minimum period between successive treatments was 7 days. Participants were compensated for their participation by means of a monetary reward.

Pharmacokinetic assessment

Two blood samples (10ml) were collected on every testing day 1.5 post-drug. MDMA and MDA concentrations were determined afterwards in plasma and whole blood. For plasma collection, blood centrifuged after collection, for 15 min at ca. 2.000 rpm at ca. 4^{o}C. The samples were stored at -20°C until analyses for pharmacokinetic assessment. Furthermore, bloodspots were collected by finger prick for a special drug assessment kit provided by the University of Heidelberg.

Saliva was collected every hour until seven hours post drug intake with a StatSure's Saliva Sampler(TM). After collection the samples were kept in a cooler before storing them at -20°C until analyses for pharmacokinetic assessment.

Blood alcohol concentrations were assessed every 15 minutes with a Dräger Alcotest® 7410 Plus breathalyzer.

Apparatus

Participants where required to complete test-rides in a (fixed-base) driving simulator consisting of a mock-up car with original controls (three pedals, clutch, steering wheel, safety belt, indicator and hand brake) linked to a dedicated graphics computer, registering driver behaviour while the road environment and dynamic traffic are computed at 30Hz+. Participants had a 180º view of the road environment. Other vehicles in the simulated world interact with each other and the simulator car autonomously and behave according to hierarchically structured decision rules that are based on human driving behaviour (Van Wolffelaar & Van Winsum, 1992).

Driving tasks

The road tracking task

The road tracking task is designed to measure involuntary (unconscious) response errors, or tracking errors, calculated as the standard deviation of the lateral position (SDLP; O'Hanlon, 1982). The task consists of two straight roads of approximately 10 km. In this part of the virtual ride, the participant drives through a rural area with moderate density traffic flow and a posted speed limit of 100 km/h. How well the participant manages this part of the route is assessed by measuring the SDLP.

In the road tracking task participants are usually instructed to maintain a lateral position in the middle of the (right) traffic lane and to drive at a constant speed. But, as Brookhuis and De Waard (1994) already pointed out, driving like this is normally not necessary and therefore the instruction is unnatural. In the current scenario the participants will therefore get no other instructions but to drive as they would normally do.

Car following

Perception and attention errors are the most frequently cited errors leading to drug and/or alcohol related driving accidents in epidemiological data (Moskowitz, 1984). Decreased attention and or perception may impact the driver's ability to react properly to manoeuvres of other drivers. Such a situation can be when a driver has to respond to changes in speed of a car in front of him or her. To measure this so called car following performance Brookhuis et al. (1994) developed the car following performance test.

In this scenario the following car (i.e. the participant) is instructed to follow a lead car at a short but safe distance. The lead car is programmed to accelerate and decelerate in a cycle of between 20 and 40 seconds (e.g. between 0.025 and 0.05 Hz). Within this frequency band this frequency is varied randomly. The response of the participant to the speed changes of the lead car is measured by assessing the coherence (the extent to which the pattern of speed changes of the lead and follow car correspond), the gain (amplification factor between the two signals, or to what extent (amplitude) the reaction is conducted; when there is an over-reaction the gain is larger than 1 while at an under-reaction the modulus is smaller than 1), and most important, the delay (the time the following car needs to respond to the lead car; see Brookhuis et al., 1994, De Waard & Brookhuis, 2000).

Traversing non-signalised crossroads

The ability to estimate the relative speed of other vehicles and accurate awareness of gaps when

crossing a road is essential for safe driving. In a split second an achievable manoeuvre can turn out to be a looming accident because the driver judged the time available for safe crossing to be longer than the actual available time. Safe road crossing is a complex task that requires not only the mentioned perception of time and speed but also a fine co-ordination to synchronize this perception with the onset of movement. These are all factors that can be affected by drugs and alcohol.
In the virtual driving environment there are several scenarios used to measure the driver's ability to safely traverse a crossing; the gap acceptance scenario and the scenarios described under violations of traffic laws. The latter are scenarios that measure both the ability to safely traverse a crossing and the ability to comply with traffic regulations.

Gap acceptance
Brookhuis et al. (2004) found that the judgment of safety is deteriorated in drug affected drivers and therefore the perception of risk is reduced. The gap acceptance task is widely used to asses risk taking in traffic (e.g. Adams, 1995). In the gap acceptance scenario the participant has to cross a junction and is faced with traffic coming from both the left and right hand side, or from the opposite side of the road in the case of a y-junction. In this scenario the gaps in between cars increase with 1 second for each new car. The driver has to choose the appropriate gap to traverse the crossing. A driver's perception of an appropriate gap is dependent on the expected waiting time for a gap involving negligible risk (Adams, 1995). The driver weighs the waiting time versus the risk of causing an accident and comes to a decision to either choose a small risky gap but short waiting time or a larger, saver gap but longer waiting time.
The parameters included to assess the drivers risk taking are the size of the chosen gap in seconds and the distance to the car approaching the driver while traversing the crossing. As said, the driver's behaviour regarding to other traffic participants can be assessed as well. The driver can choose a fairly safe gap but because of poor initiation of movement, for example, is still causing nuisance to other traffic participants. This can be assessed by measuring the deceleration of the car approaching the driver while traversing the crossing.

Violating traffic regulations
Analyses of driver records of patients admitted to substance abuse showed that drug users had significantly more traffic violations than a non-drug control group (MacDonald et al., 2004). This is an important finding since there seems to be a clear relationship between violations of traffic laws and the risk of being involved in a traffic accident (Parker et al., 1995; Rothengatter, 1991). In the virtual driving environment two types of violations are assessed; violating the posted speed limit and running a red light.

Running a red light
Red-light running is a frequent and highly dangerous driving act. Research in the United States showed that running red lights and failing to stop for stop signs and yield signs are the most frequent causes of urban crashes (Retting et al., 1995). There are several factors that are of importance to be able to react to a traffic light. The driver has to pay attention to the traffic light which is usually on the side of the road or above it. Furthermore, the driver has to be able to react quickly and has to be

willing to comply with traffic regulations. These are all factors that can be affected by drugs and or alcohol, in the sense of risk taking behaviour.

For the assessment of alcohol impaired driving a scenario developed by De Waard et al. (1999) was used. In this scenario the participant approaches a crossing with green traffic light at a posted speed limit of 50 km/h. Two seconds before the participant would pass the traffic light the light turns to amber. To equalize the situation for all speeds the traffic light is adjusted to the speed of the participant in a linear fashion in such a way that when the participant drives faster the traffic light will turn amber earlier. In this manner the situation remains critical even if the participant does not comply with the posted speed limit. When participants keep the same speed they will drive through red traffic light, when they speed up they will drive through amber light and if they want to stop they have to brake firmly. The choice of the participant can be determined by assessing the colour of the traffic light at the moment of crossing (De Waard, Van der Hulst, & Brookhuis, 1999).

Reaction to unexpected events

According to Quimby (1986), drivers who take longer to detect and react to potential hazards have more accidents. Furthermore they are more prone to react impulsively and inattentively. Alcohol intoxication and sedation by (medicinal) drugs has been associated with significant deterioration in attention and reaction time (Kelly et al., 2004). This means that alcohol and or drugs affected drivers are expected to detect a potential hazard more slowly and moreover have a deteriorated ability to react to it quickly. In the virtual driving environment three scenarios are used to measure the driver's reaction to unexpected events; a car failing to give way, a car that is suddenly pulling out of a car park, and cars suddenly coming to a standstill on the motorway.

Car pulling out of a parking lot

In this scenario the participant is driving on a straight road and is passing a lay-by with parked cars when suddenly a car pulls out of a parking lot. The participant is faced with a critical situation in which he or she has to react quickly to avoid a collision. How the driver handles this situation is assessed by measuring the deceleration and the minimal TTC.

Speed management

Choice of speed was assessed as well. Participants under the influence of sedating drugs or alcohol might lower their speed to compensate for sedating effects. On the other hand stimulating drugs that reduce hazard perception and increase risk taking might make the participant want to speed up, which would lead to higher average speed and an increase in speed variability (see also Brookhuis et al., 2004). Another compensating mechanism in speed management is (deliberately) varying speed. In the case of severe sedation a participant might temporarily increase speed to increase feelings of arousal as can be seen in an increase in the standard deviation of speed (cf. Brookhuis, 1998). Violation of the posted speed limit, standard deviation of speed and overall duration of the ride are therefore important measurements of drug effects on driving at the strategic level and can be assessed throughout the entire route.

Self report measures

Karolinska Sleepiness Scale (KSS; Reyner & Horne,1998)

The KSS is a subjective rating scale, assessing sleepiness with scores that range from 1 (extremely alert) to 9 (extremely sleepy, "I have to fight not to fall asleep"). The rate is reported as an absolute rate, which means that the participant has to report the experienced sleepiness during a specific preceding time period. Participants were asked to rate their alertness before the driving tests and after to asses whether the driving task influenced their alertness and if this was dependent on drug condition.

Rating Scale Mental Effort (RSME; Zijlstra 1993)

On this scale participants have to indicate the amount of effort they invested in the driving task. The scale is designed to reflect operator effort but has found to be sensitive to task related and state related effort as well (De Waard, 1996).

Driving Quality Scale (DQS; Brookhuis et al., 1985)

On this scale the participants have to (self) assess their own performance. Driving quality will be rated by a driver on a 12-centimeter visual analogue scales (0= poor, 6= normal, 12= excellent).

Treatment evaluation Questionnaire (TQ)

At the end of each test day, the participants were asked to guess which treatment they had received: MDMA / placebo, alcohol / placebo.

Data analysis

All statistical analyses were conducted by means of SPSS 16 for Windows. Normally distributed data were subjected to a general linear model (GLM) univariate repeated measures analysis. If the Mauchly's test indicated that the assumption of sphericity was violated the degrees of freedom were corrected using the Greenhouse-Geisser correction. On the non-normally distributed data Friedman's tests were performed and contrasts were explored with the Wilcoxon exact test. Dichotomous data were subjected to a Cochran's test. Missing values were corrected by replacing them with the mean of the participant over all conditions. Due to technical problems there were a few outliers in the gap acceptance data. Outliers could be recognized as extreme low gap times (e.g. 1 sec). If the Gap time was extremely low it was treated as a missing value.

Results

Alcohol levels

In the study we aimed for an alcohol level of 0.5 ‰ in both the alcohol only and the alcohol and MDMA condition. The mean levels that were established as measured by breath analyses were 0.46 ‰ (SD=0.10) for the alcohol only condition and 0.50 ‰ (SD=0.11) for the alcohol and MDMA condition.

Pharmacokinetics

Saliva concentrations of MDMA and MDA where determined every hour after drug intake up to seven hours post drug. As can be seen in figure 1, MDMA in saliva peaked two hours after intake and decreased in the two hours following, hereafter the level was stable for the remaining hours that were measured.

MDMA in serum ranged from 0.00 - 388.57 in the MDMA condition and from 0.00 – 339.40 in the MDMA plus Alcohol condition (see Table 1 below).

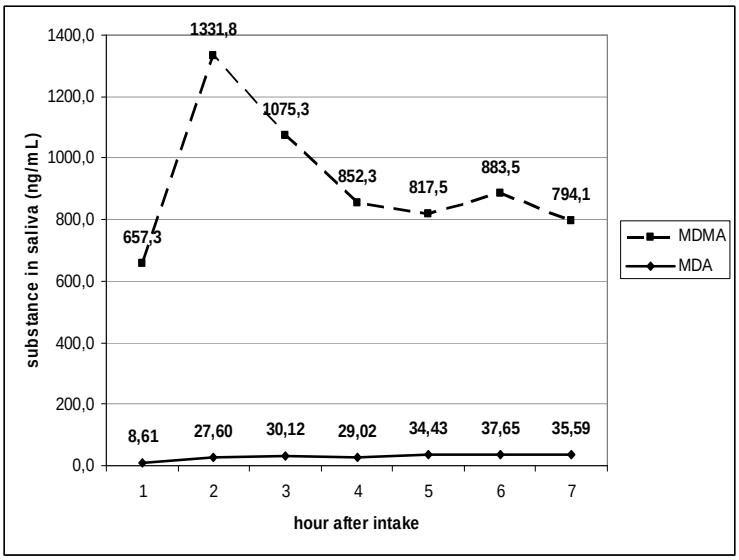

Figure 1: Average ng /ml MDA and MDMA in saliva per hour after intake.

Table 1: MDMA and MDA concentrations in serum (ng/ml) for both the MDMA and the MDMA plus alcohol condition

	Drug condition	
	MDMA condition	MDMA & Alcohol Condition
MDMA		
Range (min-max) Mean (sd)	0.00-388.57 136.41 (133.46)	0.00-339.40 127.02 (117.92)
MDA		
Range (min-max) Mean (sd)	0.00-11.77 3.33 (3.5)	0.00-8.62 3.24 (3.21)

Road tracking

As expected, there was a main within subjects effect of condition on weaving as measured as the standard deviation of the lateral position (SDLP; F (2.72) =6.16, p< 0.002). Post hoc tests reveal that there was no significant difference in SDLP between the combined alcohol and alcohol condition and placebo (F(1)= 1.12, p=n.s). However, in the alcohol condition and in the MDMA condition SDLP significantly differed from placebo (Alcohol condition; F (1) = 5.3, p< 0.05) and (MDMA condition; F (1) = 5.3, p<0.05). As can be seen in figure 2, SDLP is higher in the alcohol condition compared to placebo whereas SDLP is smaller in the MDMA condition as compared to placebo. This interaction effect of alcohol (0.5 ‰ and placebo) and MDMA (100 mg and placebo) was significant (F(1)= 15.17, p<0.001).

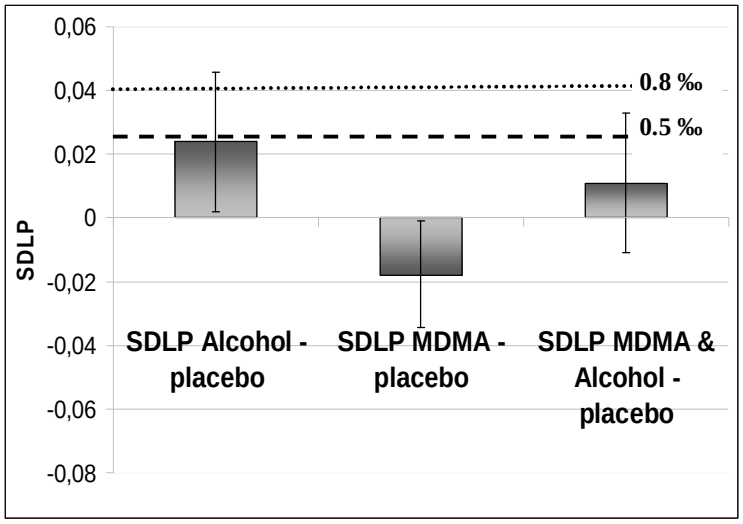

Figure 2: SDLP, mean difference with placebo and 95 % CI per condition for road tracking and equivalence to alcohol 0.5 ‰ and 0.8 ‰

When comparing the SDLP of two drug conditions to the alcohol condition, again there was no significant difference with the combined alcohol MDMA condition (F (1) = 1.61, p=n.s). However, when looking at the equivalence with the 0.5 ‰ and 0.8 ‰ criterion level (see figure 2) one can see that the upper bound of the 95 % confidence interval lies between the 0.5 ‰ and 0.8 ‰ equivalence line. This indicates that some participants showed an impairment that was above the criterion level of 0.5 ‰. Furthermore, the SDLP in MDMA condition was significantly lower compared to the alcohol condition (F (1) =15.17, p<0.001).

SDLP did not correlate significantly with MDMA in serum for both the MDMA alone (r= -.154, p=n.s) and the MDMA, alcohol combined condition (r=-.152, p=n.s). The same was true for the correlations with MDMA in saliva ((r= -.026, n.s); r= -.252, n.s) for the combined alcohol & MDMA condition.

Car following

In this scenario the following (i.e. the participants') car was instructed to follow a lead car at a short but safe distance. The response of the participant to the speed changes of the lead car was measured by assessing the coherence, the gain and the delay. As can be seen in table 2 there was no main within subject's effect of drug condition on any of the car following measures.

Overall the coherence was low, but even when correcting for the low coherence by analyzing

participants with a coherence of above 0.75 only, there was no main effect of drug conditions on any of the car following measures. Also for the time head way (THW), there were no significant main effects of drug condition on average or minimum THW.

Table 2. Car following measures. Average and 95 % CI of coherence, delay (in seconds) and gain for different drug conditions and within subjects' effects.

	Drug condition Placebo	Alcohol	MDMA	MDMA & Alcohol	Friedman's ANOVA
Coherence	0.74 (0.67- 0.86)	0.81 (0.75 - 0.87)	0.75 (0.65- 0.85)	0.79 (0.73- 0.86)	(X^2 (3) = 1.80, p= n.s
Gain	5.23 (4.15- 6.59)	4.81 (3.86- 5.76)	5.37 (4.15- 6.59)	4.64 (3.76- 5.51)	(X^2 (3) = 1.86, p= n.s
Delay	0.73 (0.61- 0.84)	0.77 (0.63- 0.90)	0.75 (0.65- 0.84)	0.79 (0.70- 0.87)	(X^2 (3) = 0.86. p= n.s
Average THW	2,45 (1,97- 2,92)	2,19 (1,81- 2,56)	2,18 (1,82- 2,54)	2,18 (1,75- 2,61)	(X^2 (3) = 0.54. p= n.s
Minimum THW	1,16 (0,88- 1,44)	0,89 (0,64- 1,15)	0,97 (0,81- 1,14)	0,99 (0,76- 1,23)	(X^2 (3) = 2,37 p= n.s

Traversing unsignalised crossroads

Gap acceptance at the y-junction

In the Gap acceptance task were participants had to traverse a y–junction while cars approached them from the crossing road. The time between the approaching cars was the dependent variable for risk assessment. It was expected that participants would accept a smaller gap time when under the influence of MDMA as compared to placebo. However, no significant main effect of drug condition on gap times was found (see table 3). Also, the distance between the participants' car and the car of the autonomous agent approaching at the moment the participant is crossing the junction was not dependent on drug condition (see table 3).

Gap acceptance at a normal junction

There was no main effect of drug condition on gap acceptance at the normal junction, neither for gap time, nor for the accepted distance to the upcoming cars in this task (see table 3).

Table 3. Gap acceptance measures at the y- and normal junction.. Average and 95 % CI of gap time (in seconds) and distance to approaching car (m) for different conditions and within subjects' effects.

	Drug condition **Placebo**	**Alcohol**	**MDMA**	**MDMA & Alcohol**	GLM repeated measures
Gap time y	3.95 (3.64- 4.26)	4.05 (3.69- 4.41)	4.08 (3.77- 4.39)	4.03 (3.63- 4.43)	(F(2.5) = 0.25, p=n.s)
Distance to car y	30.63 (26.69- 34.57)	32.22 (27.80- 36.64)	32.31 (28.25- 36.38)	32.91 (28.17- 37.65)	(F(2.5) = 0.37, p=n.s)
Gap time	4.38 (3.76- 4.99)	4.59 (3.93- 5.25)	4.24 (3.63- 4.85)	4.42 (3.76- 5.09)	(F(2.5) = 0.57, p=n.s)
Distance to car	31.29 (25.63- 36.95)	37.04 (30.21- 43.88)	31.86 (26.28- 37.45)	36.16 (28.50- 43.83)	(F(2.2) = 1.92, p=n.s)

Violating traffic regulations

Running a red light

For the assessment of drug impaired driving in the red light scenario developed by De Waard et al. (1999) participants could either keep the same speed and drive through the red traffic light or speed up and drive through an amber light or stop. The choice of the participant was determined by assessing the colour of the traffic light at the moment of crossing and comparing the reaction to the scenario between drug and placebo conditions. As can be seen in figure 3, most participants had the same reaction to the scenario for both the drug condition and placebo condition (displayed as the unimpaired group). This could be running a red light both times or running a green light. However, in most cases participants were running a red light both times (73 % for the alcohol condition, 72 % for the MDMA condition and 85 % MDMA & Alcohol condition). This difference between the percentage of participants being unimpaired or impaired was significant for the MDMA condition (binominal test, $p < 0.05$) and the MDMA & Alcohol condition (binominal test, $p < 0.05$), but not for the alcohol condition (binominal test, p = n.s). This indicates that although the reaction of most participants in the MDMA and combined MDMA & Alcohol condition was unimpaired, in the alcohol condition the percentage of participants who were unimpaired did not differ significantly from the percentage of participants who were impaired.

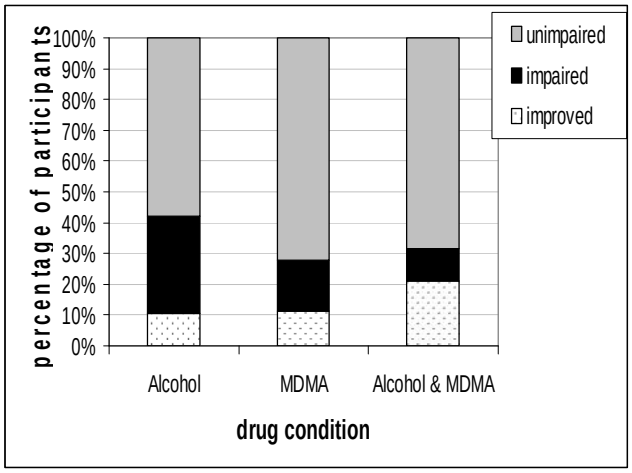

Figure 3: Percentage of participants who showed impairment in the red light scenario compared to placebo for all drug conditions.

Car pulling out of a parking

In this scenario the participant is driving on a straight road and is passing a lay-by with parked cars when suddenly a car pulls out of a parking place. The participant is faced with a critical situation in which he or she has to react quickly to avoid a collision. How the driver handles this situation is assessed by measuring the minimal TTC.

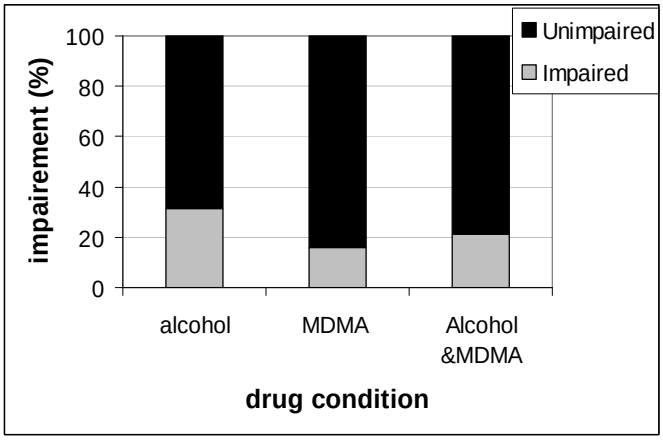

Figure 4: Percentage of participants who showed impairment in critical TTC"s compared to placebo for all drug conditions.

There was no main effect of drug condition on the average minimum TTC (X^2 (3) = 4.15, p= n.s). When looking at the difference in the percentage of critical TTC's (TTC less then 1.5 sec) between the drug conditions and placebo we can see that most participants did not show impairment. The difference between the percentage impaired and unimpaired reactions was significant for the MDMA condition (binominal test, p< 0.001) and the combined MDMA & alcohol condition (binominal test, p< 0.001) and almost significant for the alcohol condition (binominal test, p= 0.052). This means that most participants reacted fast enough to the car suddenly pulling out of the parking lay to avoid a critical situation. However, as can be seen in figure 4 there was also a percentage that were not able to react fast enough. This percentage was highest in the alcohol condition.

Speed management

Choice of speed was assessed at all road types (50 km/h,100 km/h and 120 km/h). It was expected that under influence of alcohol participants might change their average speed to compensate for sedating effects. On the other hand it was expected that MDMA would reduce hazard perception and might make the participant want to speed up (Brookhuis et al., 2004).

As can be seen in table 4 the expected speed changes were not found. Participants kept the posted speed on all road types. However, on

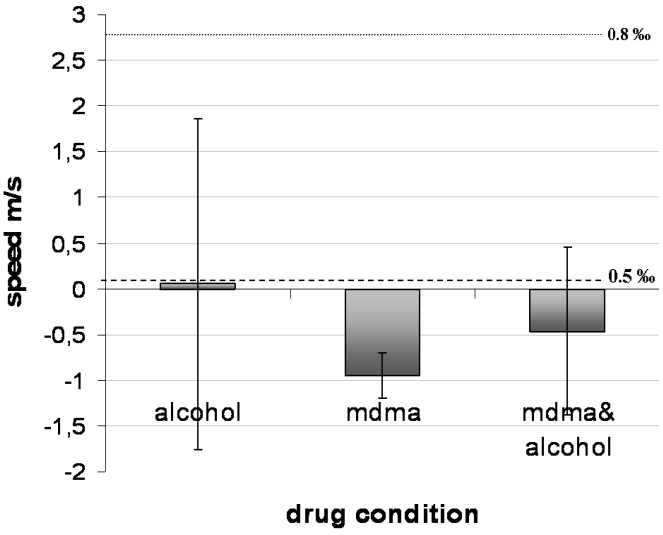

Figure 5: Average speed difference with placebo condition and 95 % CI per condition for the rural road (posted speed 27.8 m/s) and equivalence to alcohol 0.5 ‰ and 0.8 ‰

the rural roads were they could drive more freely, there was a main effect of drug condition on speed (see table 4). When looking at figure 5 it can be seen that this difference stems mainly from the difference between the MDMA and placebo condition (*T= 10.70, p< 0.05).* In the other drug conditions the speed did not differ significantly from the placebo condition. Also, the speed in both the MDMA and the combined MDMA alcohol condition did not differ significantly from the alcohol condition (MDMA condition; F(1) = 1.38, p= n.s; Combined condition F (1) = 0.32, p=n.s).

Another compensating mechanism in speed management is deliberately varying speed. In the case of sedation such as in the alcohol condition a participant might temporarily increase speed to increase feelings of arousal. This can be seen in an increase in the standard deviation of speed (Brookhuis, 1998). As can be seen in table 4, there was a main effect of drug condition on the sd speed when participants were driving on the rural road. When looking at figure 6, it can be seen that the sd speed in the alcohol condition was higher then the sd speed in the placebo condition (*T*= 11.00, P<0.05). The sd speed in the other drug conditions did not differ significantly from the sd speed in the placebo condition. When comparing the sd speed of the MDMA condition and the combined alcohol and MDMA condition to the alcohol condition there was no significant difference between the sd speed in the alcohol condition and the combined alcohol and MDMA condition (*T* (1) = 8.88, p= n.s). However, the sd speed in the MDMA condition was significantly lower then the sd speed in the alcohol condition (*T*=10.76, p<0.05). The interaction effect of alcohol (0.5 ‰ and placebo) and MDMA (100 mg and placebo) was significant (*T (1) = 10.67, p<0.005*).

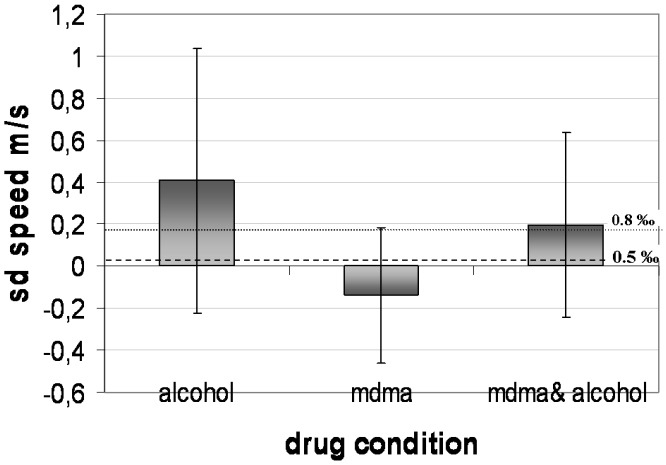

Figure 6: Average sd speed difference with placebo condition and 95 % CI per condition for the rural road (posted speed 27.8 m/s) and equivalence to alcohol 0.5 ‰ and 0.8 ‰

Table 4. Speed management. Average and CI speed and SD speed for village (13.9 m/s), rural (27.8 m/s) and motor way (33.33 m/s) driving. All for different drug conditions.

	Drug condition **Placebo**	**Alcohol**	**MDMA**	**MDMA & Alcohol**	
Speed Village	12.97 (12.46- 13. 48)	13.05 (12.47- 13.63)	13.40 (12.90- 13.89)	13.21 (12.44- 13.98)	F(2.77)= 0.94, p= n.s
Sd speed village	1.56 (1.38- 1.74)	1.61 (1.47- 1.75)	1.41 (1.21- 1.61)	1.69 (1.47- 1.91)	F(2.67)= 2.75, p= 0.06
Speed Rural road	29.39 (28.10- 30.68)	29.50 (27.78- 31.10)	28.44 (27.60- 29.28)	28.92 (27.77- 30.07)	(X^2 (3) = 8.2, p< 0.04)
Sd speed rural road	0.84 (0.49-1.20)	1.25 (0.70- 1.81)	0.71 (0.56- 0.85)	1.04 (0.69-1.39)	(X^2 (3) = 10.33, p< 0.02)
Speed Motor way	33.93 (31.94- 35.64)	33.26 (30.96- 35.57)	32.93 (31.48- 34.39)	33.75 (31.53- 35.98)	(X^2 (3) = 4.45, p= n.s)
Sd speed motor way	1.74 (1.26- 2.23)	1.84 (1.30- 2.39)	1.76 (1.15- 2.36)	2.00 (1.28- 2.73)	(X^2 (3) = 0.57, p= n.s)

Self report measures

Karolinski Sleepiness Scale (KSS ; Reyner & Horne,1998).

The KSS assessed the participants' own feeling off alertness before and after the driving session. Scores ranged from 1 (extremely alert) to 9 (Sleepy, I have to fight not to fall a sleep). As can be seen in figure 7, the average ratings of alertness before the driving test were equal over all conditions (X^2 (3) =0.36, p= n.s.). However, when comparing these ratings of alertness before the driving session with ratings after the driving session participants gave higher average ratings after the driving session as compared to before driving session (X^2 (7) =32.81, p<0.001). This indicates that they rated themselves as less alert after the driving sessions. Analyses of contrasts reveal that this difference was significant for the placebo (*T* =3.5, p<0.05), alcohol (*T* =3.5, p< 0.001) and combined alcohol and MDMA (*T* =6.91, p< 0.05) drug

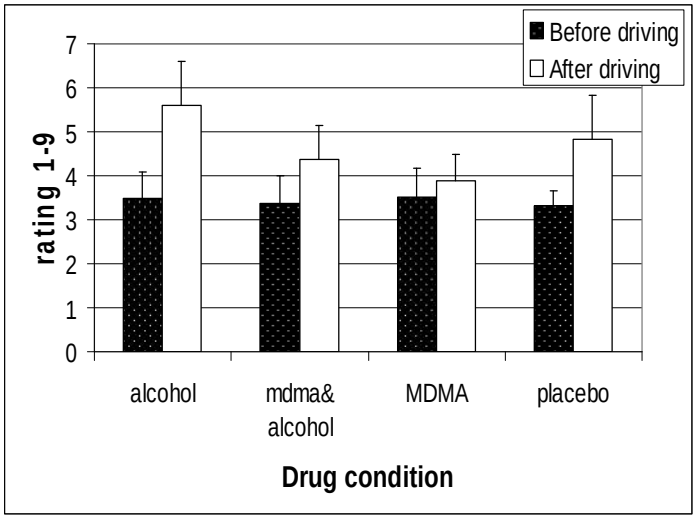

Figure 7. Average ratings on the KSS and 95 % CI per condition

conditions but not for the MDMA only condition (T = 6.62, p= n.s).

Rating Scale Mental Effort (RSME; Zijlstra 1993).

The RSME assessed whether or not there was a difference in mental effort participants had to invest in the driving session for the different drug conditions. There was no significant overall effect of drug condition on the RSME (X^2 (4) =.84, p= n.s).

Table 5. Average ratings on the RSME and standard 95 % CI for different drug conditions

	Drug condition Placebo	Alcohol	MDMA	MDMA & Alcohol
Average ratings	35.79	46.24	39.26	38.85
95 % CI	(12.5-47.1)	(32.9-59.5)	(30.4-48.2)	(25.3-52.4)

Participants assesements of the influence of the drugs on their driving performance

We asked participants in the introduction session before all the test sessions begun how they thought the different drug conditions would influence their driving performance on a scale ranging from 0 (worse) to 12 (better). As can be seen in figure 8 participants predicted their performance to be worse then normal. Furthermore, participants thought their driving would deteriorate more in the combined alcohol and MDMA condition as compared to the alcohol (T = 9.06, p< 0.009) and the MDMA (T = 4.38, p< 0.05) condition. After the testingday was over we asked participants if they kwew what condition they were in and also, if they thought that the drug they had might have influenced their driving performance. As can be seen in figure 8, participants thought that the drug condition that they had been in would still make them drive a little worse then normal, however this time they expected that the influence on their driving performance did not differ between conditions (X^2 (2) =4.67, p=n.s). When comparing the subjective ratings of influence of the different drug conditions on driving performance before participants had driven to when they had experienced how it was to drive under the influence, we can see that participants rated the influence of the drugs on their driving worse before they had driven under the influence then after. This difference was significant for both the MDMA (T = 10.12, p< 0.009) and the combined mdma/ alcohol condition (T = 7.41, p< 0.008), but not for the alcohol condition (T = 8.5, p= n.s).

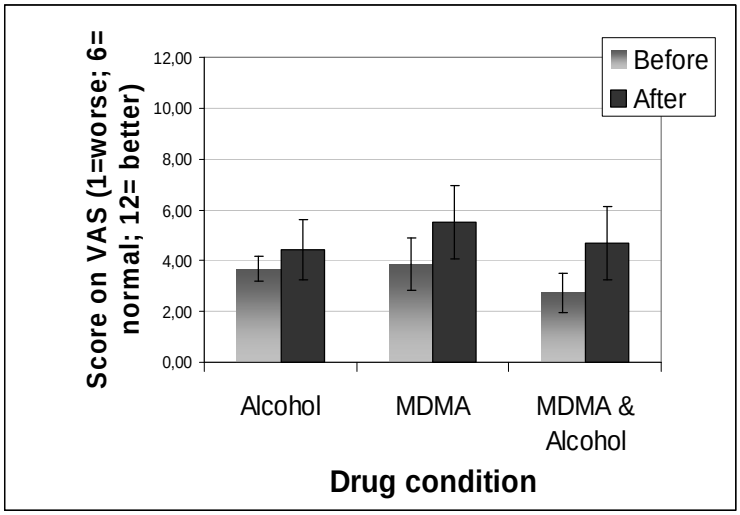

Figure 8 : Scores on a VAS Before driving tests (question: how do you think the drug will influence your driving) vs after driving tests (question: how do you think the drug influenced your driving) for the participants who guessed the condition they were in.

Driving Quality Scale (DQS; Brookhuis et al., 1985)

After every driving session we asked participants how they rated their driving performance on the DQS ranging from 0 (poor) to 12 (excellent). As can be seen in figure 9 the participants rated their performance in all drug conditions as slightly worse as compared to the placebo conditions. However, there was no significant main effect (X^2 (4) =1.68, p= n.s.).

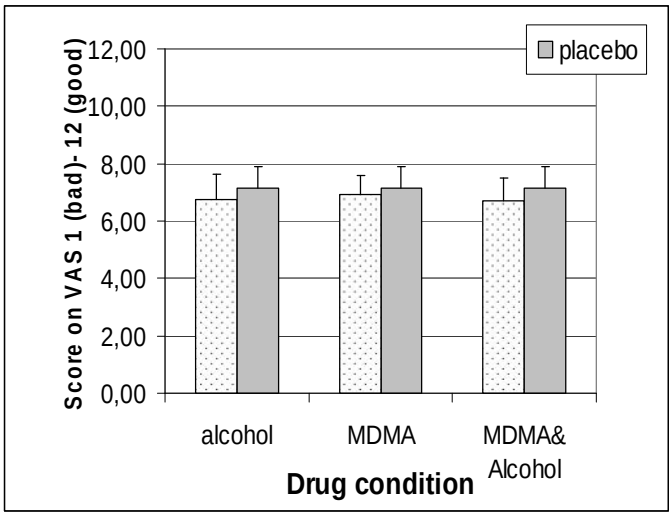

Figure 9: Average ratings on the DQS after driving and 95 % CI per drug condition

3.4.4 Treatment evaluation Questionnaire (TQ)

At the end of every session participants were asked in which condition they thought they were in. Figure 10 displays the percentage of participants who guessed the right condition per drug condition.

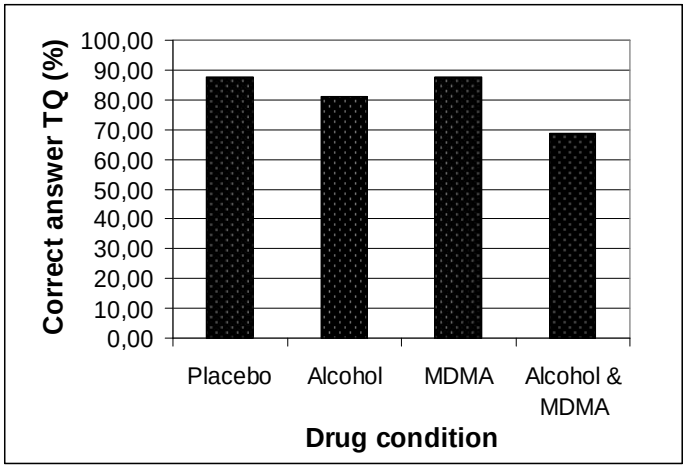

Figure 10: percentage of right answers in the TQ after driving per drug condition

Discussion and conclusions

The effect of alcohol (alone) 0.5 ‰ on weaving was very similar to the effect of alcohol 0.5 ‰ found in the alcohol study. Moreover, the increase in SDLP of about 2.4 cm is almost identical to findings in other simulator- (Thompson et al., 2010) and on-road-driving studies (Kuypers et al., 2006; Ramaekers et. al., 2006). As reported before in other studies (Kuypers & Ramaekers, 2006; Ramaekers et al.,2006), SDLP was positively affected by MDMA (alone).

The moderating effect of MDMA on alcohol as found by Kuypers et al. (2006) failed to reach significance in the current study. However, the trend effect for the SDLP difference to placebo when MDMA was co-administered with alcohol 0.5 ‰ as compared to the reference study indicates that combined use may lead to impairment in SDLP that is above the criterion level of 0.5 ‰ or even 0.8 ‰ for subset of participants. The equivalence test confirms this thought, since the upper bound of the 95 % confidence interval of SDLP when MDMA was co-administered with alcohol lies outside both the 0.5 ‰ and 0.8 ‰ equivalence lines. However, the confidence interval spreads over the null line as well, indicating that a subset of participants will not be impaired or even improved in SDLP performance as compared to placebo. These individual differences in performance are striking. Apparently, participants were differentially influenced by the different drug conditions. The lack of association between drug concentrations and performance confirms this idea.

Brookhuis et al. (2004) reported in increase in speed when participants were under the influence of MDMA in build up areas and on the motor way. In the current study, however, participants kept the posted speed on all road types and in the MDMA condition even decreased speed a bit more when driving on the rural road. Furthermore, SDSP increased when under the influence of alcohol but decreased when under the influence of MDMA. This is in line with Kuypers & Ramaekers (2006), but contrary to the increase in SDSP for driving under the influence of MDMA that Brookhuis et al. (2004) reported. However, variance in several parameters was increasing when driving under influence. Specifically regarding speed, which is definitely related to accident likelihood, the variance is similarly increased when under influence of alcohol and alcohol combined with MDMA.

Since the aim of the study was to set risk thresholds for driving under the influence of MDMA in more complex driving tasks as well, several scenario's were presented to asses risk taking. However, in almost all scenarios the expectations for what MDMA would do were not met, at least not with respect to average values. Also, a subset of the participants predicted their driving in the combined condition to be worst, while afterwards they rated their performance to be not so bad. Both in the MDMA and in the combined condition the predicted ratings differed significantly from the experienced ratings. This could be due to behavioural compensation.

Since the scenario's measuring complex driving performance are so dynamic they allow for different strategies to compensate for the intoxicating effects. For example, where one participant might have reduced speed to have an increased reaction time to unexpected events, another might have adopted an alternative strategy in which altering speed was not necessary. For more automated driving tasks, the compensatory strategies are limited and may therefore be more sensitive to drug induced effects.

It is known that a compensatory response to the intoxicating effects of alcohol/ drugs is triggered by the awareness and subjective discomfort of reduced performance efficacy (Fairclough & Graham, 1999). Although the study was double blind, the majority of the participants still guessed the condition they were in correctly and may perhaps have responded by compensating for the impairing effects. However, when comparing self assessment results with the automated driving results we could conclude that in case of alcohol consumption the self evaluation led to wrong conclusions. In this condition participants rated their performance before and after driving under the influence as the

same: that is, slightly worse than normal. Since their driving was actually seriously deteriorated, this conclusion was a falsely positive assessment of the situation. In the case of MDMA, the opposite was the case. The driving performance of the MDMA users was better than their self-assessment

In conclusion, effects were mainly found on automated driving performance such as weaving and speed. These were negatively influenced by alcohol and positively by MDMA.
Combined use may lead to impairment in SDLP that is above the criterion level of 0.5 ‰ or even 0.8 ‰ for subset of participants. However, a subset of participants might not be impaired or even improve in performance as compared to placebo. The lack of association between drug concentrations and performance confirms the idea that participants might have been differentially influenced by the different drug conditions. Furthermore, compensation strategies might differ between individuals further complicating the effect of the substances on driving performance.

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Appendix: Experimental Designa and Porcedure

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Experimental design

- 20 regular MDMA users (non abusive/ healthy)
  - 11 male and 9 female
  - mean age 30.8 years (sd= 5.6)
- Experienced drivers
  - drivers licence 9.5 years (sd=5.7)
- 4 conditions cross-over (repeated measures)
  - *MDMA 100 mg + Alcohol Placebo*
  - *MDMA 100 mg + Alcohol 0.50 ‰ (sd=0.11)*
  - *MDMA Placebo + Alcohol Placebo*
  - *MDMA Placebo + Alcohol 0.46 ‰ (sd=0.10)*
- Double blind

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Procedure

Time line

0.00	Breath analyses, Urine testing & Questionnaires
0.30	MDMA intake
1.00	Alcohol intake
1.15	Breath analyses
1.20	Alcohol intake
1.30	Breath analyses + Blood sampling
2.30	Driving test

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Chapter 4

Effects of dexamphetamine on simulated driving performance before and after sleep deprivation

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Abstract

Stimulant drugs are often used to promote wakefulness, yet their effects on driving performance during sleep deprivation have been poorly studied in experimental studies. For this mean a double blind, placebo controlled experiment including eighteen healthy, male volunteers was conducted, aiming to assess the effects on fundamental driving parameters during simulated driving of three doses of dextroamphetamine and further to assess the interaction between the stimulant drug and sleep deprivation

The results showed that, in line with what could be expected of this substance, participants in the study felt more alert when taking a dose of dextroamphetamine than when taking placebo and the effect was stronger for the higher dose. However, the data did not show any evidence that taking dextroamphetamine prevented the subjects from successively becoming sleepier during the night. A significant main effect of dose was found for three out of the five primary driving performance indicators.Taking the lower dose caused improved driving with respect to crossing car reaction time, coherence and delay, the results for the higher dose were mixed (sometimes driving was improved compared to placebo and sometimes it was about the same). There were no significant effects of dose on any of the ten secondary performance indicators.
It is important to note that no interactions were found between dose and sleep deprivation for any of the performance indicators. For the indicators where sleep deprivation lead to impaired driving, this means that there were no evidence in our data that the impaired driving was compensated by taking a dose of dextroamphetamine.

The experiment has been approved by the Regional board of Ethics in Linköping and granted authorisation by the Medical Products Agency.

Introduction

Stimulant drugs are often used to promote wakefulness, particularly during periods of sleep deprivation. This is usually true for illicit stimulants such as Ecstasy (MDMA) which are usually taken over the night (e.g. during rave parties), but also for prescription stimulants when taken by (truck) drivers to overcome sleepiness during prolonged periods of driving or during night-time. Traditional stimulants that are used by drivers to promote wakefulness include prescription amphetamines such as dextroamphetamine and methylphenidate which are registered for treatment of attention deficit hyperactivity disorder.

The effects of stimulant drugs on driving performance during sleep deprivation have been poorly studied in experimental studies. Yet the use of amphetamines to prolong wakefulness and overcome sleep of drivers may well increase a driver's crash risk, particularly during the drug elimination phase of the body. Therefore, an experiment was conducted with the aim of increasing the knowledge of the effects of dextroamphetamine on driving performance.

The experiment took place at VTI's third generation driving simulator and the aims of the experiment was to assess the effects of dextroamphetamine on fundamental driving parameters during simulated driving and to assess the interaction between the stimulant drug and sleep deprivation.

The participants in the study was healthy volunteers and they drove under three conditions of dextroamphetamine intake (placebo, 10 mg and 40 mg) and with three levels of sleep depravation for each condition (alert, slightly sleep deprived and sleep deprived).

The experiment has been approved by the Regional board of Ethics in Linköping and granted authorisation by the Medical Products Agency. The latter was needed since the experiment was regarded as a clinical trial.

Method

Subjects

It was mutually decided within the project, based on previous experience, that at least 18 subjects were required for the experiment. During planning it was realized that it was possible to do four persons per day which led to the recruitment of 20 persons (four persons per night during five days). The extra cost for adding two extra persons was very small and it gave some margin of error in case there were drop outs among the subjects.

Selection criteria

The subjects were recruited from the VTI test subject database. Inclusion criteria were: male, 23 – 40 years old and experienced drivers (driving 1 000 to 5 000 km per year and has held a valid driving license for at least five years). Exclusion criteria were problems with motion sickness, history of alcohol or drug abuse or regularly use of medicine. They should also not be large consumers of tobacco or be afraid of needles.

Screening

Before the experiment the test subjects must pass a medical examination including a physical examination as well as an interview with the physician in charge to make sure that they were suitable for this kind of experiment. All twenty subjects were approved.

Briefing and debriefing subjects

In experiments like this it is of utter importance that all subjects are fully informed of the procedure and potential risks. To make sure that everyone was aware of what they agreed to be part of they were first given information over the phone when recruited, then they were then given more detailed, written information a few weeks in advance with contact details in case of questions. Finally this was discussed during the interview with the physician in charge. The written information was then signed by the subjects as informed consent.

A few weeks after the experiment was completed all subjects had a follow-up medical examination including an interview.

Procedure

A randomized, double-blind, placebo-controlled, crossover design was used. Three different doses were combined with three levels of sleep deprivation, resulting in a total of nine conditions.

Each subject participated in the experiment at three occasions at intervals of seven days. At each occasion, the subjects were given one of three doses: placebo, 10 mg or 40 mg of dextroamphetamine. Each test occasion lasted from afternoon to next morning and included four subjects, who had three driving sessions each: one in late afternoon, one at night and one in the morning. All subjects had their driving sessions at the same day and time each week.

The timetable for the first subject to arrive on each test occasion is given in Table 1. The timetables for the three other subjects were delayed one, two and three hours, respectively. Otherwise the procedure was the same.

The subjects were not allowed to eat anything from the time they arrived until after the first driving session. They were not allowed to sleep or to drink any caffeine-containing beverages during the whole test occasion. The time between the driving sessions was spent in a room next to the simulator room, where the subjects could eat, talk to each other, watch television, use the internet etc. Throughout the night there was at least one nurse present as well as the experiment leader and the physician in charge was always stand by. After the last driving session, they subjects were sent home by taxi.

Tests

There were a number of tests carried out before, between and after each driving session. Upon arrival the subjects were asked to rate their sleepiness level using the Karolinska Sleepiness Scale, KSS (Åkerstedt and Gillberg 1990). The KSS ranges from 1-9 where 1= very alert, 5=neither sleepy nor

alert, 7=sleepy but no effort to remain awake, and 9=very sleepy, an effort to stay awake, fighting sleep.

Prior to administration of dextroamphetamine (or placebo) the subjects were tested for ethanol using a breathalyzer and drugs of abuse using a urine screening test. At this point the subjects' blood pressure and pulse were also registered and a pre-dose blood sample was taken. After drug administration the subjects filled out a background questionnaire (only at the first test occasion).

Immediately prior to their first driving session blood pressure and pulse was measured once again and sleepiness was self rated. Blood samples for plasma and whole blood was drawn and a urine sample was obtained. At this point the first dried bloodspot sample was also taken.

After the first driving session (which lasted for approximately 45 minutes) a KSS rating was given, blood pressure and pulse was measured and a urine sample was taken as well as a blood sample (plasma). The subjects then filled out a questionnaire regarding the driving session they just had gone through.

Four hours after the first driving session it was time for the second session and blood pressure, pulse and KSS was registered before the drive. Also, a urine sample and a blood sample (plasma) were taken.

After the second drive there were no test except for KSS and a questionnaire. For the third and last drive (again four hours after the second drive) it was the same procedure as for drive number two.

After the last drive the last week the drivers were given an extended questionnaire to take home and fill out. This questionnaire covered their experience throughout the whole experiment.

Table 1 Time table for the first subject at each test occasion.

Time	Event
16.00	Arrival. Tests: breath alcohol, blood pressure, pulse, blood plasma*, urine***, KSS.
16.30	Drug intake.
	No food intake was allowed before the first drive.
17.45	Tests: blood pressure, pulse, blood plasma*, whole blood*, bloodspot*, urine**.
18.00	Driving session 1. KSS before and after.
18.45	Tests: blood plasma*, urine**, questionnaire.
	Dinner and free time.
22.45	Tests: blood pressure, pulse, blood plasma*, urine**
23.00	Driving session 2. KSS before and after.
23.45	Questionnaire.
	Sandwich and free time.
03.45	Tests: blood pressure, pulse, blood plasma*, urine**
04.00	Driving session 3. KSS before and after.
04.45	Questionnaire.
	Taxi home.

*) dextroamphetamine

**) pH, dextroamphetamine

***) pH, cannabis, opiates, cocaine, amphetamine, benzodiazepines

Scenario

The route designed for this experiment covered both rural and urban roads with speed limits 50 and 70 km/h. In the urban sections there where both signal controlled intersections and intersections with right of way. All in all, the route was 44.6 km long and took approximately 45 minutes to drive. The subjects were instructed to consider the drive as their daily route to work and that they should drive as they normally would in these conditions.

The experimental design in this study required that the test subjects drove the same scenario on nine occasions which means that large learning effects can be expected. In an attempt to minimize the learning effects all subjects conducted a test drive before the experiment started. This meant that they to some extent already had familiarised themselves with the route and the scenario. To further minimize the learning effects the events they encountered did not occur at the same spot every time but could happen on several different places. For a description of the events, see section "performance indicators and events".

At the end of the drive there was a car following event. Before that event, the driver stopped the vehicle and was given new instructions, which was to follow the vehicle in front. In this event, roads with speed limit 90 km/h were also included.

Test site

The study was carried out at VTI's driving simulator III (VTI's third generation moving base driving simulator). It was used to create realistic sensations in a laboratory environment, including a:

Cut-off passenger car cab

Computerised vehicle model

Large moving base system

Vibration table

PC-based visual system

PC-based audio system

The driving simulator is shown in Figure 1. The simulated car had a manual gearbox with 5 gears. The time delay introduced in the simulator is very short (40 ms), which is important when focusing on the control and manoeuvring aspects of driving. The noise, infra-sound and vibration levels inside the cabin corresponded to those of a modern vehicle. The car body used in this experiment was a SAAB 9-3.

The driving simulator model has been extensively validated. Simulation results have been compared to field test results of most standard vehicle dynamics manoeuvres (steady state driving in a circle, step input on the steering wheel and frequency response) with good correspondence. This work has been documented in a number of reports (Jerand 1997; Aurell, Andersson et al. 1999; Aurell

2000). Furthermore, a number of validation studies with an earlier version of a simulator used with focus on driver behaviour have been carried out successively (Harms 1993; Alm 1995; Törnros, Harms et al. 1997; Törnros 1998).

Figure 1 Moving base driving simulator at VTI.

Between the test drives the subjects spent their time in a room with TV and DVD as well as computers with internet access. All medical examinations were carried out in an adjacent room with a bunk bed and a toilet.

Performance indicators and events

A number of performance indicators based on driving data were calculated for the analysis. These were based on both normal driving such as mean speed and standard deviation of lateral position but also on specific events. These events where designed to trigger behaviour that would indicate a change in behaviour, reaction and risk taking. All indicators were analysed but only a selection is presented in the results chapter.

Normal road, 50 km/h

The first and last 250 m of the 2500 m long normal (i.e., without any events) road was removed and the following measures were determined for the remaining 2000 m:

Mean speed

Standard deviation of speed

Standard deviation of lateral position (SDLP)

Normal road, 70 km/h

The first and last 250 m of the 2500 m long normal (i.e., without any events) road was removed and the following measures were determined for the remaining 2000 m:

Mean speed

Standard deviation of speed

Standard deviation of lateral position (SDLP)

Intersections

Intersections without traffic lights or events:

Minimum speed

Event: bus turning out

In this event a bus is standing still at a bus stop and when the driver is approaching the bus it starts to move. The driver has no other choice than to brake. To make this event less obvious for the test persons there were buses standing at other bus stops throughout the route and which bus started to drive varied for each time. Measures for this event are:

Reaction time to brake (from when the bus starts moving)

Minimum time-to-collision (TTC)

Event: moped

In this event the driver catches up with a moped driving at a slower speed. At the same time there is oncoming traffic which gives the driver the choices to wait until the oncoming traffic has passed or to overtake. Measures of interest are:

Speed (momentary) when passing the moped

Distance (lateral) to moped when passing

Event: traffic light

In this event the driver approaches a traffic signal and then it turns to yellow and then red. The event is triggered on time to traffic light (TTL) and there are three traffic lights with TTL 3 s and two with TTL 4.5 s. For these events the following measures were determined:

Reaction time to brake (from when traffic light turns yellow)

Red/yellow light running

Event: crossing car

In this event the driver drives through an intersection with right of way when a car from the right crosses in front. The event takes place in an urban area so the crossing car is hidden for the driver by houses and the crossing car is triggered by Time to intersection = 4s. The measure here is:

Reaction time to brake (from when time-to-intersection = 4 s)

Event: approaching car

This event is the same as the crossing car but with the difference that the car stops just before crossing the path of the test driver. Measure:

Reaction time to brake (from when time-to-intersection = 4 s)

Event: road work

In this event the driver arrives at a road work where he has to use the oncoming lane to pass (see Figure 2). At the same time there is a queue of oncoming traffic with increasing time gap between the vehicles. Measure of interest here is what time gap the driver accepts for passing.

However, this event was removed from the analysis since almost all drivers waited until the whole queue of oncoming cars had passed.

Figure 2 The road work

Event: car following

Once the test drivers had driven the test route they stopped and the car following event started. The subjects' task was to follow a car in front of him at a safe and constant distance. The performance of the car-following-test is estimated by parameters suggested in Brookhuis et al. (2007).

Coherence: a measure of squared correlation indicating the accuracy of the drivers' speed adaptations, ranges from 0 (no coherence) to 1 (perfect coherence).

Gain: amplification factor between the two signals. If the subject overreacts to decelerations and accelerations, the gain will be larger than 1.

Delay: phase shift between the two signals. Indicates how long it takes the test car driver to react to the decelerations and accelerations of the lead car.

Statistical analysis

Overall treatment effects by means of superiority testing

To study the overall treatment effect, the data was analysed using the method of analysis of variance

(ANOVA) and the models were chosen to correspond with the design of the study.

The main objective of the study was to investigate the subjects driving performance, but we also studied how different doses of dextroamphetamine affected the subjects' self reported sleepiness.

The following factors are used in the models

α = dose (0 = placebo, 1 = 10 mg, 2 = 40 mg),

θ = blood concentration of dextroamphetamine (0, 0-20, 20-40, 40-60, 60-80, 80- ng/ml)

β = test occasion (1, 2 or 3)

γ = drive (0, SD1 and SD2, reflects three levels of sleep deprivation)

ζ = subject (1, …, 18)

All factors except for subject are regarded as fixed factors. Subject is regarded as a random factor.

Two different models are used

1. $Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \zeta_l + \alpha\gamma_{il} + \varepsilon_{ijklm}$

2. $Y_{ijklm} = \mu + \theta_i + \beta_j + \gamma_k + \zeta_l + \varepsilon_{ijklm}$

where μ is the mean effect and ε is an error term. Y could be either a driving performance indicator or KSS.

The difference between the two models is that actual concentration is used instead of dose in the second model. The reasons for introducing actual concentrations in addition to dose are that concentrations may differ between subjects even when they get the same dose and that the concentration of dextroamphetamine in the blood is not constant but follows a curve that first rises during the uptake and then falls.

Sleepiness is analysed by the first model and driving performance is analysed by both models. When analysing driver performance, occasion and drive reflects the learning effects due to repeated driving sessions. Moreover, drive also reflects the different states of alertness. Subject is entered to account for individual differences. The interaction $\alpha\gamma$ is in the model to reflect that there might be a different development of the blood concentration during the night for different doses. When blood concentration is entered instead of dose, there is no need for the interaction term since the different changes in concentration are directly reflected in the factor.

In Figure 3, it is illustrated how the actual blood concentration of dextroamphetamine varies between subjects for each dose. When the dose is 10 mg of dextroamphetamine, the concentration varies between almost 0 and 40 ng/mL and when the dose is 40 mg of dextroamphetamine, the concentration varies between 20 and 140 ng/mL. For the first drive, the blood concentration was measured both before and after the driving session and a mean of these concentrations were used in the analysis. For the second and third drive the blood concentration was only measured before the driving session.

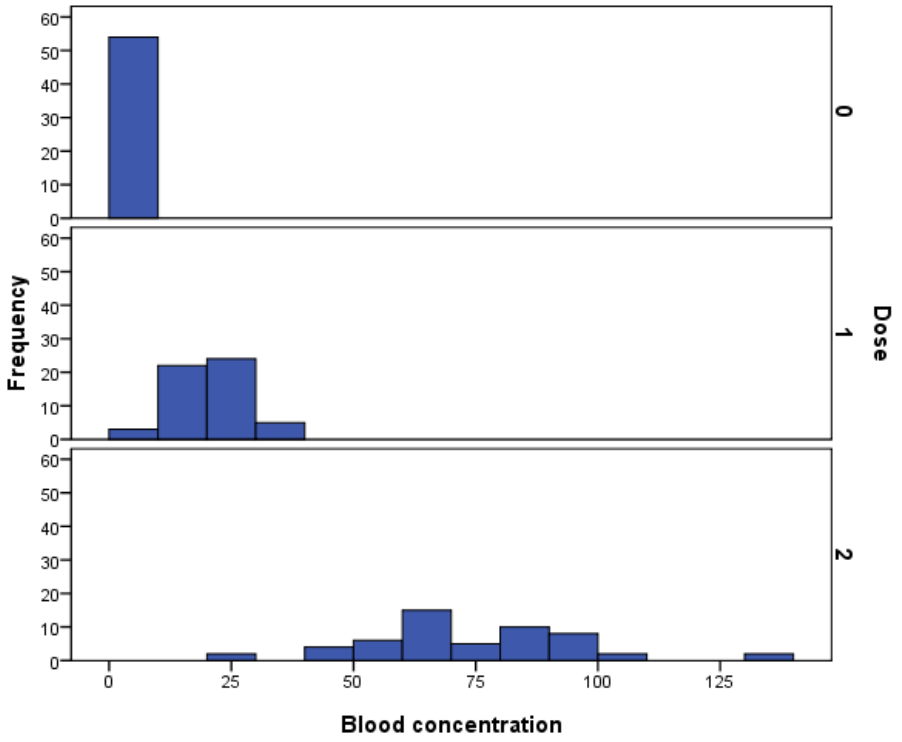

Figure 3: Distribution of blood concentration of dextroamphetamine for each dose (0, 1 = dose 10 mg, 2 = dose 40 mg).

Different performance indicators

To measure the driving performance for the different test subjects, a number of performance indicators are used as response variables, see section “Performance indicators and events” for a description. The performance indicators are divided into primary and secondary indicators and the primary indicators are studied in more detail. The primary performance indicators are: crossing car reaction time and SDLP at 70 km/h from the first part of the experiment and coherence, gain and delay from the car following event.

Pairwise comparisons

If the factor of interest: dose or blood concentration, is shown to be significant in the ANOVA analysis, pairwise comparisons between different levels of the factor are made. The comparisons are based on the estimated marginal means which compensate for an unbalanced design if that is the case. The Bonferroni adjustment for multiple comparisons is used.

Equivalence testing

To study similarities with driving under the influence of alcohol an equivalence testing of drug effects relative to an alcohol criterion are made. The alcohol comparison criteria was SDLP when driving with BAC 0,05 mg/ml. The drug effect was calculated as the difference between the effect when a dose

was given and the effect when placebo was given. Equivalence testing assesses whether the alcohol criterion values fall within the 95 % confidence interval for the drug effect. If yes, then the drug effect is equivalent to a BAC of 0,05 mg/ml (and thus relevant for traffic safety).

The effect on SDLP when driving with BAC 0,05 mg/ml was calculated in a previous alcohol calibration study described below:

The test was performed in VTI car driving simulator III and the simulated road was a single carriageway road with mild curvature. The road width was 11 m (each lane 3.75 m and hard shoulder 1.75 m on each side) and speed limit was 90 km/h. No other traffic was present.

Eight persons equally split by gender participated in the study. The subject had the following characteristics: mean age 29, driving licence held for at least five years, driving experience 10,000 - 50,000 km annually, in good health, no alcohol problems, alcohol habits average or less, not using medicines, not pregnant and no liability for motion sickness.

Three conditions were included: sober, low BAC, high BAC. BAC was measured indirectly with a breath alcohol analysis device (Alert J4X Digital Alco tester, Paramint AB). Measurements were taken before and after the test drives. The average value of the last measurement before the test drive and the first measurement after the test drive was used as the BAC value. It turned out that the average low BAC was 0.025 mg/ml and the average high BAC was 0.069 mg/ml (and, of course, 0.00 mg/ml in the sober condition).

A repeated measures design was used. Each participant drove the same road stretch three times, once when sober, once after having consumed a moderate amount of alcohol, and once after having consumed a large quantity of alcohol. The order between the three conditions was rotated across participants. The three test drives were performed on different days. When drinking alcohol, the next test drive was performed two days later. Otherwise, the time interval between conditions was one day.

Each test drive took 30 minutes to complete. The participants were informed that the speed limit was 90 km/h. They were instructed to drive as they would in a similar road and traffic environment.

Results regarding changes in SDLP are shown in Figure 4. When controlling for learning effects (Keppel, 1991), the effect of alcohol intake was significant ($F(2,12)=4.74$; $p<.05$). Pairwise comparisons (Tukey) showed that only one comparison turned out significant – that between sober and high BAC ($q=4.08$; $p<.05$).

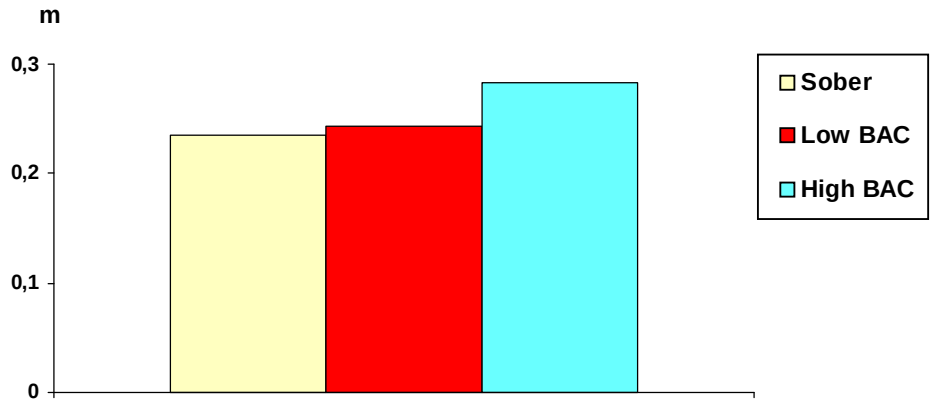

Figure 4: SDLP – effect of alcohol consumption

In order to estimate the size of the effect of alcohol intake at BAC = 0.05 mg/ml, a quadratic regression was performed on the data. The resulting function was: SDLP = .236 + .034 * BAC + .032 * BAC2. According to this function SDLP at BAC 0,0 will be 23.6 cm. SDLP at BAC 0.05 will be 26.1 cm. The effect of alcohol at BAC = 0.05 mg/ml is approximately 2.5 cm.

Concentration vs. change in SDLP

To study blood concentration of dextroamphetamine versus change in SDLP we made plots of changes in SDLP and conducted binomial tests of observations showing impairment versus no impairment. These comparisons were made for different combinations of dose and sleep deprivation as well as for different combinations of concentration class and sleep deprivation.

Results

Experiment completion

The whole experiment was carried out in three weeks starting each week at four o'clock Sunday afternoon and ending at eight o'clock Friday morning. Four subjects were present every night giving 20 subjects in total. There where however two subjects that dropped out after the first experiment. One of them suffered of simulator sickness and could not carry on. All subjects where screened for this but only during daytime. Most likely the sleep depravation and/or the medicinal drug triggered the simulator sickness. The other drop out chose to not complete the experiment and even though he did not need to motivate the decision he voluntarily explained that he couldn't take the needles. This was also screened for but only by asking the subjects. However, 18 subjects completed the whole experiment which was according to the original plan.

There is also a partial loss of data for two drivers who fell asleep during the car following even on the last drive when driving with placebo. Thus these two drives ended a few minutes earlier than planned.

Superiority testing

Effect of dose on sleepiness

Sleepiness was measured by the Karolinska Sleepiness Scale, KSS. The results from the analysis of variance showed a significant difference in sleepiness between different levels of dose, and also between different subjects and driving sessions (Table 2). The effect of different doses is shown in

Table **3**, and a higher dose leads to increased alertness. Pairwise comparisons showed a significant difference between all pairs of dose.

Table 2: Results from variance of analysis on sleepiness (KSS). P-values for tests of factor effects.

	Intercept	Dose	Occassion	Drive	Subject	Dose*drive
KSS before drive	<0.001	<0.001	0.07	<0.001	<0.001	0.53

Table 3: Mean level of sleepiness (KSS) for different doses of dextroamphetamine. The mean levels are adjusted for unbalance in the design.

	Dose		
	0 mg	10 mg	40 mg
Mean level	5.47	5.00	4.07

The effect of dose and drive is illustrated in Figure 5. We can see that an increased sleepiness is experienced during the night (drive increases from 0 to 1 and 2), and that the effect of dextroamphetamine is that the subjects experience a lower level of sleepiness.

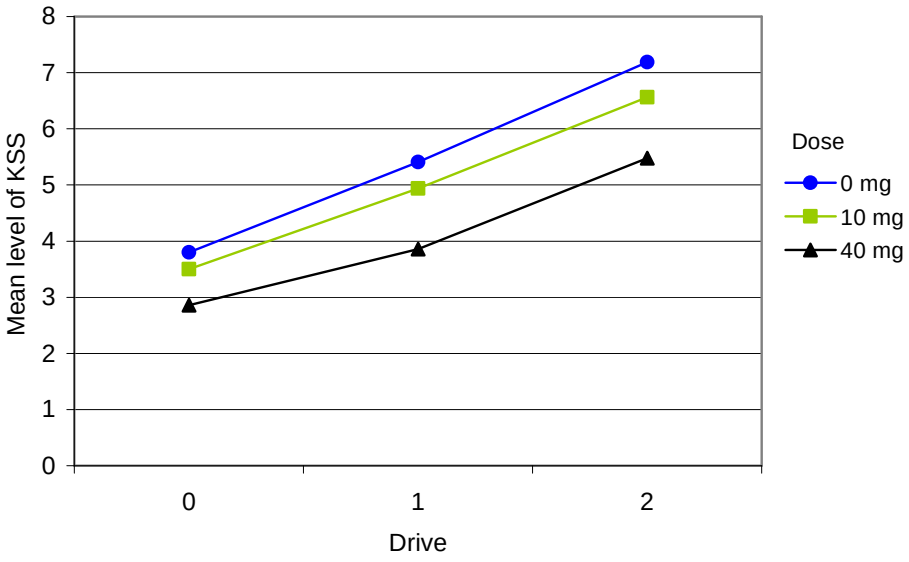

Figure 5: Mean levels of sleepiness (KSS) before each drive. The mean levels are adjusted for unbalance in the design.

Effect of dose on variables reflecting driver performance

The driver performance is studied by means of the 15 primary and secondary performance indicators described in section "Performance indicators and events".

Primary performance indicators

The results for the primary indicators and how they are affected by different doses of dextroamphetamine are summarised in Table 4 and Table 5. Table 4 shows the p-values for the tests of factor effects. Three of the primary performance indicators show a significant effect of dose. These indicators are crossing car reaction time and two of the indicators from the car-following event: coherence and delay. All indicators except gain show significant effects of drive. The most consistent result is the significant effect of subject for all variables. This was expected since we know from previous experience that there are large individual differences in driver performance. The mean levels for different doses and levels of sleep deprivation are shown in Table 5.

The mean reaction time for the event crossing car was 2.17 s when no dose was given and just below 2.0 s when 10 or 40 mg was given. The pairwise differences between dose 0 and 10 and between 0 and 40 are significant. Considering the performance indicator coherence, a significant difference between placebo and dose 10 mg was shown, with a lower value of coherence for placebo. The indicator delay (which reflects reaction time) also show a significant difference between placebo and dose 10 mg, with a higher value for placebo (see Table 5).

Table 4: Results from analysis of variance on driver performance for primary performance indicators. The analysis is based on the model with dose as a factor. P-values for tests of factor effects.

Response variable	Intercept	Dose	Occassion	Drive (sleep deprivation)	Subject	Dose*drive
Crossing car reaction time	<0.001	0.001	0.01	0.01	<0.001	0.95
Road 70 km/h, SDLP	<0.001	0.85	0.37	0.02	<0.001	0.36
Car-following: coherence	<0.001	<0.001	0.08	0.01	<0.001	0.23
Car-following: gain	<0.001	0.68	0.22	0.97	<0.001	0.81
Car-following: delay	<0.001	0.04	0.001	0.01	<0.001	0.89

Table 5: Mean levels (standard deviation) of driver performance measures (primary) for different doses of dextroamphetamine and different levels of sleep deprivation (alert, SD1, SD2). The mean levels are adjusted for unbalance in the design.

	Dose 0 mg			Dose 10 mg			Dose 40 mg		
	alert	SD1	SD2	alert	SD1	SD2	alert	SD1	SD2
Response variable									
Crossing car reaction time	2.30	2.14	2.07	2.08	1.91	1.95	2.05	1.94	1.87
	(0.07)	(0.08)	(0.07)	(0.08)	(0.08)	(0.08)	(0.08)	(0.08)	(0.08)
Road 70 km/h. SDLP	0.21	0.21	0.25	0.22	0.20	0.23	0.22	0.21	0.22
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Car-following: coherence	0.73	0.72	0.66	0.82	0.80	0.71	0.74	0.76	0.75
	(0.02)	(0.02)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Car-following: gain	1.07	1.04	1.09	1.06	1.08	1.04	1.07	1.09	1.09
	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)	(0.04)
Car-following: delay	3.89	4.04	4.57	3.05	3.24	4.07	3.72	3.40	4.29
	(0.33)	(0.33)	(0.39)	(0.31)	(0.31)	(0.33)	(0.32)	(0.32)	(0.32)

Secondary performance indicators

Results for the secondary performance indicators and how they are affected by different doses of dextroamphetamine are summarised in Table 6 and Table 7. Table 6 shows the p-values for the tests of factor effects and Table 7 show the mean levels. None of the secondary performance indicators show a significant effect of dose and only one significant effect of drive.

Table 6: Results from analysis of variance on driver performance for secondary performance indicators. The analysis is based on the model with dose as a factor. P-values for tests of factor effects.

Response variable	Intercept	Dose	Occassion	Drive (sleep deprivation)	Subject	Dose*drive
Bus min TTC	<0.001	0.14	<0.001	0.51	<0.001	0.21
Bus reaction time	<0.001	0.98	0.01	0.93	<0.001	0.84
Intersections: minimum speed	<0.001	0.42	<0.001	0.72	<0.001	0.16
Road 50 km/h, mean speed	<0.001	0.13	0.01	0.84	<0.001	1.00
Road 50 km/h, SDLP	<0.001	0.77	0.41	0.09	<0.001	0.59
Road 50 km/h, SD	<0.001	0.22	0.16	0.01	<0.001	0.97
Road 70 km/h, mean speed	<0.001	0.86	<0.001	0.14	<0.001	0.37
Road 70 km/h, SD	<0.001	0.55	0.18	0.21	<0.001	0.52
Speed passing moped_no wait	<0.001	0.93	0.56	0.65	<0.001	0.65
Traffic light reaction time	<0.001	0.66	0.52	0.09	<0.001	0.15

Table 7: Mean levels of driver performance measures (secondary) for different doses of dextroamphetamine. The mean levels are adjusted for unbalance in the design.

	Dose		
Response variable	0 mg	10 mg	40 mg
Bus min TTC	2.82	2.81	2.73
Bus reaction time	1.27	1.26	1.26
Intersections: minimum speed	38.99	39.08	38.30
Road 50 km/h, mean speed	56.70	55.75	57.60
Road 50 km/h, SDLP	0.20	0.19	0.19
Road 50 km/h, SD	2.20	1.93	2.08
Road 70 km/h, mean speed	75.39	75.22	75.03
Road 70 km/h, SD	2.34	2.59	2.55
Speed passing moped_no wait	61.70	61.56	62.10
Traffic light reaction time	1.13	1.11	1.15

Effect of blood concentration on variables reflecting driver performance

The driver performance was also analysed with respect to changes in actual blood concentration instead of dose.

Primary performance indicators

Results for the primary measures are shown in Table 8 and Table 9. The reason for introducing blood concentration was a concern that dose was not specific enough since the uptake of dextroamphetamine may vary between subjects, leading to different concentrations. Using actual concentration could therefore, possibly, reveal differences that were not seen between doses. However, the overall results did not change when blood concentration was used. As before, crossing car reaction time, coherence and delay showed significant effects (Table 8).

Table 8: Results from analysis of variance on driver performance (primary indicators) . The analysis is based on the model with concentration as a factor. P-values for tests of factor effects.

	Intercept	Concentration	Occassion	Drive (sleep deprivation)	Subject
Crossing car reaction time	<0.001	0.003	0.003	0.004	<0.001
Road 70 km/h, SDLP	<0.001	0.68	0.38	0.03	<0.001
Car-following: coherence	<0.001	0.006	0.05	0.03	<0.001
Car-following: gain	<0.001	0.08	0.27	0.93	<0.001
Car-following: delay	<0.001	0.05	0.001	0.02	<0.001

Table 9: Mean levels of driver performance measures for different concentrations of dextroamphetamine. The mean levels are adjusted for unbalance in the design.

	Concentration					
Response variable	0	0-20	20-40	40-60	60-80	80-
Crossing car reaction time	2.17	2.06	1.90	2.02	2.04	1.87
Road 70 km/h, SDLP	0.22	0.22	0.22	0.23	0.21	0.23
Car-following: coherence	0.70	0.75	0.79	0.75	0.77	0.74
Car-following: gain	1.06	1.04	1.07	1.06	1.17	1.03
Car-following: delay	4.18	3.67	3.33	4.43	3.38	3.81

Pairwise comparisons of crossing car reaction time showed a significant difference between blood concentration 0 (class 0) and blood concentration 20 - 40 (class 2) and between blood concentration 0 (class 0) and blood concentration 80 – (class 5), where the highest reaction time was found when the subject has taken placebo. For coherence only the difference between no concentration of dextroamphetamine in blood (class 0) and blood concentration 20-40 (class 2) was significant with lowest value of coherence (0.70) for class 0 compared to 0.79 for class 2. For the indicator delay no

significant pairwise difference was found.

Secondary performance indicators

Results for the secondary performance indicators and how they are affected by different blood concentration of dextroamphetamine are summarised in Table 10 and Table 11. Table 10 shows the p-values for the tests of factor effects and Table 11 show the mean levels. None of the secondary performance indicators show a significant effect of blood concentration.

Table 10: Results from analysis of variance on driver performance (secondary indicators). The analysis is based on the model with concentration as a factor. P-values for tests of factor effects.

	Intercept	Concentration	Occasion	Drive (sleep deprivation)	Subject
Bus min TTC	<0.001	0.71	<0.001	0.44	<0.001
Bus reaction time	<0.001	0.64	<0.001	0.92	<0.001
Intersections: minimum speed	<0.001	0.73	0.60	<0.001	<0.001
Road 50 km/h, mean speed	<0.001	0.56	0.008	0.66	<0.001
Road 50 km/h, SDLP	<0.001	0.86	0.40	0.09	<0.001
Road 50 km/h, SD	<0.001	0.22	0.12	0.004	<0.001
Road 70 km/h, mean speed	<0.001	0.78	<0.001	0.12	<0.001
Road 70 km/h, SD	<0.001	0.22	0.29	0.04	<0.001
Speed passing moped no wait	<0.001	0.71	0.48	0.63	<0.001
Traffic light reaction time	<0.001	0.32	0.54	0.10	<0.001

Table 11: Mean levels of driver performance indicators (secondary) for different concentrations of dextroamphetamine. The mean levels are adjusted for unbalance in the design.

	Concentration					
Response variable	0	0-20	20-40	40-60	60-80	80-
Bus min TTC	2.82	2.79	2.80	2.68	2.73	2.78
Bus reaction time	1.27	1.22	1.32	1.14	1.26	1.28
Intersections: minimum speed	38.99	39.54	38.73	38.39	38.56	37.90
Road 50 km/h, mean speed	56.70	55.36	56.37	56.32	57.73	57.82
Road 50 km/h, SDLP	0.20	0.19	0.19	0.21	0.18	0.20
Road 50 km/h, SD	2.20	2.00	1.85	1.68	2.09	2.27
Road 70 km/h, mean speed	75.37	74.80	75.71	75.84	74.31	75.09
Road 70 km/h, SD	2.35	2.22	3.00	2.19	2.27	2.82
Speed passing moped_no wait	61.63	61.03	62.39	61.46	59.81	64.05
Traffic light reaction time	1.13	1.10	1.12	1.02	1.20	1.15

Equivalence testing

The results of the equivalence testing are shown in Figure 6 and Table 12. The diagram in Figure 6 shows 95 % confidence interval for the drug and sleep deprivation effect and a reference line for BAC 0.05 mg/ml (alcohol criterion) based on a previous study (Table 12 shows the actual values). Since the alcohol criterion falls within the 95 % CI it is indicated that the drug effect might be relevant for traffic safety. On the other hand, we have not shown that the differences differs significantly from zero in any situation other then when driving in severe sleep deprivation in combination with placebo (alert + SD2), so the results are not clear.

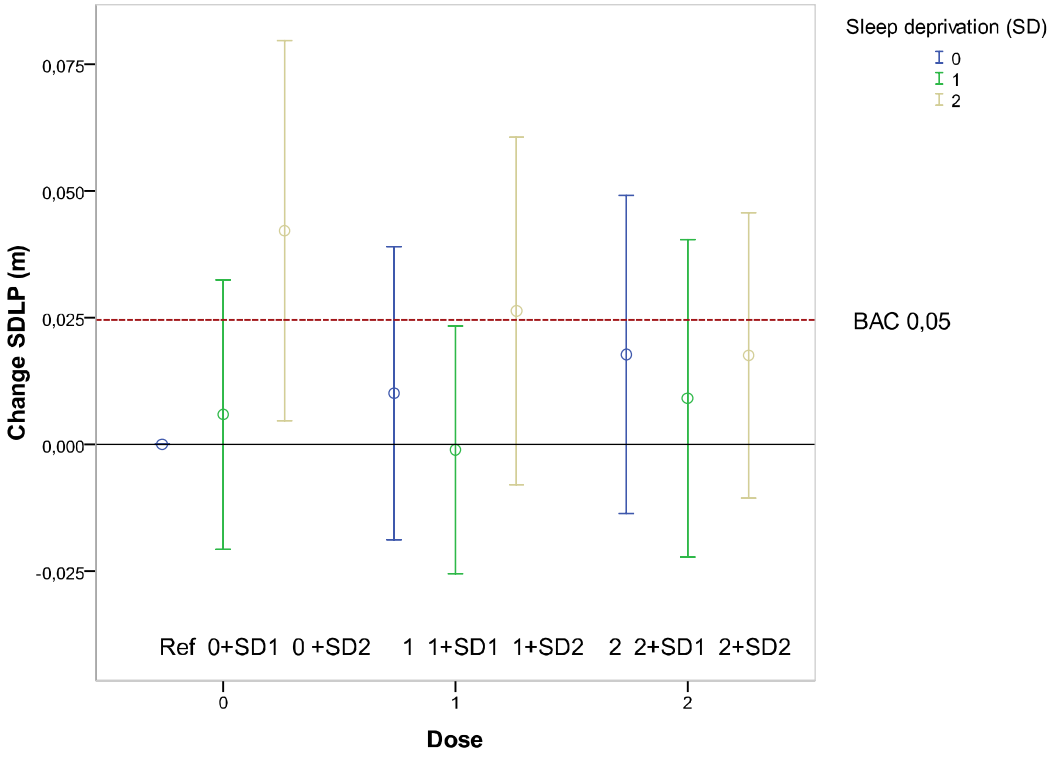

Figure 6 : 95 % confidence intervals for the change in SDLP for different levels of sleep deprivation (alert, SD1 and SD2) and different dose (0=placebo, 1 = 10 mg and 2 = 40 mg).

Table 12: Means and 95 % confidence intervals for change in SDLP for different dose (0=placebo, 1 = 10 mg and 2 = 40 mg) and different levels of sleep deprivation (alert, SD1 and SD2).

Level	0 & SD1	0 & SD2	1 & alert	1 & SD1	1 & SD2	2& alert	2 & SD1	2 & SD2
Mean	0.006	0.042	0.010	-0.001	0.026	0.018	0.009	0.017
CI	(-0.019, 0.031)	(0.007, 0.077)	(-0.017, 0.037)	(-0.024, 0.022)	(-0.006, 0.058)	(-0.011, 0.047)	(-0.020, 0.038)	(-0.009, 0.044)

Serum concentration vs. change in SDLP

In Figure 7 change in SDLP (relative each individuals state with placebo and no sleep deprivation) versus actual blood concentration are shown. No clear pattern can be seen, though there is a tendency that there are more positive than negative values.

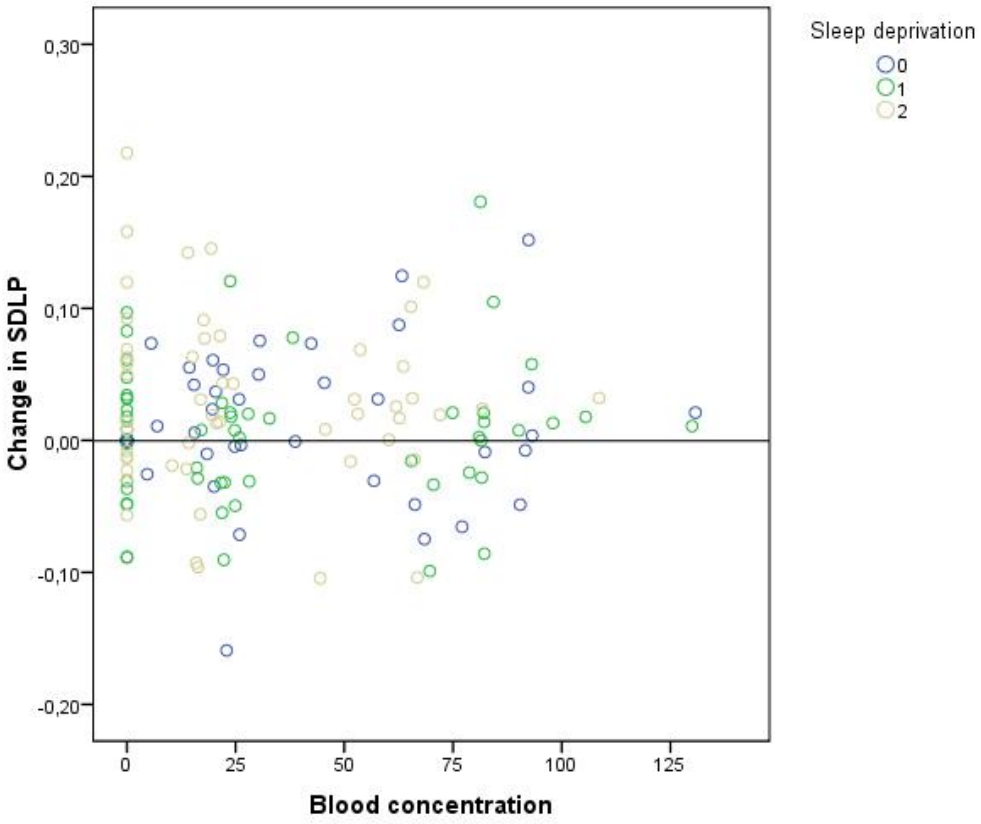

Figure 7: Change in SDLP versus blood concentration for different levels of sleep deprivation

Results from Binomial tests for separate levels of dose and sleep deprivation are shown in Table 13. Corresponding results for concentration classes instead of dose are shown in Table 14.

Only the situation with high dose and a severe level of sleepiness shows a significant result (Table 13).

Table 13: Binomial tests for separate levels of dose and sleep deprivation. The level specifies levels of different dose (0=placebo, 1 = 10 mg and 2 = 40 mg) and sleep deprivation (0, SD1 and SD2).

Level	0 + SD1	0 + SD2	1+alert	1 + SD1	1 + SD2	2+alert	2 + SD1	2 + SD2
Proportion	0.61	0.65	0.61	0.56	0.67	0.67	0.61	0.78
> 0								
p-value	0.48	0.33	0.48	0.82	0.24	0.82	0.48	0.03

The binomial test for separate levels of blood concentration and sleep deprivation do not show any significant result (Table 14). It should be noted that when the data is divided into concentration classes instead of doses, the same person may appear more than once in a class. This will affect the p-values which are calculated based on the assumption of independence. However, in this case, since we did not found any significant results, it should not have affected the interpretation of the overall results.

Table 14: Binomial tests for separate levels of blood concentration and sleep deprivation. The level specifies levels of blood concentration (0-40, 40-80, >80) and sleep deprivation (alert, SD1 and SD2).

Level	0-40	0-40	0-40	40-80	40-80	40-80	≥80	≥80	≥80
	alert	SD1	SD2	alert	SD1	SD2	alert	SD1	SD2
Proportion	0.60	0.58	0.66	0.56	0.20	0.75	0.57	0.77	1.0
> 0									
N	20	36	35	9	5	16	7	13	2
p-value	0.50	0.41	0.09	1.0	0.38	0.08	1.0	0.09	0.50

Results from the questionnaires

Final "open" questionnaire

The subjects' experiences of the medicine.All subjects could correctly state at what occasion they were given the high dose. 16 out of 18 could also distinguish the low dose from placebo. The experience of the medicine differed somewhat among the subjects. Several described that they felt "high": they became alert, talkative, social and excited. However, some described the effect as positive – they felt relaxed and comfortable – while others felt restless. Almost all subjects thought the medicine made them less drowsy. A few stated that the medicine only caused an absence of drowsiness, without increased alertness or any other effects. The experiences of the low dose were about the same as of the high dose, but the effects were much weaker and lasted for a shorter period of time. Common side effects of the drug were dry mouth/throat, reduced appetite and a tingling sensation in the body.

About a third of the subjects didn't believe that the medicine had any influence on their driving performance. Some subjects commented that since the medicine made them alert they probably drove better after intake of the medicine compared to while driving in a sleep deprived condition, but they didn't think the medicine lead to increased risk taking or impaired driving performance. Other subjects described positive effects. They meant that the medicine made them more attentive, focused and alert. During the high dose drive, some subjects had a feeling that their driving performance was really good. However, afterwards they had doubts and were afraid that their driving performance might not have been that good, after all. About a fourth of the subjects thought that the medicine had a negative influence on their driving. They meant that the high dose lead to increased speed and a risky and egocentric behaviour.

More than half of the subjects had difficulties to fall asleep the day after the high dose occasion. Some felt drowsy for a couple of days following the test, mainly after the high dose. Some reported that the dry mouth/throat and reduced appetite remained also on the day after the test. Two subjects got stomach-ache. About a third of the subjects didn't feel any medicine effects at all the day after the test.

The subjects' experiences of the simulator trial/ experiences of the scenario

The general view about the scenario is that it was quite monotonous, boring and after a few drives also very predictive. Several of the subjects thought that the events were realistic but they learnt fast where to be attentive and how to act in different events. Some subjects stated that they had expected a larger variation and less predictive events. Some thought the scenario was realistic but many also noted deficiencies, for example the motion of other vehicles, the moped driver not wearing a helmet, no other vehicles in the own lane and a truck driving through a car (which caused the subject to brake since he expected an accident!).

How the subjects changed their behaviour as their experience of the simulator and the road increased. Some meant that they became more relaxed and less attentive. Some also stated that they drove faster. Several mentioned that they learnt quickly what events to expect and how to act in the events. About a third of the subjects claimed that they didn't change their behaviour much at all, but some of them added that they might have driven a little more carefully in the beginning, before they got used to the simulator.

About using tactics during the drives

About a third of the subjects claimed that they didn't use any particular tactics and/or that they endeavoured to drive as they would in real life. Another third reported that they learnt where to expect events and consequently, they became extra attentive in some situations and more relaxed where they knew nothing would happen. Some subjects mentioned that they used some tactics in specific situations. One subject found out at what speeds to drive in the car following event. Another thought it was easier to maintain a constant distance to the lead car if the distance was short. One subject tried to fight his drowsiness by decreasing the temperature and adjusting the seat into an uncomfortable position. Yet another one stepped on the gas in order to reach the road work and moped before the

oncoming traffic (without success). One subject drove faster the two last nights. A few mentioned that they tried to brake carefully in order to avoid large simulator movements.

The subjects' experiences of the simulator drivings(tough, boring, motion sickness etc)

A common opinion was that the driving was okay but boring and monotonous. Five subjects felt motion sick and a few others reported dizziness. Some subjects commented on technical issues (see below).

Comments on the simulator technology

Some subjects were very positive about the simulator and thought it was realistic. There were also a lot of comments on different aspects of the technology. About half of the subjects thought that the motion of the car – especially at braking and acceleration – was not very realistic. Others mentioned the graphics, both technical aspects such as resolution and oddities in the scenario such as wheels that are not moving, vehicles without drivers and incorrect signposting. Several subjects complained about the climate control – it was too cold in the car. Some mentioned difficulties in estimating distances since the simulator scenario is not in 3D.

About the subjects talking to each other about the simulator driving

There have been some discussions about the moped and road work events, but the general opinion is that these discussions did not have any influence on the subjects' driving. There have also been discussions about the simulator technology, the medicine and if someone for example fell asleep during the drive.

About the contact with the VTI staff

All subjects were satisfied with the VTI staff.

About the information given prior to the test

One subject had wanted more information about the aim of the study. The others thought that the information was sufficient.

About contact with the nurses

The nurses have been highly commended by the subjects. Some subjects experienced minor problems with the blood sample collection (difficulties with needle insertion, bruises).

About the wait between the drives

Most of the subjects thought the wait was okay. Some thought it was long-winded and boring. The internet access was appreciated. Some had wanted a TV, a (functioning) DVD player and newspapers. Several subjects mentioned the good food that was served.

About the meaningfulness in participating in the study

Most subjects thought their participation was meaningful (no one gave a negative answer). Several subjects thought it was interesting to drive while very drowsy. Others mentioned that it was interesting to see how they reacted to the medicine. Some were curious on the simulator and others mentioned that they have got knowledge that can be useful in their profession. Some thought it felt meaningful to contribute to the research.

About the reasons to participate

More than half of the subjects stated that money was (one of several or the only) reason to participate in the study. Others gave interest in research, the simulator or the medicine as reason. Some mentioned that they wanted to get a better understanding of professional drivers' situation.

Interest in getting information after the test

Several subjects wanted to know at what occasion they got what dose. Also quite many wanted to know about their driving performance. There is also an interest in getting the results from the study.

Interest in participating in other tests

All subjects were interested in participating in other tests.

Other comments

One subject will ask the physician about long-term side effects of the medicine. Two subjects wondered why they didn't have to show any identity card to the test leader prior to the test. One subject wants to know if the remuneration is exempt from tax. One subject would have wanted a medicine that neutralized the effect of the amphetamine after the test.

Discussion/conclusion

The aims of the present study were to assess the effects of dextroamphetamine on fundamental driving parameters during simulated driving and to assess the interaction between the stimulant drug and sleep deprivation.

First of all, the results showed that participants in the study felt more alert when taking a dose of dextroamphetamine than when taking placebo and the effect was stronger for the higher dose. This is in line with what could be expected of this substance. However, the data did not show any evidence that taking dextroamphetamine successfully prevented the subjects from becoming sleepy during the night, there were no significant interaction between time of drive and dose with respect to self rated sleepiness.

The results for driving performance indicators were less clear. A significant main effect of dose (placebo, 10 mg and 40 mg of dextroamphetamine) were found for three out of the five primary indicators. Taking the lower dose leads to an improved driving with respect to crossing car reaction time, coherence and delay, the results for the higher dose is mixed (sometimes driving is improved compared to placebo and sometimes it is about the same). There were no significant effects of dose

on any of the ten secondary performance indicators.

Regarding sleep deprivation (alert, slightly sleep deprived, sleep deprived), a main effect was found for four of the primary indicators and three of the secondary indicators. The results showed overall impaired driving with respect to for example SDLP and delay when the sleep deprived conditions were compared to the alert condition. However, improved driving was also found, with respect to crossing car reaction time.

It is important to note that no interactions were found between dose and sleep deprivation for any of the performance indicators. For the indicators where sleep deprivation lead to impaired driving, this means that there were no evidence in our data that the impaired driving was compensated by taking a dose of dextroamphetamine.

The results did not change when actual blood concentration was considered instead of dose.

There are several circumstances that may have affected the results of the experiment. For example, the subjects drove the same route ten times (three conditions times three levels of sleep depravation plus one familiarization drive). There was thus a large risk for learning effects and the analyses also showed a learning effect from week to week on several performance indicators. This effect might also have affected the sleep deprivation condition, that is when the driver became more and more sleep deprived he or she also got more familiar with the driving scenario.

Apart from the learning effect there was also large individual variation in how the subjects responded to the treatment which further complicated the analysis. This was expected since we know from previous experience that there are large individual differences in driver performance. This is also reflected in the comments from the questionnaire made by subjects:

- ”I felt focused, attentive and alert”
- ”I didn’t feel anything at all”
- ”I felt hyperactive and drove badly”

It should also be noted that the doses used were medicinal doses which are expected to effect driving ability but not to the same level as if abuse doses were used.

An equivalence test was made to study whether the combined effect of drug and sleep deprivation was comparable to a BAC of 0.5 with respect to SDLP. The results showed that the 95 per cent CI included the alcohol criterion as well as zero. This indeed indicates a large variation between individuals. For the placebo condition, the confidence interval for sleep deprivation did not include zero and could therefore be interpreted as comparable to a BAC of 0.5.

To summarize there where few significant results and they showed both improved and worsened driving performance. The effects are however rather small and one could speculate that simple tasks such as a pure reaction task is improved (i.e. crossing car reaction time) while for more complex tasks where the situation demands assessment like the bus event performance there is no improvement. It is however impossible to go further than a speculation based on this study.

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Chapter 5

Effects of dexamphetamine with and without alcohol on simulated driving performance

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Abstract

Objectives: The aim of the study was to assess the effects of dexamphetamine with and without alcohol on simulated driving and driving-related cognitive performance.

Method: in a randomized, cross-over, placebo-controlled study, 18 subjects were administered each of the following treatments: 10 mg dexamphetamine+0.8 g/kg alcohol, 10 mg dexamphetamine+alcohol-free drink, 0.8 g/kg alcohol+placebo, and placebo+ alcohol-free drink. A driving simulator was used to assess fundamental driving skills, risk taking behaviour and situation awareness. Driving-related performance was assessed using vigilance and divided attention tasks and subjects completed rating scales on sleepiness, driving quality, mental effort, and mood.

Results: Mean BAC-levels during the simulated driving were 0.91 ‰ at the start and 0.64 ‰ at the end of the test. Mean Standard Deviation of Lateral Position (SDLP) was significantly larger in the alcohol than in the placebo condition. Accepted gap time was significantly shorter and accepted gap distance was significantly smaller in the alcohol condition. Subjects using alcohol also showed significant higher average and maximum speed and violation of speed limits. Similar to the alcohol condition, a significant higher percentage of subjects in the dexamphetamine+alcohol condition did not stop for the red traffic lights, or collided onto a vehicle. Performance of vigilance and divided attention tasks was significantly impaired in the alcohol condition and impaired to a lesser degree in the dexamphetamine+alcohol condition. Dexamphetamine did not significantly affect primary and secondary measures driving performance when given alone, although some trends towards improvement could be observed.

Conclusion: 0.8 g/kg Alcohol significantly affected driving skills at control level (SDLP), strategical level (speed), and manoeuvring level (gap acceptance, reaction to traffic light, violations). Ingestion of 10 mg dexamphetamine alone had no significant effect on traffic safety parameters and no evidence was found that addition of 10 mg dexamphetamine to alcohol potentiates the effects of alcohol on risk taking behaviour. Red-light running and collisions were significantly more frequently observed in the alcohol and alcohol+ dexamphetamine conditions. The findings of the present study justify the conclusion that drivers using 0.8 g/kg alcohol, or the combination of dexamphetamine with alcohol, pose a considerable traffic safety risk.

Introduction

The road transport system is one of the most hazardous and most expensive in terms of human lives in the European Union. There is substantial evidence that consumption of psychoactive substances such as alcohol, recreational drugs and several prescription and "over-the-counter" drugs may endanger traffic safety (e.g. Movig et al., 2004; Ramaekers, 1998). Driving under the influence of alcohol and/or drugs is considered to be a major cause for road accidents (Movig et al., 2004). The combination of an increasing frequency of drug and medication use in Europe and an ever increasing traffic complexity and density may further compromise future traffic safety. In order to find solutions to tackle this complex problem, it is important to gain a better knowledge of the various aspects of this problem. The European project DRUID (Driving under the Influence of Drugs, Alcohol and Medicines) has been implemented to find answers to questions concerning the use of drugs or medication that affect people's ability to drive and is geared to provide scientific support to the EU transport policy, which was aimed at a 50 % reduction in the number of road fatalities in the EU by the year 2010.
The aim of DRUID is to establish guidelines and providing measures to combat impaired driving under the influence of drug, alcohol and/or medication. In this context 15 reference studies of the impact on fitness to drive for alcohol, illicit drugs and medicines will be performed in work package 1 of DRUID.

The present report describes the results of one of these intervention studies performed by TNO. In this study the effects of 10 mg dexamphetamine, 0.8 g/kg alcohol, and the combination of 10 mg dexamphetamine and 0.8 g/kg alcohol on driving behaviour and driving-related cognitive functioning have been assessed using a driving simulator.

Rationale

Alcohol

The effects of alcohol on driving performance are well documented and impaired performance has been evidenced for doses as low as 0.3‰ (for a review see Moskowitz and Robinson, 1986). For the effects of alcohol on driving behaviour, a huge body of empirical results and theoretical considerations has been accumulated over the past 100 years. Additionally, the effects of different legal thresholds on driving safety are well understood. An alcohol dose of 0.8 g/kg is likely to result in a BAC of around 0.1 %, which is considerably higher than the limits used in Road Traffic legislation. The expected effects of such alcohol dose on driving abilities are sedation and consequently lowered vigilance, impaired lane keeping, and risk taking behaviour.

Dexamphetamine

There is limited research available on the effects of dexamphetamine on in situ driving. In epidemiological studies, Logan (1996) and Logan et al. (1998) noted that typical driving behaviours observed in drivers under the influence of methamphetamine, which is twice as potent as dexamphetamine, include drifting out of lane, erratic driving, weaving, speeding, drifting off the road, an increase in risk taking, and high-speed collisions. There have been no experimental studies of

dexamphetamine employing simulated driving. In contrast to epidemiological and simulated driving studies, there is a considerable body of knowledge concerning the effects of amphetamines on cognition and behaviour. With regard to the effects on risk-taking behaviour, the literature is contradictory. Some studies have reported decreases in impulsive behaviours following acute doses of 10 or 20 mg dexamphetamine (de Wit et al. 2002) and others have shown increases in impulsive behaviours (Hurst et al. 1967; Evenden and Ryan, 1996). Although, there is a demonstrable relationship between amphetamine and impulsivity, it is clearly complex and dose-, situation-and/or population specific (Silber et al. 2005). Concerning cognitive performance, the literature indicates that, at lower doses, dexamphetamine appears to improve performance on some cognitive processes. De Wit et al. (2002) found that 10 mg and 20 mg dexamphetamine improved performance on measures of vigilance and memory, and decreased several forms of impulsive behaviour. Ward et al. (1997) found that 5 mg and 10 mg/70 kg dexamphetamine increased response rate on the Digit Symbol Substitution task (DSST) without affecting accuracy. These findings suggest that low doses dexamphetamine may improve driving ability. However, dexamphetamine-induced deficits on divided attention tasks have been reported by Mills et al. (2001), who found that 10 mg dexamphetamine induces 'tunnel vision', a phenomenon in which attentional processes become overwhelmed, producing a decrease in an individual's ability to gather information efficiently. This might be dangerous when driving as it increases the risk of failing to attend to potential hazards that fall outside of the driver's attentional focus. Higher doses of dexamphetamine are likely to cause clearer and more unequivocal effects. Therefore, studies of the behavioural effect of dexamphetamine often used doses higher than 10 mg. E.g. Silber et al. (2005) used oral doses of 25-35 mg and Asghar et al. (2003) used 25 mg. In both studies no health-threatening adverse effects have been reported. The effects of an oral dose of 10 mg dexamphetamine are less unequivocal and subject to inter-individual variability (Newhouse et al. 1989; Mills et al. 2001; Holdstock and de Wit, 2001).

Combination of Alcohol and dexamphetamine

In party circuits, relatively high doses of dexamphetamine are frequently used in combination with high doses of alcohol. There are no clear data concerning the effects of this combination on driving ability. One of the scarce studies of the effects of dexamphetamine and alcohol relevant for driving behaviour has been performed by de Wit and Richards (2000). They assessed the effects of 0.2, 0.4, and 0.8 g/kg ethanol, or 10 and 20 mg d-amphetamine on impulsivity and decision making, using a 'Stop Task' paradigm, which provides a measure of reaction time (RT) needed to inhibit a response. Dexamphetamine improved inhibition, only in subjects with a slow baseline RT, and 0.4 and 0.8 g/kg alcohol impaired inhibition. They concluded that dexamphetamine and alcohol have specific and distinctive effects on the ability to inhibit responses. In a within-subjects study, Holdstock and de Wit (2001) used 10 or 20 mg dexamphetamine or 0.8 g/kg alcohol, but not the combination, and found that subjects who experience pronounced stimulant-like effects from alcohol also report greater stimulant effects from dexamphetamine. In both studies no health threatening adverse effects have been reported.

Because data on the effects of a combination of dexamphetamine and alcohol are scarce and

inconsistent, and because the effects of the separate substances are variable, effects of this combination on simulated driving performance are difficult to predict. On a theoretical basis, it is expected that dexamphetamine may reduce sedation and impairment of vigilance caused by alcohol, but may potentiate risk-taking behaviour and impaired judgement, when 0.8 g/kg alcohol and 10 mg dexamphetamine are taken in combination. The combination may also potentiate 'tunnel vision' and deficits in divided attention.

Dosage of dexamphetamine

Amphetamine produces indirect sympathetic activation by releasing norepinephrine, dopamine, and serotonin from terminals in the central and autonomic nervous system. Amphetamine toxicity is potentiated when it is taken with alcohol (Ghuran and Nolan, 2000; Mendelson et al. 1995). Sympathetic activation may lead to varying degrees of tachycardia, vasoconstriction, unpredictable blood pressure effects, and arrhythmias depending on the dose given (Ghuran and Nolan, 2000). Moderate to higher doses of alcohol, such as 0.8 g/kg, may potentiate some adverse effects of dexamphetamine, particularly increase of heart rate and blood pressure and cardiac arrhythmia risk (Higgins et al. 1988; Mendelson et al. 1995; Ghuran and Nolan, 2000). Therefore, it was decided that in the present study 1) the lowest relevant dose of dexamphetamine (10 mg) was to be used in the combination with 0.8 g/kg Alcohol, 2) subjects were monitored using a 1-lead electrocardiogram (ECG) for arrhythmia detection, and 3) that a qualified physician was be present during the trials.

Aim of the study

The aim of the present study was to assess the effects of 10 mg dexamphetamine, 0.8 g/kg alcohol, and the combination of 10 mg dexamphetamine and 0.8 g/kg alcohol on driving behaviour and driving-related cognitive functioning, using a driving simulator.

Method

Subjects

Eighteen subjects participated in the study. Participants had to be recreational users of alcohol and amphetamine-like substances and had to have a driving license and two years of driving experience. Subjects were recruited by advertisements in the Trimbos institute and from the pool of volunteers of TNO Defense, Safety and Security, location Soesterberg and TNO Quality of Life, location Zeist. All subjects signed an informed consent form and participated on a voluntary basis and knew that they could withdraw at any moment of the study. Participants received a financial remuneration. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht.

Pre-study screen, eligibility and selection

The volunteers had a pre-study screening before the start of the treatment period. The pre-study screening involved:

- An interview (anamnesis) with the medical investigator about the medical history based on the completed health and lifestyle questionnaire

- A physical examination (including Blood pressure and an ECG)
- Pregnancy testing in urine
- Pre-testing in the driving simulator (simulator sickness was an exclusion criterion)
- Training of the cognitive tests

Based on the results of the pre-study screening, the medical investigator established the eligibility.

Inclusion criteria

- Healthy as assessed by the
    - health and lifestyle questionnaire
    - physical examination
    - results of a standard 12-lead ECG
- Males/Females aged ≥ 20 and ≤ 40 years at day 01 of the study
- Previous experience with the recreational use of alcohol and dexamphetamine or amphetamine derivatives
- Normal weight and BMI (≥ 18 and ≤ 28 kg/m2)
- Having a driving license and two years of driving experience
- Voluntary participation
- Having given written informed consent
- Willing to comply with the study procedures
- Willing to accept use of all nameless data, including publication, and the confidential use and storage of all data for at least 15 years
- Willing to accept the disclosure of the financial benefit of participation in the study to the authorities concerned.

Exclusion criteria

Subjects with one or more of the following characteristics were excluded from participation:

- Participation in any clinical trial including blood sampling and/or administration of substances up to 6 weeks before Day 01 of this study
- Having a history of medical or surgical events that may significantly affect the study outcome, including psychiatric disorders, cardiovascular disease and/or hypertension (systolic > 160 mmHg and diastolic > 95 mmHg).
- History of drug abuse or addiction
- Using psychotropic medication
- History of malignant hyperthermia /serotonin syndrome
- Susceptibility to simulator sickness (subjects were pre-tested in simulator)
- Heavy smoking: during the visit at TNO smoking is restricted to one break.
- Alcohol consumption (> 28 units/week for males and > 21 units/week for females)
- Pregnant or lactating or wishing to become pregnant in the period of the study
- Personnel of TNO Defence, Safety and Security, their partner and their first and second

degree relatives

- Not having a general practitioner
- Not willing to accept information transfer concerning participation in the study, or information regarding his/her health, like laboratory results, findings at anamnesis or physical examination and eventual adverse events to and from his general practitioner.

Design

The study was a double blind, placebo controlled study for dexamphetamine using a randomized cross over repeated measures design. Although everything possible has been done to blind the administration of alcohol, it is considered that alcohol can not be orally administered or consumed in a completely blinded fashion.

Study treatments

- 10 mg Dexamphetamine + alcohol-free drink (= AMP)
- 0.8 g/kg Alcohol + matching placebo for dexamphetamine (= ALC)
- 10 mg Dexamphetamine + 0.8 g/kg alcohol (= AMP+ALC)
- Matching placebo for dexamphetamine + alcohol-free drink (= PLA)

Wash-out period between the study conditions was 7 days. In order to standardize circadian influences, each subject was assessed at the same time of day in each condition.

Procedure

One week before the first trial day the subjects have been trained to perform the driving task in the simulator and the cognitive tasks. Subjects were instructed to abstain from alcohol and/or psychoactive drugs in the 24 hours prior to each trial day and to use no more than 2 caffeinated drinks from 12 h prior to each test session

After arrival on each of the four trial days, subjects were screened for alcohol in breath and for drugs in urine (Triage ® 8). Also the pH of the urine was measured. Subjects were questioned about sleep the day/night before, health and fitness, and abstinence from alcohol/drugs and more than 2 caffeinated drinks 12 h prior to the test session.

All study sessions took place in the afternoon/early evening between 14:00 and 22:00 hrs. In between the test sessions subjects were allowed to watch television or read and have a light snack. After the last test session a meal was provided. The subjects had to stay at TNO until the alcohol promillage was below 0.2 ‰. Dexamphetamine has a long half life (12 h), therefore heart rate, blood pressure, fitness, and alertness were assessed by a physician, who decided whether a subject could go home or not. If necessary, subjects would be brought to their homes or kept longer at TNO.

Administration of dexamphetamine and ethanol

The effect of ethanol is felt very fast, however the Cmax of 10 mg dexamphetamine is estimated to be 2.6 ± 2.5 h after ingestion (De Wit et al., 2000). Because it was pursued that the Tmax would cover the 50 min simulated driving test, the best time for testing the driving capabilities was considered to be approximately two hours after ingestion of dexamphetamine. Time of ingestion of

dexamphetamine was then defined as t=0.
The ethanol ingestion has been titrated to be 1‰ on t=120 minutes according to the method described in De Wit et al (2000):

t=0	orange juice + 10 mg d-amphetamine or placebo
t=41 min	0.2 g/kg ethanol in orange juice
t=73 min	0.2 g/kg ethanol in orange juice
t=105 min	0.4 g/kg ethanol in orange juice

Experimental Time Schedule
Each trial day tests were performed at the following experimental times:

T= -10	baseline session for VigTrack vigilance task
T=0	ingestion of 10 mg dexamphetamine or placebo
T=120-170	simulated driving test
T=180-200	cognitive tasks, rating scales
T=240-260	cognitive tasks, rating scales
T=300	end of study sessions

Apparatus
Participants were required to complete test-rides in a moving-base driving simulator consisting of a BMW316 car with original controls linked to a dedicated graphics computer, registering driver behaviour while computing the road environment and dynamic traffic at high rate (see annex A for technical specifications). Participants had a 180 º view of the road environment. Other vehicles in the simulated world interacted with the simulator car autonomously and behaved according to hierarchically structured decision rules that are based on human driving behaviour.

Driving tasks

A scenario as developed and used by the University of Groningen (Veldstra et al., 2010) was used to test the effects anticipated to occur after alcohol and dexamphetamine consumption. A graphical outline of the scenario is presented in annex B. The simulated ride had duration of 50 minutes and was varied in road type (urban, rural, highway) and traffic density (normal, high and low traffic density). At the start of the ride on each of the four trial days participants were instructed to drive straight on unless instructed otherwise. To reduce predictability of critical events four parallel scenarios were used: in each treatment condition the subject started at a different point in the virtual environment (out of four defined standard points) and encountered events at a different point.
During the ride performance has been assessed by measuring skills at a strategical level (speed), manoeuvring level (gap acceptance, car following, reaction to traffic light and critical situations), and control level (lateral and longitudinal control). A calibration study using the identical scenario has been performed by Veldstra et al. (2010) and detailed information on the driving task scenario is described

in their report. In the context of the present study we therefore refer to that report. The driving scenario included the following features:

Road tracking task

In this part of the virtual ride, the participant drives trough a rural area with moderate density traffic flow and a posted speed of 100 km/h. The participant is asked to drive as she/he would normally do (no specific instruction). How the participant manages this part of the route is assessed by measuring the SDLP.

Car following

The participant had to respond to the speed changes of the lead car. Performance was measured by assessing distance headway (DHW), which was calculated as the distance between the follow car and the lead car from bumper tot bumper and Time Headway (THW) calculated as the time interval between the two cars. Furthermore, average, SD, and minimum of time-to-collision (TTC), speed, and lateral position were measured.

Traversing unsignalised crossroads

In the virtual driving environment there are several scenarios used to measure the driver's ability to safely traverse a crossing; the gap acceptance scenario and the scenarios described under violations of traffic laws. The latter are scenarios that measure both the ability to safely traverse a crossing and the ability to comply with traffic regulations.

Gap acceptance

The parameters included to assess the drivers risk taking are: the size of the accepted gap in seconds and the distance to the car approaching the driver while traversing the crossing.

Violating traffic regulations

In the virtual driving environment two types of violations are assessed; violating the posted speed limit, failing to give right-of-way, overtaking via the emergency lane and running a red light.

Reaction to unexpected events

In the virtual driving environment three scenarios are used to measure the driver's reaction to unexpected events; a car failing to give way, a car suddenly is pulling out of a car park, and cars suddenly coming to a standstill on the motorway. How the driver handles these situations was assessed by measuring the deceleration and the minimal time to collision (TTC, Van der Horst, 1990).

Driving on the motorway

There are two general indicators of driving style that have been linked to accident risk when driving on a motorway with traffic, namely: speed and headway (Ward et al., 2003). With Headway choice the

tendency to leave short headway distances to the vehicle in front is measured as an indication of risky driving. In the virtual driving environment the participants are faced with several scenarios: first the participants have to filter into traffic to get onto the motorway, second they are driving on the motorway with normal density traffic for 15 km when suddenly the traffic comes to a standstill, from this point on the participants are driving in congested traffic for 10 km, the last 5 km is normal driving again until the participants finally leave the motorway.

Normal highway driving

The participant has to drive in normal traffic density on a two-lane motorway with a 120 km/h speed limit. Some of the other traffic participants are programmed to keep to the exact posted speed limit, some drive below or above the posted speed to simulate the natural situation. How the participants deal with this situation is assessed by measuring the number of times they change lanes and the mean and minimal time headway (THW) the participant holds to other traffic participants.

Quantitative measures of cognitive functioning

Critical Tracking Task (CTT)

The CTT measures the subject's ability to control a displayed error signal in a 1st-order compensatory tracking task. Error appears as horizontal deviation of a cursor from midpoint on a horizontal, linear scale. Compensatory joy-stick movements null the error by returning the cursor to the midpoint. The frequency of cursor deviations, and therefore its velocity, increases as a stochastic, linear function of time. The subject is required to make compensatory movements with a progressively higher frequency until the subject loses the control. The frequency at which control loss occurs is commonly called lambda-c (the critical frequency). The reciprocal of this frequency is theoretically the perceptual/motor delay lag for humans operating in closed-loop systems. The subject performs this test in five trials on each occasion and the median lambda-c is recorded as the final score (Jex et al., 1966). Total time of the test is 5 minutes

Divided Attention Task (DAT)

The divided attention task assesses the ability to divide attention between two tasks performed simultaneously. The primary task requires the use of a joystick to continuously null the horizontal movement of a cursor from the center of a display. The cursor travels in both directions with irregular velocity, on the average, 50% of that which is just controllable by the particular subject. Tracking error is measured by the absolute distance (mm) between the cursor's position and the center. The latter subtask involves monitoring 24 single-digit numbers (0-9) that are arranged around the display's periphery. The numbers change asynchronously every 5 seconds. The requirement is to react as rapidly as possible by lifting the foot from a pedal anytime a target, the numeral "2", appears. Average reaction time to targets is recorded as the second response measure (Moskowitz, 1973). Total duration of the test is 12 minutes.

Psychomotor Vigilance Task (PVT)

The psychomotor vigilance task assesses the reaction time in response to a visual stimulus (visual RT). This visual stimulus is a counter in the center of a computer screen that goes from 0 to 60 in 60 seconds (fixed inter-stimulus interval=1 ms). The counter starts at random intervals, i.e. between 2 and 10 seconds, and the subject has to react to the onset of the counter as quickly as possible by pressing a response button. Duration of the task is 10 minutes (Loh et al., 2004).

Vigilance and Tracking test (VigTrack)

The VigTrack task is a dual-task measuring vigilance performance under the continuous load of a compensatory tracking task. The task will be done on a PDA and is based on an earlier version performed on a Psion 3a handheld computer (Valk et al., 1997). This task has been successfully applied in field studies concerning effects of fatigue and sleepiness in pilots, laboratory studies on sedative effects of antihistamines, and residual effects of alcohol. During the tracking task, subjects have to steer a blue dot using a stylus and a compass-card, so that it is kept below a red dot in the centre of the display. The blue dot is programmed to move continuously from the centre of the display. While tracking, subjects have to perform the vigilance task. Inside the red dot a black square alternates with a diamond once a second. In some cases a hexagon is presented. If this is the case, subjects have to respond as quickly as possible by pressing a response button. The duration of this test will be 5 minutes and performance measures include root mean square tracking error, percentage omissions, number of false reactions, and reaction times.

Selective attention task from the usual field of view (UFOV)

In this test an object in the center of the computer screen is displayed. The object in the center is either a car or a truck. Simultaneously a car is displayed in the periphery. The car in the periphery is embedded in a field of 47 triangles or distracters. The next screen will question the subject about which object was displayed and also where the outside car was located. The length of the stimulus presentation will automatically adjust: after a correct answer it is shortened and it is lengthened after an incorrect answer. The average exposure duration at which the examinee can perform the task correctly 75% of the time is presented at the end of the test. Duration of the test is 3 minutes.

Subjective report measures

Profile of Mood States (POMS)

The POMS is a self-assessment mood questionnaire with 72 five-point Likert scale items, representing eight mood states, i.e. Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness and Elation. The subject had to indicate in how far these items are representing his/her mood (de Wit et al. 2002).

Rating Scale Mental Effort (RSME; Zijlstra, 1993)

On this scale participants had to indicate the amount of effort they invested in the driving task. The scale ranges from 0=absolutely no effort to 150=more than extreme effort. This scale is presented in

annex C.

Driving Quality Scale (DQS; Brookhuis et al., 1985)

On this scale participants had to judge the quality of their driving performance. It is a Visual Analogue Scale between the endpoint “I drove very badly” and “I drove very well”. The scale is presented in annex D.

Karolinska Sleepiness Scale

The KSS is a subjective rating scale with scores that range from 1 to 9. The rate is reported as an absolute rate, which means that the subject has to report the experienced sleepiness during a specific preceding time period, e.g. 10 minutes.

Annex E shows the KSS rating scale as modified by Reyner & Horne (1998), which was the version used in the present study.

Statistical analysis

All statistical analyses were conducted by means of Statistica Data Analysis Software (StatSoft®). Treatment effects were investigated with repeated measures ANOVA. If the analysis of variance indicated a condition effect ($p < 0.05$), comparisons between conditions means of the parameters was performed using planned comparison, or Tukey HSD tests. Relationships between different variables and methods were investigated by using correlational computations (Pearson product-moment or Spearman Rank correlation coefficients). Data were corrected for outliers, who were not replaced. Missing values were only replaced with the mean of the subject over all conditions when data of three of the four conditions were available. In all statistical tests performed, a significance level of 0.05 was used.

Results

Subjects

18 Subjects started in this study. During the study, there were two drop-outs: one subject felt sick on the first trial day and did not wish to continue the study and one subject withdrew due to medical reasons not-related to the study. Drop-outs were not replaced.

Data sets of 16 volunteers (4 female and 12 male) were available for analysis.

Mean age of the subjects was 25.7 yr (range 21-37) and mean weight was 76.1 kg (range 50.1-106.6). On average, subjects held a driving licence for 4.3 years and drove 5600 km/yr on average.

Blood alcohol concentration (BACs)

Alcohol levels were measured in breath by the Dräger Alcotest 6510 (Lubeck) and transformed to BAC (table 1).

Condition	T=120 ‰ (range)	T=170 ‰ (range)	T=200 ‰ (range)	T=240 ‰ (range)	T=260 ‰ (range)
DEX+ALC	0.91 (0.63-1.22)	0.64 (0.47-0.77)	0.55 (0.39-0.66)	0.35 (0.22-0.58)	0.23 (0.10-0.43)
ALC	0.85 (0.61-1.13)	0.64 (0.46-0.77)	0.57 (0.41-0.64)	0.37 (0.18-0.51)	0.26 (0.08-0.41)

Table 1. *Blood alcohol concentrations (‰) and ranges ( ) measured at the start of the driving test (T=120), at the end of the driving test (T=170), at the end of the first cognitive test session (T=200), at the start of the second cognitive test session (T=240), and at the end of that session (T=260). DEX+ALC: dexamphetamine+alcohol condition; ALC: alcohol+placebo condition.*

d-Amphetamine concentration

Prior to the simulated driving test, 115 minutes after administration of the d-amphetamine or placebo, 10 mL blood was taken by venapuncture to determine d-amphetamine levels. Levels of d-amphetamine in blood and plasma have been analyzed by Prof. Gisela Skopp, University of Heidelberg (table 2).

	T=115		
Condition	Dried Blood Spot ng/mL (range)	Blood ng/mL (range)	Plasma ng/mL (range)
DEX+ALC	21.8 (10.9-38.2)	20.7 (11.9-39.1)	24.1 (13.2-41.1)
DEX	21.8 (13.2-43.9)	20.8 (11.8-40.7)	22.6 (14.2-40.3)

Table 2. *Concentrations (ng/mL) and ranges ( ) of d-amphetamine in the conditions dexamphetamine + alcohol (DEX+ALC) and dexamphetamine + alcohol-free drink (DEX).*

Primary driving tasks

Road tracking – SD of Lateral Position

The SDLP has been assessed during monotonous driving on a road where the speed limit was 80 km/h and monotonous driving on the motorway (speed limit 100 km/h). Three subjects were excluded from the analysis due to off-road driving, or inconsistency of their SDLP data (1 ALC, 1 DEX+ALC, 1 PLA). Mean SDLP was 32% (4.9 cm) higher in the alcohol condition (ALC) than in the placebo condition (PLA). The differences between the alcohol and the placebo condition were significant at 80 km/h ($F_{1,12}$=7.12; p=0.020) as well as at 100 km/h ($F_{1,12}$=6.49; p=0.026). SDLP in the alcohol condition was also higher than in the dexamphetamine (DEX) and dexamphetamine+alcohol (DEX+ALC) conditions, but these differences did not reach statistical significance. Results are presented in table 3. There was no significant DEXxALC interaction effect (table 4). Equivalence testing of treatments is presented in tables 5 and 6: mean differences between ALC and PLA are within 95% CI limits of the other treatments.

	Mean ±SD per treatment (cm)			
	DEX	DEX+ALC	ALC	PLA
SDLP 80 km/h	17.8 ±5.6	17.2 ±5.3	20.3 ±8.1	15.4 ±2.7
SDLP 100 km/h	18.8 ±7.9	17.7 ±8.2	20.1 ±6.8	15.2 ±4.1

Table 3. *Mean Standard Deviation of the lateral position (SDLP) and its standard deviation (SD) measured during monotonous driving on roads where the speed limit was 80 km/h and 100 km/h respectively. Daylight conditions and no other traffic on the road.*

ANOVA – differences with PLA

	ALC	DEX	DEXxALC
SDLP 80 km/h	<.05	ns	ns
SDLP 100 km/h	<.05	ns	ns

Table 4. *Superiority testing of differences with placebo. Significance is indicated by p-values*

SDLP - 80 km/h (m) – differences with placebo scores

	DEX	DEX+ALC	ALC
mean	0,0242	0,0184	0,0496
standard deviation	0,0515	0,0630	0,0670
median	0,0107	0,0024	0,0433
95% CI lower	-0,0070	-0,0197	0,0091
95% CI upper	0,0553	0,0565	0,0901

Table 5. *Equivalence testing. SDLP scores: differences with placebo at 80 km/h.*

SDLP-100 km/h (m) – differences with placebo scores

	DEX	DEX+ALC	ALC
mean	0,0363	0,0257	0,0494
standard deviation	0,0781	0,0974	0,0699
median	0,0326	0,0220	0,0171
95% CI lower	-0,0109	-0,0332	0,0071
95% CI upper	0,0834	0,0845	0,0916

Table 6. *Equivalence testing. SDLP scores: differences with placebo at 100 km/h.*

Road tracking – Speed

Driving on a road where the speed limit was 50 km/h showed a treatment effect concerning average speed (trend: F3,36=1.92; p=.072), the SD of speed (F3,36=3.02; p=.042), and the maximum speed (F3,36=3.86; p=.017). Subjects using ALC drove with higher average speed (56 km/h) than those using DEX or PLA (p<.05), had a higher SD of speed than in the PLA condition (p<.05), and drove with a higher maximum speed (63 km/h) than in the DEX and PLA conditions.

A treatment effect was found for the S.D. of speed when driving on a road with a speed limit of 80 km/h (F3,39=4.05; p=.01). Subjects showed significantly higher SD of speed when using DEX/ALC

than in the other treatments (p<.05).When driving on the motorway with a speed limit of 100 km/h subjects with DEX+ALC treatment showed higher S.D. of speed than in other treatment conditions (F3,39=3.47; p=.0025).

Time to Line Crossing (TLC) and number of Line Crossings

No significant differences could be demonstrated between the treatment conditions concerning the average and minimum TLC, or the number of line crossings.

Gap acceptance

There were two gap acceptance challenges in a village environment (speed limit 50 km/h) in which participants: 1) had to traverse a normal junction and were faced with traffic coming from the left and right side (gap LR) 2) had to cross upcoming traffic at a Y-junction (gap Y). In both gap acceptance challenges a significant treatment effect was found concerning accepted gap time (F3,42=5.13; p=.0041 and F3,42=3.88; p=.016 respectively) and accepted gap distance (F3,42=3.87; p=.016 and F3,42=4.41; p=.0087 respectively). Compared with the other treatments, accepted gap time and accepted gap distance were significantly shorter when subjects had used ALC treatment, except for DEX+ALC treatment which showed no significant difference with ALC concerning accepted gap distance (fig. 1 and 2; table 7).

In both gap acceptance challenges significant DEXxALC interaction effects were observed concerning minimum accepted gap times (F1,14=13.03; p=0.0028 and F1,14=10.84; p=0.0053) and accepted gap distances (F1,14=8.27; p=0.0122 and F1,14=8.73; p=0.0104) (table 8).

Mean ±SE per treatment

	DEX	DEX+ALC	ALC	PLA	P
Gap LR – time (sec)	6.7* ±0.4	6.1 ±0.4	5.3 ±0.2	6.6* ±0.5	<.01
Gap LR – distance (m)	57.0* ±6.5	47.7 ±7.7	31.6 ±4.5	52.2 ±6.5	<.05
Gap Y – time (sec)	5.5* ±0.1	5.5* ±0.2	4.7 ±0.3	5.3 ±0.3	<.05
Gap Y – distance (m)	43.9* ±5.1	37.7 ±5.9	24.1 ±4.3	42.6* ±5.6	<.01

Table 7. *Means of accepted gap time and accepted distance to car in two gap acceptance challenges (Gap LR and Gap Y). * Asterisks indicate comparisons of ALC treatment with other treatments to which the p-values apply.*

ANOVA – differences with PLA

	ALC	DEX	DEXxALC
Gap LR – time	<.05	ns	<.01
Gap LR – distance	<.05	ns	<.05
Gap Y – time	<.05	ns	<.01
Gap Y – distance	<.05	ns	<.05

Table 8. *Superiority testing of differences with placebo. Significance is indicated by p-values*

Equivalence testing (tables 9, 10, 11, and 12) shows that the mean differences between ALC and PLA are outside the 95% CI limits of DEX and DEX+ALC, except for DEX+ALC at the normal junction with traffic coming from the left and right side (gap LR).

Gap Time (s) – LR differences with placebo scores

	DEX	DEX+ALC	ALC
mean	0,07	-0,53	-1,33
standard deviation	1,33	1,73	1,99
median	0,00	0,00	-1,00
95% CI lower	-0,67	-1,49	-2,43
95% CI upper	0,81	0,42	-0,23

Table 9. *Equivalence testing LR challenge: accepted gap time (s) -differences with placebo.*

Gap Time (s) – Y differences with placebo scores

	DEX	DEX+ALC	ALC
mean	0,27	0,27	-0,53
standard deviation	1,28	1,10	1,06
median	0,00	0,00	0,00
95% CI lower	-0,44	-0,34	-1,12
95% CI upper	0,98	0,88	0,05

Table 10. *Equivalence testing Y-junction challenge: accepted gap time (s) - differences with placebo.*

Gap Distance (m) – LR differences with placebo scores

	DEX	DEX+ALC	ALC
mean	4,79	-4,50	-20,67
standard deviation	26,62	35,38	26,33
median	-0,26	-7,52	-19,48
95% CI lower	-9,95	-24,09	-35,25
95% CI upper	19,54	15,10	-6,08

Table 11. *Equivalence testing LR challenge: accepted gap distance (m) - differences with placebo.*

Gap Distance (m) - Y differences with placebo scores

	DEX	DEX+ALC	ALC
mean	1,30	-4,93	-18,57
standard deviation	22,70	24,07	19,39
median	2,37	-9,35	-14,90
95% CI lower	-11,26	-18,25	-29,31
95% CI upper	1,30	-4,93	-18,57

Table 12. *Equivalence testing Y-junction challenge: accepted gap distance (m) - differences with placebo.*

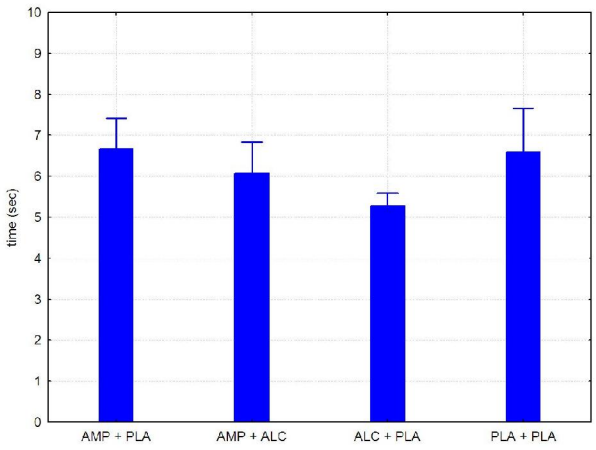

Figure 1. Gap acceptance with traffic coming from left and right side (Gap LR): mean accepted gap time (s). Whiskers denote 0.95 confidence intervals.

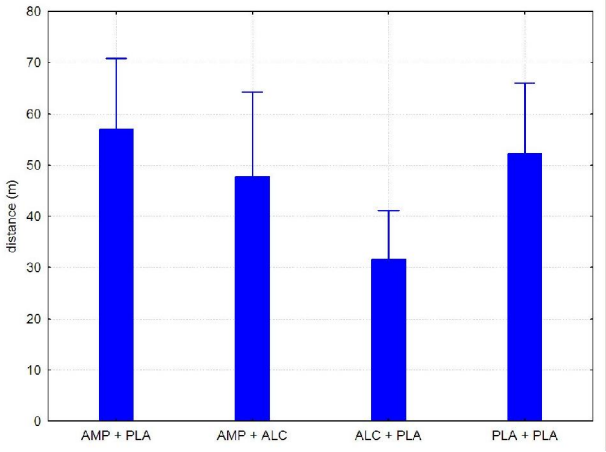

Figure 2. Gap acceptance with traffic coming from left and right side (Gap LR): mean accepted gap distance (m). Whiskers denote 0.95 confidence intervals.

Traffic lights

At two traffic lights (at the 3rd and 8th junction respectively) where the drivers had to stop for the red light, drivers in the DEX+ALC and the ALC conditions passed the red traffic light without stopping in

64% and 59% of the cases, while in the DEX and PLA conditions drivers did not stop in 30% of the cases (chi-square =10.8496, df=3, p= .0126). Concerning the differences with placebo, a significant DEXxALC interaction effect was found (F1,13=6.30; p=0.026), while the mean difference between ALC and PLA was within 95% CI limits of the other treatments (table 13).

Red Traffic Light running (#) - differences with placebo scores

	DEX	DEX+ALC	ALC
mean	0,036	0,321	0,321
standard deviation	0,535	0,372	0,464
median	0,000	0,250	0,500
95% CI lower	-0,274	0,106	0,533
95% CI upper	0,354	0,536	0,590

Table 13. Equivalence testing concerning red traffic light running: number (#) of violations at two traffic lights - differences with placebo.

Car following

There were two occasions where car following behavior was assessed. On a road where a speed limit of 80 km/h applied, participants had to follow a car which accelerates and decelerates in a programmed frequency. On the second occasion a challenge similar to car following occurred at the highway but here the participant had to react to the brake lights. Distance headway (DHW) was calculated as the distance between the follow car and the lead car from bumper tot bumper and Time Headway (THW) as the time interval between the two cars. During both challenges, no significant differences could be demonstrated between the treatment conditions concerning average, S.D., and minimum of time-to-collision (TTC), headway (DHW), time headway (THW), speed, and lateral position.

Giving right-of-way

In this scenario the participants were driving on a road towards a normal junction at a posted speed of 50 km/h. The driver coming from the right has priority over the participant. A treatment effect was found when approaching the junction where the subject had to give way (F3,36=5.53; p=.0032). Subjects using ALC had significantly higher maximum speed approaching the junction than in the DEX (p<.05), DEX+PLA (p<.01), and PLA (p<.05) condition. There were only two cases in which no right of way was given (min. velocity >8.5 m/s, braking percentage <5, and deceleration <1 m/s2). There were no significant differences between treatments concerning the number of participants coming to a standstill.

Stop sign

A treatment effect was found when approaching the stop sign (F3,39=3.13; p=.04). Subjects showed significantly higher S.D. of speed when using ALC than in the PLA or DEX treatment conditions

(p<.05). They also had a higher speed approaching the stop sign, but this was not statistically significant. There were no significant differences between treatments concerning the number of participants coming to a standstill.

Car pulling out of a parking

The reaction to a car suddenly pulling out of a parking lay was assessed by measuring the maximal deceleration and the time to collision (TTC). Max. deceleration was higher in the PLA condition (8.8 ± 2.2 m/s2) than in the DEX (5.5 ± 2.4 m/s2), DEX+ALC (6.1 ± 2.2 m/s2), and ALC condition (4.5 ± 2.2 m/s2), however the differences were not statistically significant. The minimum TTC was lower in the DEX condition (0.9 ± 0.2 s), than in the DEX+ALC (1.6 ± 0.2 s), ALC (1.4 ± 0.2 s), PLA condition (1.1 ± 0.2 s). Differences were not significant (F3,29=2.19; p=.11).

Filtering into traffic

Subjects had to filter into the motorway traffic between two trucks. Data of ramp entry velocity, velocity when merging, merging distance, and THW to the lead truck when merging is presented in table 14. There was no statistically significant effect of treatment condition.

	Mean ±SE per treatment			
	DEX	DEX+ALC	ALC	PLA
Ramp entry velocity (m/s)	85.9 ±12.3	68.9 ±11.3	78.2 ±11.3	70.2 ±11.4
Velocity when merging (m/s)	104.1 ±8.7	83.0 ±8.0	88.8 ±8.0	87.3 ±8.0
THW lead when merging (s)	3.44 ±0.74	1.65 ±0.65	1.43 ±0.65	0.79 ±0.64
Merging distance (m)	154.5 ±15.9	105.7 ±14.6	123.2 ±14.6	125.2 ±15.2

Table 14. *Filtering into traffic. Significance levels Ramp entry velocity: F3,46=0.44 p=.725; Velocity when merging: F3,46=1.18 p=.328; THW lead: F3,45=2.55 p=.067; Merging distance: F3,46=1.72 p=.18.*

Normal Highway Driving

Although the posted speed limit was 120 km/h, participants in the ALC condition had an average speed of 123 km/h, while in the other conditions the average speed was between 116 and 120 km/h. Differences were however not statistically significant. There were also no significant effects on the number of lane changes and mean THW that was held to other road users.

Accidents and dangerous actions

When encountering a traffic jam at the highway, traversing unsignalised crossroads, or filtering into the traffic some participants collided onto a vehicle. In total there were 18 collisions, which were

distributed as follows:

- dexamphetamine (DEX): 1 case
- alcohol + dexamphetamine (DEX + ALC): 6 cases
- alcohol (ALC): 8 cases
- placebo (PLA): 3 cases

Compared with placebo, significantly more accidents were observed in the ALC and the DEX+ALC conditions. A significant DEXxALC interaction effect was found (F1,13=6.06; p=0.029)). The mean difference between ALC and PLA was within the 95% CI limits of the DEX+ALC treatment, indicating that the effect of DEX+ALC is comparable to the effects of ALC on the number of collisions (table 15).

In two cases, both in the alcohol (ALC) condition, participants overtook a car by driving over the emergency lane, instead of taking over via the left lane.

Collisions (#) - differences with placebo scores

	DEX	DEX+ALC	ALC
mean	-0,071	0,214	0,429
standard deviation	0,475	0,579	0,852
median	0,000	0,000	0,500
95% CI lower	-0,345	-0,120	-0,063
95% CI upper	0,203	0,549	0,920

Table 15. *Equivalence testing concerning the number (#) of collisions - differences with placebo.*

Cognitive Tasks

The first test sessions of the cognitive tasks were performed 10 minutes after completion of the simulated driving task (T=180) and a second session was performed one hour later (T=240). Each session included the complete set of tasks. The sequence of the different tasks was balanced over the subjects to minimize order effects. A baseline session (prior to driving) was performed for the VigTrack only. The first cognitive test session was performed with mean BACs between 0.64 and 0.55 ‰, while mean BACs during the second session were between 0.37 and 0.23 ‰.

Critical Tracking Task (CTT)

The critical tracking frequency showed no significant differences between the treatments.

Divided Attention Task (DAT; fig.3 and 4).For each treatment the mean of the results of the two sessions was used to perform the statistical analysis of differences between treatments. Results of the separate sessions are presented in table 16. Performance on the DAT was significantly impaired for subjects using ALC. Compared to the other conditions, they showed a larger tracking error (fig. X; F3,39=5.15; p=.0043), higher reaction time (F3,39=4.04; p=.0136), higher number of missed targets (F3,39=5.05; p=.0047), a lower number of hits (F3,39=5.05; p=.0047), higher number of control losses (fig. XY; F3,39=6.39; p=.0013), and more false alarms (F3,39=4.04; p=.0136). The number of false

alarms was higher when subjects used DEX+ALC compared to DEX (p<.05).

Correlations between DAT scores and BACs or dexamphetamine levels in blood ranged from r=.15 to r=.21 and did not reach statistical significance.

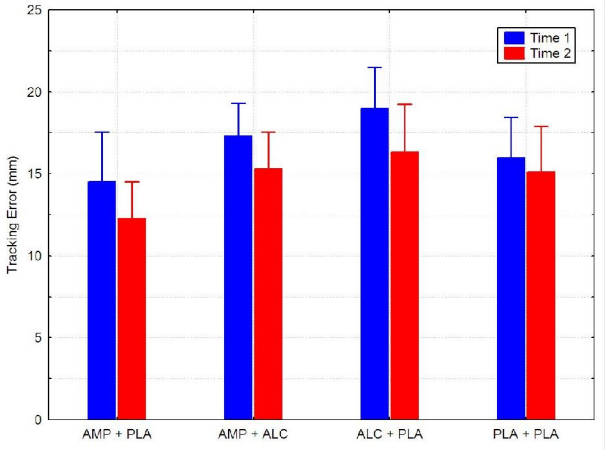

Figure 3. Divided Attention Time: mean tracking error (mm) in the first (Time 1) and second (Time 2) session. Whiskers denote 0.95 confidence intervals.

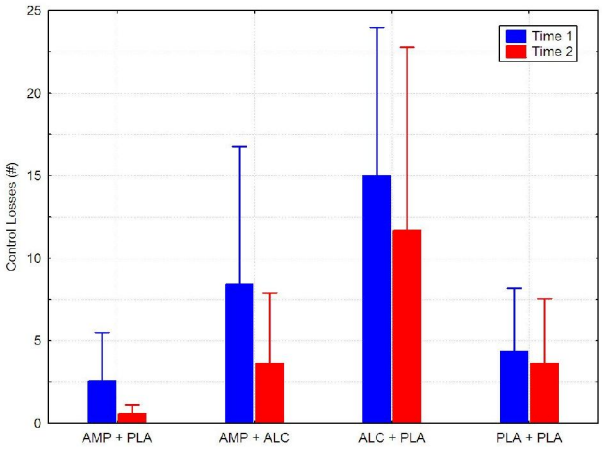

Figure 4. Divided Attention Time: mean number of control losses in the first (Time 1) and second (Time 2) session. Whiskers denote 0.95 confidence intervals.

	DEX Mean ±SE	DEX+ALC Mean ±SE	ALC Mean ±SE	PLA Mean ±SE
DAT 1 – Mean BAC 0.6 ‰				
tracking error (mm)	14.5 ±1.4	17.3 ±0.9	19.0 ±1.2	16.0 ±1.2
reaction time (msec)	1766 ±93	1818 ±107	2071 ±124	1931 ±110
# hits	44.8 ±1.1	42.6 ±2.4	41.1 ±2.3	44.4 ±1.4
control losses	2.6 ±1.3	8.4 ±3.9	15.0 ±4.2	4.4 ±1.8
missed	3.2 ±1.1	5.4 ±2.4	6.9 ±2.3	3.6 ±1.4
false alarms	1.1 ±0.4	3.3 ±1.1	3.1 ±1.1	1.4 ±0.4
DAT 2– Mean BAC 0.3 ‰				

tracking error (mm)	12.3 ±1.4	15.3 ±1.0	16.3 ±1.3	15.1 ±1.3
reaction time (msec)	1759 ±111	1776 ±95	1842 ±84	1863 ±103
# hits	45.4 ±1.1	43.5 ±1.7	39.9 ±2.9	42.7 ±2.1
control losses	0.6 ±0.3	3.6 ±2.0	11.7 ±5.1	3.6 ±1.8
missed	2.6 ±1.0	4.5 ±1.7	8.1 ±2.9	5.3 ±2.1
false alarms	1.1 ±0.4	2.1 ±0.5	1.7 ±0.4	1.9 ±0.4

***Table 16**. Divided Attention Task: scores of all variables during the first session (DAT 1) and second session (DAT 2) and mean BAC levels.*

Psychomotor Vigilance Task (PVT)

For each treatment the mean of the results of the two sessions was used to perform the statistical analysis of differences between treatments.

The number of lapses of attention was highest when subjects had used ALC (trend: $F_{3,42}=2.75$; $p=.055$). The ALC treatment showed significantly more lapses than DEX treatment ($p<.05$). There were no significant differences between treatments with regard to mean reaction time. No significant correlations were found between PVT scores and BAC or dexamphetamine levels in blood.

Vigilance and Tracking test (VigTrack; table 17; fig. 5 and 6). Scores on this task were calculated as difference scores compared to the scores obtained in the baseline session prior to treatment administration (T=-10). Subjects using ALC showed significantly impaired vigilance performance compared to the other conditions. This was signified by a larger tracking error ($F_{3,45}=5.01$; $p=.0044$), longer reaction times ($F_{3,45}=8.38$; $p=.00016$), and a higher percentage of omissions (trend: $F_{3,45}=2.55$; $p=.067$). Tracking performance of subjects using ALC showed 50% impairment compared to baseline values. Subjects using dexamphetamine alone (DEX) showed the best performance, although this was not statistically significant.

Correlations between VigTrack scores and BACs or dexamphetamine levels in blood ranged from r=.13 to r=.25 and did not reach statistical significance.

	DEX Mean ±SE	DEX+ALC Mean ±SE	ALC Mean ±SE	PLA Mean ±SE
VigTrack 1– Mean BAC 0 ‰				
RMS tracking error	20.5 ±3.0	18.8 ±2.3	19.8 ±3.8	19.3 ±2.2
reaction time (msec)	686 ±22	674 ±17	676 ±15	689 ±19
% correct responses	98.3 ±1.1	96.9 ±2.3	96.9 ±2.2	98.6 ±0.7
VigTrack 2– Mean BAC 0.6 ‰				
RMS tracking error	20.9 ±3.1	25.9 ±3.4	30.1 ±4.9	23.7 ±3.5
reaction time (msec)	711 ±26	747 ±29	802 ±31	733 ±27
% correct responses	97.4 ±1.2	94.1 ±2.6	90.9 ±2.9	95.8 ±2.1
VigTrack 3– Mean BAC 0.3 ‰				
RMS tracking error	19.2 ±2.8	21.9 ±3.1	29.8 ±5.3	20.8 ±3.0
reaction time (msec)	701 ±27	719 ±29	769 ±28	716 ±28
% correct responses	97.0 ±1.8	96.5 ±1.6	89.9 ±4.5	95.8 ±2.9

Table 17. *Vigilance and Tracking task and mean BAC levels. Scores of all variables during the first baseline session (VigTrack 1), second session (VigTrack 2), and third session (VigTrack 3).*

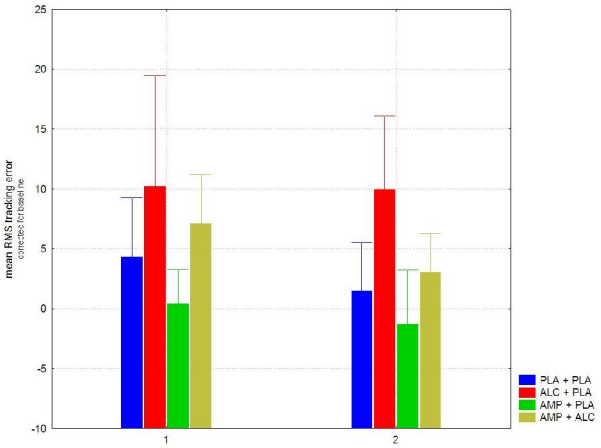

Figure 5. Vigilance and Tracking task: mean RMS tracking error (difference scores compared with baseline) in the first (1) and second (2) session. Whiskers denote 0.95 confidence intervals.

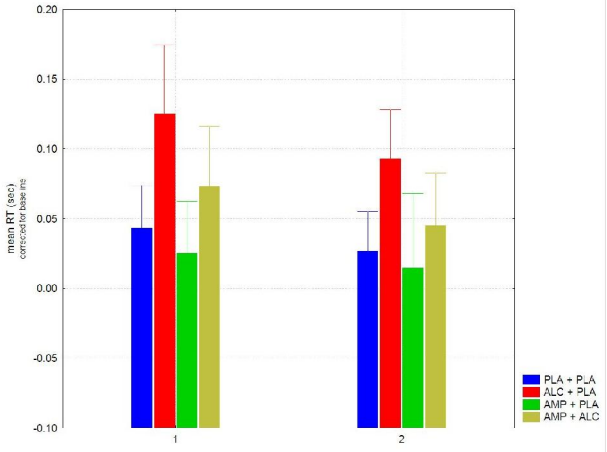

Figure 6. Vigilance and Tracking task: mean reaction time (s) (difference scores compared with baseline) in the first (1) and second (2) session. Whiskers denote 0.95 confidence intervals.

Selective attention task from the usual field of view (UFOV)

No significant differences between treatments were found on this task.

Subjective rating scales

Subjects completed the subjective rating scales immediately after ending the simulated driving test a. The KSS and POMS were also completed in the second session at T=240. Scores on the Karolinska Sleepiness Scale, Mental Effort Scale and Driving Quality Scale are presented in table 18.

Karolinska Sleepiness Scale (KSS)

For each treatment the mean of the results of the two sessions was used to perform the statistical analysis of differences between treatments. Results of the separate sessions are presented in table 18.

There were significant treatment effects on sleepiness scores ($F_{3,45}=6.82$; $p=.0007$) Subjects using dexamphetamine alone (DEX) or in combination with alcohol (DEX+ALC) were less sleepy than subjects using placebo (PLA) or alcohol alone (ALC) ($p<.01$ for both dexamphetamine containing treatments).

Mean ±SD per treatment

	DEX	DEX+ALC	ALC	PLA
KSS 1	4.0 ±1.4	4.9 ±1.6	6.4 ±2.0	6.2 ±2.3
KSS 2	4.2 ±1.3	4.9 ±1.5	5.2 ±1.4	5.0 ±1.4
Mental Effort Scale	36.3 ±18.7	44.4 ±18.7	55.1 ±18.7	56.5 ±18.7
Driving Quality Scale	4.0 ±1.5	3.1 ±1.4	2.4 ±1.1	2.9 ±1.4

Table 18. *Scores on Karolinska Sleepiness Scale (KSS), Mental Effort Scale and Driving Quality Scale. KSS 1=first session; KSS 2=second session.*

Mental Effort Scale

Subjects using DEX showed the lowest level of subjectively estimated mental effort during driving (n.s.)

Driving quality scale

Subjects using DEX showed higher subjectively estimated driving quality than those who used ALC ($p<.05$).

Profile of Mood States (POMS)

Significant treatment effects were found indicating that subject using DEX reported to feel less fatigued, more energetic, more cheerful, less depressed, and more clear-headed than when they had used ALC or PLA (all differences: $p<.05$)

Blood pressure and heart rate

Blood pressure and heart rate were measured prior to the start of the driving test and at the end of each trail day (table 19). There were no significant clinical or statistical differences between treatments concerning systolic blood pressure and heart rate. Continuous ECG monitoring revealed paroxysmal supraventricular tachycardia (SVT) in two cases using the combination of d-amphetamine and alcohol. Both SVTs were asymptomatic and recovered spontaneously.

	DEX	DEX+ALC	ALC	PLA
Mean SBP difference (mmHg) ± SD	4.1 ±1.3	5.0 ±1.8	2.6 ±0.9	2.1 ±1.2
Mean HR difference (bpm)	3.2 ±2.8	5.3 ±2.5	3.9 ±1.8	2.0 ±1.5
Cardiac arrhythmia (#cases)	0	2	0	0

***Table 19**. Mean systolic blood pressure (SBP) and mean heart rate (HR): differences calculated between pre-driving and end of trial day values. Cardiac arrhythmias concerned asymptomatic supraventricular tachycardias.*

Adverse effects

Adverse effects are presented in table 20. There were no significant clinical or statistical differences between treatments and all adverse effects disappeared spontaneously after the trial day.

	DEX	DEX+ALC	ALC	PLA
Nausea	0	2	4	1
Headache	1	1	2	2
Dizziness	0	1	2	2
Gastric discomfort	0	1	1	0
Blurred vision	0	0	1	0

***Table 20**. Adverse effects: number of cases per treatment condition.*

Discussion

The aim of the present study was to assess the effects of 10 mg dexamphetamine, 0.8 g/kg alcohol, and the combination of 10 mg dexamphetamine and 0.8 g/kg alcohol on driving behaviour and driving-related cognitive functioning, using a set of measures within a scenario developed to investigate the effects of stimulants, like dexamphetamine and/or alcohol on simulated driving performance. The scenario used in the present study has been developed by the department of Traffic and Environmental Psychology of the University of Groningen (RUG), who used this scenario to study the dose related effects of 0.3, 0.5, and 0.8 ‰ alcohol blood levels on simulated driving performance (Veldstra et al., 2010). Their results can be considered as a 'calibration' of this experimental scenario for the effect of alcohol and will be used as such in the discussion of our results, although it is considered that the TNO driving simulator is not identical to the simulator used by Veldstra et al.

Although in both simulators the field of view is 180º, an obvious difference is that the TNO simulator is moving-based, while the RUG simulator is fixed-based. Further differences may include controls and registering software.

Effects of 0.8 g/kg alcohol

In the alcohol condition, the mean BAC was between 0.85 and 0.64 ‰ and these are levels known to cause impairment of driving performance (Moskowitz and Robinson, 1986). Literature provides evidence that at the control level the effects of alcohol are marked by an increase of the SDLP with consequent increase in the amount of weaving (e.g. Louwerens et al., 1987). This was confirmed by the present study, in which we found that during monotonous driving the SDLP in the alcohol condition was significantly larger than in the placebo condition. Mean differences in SDLP between the alcohol and placebo conditions can be considered to be relevant (4.9 cm) and were in the same order as was found by Veldstra et al. (2010) in their 'calibration' study.

It was found that accepted gap time was significantly shorter and accepted gap distance was significantly smaller in the alcohol condition than in other conditions, except for dexamphetamine + alcohol condition which showed no significant difference with alcohol concerning accepted gap distance. These effects were not significant in the 'calibration' study of Veldstra et al. (2010), although the order magnitude of the mean gap times and gap distances found in their study was quite similar to the values found in the present study. Although non-significant, Veldstra et al. found a trend that participants accepted a smaller distance when their alcohol level was higher, which is in accordance with our finding. The gap times found in the present study may be considered to be rather high (between 4.7 and 6.7 s). This may be explained by the fact that due to a software problem the first 3 cars in the gap acceptance scenario, which should be presented with intervals of 1 second, appeared without interval between them. Therefore, the first opportunity to accept the gap was 1 second (which was done in 1 case) and the next was 4 seconds. Nevertheless, our findings concerning gap acceptance provide evidence that participants using alcohol took more risks by choosing shorter gap times and smaller gap distances.

As indicated by the average speed levels, speed limits of 50 km/h and 120 km/h were violated by participants using alcohol and they showed significantly higher average speed, higher SD of speed and higher maximum speed than in the dexamphetamine and placebo conditions. When approaching a junction where the subject had to give way to a car coming from the right, subjects using alcohol had a significantly higher maximum speed approaching the junction. When approaching a stop sign, participants showed significantly higher SD of speed than in the dexamphetamine or placebo conditions.

It appeared that participants using alcohol showed more risky and irresponsible behaviour than in the dexamphetamine or placebo conditions. In the alcohol condition, a significant higher percentage of drivers did not stop for the red traffic lights (59%) than in the dexamphetamine and placebo conditions (both 30%). In two cases, both in the alcohol condition, participants overtook a car by using the emergency lane, instead of taking over via the left lane. Moreover, participants using alcohol caused more collisions (8) than in the dexamphetamine (1) or placebo (3) conditions.

Participants using alcohol also reported the poorest subjective driving quality.

No significant effects of alcohol were found in the car following, car pulling out of a parking, and filtering into traffic challenges, which is in agreement with the findings of Veldstra et al. in their alcohol 'calibration' study..

Alcohol affected driving skills at control (SDLP), strategical (speed), and manoeuvring levels (gap acceptance, reaction to traffic light, violations). In contrast with the alcohol 'calibration' study of Veldstra et al. (2010), we found some significant effects on the manoeuvring level. This contrast may be explained by differences in pre-test training of the participants. Some authors state that 'the more accustomed subjects were to a test, the less the results were affected by alcohol' (Reisby and Theilgaard, 1969). Participants in the study of Veldstra et al. may have been more accustomed to the simulated driving task (i.e. better trained) than the subjects in the present study.

Findings concerning cognitive task performance provided clear evidence for the negative effects of alcohol on vigilance and divided attention.

It is concluded that the results of the present study support existing scientific consensus that 0.8 g/kg alcohol negatively affects driving performance, traffic safety, and cognitive functioning.

Effect of 10 mg dexamphetamine alone

It was found that participants using 10 mg dexamphetamine alone caused the least number of collisions (1), less passing of red traffic lights, and showed the best performance on the divided attention and vigilance tasks. These findings are in agreement with Ward (1997), who found that doses of 5 and 10 mg dexamphetamine increased the response rate on the Digit Symbol Substitution Task without affecting accuracy.

Effect of 10 mg dexamphetamine + 0.8 g/kg alcohol

Data on the effects of a combination of dexamphetamine and alcohol are scarce and inconsistent. On a theoretical basis, it was expected that dexamphetamine may reduce sedation and impairment of vigilance caused by alcohol, but may potentiate risk-taking behaviour, impaired judgement, 'tunnel vision', and deficits in divided attention when 0.8 g/kg alcohol and 10 mg dexamphetamine are taken in combination.

The BAC levels during the simulated driving test in this condition were between 0.91 and 0.64 ‰, and are considered high enough to affect driving performance. The mean dexamphetamine blood concentration in this condition was 20.7 ng/mL. This equals the mean blood concentration of dexamphetamine in the dexamphetamine alone condition (20.8 ng/mL) and is in agreement with the predicted concentration after a dose of 10 mg (Wong et al., 1998).

Concerning the simulated driving performance, it appeared that the addition of 10 mg dexamphetamine mitigated many of the harmful effects found in the alcohol condition. Although the addition of dexamphetamine appeared to reduce impairment caused by alcohol, these effects were not significant. There were only few indications for increased risk taking associated with the combination of dexamphetamine + alcohol. Subjects showed significantly higher standard deviations of speed when driving on roads with speed limits of 80 and 100 km/h. Similar to the alcohol condition,

a significant higher percentage of drivers in the dexamphetamine + alcohol condition did not stop for the red traffic lights (64%), or collided onto a vehicle (6 cases).

In the divided attention task (DAT) there were significantly more false alarms when using dexamphetamine + alcohol. For the rest, participants using dexamphetamine + alcohol had better, although non-significant, tracking performance and reaction times on the divided attention task (DAT) and vigilance and tracking task (VigTrack).

Subjects using dexamphetamine alone, or in combination with alcohol were significantly less sleepy than subjects using placebo, or alcohol alone. Participants using dexamphetamine alone felt less fatigued, more energetic, more cheerful, less depressed, and more clear-headed than in any other condition.

It was to be expected that the effects of alcohol on tracking performance, reaction times, and sleepiness might be mitigated by 10 mg dexamphetamine. It is, however, remarkable that the addition of dexamphetamine also seemed to mitigate several aspects of risky traffic behaviour associated with the use of 0.8 g/kg alcohol, instead of potentiating risk taking behaviour. This may be explained by the fact that in the present study a relative low dose (10 mg) of dexamphetamine was used instead of higher doses which are known to cause risk taking behaviour and impaired judgement (Simons and Valk, 2000). The decision to use the lowest relevant dose of dexamphetamine (10 mg) was based on medical considerations taking into account that moderate to higher doses of alcohol, such as 0.8 g/kg, may potentiate some adverse effects of dexamphetamine, particularly increase of heart rate and blood pressure and cardiac arrhythmia risk (Higgins et al. 1988; Mendelson et al. 1995; Ghuran and Nolan, 2000). Doses of 5 to 10 mg dexamphetamine are used in the US Air Force to improve performance of fatigued pilots (Emonson and Vanderbeek, 1995) and are known to reduce the effects of fatigue and sleep deprivation without adverse effects such as stimulating risk taking behaviour (Caldwell and Caldwell, 1997). Therefore it is hypothesized that addition of 10 mg dexamphetamine favoured the reduction of sedation and disinhibition caused by alcohol, while this dose might have been too low to cause impaired judgment and stimulation of risk taking behaviour.

Above reasoning is supported by the finding that in the present study participants using 10 mg dexamphetamine alone caused the least number of collisions (1), less passing of red traffic lights, and showed the best performance on the divided attention and vigilance tasks. These findings are in agreement with Ward (1997), who found that doses of 5 and 10 mg dexamphetamine increased the response rate on the Digit Symbol Substitution Task without affecting accuracy.

It is known that in the ‘party circuit’ the amphetamine doses that are used in combination with alcohol may be much higher than the dose used in the present study. Although our findings show that the combination of dexamphetamine and alcohol might mitigate some effects of alcohol, most differences between these two conditions were not significant. Moreover, red-light running and collisions, both highly dangerous driving acts, were observed significantly more frequent in the alcohol condition and in the alcohol + dexamphetamine condition. Therefore, individuals using alcohol, or the combination of dexamphetamine with alcohol, should not be allowed to participate in traffic.

Conclusion

Findings of the present study support existing scientific consensus that 0.8 g/kg alcohol negatively affects driving performance and traffic safety. Alcohol affected driving skills at control (lateral position), strategical (speed), and manoeuvring levels (gap acceptance, reaction to traffic light, violations).

Although not significant, the combination of dexamphetamine and 0.8 g/kg alcohol seemed to mitigate several aspects of risky traffic behaviour associated with the use of 0.8 g/kg alcohol, instead of potentiating risk taking behaviour. This may be explained by the use of a relative low dose (10 mg) of dexamphetamine in the present study. Such dose may have favoured the reduction of sedation and disinhibition caused by alcohol, while the dose might have been too low to cause impaired judgment and stimulation of risk taking behaviour.

In addition to the impairing cognitive effects and effects on lateral position, speed, and gap acceptance, red-light running and collisions, both highly dangerous driving acts, were much more frequently observed in the alcohol and alcohol + dexamphetamine conditions than in the other conditions.

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The findings of the present study justify the conclusion that individuals using alcohol, or the combination of dexamphetamine with alcohol, should not be allowed to participate in traffic.

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Annex A – Technical specifications TNO driving simulator

The simulator is a set of separate modules. Each models has is specific function in the total simulation. The modules are communicating over Ethernet. The communication interface, data packet, is specified. In this way it's possible to exchange modules if another implementation is necessary without breaking the simulation.

Modules:

Mockup.

Physical BMW 316i body. Steering wheel angle and pedal positions are measured and send to the Car model. Steering wheel and pedals are equipped with control loading to simulate power steering and pedal characteristics. The Mockup is controlled by a CCit system. This is a P-104 based linux system that runs realtime Matlab-Simulink.

Car Model.

The output of the Mockup, steering wheel angle and pedal positions, are used in the Car Model to calculate the motion of the "virtual" car. The forces on the steering wheel and pedals are also calculated and send to the Mockup. A motion filter calculates the displacement for the Motion system. The model runs on Windows computer in a Matlab-Simulink environment at 400Hz controlled by a C++ shell for timing and interface.

Supervisor.

This is the director of the whole simulator. All tasks of the Supervisor:

Controlling participant information.

Loading scenarios.

Starting and stopping the necessary software on the other modules.

Storing experimental data during the simulation.

Monitoring the status of all other modules.

Calculating other traffic. Other traffic is interacting with the Mockup.

Calculating the sound around the Mockup.

Routing the data.

The Supervisor is a C++ application running on a Windows computer running at 200Hz.

Sound System.

Produces the sound in and around the Mockup. This includes other traffic and car noise. Outside the Mockup there are 4 speakers to produce 3D sound of objects around the Mockup. Inside there are 5 speakers. At every wheel one speaker for wheel/wind noise. One in the center of "engine" compartment for engine sound and body vibration.The Sound System is a state machine playing the requested sound files. This can be once or in a loop. The engine and wind noise sounds are on the fly mixed sound files.

Visualization.

The visualization is a combination of hard en software components. One train of Image Generator, Warping/Blending and Projector is called a channel. The total picture (180° front view and 120° back view) is created with multiple channels. The update frequency of each channel is 60Hz.

Image Generator.

Software to produce the 3D environment. The viewpoint inside the 3D environment is the position and orientation outcome of the Car Model.

Warping and Blending.

The image is corrected for the shape of the projection screen. The front projection screen is a cylindrical screen. The rear mirrors are projected from an angle. The edges of the image can be blend to create one seamless picture with multiple channels.

Projector / Display.

For the front view tree wide-angle high resolution (1900x1200) projectors are used to create a 180° view.

The back projection is split into tree parts. Two projection screens (1.6m wide x 1.15m high) are used for the outside mirrors. The inside mirror is displayed on a 32" LCD panel.

The visualization runs on a Windows computer with OpenScenGraph 2.8 as database manager. The graphics are generated by high-end Nvidia Quadro graphics cards.

Motion System.

The Mockup is mounted on a 6 Degrees Of Freedom Motion platform to give additional feedback to the driver. The feedback can be onset acceleration in case of accelerating or braking, or roll in case of cornering. To generate the feeling of driving, road rumble is set on top of the other movements. The motion has a maximum range of +/- 20cm and +/- 20°.

The Motion System is controlled by a motion control computer running at 50 Hz.

Secondary Task.

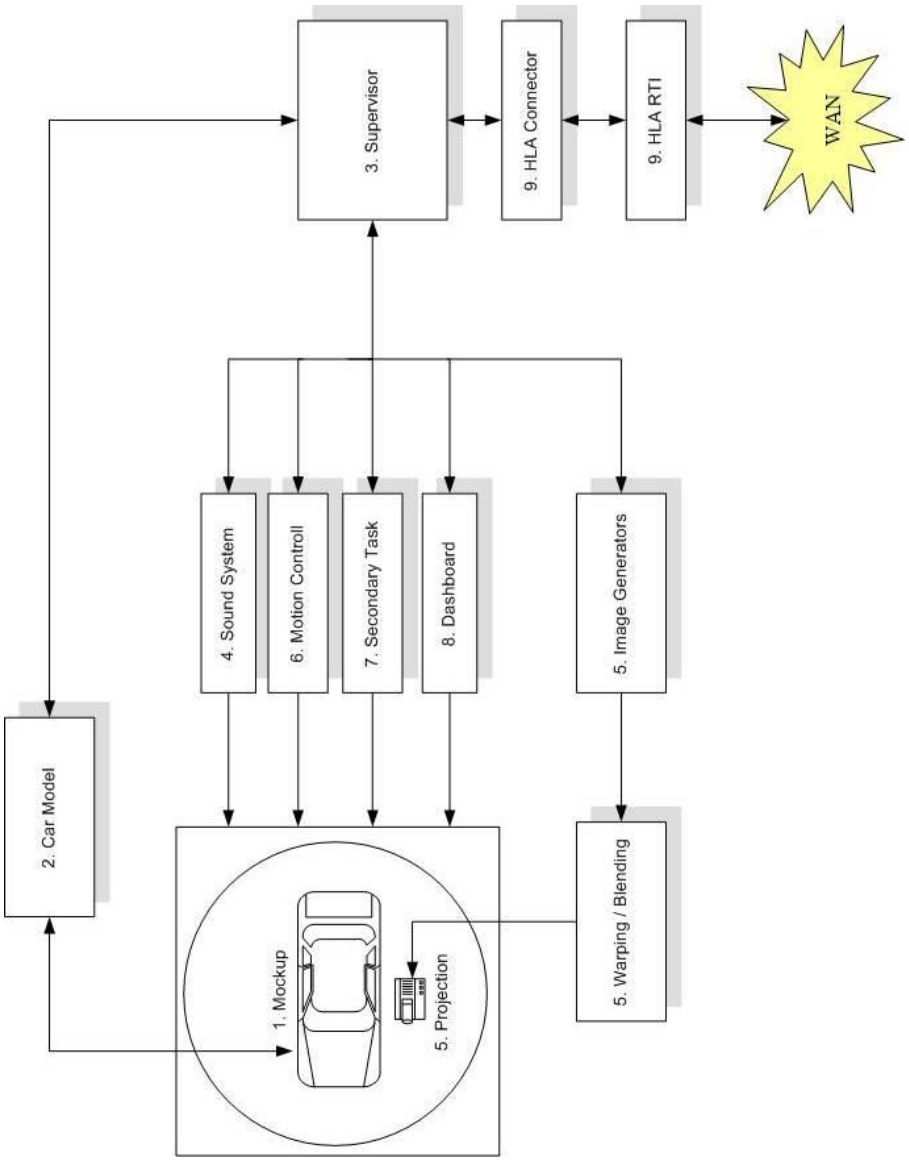
To measure the workload of the driver a Secondary Task can be added to the experiment. Every application suitable for this purpose can be used. The application is presented on a 12" touch screen, mounted on the dashboard of the car.

Dashboard.

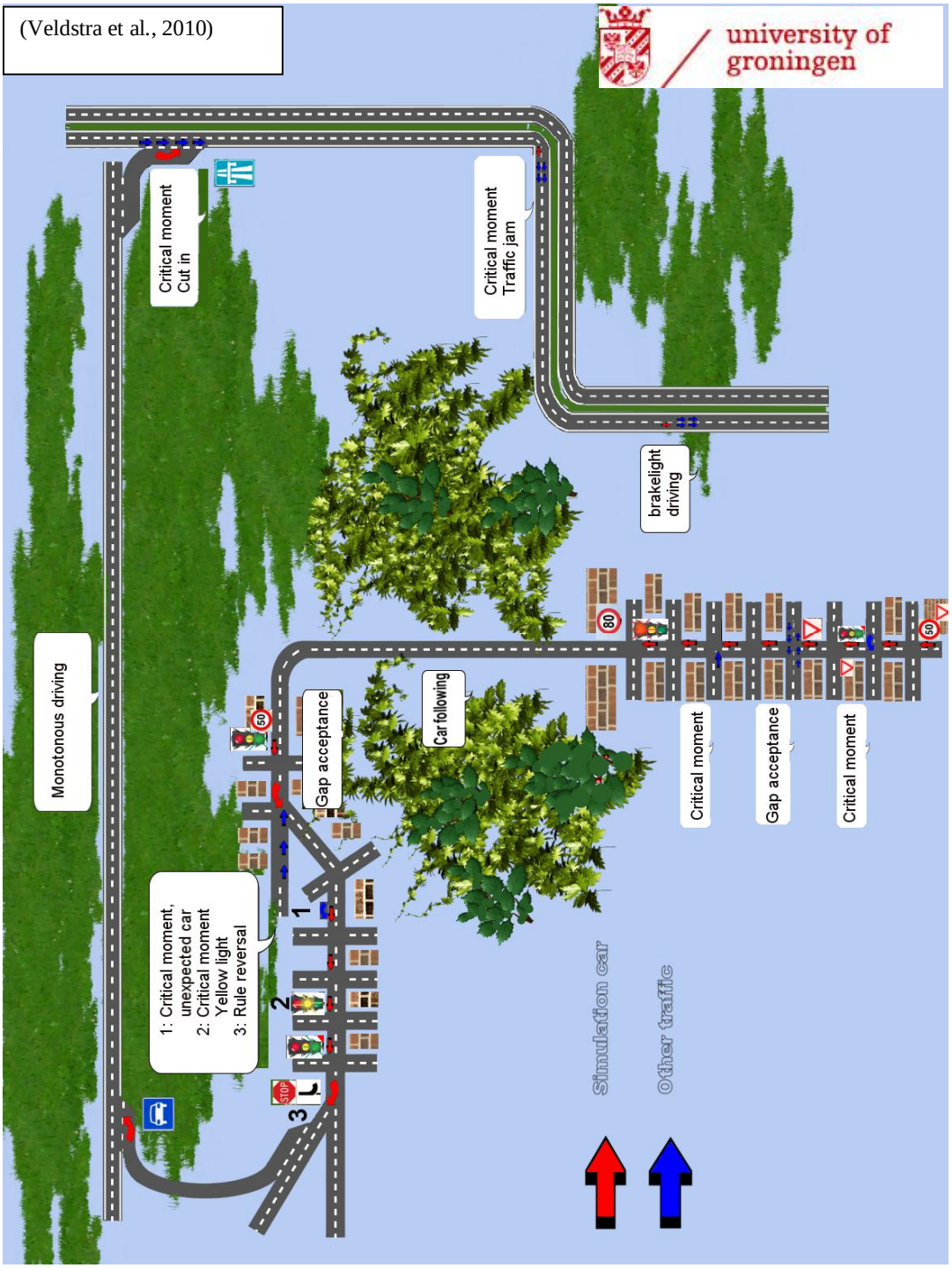
A display in front of the driver, mounted in the dashboard, displays information about the state of the car and speed and rpm. This display is freely configurable. The Update rate is 25Hz.

HLA System.

For external communication to other simulators or learning environments, this simulator is capable to communicate trough HLA. A subset of the RPR FOM is implemented. The system has a build-in dead reckoning algorithm to compensate for the lower HLA data communication. The HLA System is communicating with the simulator at 200Hz. The update rate trough HLA is 60-90Hz.


3. Supervisor
9. HLA Connector
9. HLA RTI
WAN
4. Sound System
6. Motion Controll
7. Secondary Task
8. Dashboard
5. Image Generators
2. Car Model
5. Warping / Blending
1. Mockup
5. Projection

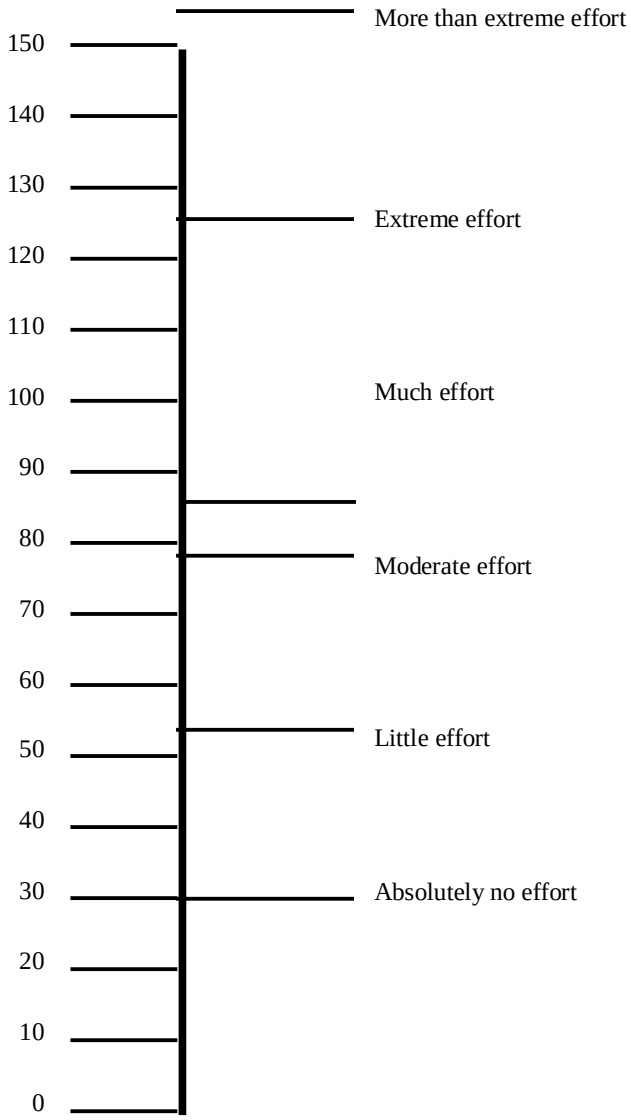
Annex B – Graphical representation of the driving scenario



Annex C

Rating Scale Mental Effort

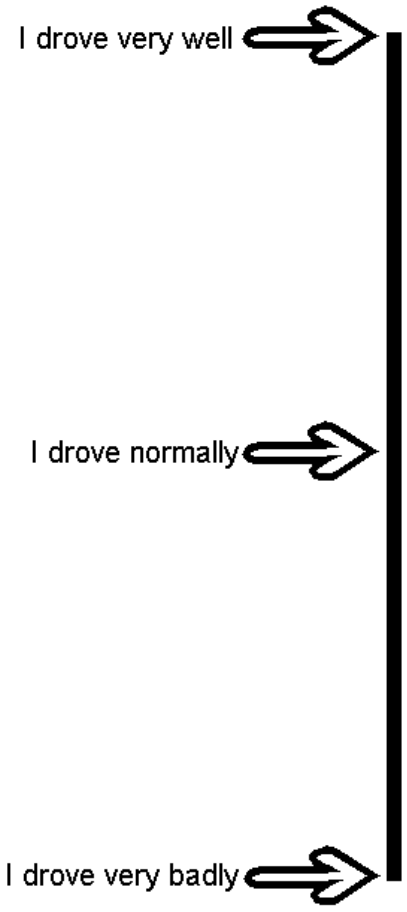
Instruction:
Please indicate the amount of effort it has cost you to perform the driving task by putting a cross (X) at the applicable level of effort on the vertical line below.



Annex D – Driving quality scale

Driving Quality Scale

Could you please indicate, by marking the vertical line with a cross, how you think you just drove



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Annex E – Karolinska Sleepiness Scale

Rating	KSS (Reyner & Horne, 1998)	KSS – Dutch version
1	Extremely alert	Extreem alert
2	Very alert	Zeer alert
3	Alert	Alert
4	Rather alert	Vrij alert
5	Neither alert nor sleepy	Niet alert en niet slaperig
6	Some signs of sleepiness	Een beetje slaperig
7	Sleepy, but no effort to keep alert	Slaperig, maar nog geen moeite om alert te blijven
8	Sleepy, some effort to keep alert	Slaperig, moeite om alert te blijven
9	Very sleepy, great effort to keep alert, fighting sleep	Zeer slaperig, grote moeite doen om alert te blijven, vecht tegen de slaap

Table. The Karolinska Sleepiness Scale, as modified by Reyner & Horne (1998). Dutch version presented in right panel.

Chapter 6

Blood to plasma ratios of amphetamine and MDMA and analysis of dried blood spots

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Abstract

Aims: Blood is prevalently used for analysis in toxicology including DUID cases whereas analysis on pharmacokinetic samples is performed on separated serum or plasma, as a rule. Most drugs, however, do not equally distribute between blood and plasma. Dried blood spots (DBS) are increasingly used in drug analysis due to their ease of collection, shipping and storage as well as reducing the risk of infection. Therefore, the following objectives were pursued: a. determination of drug concentration in whole blood and corresponding plasma samples to estimate *ex vivo* blood to plasma (b/p) ratios, and b. comparison of drug levels in whole blood and corresponding DBS.

Methods: Analytes were amphetamine, MDMA und MDA. Analysis was performed on whole blood, plasma and DBS by LC/MS/MS following evaluation of the analytical method according to international guidelines. B/p ratios were derived from *in vitro* partition experiments (different hematocrit values) and from corresponding blood and plasma samples (*ex vivo* studies, n=63 for MDMA, MDA, n=29 and 60 (2 studies) for amphetamine). Bland Altman analysis was used to test agreement of concentrations determined from whole blood and corresponding DBS.

Results: B/p ratios of amphetamine, MDMA and MDA were dependent on the hematocrit value, but not on the concentration (≤ 500 ng/mL). B/p ratios of MDMA and MDA averaged 1.16 +/- 0.13 and 1.27+/-0.20, respectively. Mean ratios of amphetamine were 0.89 +/- 0.10 and 0.91 +/- 0.12. Bland Altman analysis revealed that < 5% of the concentration differences (DBS-blood) were not within the limits of “agreement” for both MDMA and amphetamine. Mean differences were 1.4 ng/mL for MDMA and -0.63 ng/mL / 1.03 ng/mL for amphetamine using DBS instead of whole blood for analysis which is considered acceptable.

Conclusions: Dividing the concentration of MDMA or amphetamine in blood by 0.78 or 1.11, respectively, may give a reasonably good estimate of the coexisting concentration in plasma. For law enforcement purposes, it is recommended to consider inherent biological variations in the b/p relationship. There is sound evidence that the DBS assay has potential as a precise and inexpensive option for the determination of amphetamine and amphetamine derivates in small blood samples.

Introduction

Distribution of drugs between whole blood and serum or plasma

Drug analysis in forensic and postmortem toxicology including DUID (driving under the influence of drugs) cases is usually performed on whole blood whereas serum or plasma is preferably used in clinical facilities and pharmacological studies. Blood is a complex biological fluid consisting of a buffered clear fluid containing proteins, fats, solids and suspended cells. The major constituents – the red cells – can be separated from the clear fluid by centrifugation. If blood is allowed to stand without the addition of an anti coagulating agent, then red cells will clot and the resultant fluid can be decanted. If anticoagulants are added, plasma can subsequently be prepared. Serum is in most respects similar to plasma except that it does not contain soluble factors that lead to blood clotting (1).

The proportion of blood volume that is occupied by red cells is referred to as the hematocrit value (%). Normally, it averages 48% for men and 38% for women, and ranges from 35–54% in blood from healthy adults (2). Most drugs are not equally distributed between the sub compartments of blood; hence, the concentration in serum or plasma may differ from that in whole blood. Blood to plasma (b/p) concentration ratios may not only vary between different compounds with the same core structure, but also between the parent drug and corresponding metabolites (3) or depend on the hematocrit value (4, 5). To know the distribution of drugs into the major sub compartments of blood is mandatory in order to reliably compare whole blood to plasma or serum levels that have been derived from controlled pharmacokinetic studies.

Generally, distribution of compounds between whole blood and plasma is determined using *in vitro* or, to a much lesser extent, *ex vivo* procedures (5-7). In the conventional *in vitro* method, the drug is incubated with a whole blood specimen at a known hematocrit value – which does not apply to DUID samples. Following equilibration, an aliquot of the whole blood specimen is put on the side while plasma is prepared from the remaining sample. It is not known how far off from the true values the *in vitro* determined ones are likely to be. In the *ex vivo* method, the blood sample taken from a drug user for analysis is portioned, and plasma is prepared from an aliquot of the original specimen. In both methods, drug concentrations in whole blood and plasma will be measured separately using separate standards for whole blood and plasma, respectively.

This investigation compares b/p distribution ratios of methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), d-amphetamine (from 2 different studies) in both an experimental setting and using authentic samples from healthy volunteers conducting a DUID experiment under the influence of MDMA or d-amphetamine (which is subsequently called amphetamine), respectively.

Partners were instructed to collect 5-10 mL blood from the median cubital vein or the anterior forearm by venipuncture into a vacuum tube or into a syringe and needle with subsequent transfer to a Vacutainer™ or a Monovette™ containing potassium oxalate/sodium fluoride as an anticoagulant/preservative (e.g. grey top Vacutainer™, DIN ISO 6710). Plasma should be prepared by centrifuging the sample at 2000-3000 g for 10-15 minutes at 20-22°C or at 4°C (labile compounds). Samples should be stored frozen and shipped on dry ice.

Dried blood spots

Dried blood spots (DBS) have routinely been used in neonatal metabolic screening for over two decades, and have recently established themselves as a valuable tool in therapeutic drug monitoring (8-13). Despite a limited

sample size of 10-100 µL blood, analysis of DBS specimens has become feasible with the advent of increasingly sensitive MS technologies (14). DBS can be stored at room temperature and shipped by regular mail, in contrast to whole blood or plasma specimens. Use of DBS is an appropriate method to reduce virus infection risk to a minimum which is a major concern handling samples of drug users (9, 11). Being readily accessible also in subjects with limited venous access, such as e.g. injecting drug users, it represents a valuable and less invasive alternative to taking of a blood sample. In addition, the use of DBS makes labile compounds such as ester type drugs less susceptible to degradation (15).

A blood spot card was designed for collection of DBS in the present investigation. The face and the back of the card are shown in Figure 1. The 903 specimen collection paper (GE Healthcare, Dassel, Germany) used for the custom made card is an FDA listed class II medical device in the US. It is manufactured from 100% pure cotton linters with no wet-strength additives. Both, the manufacturing and post-printing quality of the paper were checked. Figure 2 gives an overview on the study design.

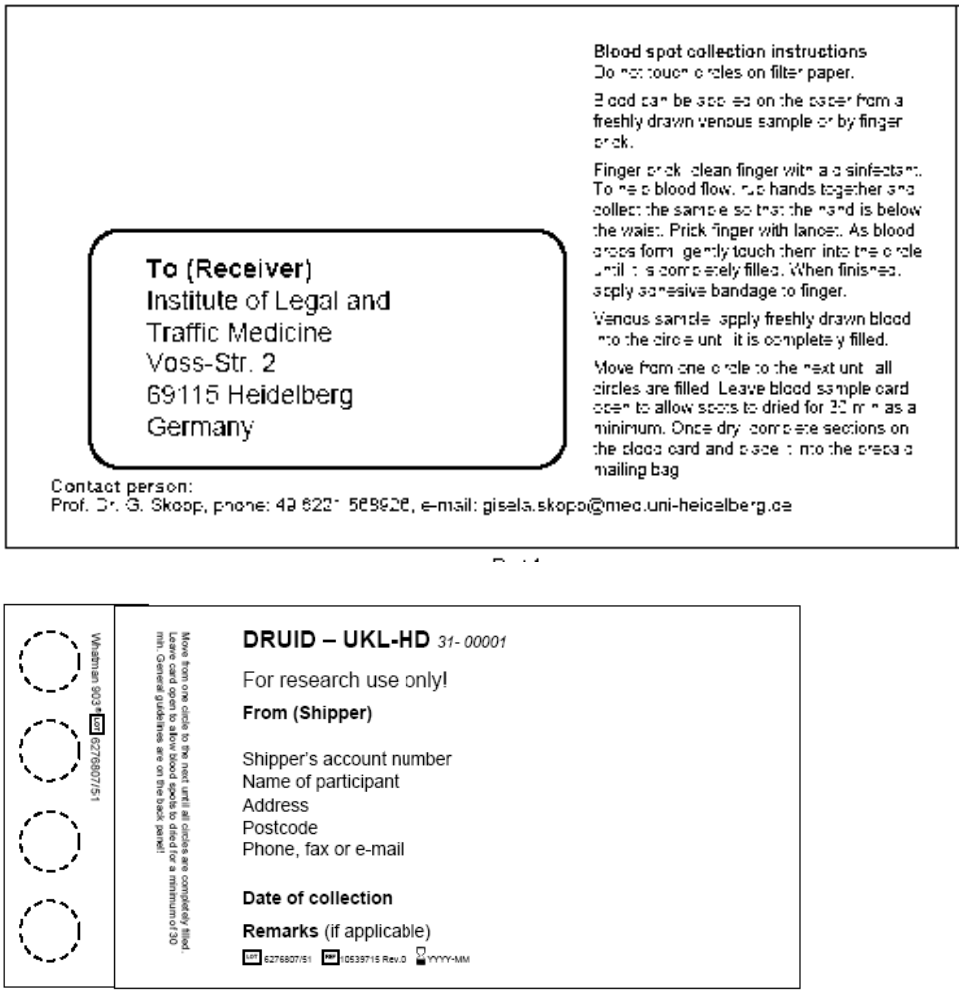

Fig. 1: Blood spot card, face and back of the card

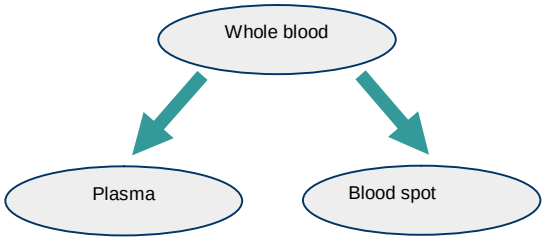

Fig. 2: Plasma and blood spot specimens are obtained from the corresponding whole blood specimen for measurement of blood/plasma ratios and to evaluate measurement from DBS

Measurement of drug concentration in whole blood and corresponding plasma samples enables an estimation of *ex vivo* b/p ratios. The knowledge of these ratios and of their range is mandatory to valuably compare drug concentrations measured either in whole blood or plasma.
A comparison of drug levels in whole blood and corresponding DBS allows demonstrating whether drug measurement from DBS is as accurate as that from the whole blood specimen. DBS analysis will make handling of toxicological samples much easier.

Blood to plasma ratios – a short review of the current literature

Temperature, pH, hematocrit (hk) value and drug concentration have been shown to influence the distribution of a drug among blood and plasma.

Table 1: Blood to plasma ratios (4, 7, 16, 17)

Drug	Blood/plasma concentration ratio
Amitriptyline	1.0-1.1
Nortriptyline	1.5-1.7
Cocaine	1.00
Diazepam	0.70
Oxazepam	1.00
Ethanol	0.74-0.90
Methadone	0.75 / 1.00*
Morphine	1.02
Morphine-3- and -6-glucuronide	dependent on the hematocrit value
Tetrahydrocannabinol (THC)	0.55 / 0.66*
11-Hydroxy-THC	0.57 / 0.63*
11-Nor-9-carboxy-delta9-THC	0.62 / 0.59*

*: depending on the particular reference

Distribution ratios are generally derived from *in vitro* partition experiments where plasma water, plasma proteins and red blood cells are pooled. Some caution is advisable using these data. When spiked blood is diluted with autologous plasma water, erythrocytes may discharge the compound over proportionally compared to plasma proteins. Concentration ratios between blood and plasma may vary from 0.5 to 2.0 such as e.g. for phenytoin and maprotiline, respectively. Also, non-linear, concentration dependent variations of the blood/plasma distribution have been observed, e.g. for topiramate (16).

Blood to plasma ratio of MDMA and MDA

Experimental study

Study design: A preliminary study revealed that the b/p ratio was not dependent on the concentration (50-500 ng/mL) (18). Fresh blood was drawn by venous puncture from a healthy volunteer (S-Monovette) and centrifuged. Plasma (supernatant) and packed erythrocytes were combined to yield different hk values (n=4). Each of the samples was spiked with MDMA and MDA (200-250 ng/mL, respectively). Following equilibration (30 min), an aliquot of each sample was directly extracted while the remaining liquid was again centrifuged. Five aliquots (100 µL, each) of whole blood and recovered plasma were extracted independently. *Extraction*: Briefly, 1 mL NaOH (0.01 M) and deuterated standards (10 ng of MDMA-d_5 and MDA-d_5) were added to a 100 µL aliquot of samples or calibration standards. Supplemented samples were extracted with ethyl acetate (1.5 mL, 30 min on a shaker) and centrifuged. The organic layer was transferred to a silanised vial, acidified with 50 µL of methanol/hydrochloric acid (MeOH/HCl 49:1 v/v) and evaporated to dryness. The residue was reconstituted with mobile phase (50 µL of 4 mM ammonium acetate buffer pH 3.2/methanol/ acetonitrile (65:7:28 v/v/v)).

Analysis:

Analysis was performed on an API 4000 tandem mass spectrometer with a TurboIon ionisation source operating in the positive-ion mode (Applied Biosystems) which was interfaced to a HPLC pump equipped with an auto sampler (1100 Series, Agilent). Processed samples were eluted from a Zorbax Eclipse XDB-C8 column (2.1 x 150 mm, particle size 5 µm, Agilent) at a flow rate of 220 µL/min (mobile phase, see above) following injection of 10 µL. Data were acquired in multiple reaction monitoring mode, and the following transitions were used for quantification: MDMA, m/z 194→163, MDA, m/z 180→163; MDMA-d_5, m/z 199→165 and MDA-d_5, m/z 185→168.

Evaluation: The extraction efficiency for MDMA and MDA was >95% of the spiked concentration, for both blood and plasma. All calibrators were within a 10% range of the target concentration; standard deviations were below 7%. Calibration lines (5-1000 ng/mL for each analyte) were linear (correlation coefficients r>0.995 for MDMA and MDA in blood or serum, respectively). In addition, ion suppression/enhancement and carry could not be observed. Benchtop stability (+/- 2.5%) of the extracts at ambient temperature was > 24 hours. The lower limit of detection (LLOD) and quantitation (LLOQ) was estimated from the calibration curves according to DIN 32465 at a probability of 95% (19), and was 1.9 ng/mL and 5.5 ng/mL for MDMA and 2.9 ng/mL and 5.6 ng/mL for MDA in blood and serum, respectively.

Table 2: Hematocrit values (%) and mean +/- standard deviation (n=5) of blood to plasma (b/p) concentration ratios for MDMA and MDA

Hematocrit value (%)	b/p ratio MDMA	b/p ratio MDA
25.7	1.12 +/- 0.04	1.05 +/- 0.05
38.3	1.22 +/- 0.14	1.17 +/- 0.04
44.8	1.29 +/- 0.02	1.19 +/- 0.05
45.0	1.32 +/- 0.06	1.23 +/- 0.12

Results

Mean b/p concentration ratios are summarized in Table 2. Data revealed a linear relationship of b/p concentration ratios with hk values for both MDMA and MDA (b/p = 0.0097hk + 0.8661, r=0.984 for MDMA; b/p = 0.0083hk + 0.8393, r = 0.975 for MDA). Ratios were slightly higher for MDMA than those for MDA.

B/p concentration ratios of MDMA and MDA determined from authentic samples

Study design

Blood and corresponding plasma samples from 16 healthy volunteers of a driving experiment were provided from the Experimental Psychopharmacology Unit, Maastricht University. Samples were taken on four different occasions following administration of either MDMA (25, 50 or 100 mg) or placebo. One participant providing blood and serum samples on only three of the four occasions, 63 samples in total were obtained for blood and serum, respectively. Samples were stored frozen at -20°C for approximately two weeks until analysed.

Extraction and analysis of hemolysed specimens and plasma samples were performed as described above. Each sample was extracted twice on 2 different occasions. Mean values were used with differences < 10% among single values. Findings where the concentrations in either blood or plasma or both were below LLOD were not considered for calculations; those with concentrations between the LLOD and the LLOQ were labelled "positive" but not considered for calculations either.

Results

MDMA concentrations in the 63 authentic samples ranged from not detectable to 310 or 236 ng/mL in blood or in plasma, respectively. Whenever MDMA could be determined in the plasma specimen, it was also present in the blood, and vice versa. Two samples showed concentrations between the LLOD and the LLOQ in both plasma and blood. Of the total 42 samples in which MDMA could be quantified, 36 showed a b/p ratio > 1.0, and for six samples the ratio was < 1.0. Overall, b/p ratios for MDMA ranged from 0.84-1.35, the average being 1.16 +/- 0.13**.**

In 27 plasma samples the MDA concentration was between the LLOD and the LLOQ, whereas for the blood samples a positive finding could be obtained in 15 cases. MDA concentrations were up to 10.4 ng/mL in blood and 16.3 ng/mL in plasma, respectively. All of the 10 samples from which MDA could be determined and quantified had a b/p ratio > 1.0. The average b/p ratio was 1.27+/-0.20 (range: 1.01-1.77). *Conclusion:* The present study revealed b/p concentration ratios > 1 for both MDMA and MDA with a slightly higher mean ratio for MDMA than for MDA, no matter whether results were derived from supplemented or authentic samples. A b/p ratio > 1 demonstrates an additional binding to or greater solubility in the red blood cell than can be accounted for simple distribution in the sub compartments of blood. MDMA and MDA partitioning under *in vitro* and *ex vivo* conditions gave equivalent results which is in accordance with passive diffusion of the analytes as the underlying process.

A linear relationship between the hk value and the b/p ratio of both MDMA could be established from the experimental data. Based on the normal range of hk values (35-54%) (2), b/p ratios are expected to range from 1.20-1.39 for MDMA and from 1.14-1.28 for MDA within the normal range. These estimates are quite in line with the range of b/p ratios covered by the authentic samples.

Samples of whole blood that have been taken by the police for drug analysis are often hemolysed and sometimes contain clots. Therefore, plasma is less often analysed, and determination of the hk value is generally not possible. The whole blood samples from the field study largely addressed the conditions of DUID specimens. From the present results, the MDMA plasma concentration could thus be estimated from a whole blood concentration with adequate accuracy using a factor of 0.78. If it is advisable to consider inherent biological variations in the b/p relationship, e.g. for law enforcement purposes, the standard deviation or range should be considered.

For MDA, this process bears certain crucial limitations. The concentrations determined from field study specimens were very low. The concentration used in the *in vitro* setting was much higher, in order to get reliable and quantifiable results. While the experimental setting would suggest a factor of about 0.79 to calculate the plasma concentration on the basis of a whole blood sample, the distribution of coefficients determined from the authentic samples was scattered over a large range of values (0.56-0.99). Nevertheless, reports of MDA

concentration being 5-10% of the corresponding MDMA blood or plasma levels could be confirmed.

Blood to plasma ratios for amphetamine

Experimental study

Study design

A preliminary study revealed that the b/p ratio was not dependent on the concentration (50-500 ng/mL). Four blood samples with different hk values were prepared as described previously, which were spiked with amphetamine (ca. 400 ng/mL). Following equilibration (30 min), a portion of each sample was removed while the remaining liquid was centrifuged. Five aliquots (100 µL, each) of whole blood and recovered plasma were extracted independently.

Extraction

Processing of hemolysed specimens and plasma samples was performed as described above for MDMA and MDA except that 250 ng of deuterated amphetamine (amphetamine-d_5) was added as the internal standard.

Analysis

Analysis was performed on an API 4000 tandem mass spectrometer with a TurboIon ionisation source operated in the positive-ion mode (Applied Biosystems) which was interfaced to a HPLC pump equipped with an auto sampler (1100 Series, Agilent). Samples were eluted from a Zorbax Eclipse XDB-C8 column (2.1 x 150 mm, particle size 5 µm, Agilent) at a flow rate of 220 µL/min (mobile phase: 4 mM ammonium acetate buffer pH 3.2/methanol/acetonitrile (62:7.6:30.4 v/v/v) following injection of 8 µL of the processed specimen. Data were acquired in multiple reaction monitoring mode, and the following transitions were used for quantification: amphetamine, m/z 136→91, amphetamine-d_5, m/z 141→124.

Evaluation

The extraction efficiency for amphetamine was >95% of the spiked concentration, for both blood and plasma. All calibrators were within a 10% range of the target concentration; standard deviations were below 6%. Calibration lines (5-500 ng/mL for each analyte) were linear (correlation coefficient r>0.999 in blood or serum, respectively). Ion suppression/enhancement and carry could not be observed. Benchtop stability (+/- 2.5%) of the extracts at ambient temperature was > 24 hours.

The LLOD and LLOQ were estimated from the calibration curve (5-50 ng/mL) according to DIN 32465 at a probability of 95% (19), and were 0.6 ng/mL, 0.7 ng/mL and 2.3 ng/mL, 2.6 ng/mL for blood and plasma, respectively.

Results

Mean b/p concentration ratios are summarized in Table 3. Data revealed a linear relationship of b/p

concentration ratios with hk values for amphetamine (b/p = 0.0053hk + 0.5998, r= 0.996). Ratios were consistently lower than those of MDMA and MDA.

Table 3: Hematocrit values (%) and mean+/-standard deviation (n=5) of blood to plasma (b/p) concentration ratios for amphetamine

Hematocrit value (%)	b/p ratio of amphetamine
29.8	0.76 +/- 0.04
38.1	0.80 +/- 0.04
44.9	0.83 +/- 0.03
54.2	0.89 +/- 0.05

B/p concentration ratios of d-amphetamine/amphetamine determined from authentic samples

Study design: Blood and corresponding plasma samples were provided from studies of TNO, The Netherlands and of VTI, Sweden. Twenty nine samples in total were obtained from TNO, and 60 samples were from VTI, for blood and plasma, respectively. Samples were stored frozen at -20°C for up to 6 weeks until analysed. *Extraction and analysis* of hemolysed specimens and plasma samples were performed as described above (4.1). Each sample was extracted twice on 2 different occasions. Mean values are used with differences < 10% among single values. Findings where the concentrations in either blood or plasma or both were below LLOD were not considered for calculations; those with concentrations between the LLOD and the LLOQ were labelled “positive” but not considered for calculations either.

Table 4: Number of positive samples (%), concentration range (ng/mL), mean values (ng/mL) and blood to plasma concentration (b/p) ratios determined from blood and plasma samples of both studies, 0: not detectable, SD: standard deviation

	Study of TNO, The Netherlands	**Study of VTI, Sweden**
positive findings in blood	n=29 (100%)	n=37 (62%)
positive findings in plasma	n=29 (100%)	n=37 (62%)
Concentration range, blood	10.75-40.65	0-123.5
concentration range, plasma	12.20-41.13	0-112.0
mean +/- SD, blood	20.61 +/- 7.08	21.78 +/- 29.60
mean +/- SD, plasma	23.21 +/- 7.60	22.92 +/- 29.40
b/p, range	0.64-1.14	0.65-1.10
b/p mean +/- SD	0.89 +/- 0.10	0.91 +/- 0.12

Results

Analytical data from the 2 studies were separately analyzed (Table 4). Whenever amphetamine could be determined in the plasma specimen, it was also present in the blood, and vice versa. The levels determined from

samples obtained from TNO were all above the LLOQ, whereas amphetamine could be quantified in 62% (n=37) of samples from VTI. The b/p ratios estimated from corresponding blood and plasma specimens are given in Table 4. Of the total 29 samples from TNO 5 showed a b/p ratio > 1.0, and for 24 samples the ratio was < 1.0. The b/p ratio was > 1.0 for 6 samples from VTI, 1.0 for 2, and < 1.0 for 29 samples. *Conclusion:* The present study revealed mean b/p concentration ratios < 1 for amphetamine, no matter whether results were derived from supplemented or authentic samples. The b/p ratios of amphetamine in the experimental setting are not significantly different from those of both *ex vivo* studies despite their substantial different concentration ranges.

A linear relationship between the hk value and the b/p ratio of amphetamine (y=0.0053•x + 0.6018, x: hematocrit value (%), y: b/p ratio) could be established from the experimental data. Based on this relationship, b/p ratios of amphetamine are expected to range from 0.79-0.89 within the normal range of hematocrit values. This range is quite in line with the range of b/p ratios covered by the authentic samples. A b/p ratio < 1 demonstrates that amphetamine does not additionally bind to or exhibits greater solubility in the red blood cell than can be accounted for simple distribution in the sub compartments of blood. Dividing the concentration of amphetamine in blood by 1.11 may give a reasonably good estimate of the coexisting concentration in plasma. If it is advisable to consider inherent biological variations in the b/p relationship, e.g. for law enforcement purposes, the standard deviation or range should be considered (Table 4).

Dried blood spot analysis – a short overview on previously published data of drug analysis

At present, the DBS technology has been used to determine drugs that are effective in the therapy of the epilepsies, antimicrobial agents or agents to reduce the viral load of HIV infected persons (Table 5). Reviews have been published by Mei et al. (11) Edelbroek et al. (9) and Li & Tse (14). A broad range of analytical methods including radio immunoassays or liquid chromatography coupled to UV-, fluorimetric or mass spectrometric detection has been used for DBS analysis.

Prior to extraction the spot has been cut or punched out. For isolation, organic solvents or buffers have been used, in combination with tenside solutions, heat or ultrasonication. Imprecision and accuracy of published analytical methods are comparable to those performed on blood or plasma, whereas a clinical or a full analytical validation is so far being rarely reported.

Table 5: Analysis using dried blood spots. Isolation of the analyte(s), assay, extraction efficiency, imprecision, accuracy, clin. val.: clinical validation, ref.: reference, SPE: solid phase extraction, n.s.: not specified, LC: liquid chromatography, UV: ultraviolet light, MS/MS: tandem mass spectrometry, RIA: radioimmunoassay, fluo: fluorescence detection

Analyte(s)	Isolation	Assay	Extraction efficiency	Impre-cision	Accu-racy	Clin. val.	Refe-rence
Antiepileptic agents	MeOH/ace-tonitrile 3:1	LC-UV	95-100%	2-4%	101-110%	Blood/ serum	20
Anti-HIV drugs	MeOH/0.2 M $ZnSO_4$	LC-MS/MS	62-94%	7.8-11.5%	92-113%	Plasma	21
Chloroquine	0.9 M NH_4OH, SPE	LC-UV	72-92%	3.6-8.6%	98-104%	Blood (n=10)	22
Cyclosporine	Tris/Tween	RIA	n.s.	11.5%	n.s.	Blood	23
Sisomicin	Phosphate buffer	LC-fluo	93.6%	10.5-16.4%	93-100%	Plasma (n=5)	24
Tacrolimus	MeOH/Ace-tonitrile 4:1	LC-MS/MS	78±3.5%	5.1-7.4%	88-92%	Blood (n=24)	25

DBS vs whole blood: MDMA and MDA

Experimental study

Study design

First, a study on the dependency of the blood spot size on the hk value has been performed. One hundred µL aliquots (n=10) from freshly drawn blood samples or from blood samples with different hematocrit values (24.0-68.3%, n=9) were spotted on the paper. The diameter of each spot was measured independently by 2 individuals using a calliper gauge.

Partners were instructed to apply 100 µL of whole blood from a freshly drawn sample to each of the 4 circles of the blood spot card using a pipette (Fig. 1). As an alternative, whole blood from a finger prick could be applied to the paper. Care should be taken to completely fill the circles without touching the paper. The card was allowed to dry at ambient temperature for a minimum of 30 minutes folding it along the line where the filter paper and the form are stuck together. Then, the card was placed into a plastic bag containing a desiccant pad. The

DBS was stored at room temperature protected from light.

Extraction

Spots were cut out and placed into appropriate tubes. Extraction was performed as described for blood or plasma. Extraction efficiency could not be improved applying ultrasonication.

Analysis

Analysis was performed as described above for plasma and whole blood specimens by liquid chromatography/tandem mass spectrometry.

Results

Dependency of the blood spot size on the hematocrit value. The diameter of the blood spot decreased as the hk value increased, and a linear correlation could established: diameter (mm) = - 0.0893·hk (%) + 20.06 (Table 6).

Table 6: Dependency of blood spot diameter (mm) on the filter paper (n=10) on the hematocrit value (%); mean (mm) and standard deviation (sd, mm)

hematocrit / diameter	24%	31.3%	33.3%	44.0%	45.0%	50.3%	53.0%	64.6%	68.3%
mean	17.6	17.3	17.1	16.6	16.3	15.4	15.2	14.3	13.8
sd	0.70	0.67	0.32	0.52	0.48	0.70	0.79	0.48	0.63

Evaluation of DBS analysis: Validation data of MDMA analysis from DBS are summarized in Table 7. As 100 µL of blood had been spotted on the paper, DBS concentrations are given in ng/mL to keep a comparison between DBS and blood simple and comprehensible.

Table 7 Validation data of MDMA analysis from DBS, comparison of matrix effects and process efficiency according to Matuszewski et al. (26) in spots and blood (blood specimens from 5 different sources)

Calibration was performed using supplemented DBS	Matrix effect / process efficiency: DBS vs. blood
Extraction efficiency > 95%	Set 1: pure substance in mobile phase (n=5)
Linearity: 10-1000 ng/mL, r > 0.995	
Imprecision: 2-7%	Set 2: DBS / blood spiked after extraction
	Set 3: DBS / blood spiked before extraction

Accuracy > 90%	Matrix effect (%, set2 / set1)
LLOQ: 7.0 ng MDMA/spot	92.1 ± 4.1 DBS / 54.2 ± 12.0 blood
No carry-over could be observed	Process efficiency (%, set3 / set1)
	96.6 ± 10.7 DBS / 93.5 ± 9.3 blood

Results of DBS analysis

Concentrations in DBS ranged from not detectable to 316 ng/mL (31.6 ng/spot), and mean values were 99.0 ng/mL and 100.4 ng/mL for DBS and blood, respectively. For all samples where MDMA could be determined in the DBS, it was also present in the blood, and vice versa. Three samples showed concentrations between the LLOD and the LLOQ in both DBS and blood. In 42 samples which were considered for further analysis, MDMA concentrations were above the LLOQ for both blood and DBS.

Now, the issue has to be addressed whether DBS analysis is comparable to that in blood or by how much DBS analysis is likely to differ from conventional blood analysis. A simple scatter plot of the results of one method against those of the other may be a useful start, but it will be difficult to assess between-method differences. Therefore, a Bland-Altman difference plot was chosen as an alternative approach where the differences between the methods are plotted against their mean (27). There was no obvious relation between the difference and the mean. Therefore, the lack of agreement can be summarized by calculating the bias, estimated by the mean difference md and the standard deviation sd of the differences. If the differences are normally distributed, 95% of all differences will lie between the md-1.96·sd and md+1.96·sd (limits of “agreement”). Differences between the limits of “agreement” were not considered important which means that the measurement from either blood or DBS could be used interchangeably. The Bland-Altman difference plot of MDMA measurement from blood and DBS is shown in Figure 3.

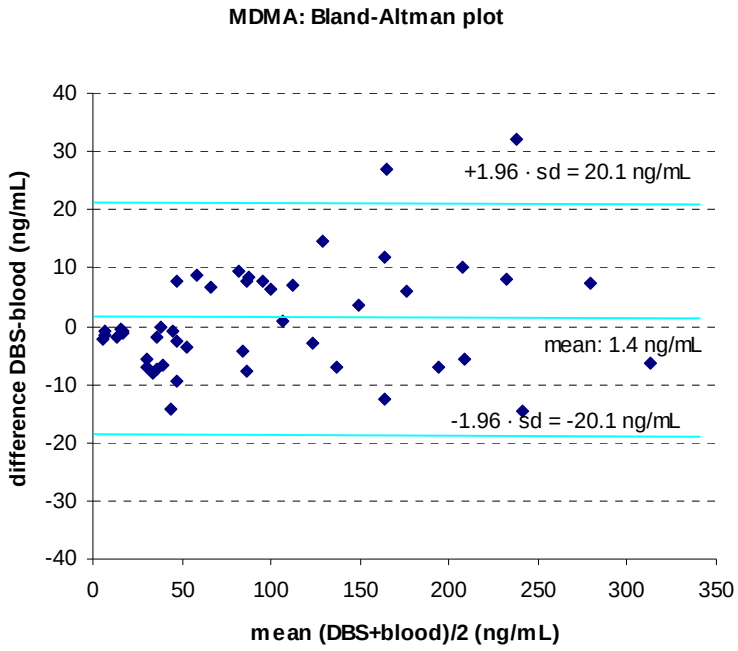

Figure 3 Bland-Altman difference plot of MDMA analysis (n=42) in whole blood and corresponding dried blood spots; mean difference=1.4 ng/mL, limits of agreement: mean difference +/- 20.1 ng/mL

Conclusion

Two out of 42 differences (4.76%) did not lie within the limits of "agreement", which is acceptable to use both methods interchangeably. There is a mean overestimation of 1.4 ng/mL (0.14 ng/spot) using DBS for analysis which is considered acceptable.

Spot vs. whole blood: amphetamine

Experimental study

Partners (TNO, The Netherlands, VTI, Sweden) were instructed to apply 100 µL of whole blood from a freshly drawn sample to each of the 4 circles of the blood spot card using a pipette (Fig. 1). As an alternative, whole blood from a finger prick could be applied to the paper. Care should be taken to completely fill the circles without touching the paper. The card was allowed to dry at ambient temperature for a minimum of 30 minutes folding it along the line where the filter paper and the form are stuck together. Then, the card was placed into a plastic bag containing a desiccant pad. DBS were stored at room temperature protected from light.

Table 8 Validation data of amphetamine analysis from DBS

Calibration was performed using supplemented DBS
Extraction efficiency > 95%
Linearity: 5-500 ng/mL, r > 0.997
Imprecision: 1.5-6.3 %
Accuracy > 90%
LLOD: 0.8 ng/mL (0.08 ng/spot)
LLOQ: 3.0 ng/mL (0.30 ng/spot)
No carry-over could be observed

Extraction

Spots were cut out and placed into appropriate tubes. Extraction was performed as described for blood or plasma (4.1). Extraction efficiency could not be further improved applying ultrasonication. *Analysis:* Analysis was performed as described above by liquid chromatography/tandem mass spectrometry (4.1).

Evaluation

Validation data of amphetamine analysis from DBS are summarized in Table 8. As 100 µL of blood had been spotted on the paper, DBS concentrations are given in ng/mL to keep a comparison between DBS and blood simple and comprehensible.

Results

The minimal, maximal and mean values as well as the standard deviation (sd) and the number of samples exhibiting values > LLOQ are given in Table 9; 0: not detectable

Table 9 Amphetamine-positive blood and DBS samples (ng/mL) – an overview; sd: standard deviation, conc.: concentration, LLOQ: lower limit of quantification

	Study of VTI (Sweden)		Study of TNO (The Netherlands)	
	Blood	DBS	blood	DBS
minimal value	0	0	10.75	10.90
maximal value	123.5	118.0	40.65	43.90
mean value	21.78	21.63	20.61	21.64
sd	29.6	29.5	7.08	7.40
conc. > LLOQ	n=37	n=37	n=29	n=29

In all samples from both studies where amphetamine could be determined in the DBS, it was also present in the blood, and vice versa. The difference between amphetamine values determined from DBS and whole blood is presented in Figures 4 and 5 using Bland-Altman difference plots. The mean difference between the methods was -0.63 ng/mL (sd 3.66 ng/mL) for samples from VTI, and 1.03 ng/mL (sd 1.17 ng/mL) for samples from TNO. Differences of samples from both sources are in good agreement with only 1 sample exceeding the upper “limit

of “agreement” (2.7%, VTI, Sweden).

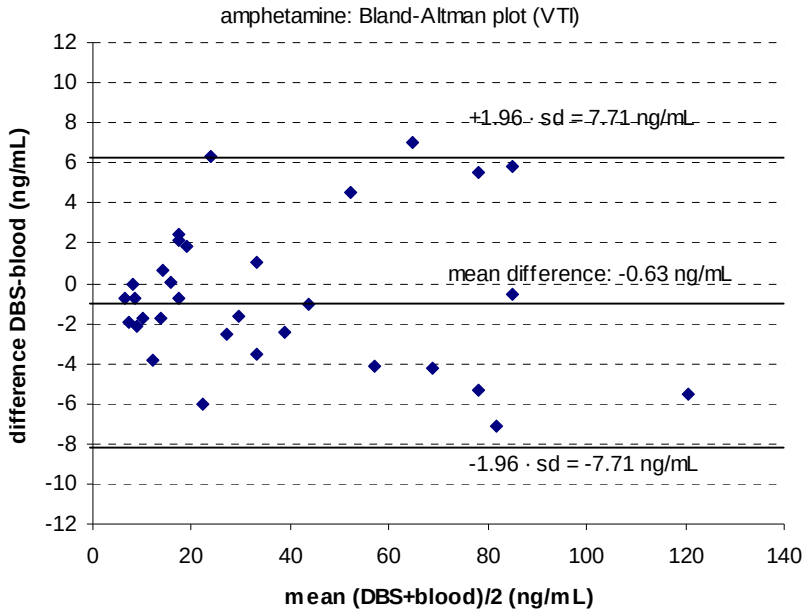

Figure 4 Bland-Altman difference plot of blood and DBS samples obtained from VTI, Sweden (n=37)

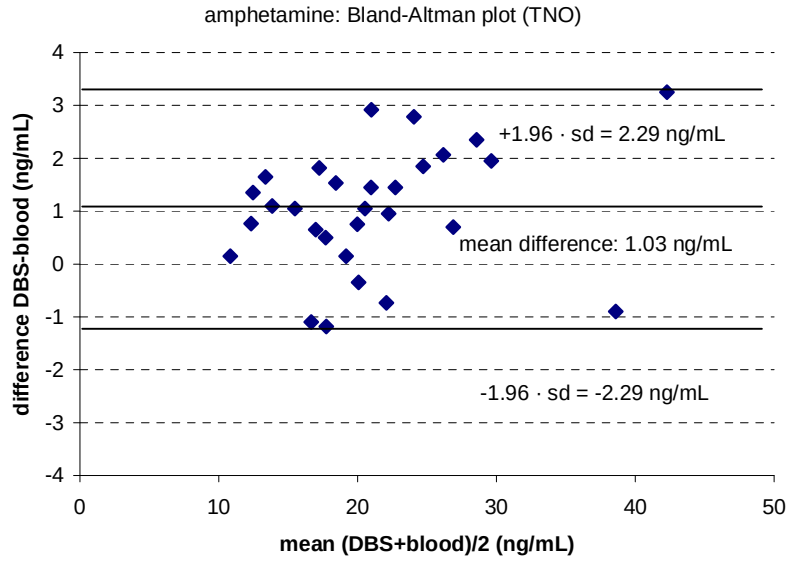

Figure 5 Bland-Altman difference plot of blood and DBS samples obtained from TNO, The Netherlands (n=29)

Conclusion

Mean differences were -0.63 ng/mL (-0.06 ng/spot) and 1.03 ng/mL (0.10 ng/spot) using DBS for analysis which is considered acceptable. As already observed for MDMA and MDA, the DBS assay has potential as a precise

and inexpensive option for the determination of amphetamine and amphetamine derivates in small blood samples. The limits of detection and quantification as well as the imprecision data were within a satisfactory range.

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