

Cognitive and physiological effects of  
3,4-methylenedioxymethamphetamine  
(MDMA or 'ecstasy') in combination with  
alcohol or cannabis in humans



**DONDERS**  
series

G.J.H. Dumont

*'Everything leads me to think that, in the near future, reality will be considered exclusively as a mere state of depression and inactivity of the mind'* S. Dali

~

*'The warm, the richly coloured, the infinite friendly world of soma-holiday. How kind, how good-looking, how delightfully amusing everyone was!'* A. Huxley *'A brave new world'*

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**Cognitive and physiological effects of 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') in combination with alcohol or cannabis in humans**

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Medische Wetenschappen

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## ***Introduction***

Psychoactive compounds are used to treat psychiatric disorders as well as for recreational purposes. The use of psychoactive substances for recreational purposes appears to be as old as civilisation, where especially alcohol and tobacco have a long standing history of socially accepted and legal recreational use, although trends in drug use vary as exemplified by for example (now illegal) opium use. In western society ecstasy, cannabis and alcohol are currently one of the most commonly used psychoactive substances for recreational purposes. Moreover, combined use of such drugs appears to be the rule rather than the exception (Parrott, Milani et al. 2007; Winstock, Griffiths et al. 2001).

Although there are only minor differences between legal and illegal psychoactive drugs from a pharmacological point of view, the use of psychoactive compounds for recreational purposes is subject to great controversy in Western society. Alcohol use for example is a common, legal and socially accepted recreational drug while the use of ecstasy is illegal and disapproved by society. Most often, addiction and greatly impaired mental and physical health are said to result from recreational use of illegal drugs and these arguments are used to support restrictive legislation. However, ecstasy use seldomly leads to addiction, while alcohol on the other hand has well-known addictive properties (Adinoff 2004). Moreover, relatively 'new' addictions such as gambling, internet, pornography and gaming underscore the fact that it is not the drug but the behaviour that shapes the addiction. Although excessive drug use (whether this concerns ecstasy or alcohol) can induce cognitive and physiological impairments, research into the long-term effects of drugs such as ecstasy generally show only small effects on cognitive function (Gouzoulis-Mayfrank and Daumann 2006a). The long-term physiologic effects of ecstasy use are less well investigated, but results suggest that impairment of physiologic function generally occurs only when drug exposure is frequent (Brody, Krause et al. 1998; Droogmans, Cosyns et al. 2007). Any substance will impair health when used carelessly, and as such, the pharmacological cliché that it is not the substance *persé* but the quantity in which it is used applies here as well.

One must however not assume that drugs are harmless, as these drugs typically induce robust acute effects, and small reports in papers as well as case

reports exemplify that drug use can be acutely hazardous to health and even lethal when used carelessly (Kalantar-Zadeh, Nguyen et al. 2006; Yoda, Crawshaw et al. 2005). Thus, research regarding the acute psychological as well as physiological effects may provide highly relevant information regarding the acute dangers of drug use, and as such may provide a rationale for harm reduction in recreational drug users and (more) appropriate legislation. Obviously, not using drugs is the easiest and most effective way of reducing harm, however, and analogous to cigarettes and alcohol, the use of ecstasy is prevalent with estimates of 40.000 current users in the Netherlands alone (Trimbos Instituut 2008). A scientific evaluation of the acute effects of MDMA, the psychoactive compound of ecstasy, thus appears warranted. However, typical recreational ecstasy users are generally mis-classified as these persons do not exclusively use ecstasy. Rather, they experiment with an abundance of psychoactive compounds and combine these substances, reportedly to alleviate some of the less desired effects and potentiate desired effects. Hence the self-proclaimed term 'psychonauts' (derived from psychoactive and astronaut) is a better description. Ecstasy is most frequently combined with alcohol (most probably due to availability) and cannabis (anecdotely to alleviate the ecstasy come-down, ie. the descending slope of ecstasy effects) (Gouzoulis-Mayfrank and Daumann 2006a).

Next to this rather pragmatic rationale for the current thesis, the powerful acute effects of recreational drugs provide new means to study and understand the way the human brain functions. As the neurobiological targets of most recreational drugs are known from animal research, this provides a powerful addition to psychopharmacologic research to study the effects of specific manipulations of the brain's neurochemistry in humans. The psychopharmacology of cannabis, for example, has only recently received extensive attention from the scientific community and already has provided many interesting leads regarding for example weight loss, pain allevation, and even cancer therapy (Pacher, Batkai et al. 2006). Methylphenidate (Ritalin), an amphetamine (streetname speed) analogue is registered as a treatment for Attention Deficit Hyperactivity Disorder (ADHD), and MDMA (ecstasy) is currently being investigated as a possible therapy for post-traumatic stress disorder (Sessa and Nutt 2007). Thus, recreational drugs may

provide new leads for potential treatments for psychiatric disorders, which also warrants further research into the pharmacology of recreationally used substances.

However, investigating the effects of recreational drugs is prohibited in many countries and, in the countries where it is allowed such as the Netherlands, extremely difficult to undertake because of restrictive legislation. Although one should most definitely be very cautious and careful when undertaking such experimental research, *'Regulation must follow science, not dictate it'* (M. Pirmohamed, NHS).

### ***Ecstasy***

Ecstasy is the streetname for 3,4-methylenedioxymethamphetamine (MDMA). MDMA was first synthesized in 1912 by Merck, and was called 'Methylsafrylamin'. However, MDMA was not pharmacologically tested until 1927, and its effects were not evaluated in humans by Merck (Freudenmann, Oxler et al. 2006). Later, psychotherapists used MDMA to aid psychotherapy. Although these therapists generally reported successful use of MDMA, its actions were never scientifically evaluated. These therapists did note that ecstasy enabled patients to discuss issues that they found difficult to confront and to facilitate emotional catharsis (Sessa 2007). However, MDMA was prohibited in 1985 in the USA under the Controlled Substance Act of 1984, and despite recommendations by its own advisory board, it was not permitted to be used in a medical situation anymore. Ecstasy gained its wide spread popularity in recreational drug users after its prohibition, suggesting that the prohibition facilitated its popularity. Recently, the therapeutic potential of MDMA has been attracting renewed attention from scientist and therapists, and researchers question its drug classification (Nutt 2006), and request to legalise MDMA use for therapeutic purposes (Sessa and Nutt 2007).

Currently, there are an estimated 40000 current users of ecstasy in the Netherlands alone (Trimbos Instituut 2008). Despite the large population at risk world-wide, relatively few reports of severe adverse events with ecstasy have

emerged, although adverse events with fatal outcome have been reported, presumably in individuals who are (genetically) susceptible to ecstasies deleterious side effects (Hall and Henry 2006; Hartung, Schofield et al. 2002; Kalantar-Zadeh, Nguyen et al. 2006). Ecstasy is most popular in the club scene, most likely due to its unique behavioral effects. The behavioural effects of MDMA resemble, but are not restricted to, effects of psychostimulants (e.g. amphetamines or 'speed') as well as hallucinogenics (e.g. lysergic acid or 'LSD'), although MDMA's most characteristic effects are described as an increase in empathy and friendliness, presumably leading to streetnames such as 'love-drug'. As these effects were not observed in hallucinogens nor in stimulants, MDMA was referred to as an 'entactogen', a separate drug-class (Nichols and Oberlander 1990; Tancer 2001; Vollenweider, Liechti et al. 2002).

MDMA is typically ingested orally and rapidly absorbed. Within 30 minutes MDMA is detectable in the blood. Plasma levels peak at 1-2 hr after drug administration, and maximum behavioural and subjective effects occur around 1-2 hr and have declined by 4 hr in spite of persisting plasma levels (de la Torre, Farre et al. 2004; Green, Mehan et al. 2003). MDMA's mechanism of action involves interference with the transporters of the monoamine neurotransmitters. These pre-synaptically located transporters remove the neurotransmitter from the synapse enabling recycling of these neurotransmitters. MDMA is relatively selective for serotonin (5-HT), but also releases dopamine and noradrenaline (Liechti and Vollenweider 2001). MDMA enters presynaptic serotonin nerve cells mainly by means of the presynaptic serotonin transporter (SERT), and releases the intracellular 5-HT into the synapse by reversal of the SERT direction. MDMA also releases 5-HT from its intracellular storage vesicles via interference with the vesicular transporter (VMAT-2) similar to its actions on the SERT. Vesicular 5-HT release leads to high cytoplasmic 5-HT levels, which can be transported into the synapse by the 'reversed' SERT or even 'leak' out of the cell, thus increasing synaptic 5-HT levels (Mlinar and Corradetti 2003).

The characteristic psychological effects of MDMA (augmented social interaction, friendliness and empathy towards others) have been shown to be caused

by the enhanced serotonin neurotransmission (Thompson, Callaghan et al. 2007). In humans, pre-treatment with the 5-HT reuptake inhibitor citalopram, which effectively blocks SERT, attenuated the typical psychological effects of MDMA in healthy volunteers (Liechti and Vollenweider 2001). Physiologically, MDMA shows typical stimulant effects with increases of heart rate, blood pressure, and body temperature (Dumont and Verkes 2006; Vollenweider, Liechti et al. 2002). MDMA's stimulant effects are induced by increased dopamine and/or noradrenaline availability (Colado, O'Shea et al. 2004; Mills, Banks et al. 2003).

### *Alcohol*

Drinks containing ethanol, commonly referred to as alcohol, are regularly used in social settings. With over 4 million current users in the Netherlands, it is by far the most common drug to be used recreationally, even exceeding tobacco use (estimated number of current users 3.7 million). However, compared to ecstasy and cannabis, it is by far the most harmful, with 12.013 hospitalisations in the last year and 1.742 fatalities (only tobacco is more harmful with an estimated 19.366 fatalities) in the Netherlands (Trimbos Instituut 2008).

A single dose of oral alcohol will show a rapid increase with maximal plasma concentrations around 45-60 minutes and a steady decline afterwards. The dynamic effects of alcohol generally are congruent with its kinetic time profile. As alcohol is a sedative drug it generally impairs cognitive function, but it also can disinhibit behavior. Ethanol has many physiological effects, with complex but relatively small effects on heart rate, and typically lowers peripheral vascular resistance which facilitates heat dissipation. In unfavorable surroundings, this may induce hypothermia (Pohorecky and Brick 1988). Ethanol's mechanism of action is allosteric modulation of many transmembrane receptors, but functionally it acts foremost as a CNS depressant, depressing both excitatory and inhibitory postsynaptic potentials by potentiating the action of GABA at the GABA<sub>A</sub> receptor (Suzdak, Schwartz et al. 1988).

## *Cannabis*

Cannabis is the product of dried flowertops of the cannabis sativa plant. There are currently an estimated 363.000 cannabis users in the Netherlands (Trimbos Instituut 2008). THC, the major psychoactive compound in cannabis, is an agonist for the CB<sub>1</sub> and CB<sub>2</sub> receptors of the endocannabinoid system (ECS). The ECS is an atypical neurotransmitter system as the path of information transmission is reversed compared to 'typical' neurotransmission: Endocannabinoids (such as anandamide) are synthesized on-demand post-synaptically and diffuse back to the pre-synaptic axon terminal, where the CB<sub>1</sub> receptor is located. CB<sub>1</sub> activation in turn depresses the pre-synaptic membrane potential thus functionally silencing synaptic neurotransmission, ie. facilitating synaptic negative feedback. The CB<sub>1</sub> receptor is abundantly expressed in the central nervous system whereas the CB<sub>2</sub> receptor is expressed predominantly in the peripheral parts of the body (Ameri 1999). Probably due to the lack of CB<sub>1</sub> receptors in the brain stem areas supporting vital functions, there are few hospitalisations due to cannabis use (54 in the Netherlands in 2006, including hospitalisation for addiction (Trimbos Instituut 2008)) and cannabis intoxication rarely induces serious adverse events. However, THC is a potent stimulant of heart rate and reduces vascular resistance (Sidney 2002), which may induce transient collapse due to cardiovascular dysfunction (Ghuran and Nolan 2000). Typical desired psychological drug effects of THC are relaxation, but also mild hallucinogenic effects. At high concentrations, THC can induce anxiety (Block, Erwin et al. 1998).

THC, a highly lipophilic compound, is rapidly distributed from the blood into fatty tissue (among which the CNS), and after inhalation peak plasma concentration are reached within minutes and show a rapid decline, although cognitive and subjective effects peak around 15 to 60 minutes and last for several hours (Curran, Brignell et al. 2002; Strougo, Zuurman et al. 2008).

### *This thesis*

This thesis aimed to assess the acute effects of MDMA and ethanol or THC, two frequently used recreational drug combinations, on cognitive performance, subjective experience and physiological function. As MDMA, a psychostimulant, on the one hand, and ethanol or THC, both sedatives, on the other hand have quite distinct effect profiles, the effects of drug combinations were expected to differ from single drug effects.

Both studies recruited sixteen healthy volunteers, regular users of ecstasy and alcohol or THC, and used a four-way, double blind, randomized, crossover, and placebo controlled design. MDMA (or matched placebo) was given orally as a capsule in a single dose of 100 mg, a relevant dose in the range of normal single recreational dosages (Tanner-Smith 2006).

Ethanol (or glucose 5% as its placebo) was administered continuously by IV infusion of a 10% ethanol in 5% glucose solution for three hours. The alcohol clamp was targeted at 0.6‰, the equivalent of approximately 2-3 alcoholic beverages. This promillage is just above the legal limit for traffic participation in many Western countries and commonly used in social settings, as it is considered to be a safe and relatively moderate dose, despite significant CNS effects (Amatsaleh, Schoemaker et al. 2006). An intravenous administration route was chosen to ensure standardization of the rate and bioequivalence of ethanol administration, an important prerequisite for predictable pharmacokinetics of ethanol.

THC (4, 6 and 6 mg at 90-minute intervals) or placebo were administered by inhalation using a Volcano<sup>®</sup> vaporizer (Storz-Bickel GmbH, Tuttlingen, Germany), a validated method of intrapulmonary THC administration (Abrams, Vizoso et al. 2007; Hazekamp, Ruhaak et al. 2006). The inhalation schedule was predicted to cause THC plasma concentrations and effects roughly corresponding to the use of one marijuana cigarette (Zuurman, Roy et al. 2008).

***A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers***

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## *Abstract*

This review of the literature aims to identify the acute effects of MDMA (“ecstasy”) in healthy volunteers. The wide range of relevant but methodologically diverse tests was first grouped into clusters to allow an evaluation of tests that would otherwise have been excluded due to their low frequency of utilisation. The following three types of tests were evaluated: (1) *Functional* tests quantifying executive, attention, visual, motor, visuomotor and auditory functions, (2) *Phenomenological* tests assessing personal, subjective experiences, and (3) *Physiological* measures reflecting neurophysiological, endocrine and physiological parameters.

MDMA showed robust effects on most of the phenomenological and physiological tests. Functional tests were scarce, preventing any meaningful conclusions to be drawn from their evaluation other than that these tests should be incorporated into future acute-effect studies.

A striking dose-response relationship appeared for cardiovascular effects. At doses below 1.0 mg/kg MDMA no change was observed relative to placebo while above this dose all studies reported significant increases. Furthermore, pupil size, plasma cortisol and plasma prolactin levels proved responsive to MDMA administration. The reported subjective effects of MDMA matched the entactogenic profile.

Although interest in the action of MDMA is considerable, the existing knowledge about the cognitive effects of MDMA in humans is still rather limited and further research into the drug’s effects is recommended, also in view of potential therapeutic uses of the drug.

## ***Introduction***

In Western societies a considerable percentage of young people expose themselves to 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”) under less than ideal circumstances (Gross, Barrett et al. 2002; Parrott 2001; Schifano, Di Furia et al. 1998). The potential hazards associated with this prevalent recreational use of the drug make in-depth knowledge about the acute effects of MDMA indispensable. Since trials to assess the therapeutic potential of MDMA are underway, a thorough understanding of the acute actions of MDMA has also become essential to assure drug safety.

MDMA is rapidly absorbed following oral administration, is detectable in the blood within 30 minutes, reaches its T-max in 1-2 hours and has a half life of about 6-8 hours (Green, Mehan et al. 2003). The psychoactive effects last for approximately 2-4 hours in spite of persisting blood levels and in concordance with the persisting MDMA levels objective impairment of mental functioning lasts longer than the subjective effects (Lamers, Ramaekers et al. 2003). The pharmacokinetics and metabolism of MDMA are described in more detail elsewhere (de la Torre, Farre et al. 2000a; Green, Mehan et al. 2003).

MDMA enters presynaptic serotonin nerve cells mainly by means of the presynaptic serotonin transporter (SERT), and releases the intra-cellular 5-HT storage into the synapse by reversal of the SERT. Depletion of 5-HT from its intracellular vesicles due to interference with the vesicular transporter (VMAT-2) has also been reported (Mlinar and Corradetti 2003). Vesicular depletion leads to high cytoplasmic 5-HT levels, which can be transported into the synapse by the SERT or even ‘leak’ out of the cell, thus increasing synaptic 5-HT levels. The characteristic psychological effects of MDMA (augmented social interaction, friendliness and empathy towards others) are thought to be caused by the enhanced serotonin neurotransmission. In concordance with this, pre-treatment with the 5-HT reuptake inhibitor citalopram, which effectively blocks SERT, was found to markedly but not completely attenuate the psychological effects of MDMA in healthy volunteers (Liechti and Vollenweider 2000a).

As the characteristic effects of the drug are not observed in strict hallucinogens nor in stimulants, MDMA is referred to as an 'entactogen' (Nichols and Oberlender 1990;Parrott 2001;Ramaekers, Lamers et al. 2002;Tancer and Johanson 2000;Vollenweider, Liechti et al. 2002). It also has arousing effects presumably induced by dopamine and/or noradrenaline release (Colado, O'Shea et al. 2004;Liechti and Vollenweider 2000;Liechti and Vollenweider 2001;Mills, Rusyniak et al. 2004).

Several studies have shown MDMA to be neurotoxic in rats and primates (Jones, Duvauchelle et al. 2005;Ricaurte, Yuan et al. 2000). The process of neurodegeneration is exacerbated by high ambient temperatures and mainly occurs in fine serotonergic axons (Sanchez, O'Shea et al. 2004). This effect was explained in a report in which increased temperature was found to raise the ratio of dopamine/serotonin uptake by SERT in vitro (Saldana and Barker 2004). Dopamine degradation in the 5-HT terminal and the subsequent formation of radical oxygen species (ROS) thus might play a causal role in the drug's neurotoxicity (Colado, O'Shea et al. 2004;Escobedo, O'Shea et al. 2005), in addition to its own metabolism that also leads to ROS formation (Colado, O'Shea et al. 2004;Johnson, O'Callaghan et al. 2004;Jones, Duvauchelle et al. 2005). Although neurotoxicity has clearly been demonstrated in animals, and some studies have suggested neurodegeneration in humans (Reneman, Booij et al. 2001a;Reneman, Booij et al. 2001b), functional impairment has not been convincingly linked to the actions of MDMA (Curran 2000).

Many retrospective studies have been performed to identify functional impairment associated with the recreational use of ecstasy or 'XTC'. Although the results are inconsistent or even contradictory, memory is most frequently reported to be affected by MDMA (Daumann, Fischermann et al. 2004;Verbaten 2003;Verkes, Gijsman et al. 2001). The most consistent predictor of cognitive impairment appears to be the number of tablets per occasion, i.e. the stacking of XTC pills to prolong the drug's effects (McCann, Szabo et al. 2005). This complies with the data on acute effects, where systemic metabolism leading to the formation of ROS is necessary for neurotoxicity to develop (Colado, O'Shea et al. 2004). The stacking of pills is

likely to exhaust antioxidant resources, thereby increasing toxicity, causing subsequent axonal degeneration. This hypothesis is strengthened by the finding that co-administration of drugs that lower the hyperthermic response and/or provide radical trapping with MDMA tend to decrease this nerve damage while the entactogenic MDMA effects remain unaltered (Escobedo, O'Shea et al. 2005).

Prospective studies investigating the effects of an illegal and potentially neurotoxic drug may be rejected or amended on the grounds of ethical issues, but the interpretation of retrospective studies into MDMA-induced functional impairments is hampered by methodological difficulties making conclusions questionable. The main confounding factor is that MDMA users are generally multidrug users, either consciously or due to the impurity of the XTC pills. As a result, any functional impairment cannot be strictly attributed to MDMA use since it might partly be associated with the concurrent substance or even to the multidrug use itself. A second confounder is that due to their design retrospective studies rely on self-reported drug use. As discussed above, as the drug use might affect memory and may not be restricted to MDMA alone, and because the contents of the XTC tablets used is variable, the data and conclusions drawn are inherently controversial. And finally, pre-existing group differences are possible and remain unknown. For an in-depth discussion of this latter topic we refer to Curran *et al.* (Curran 2000).

With the present study we aimed to create a profile of the acute effects of MDMA in humans by verifying or dismissing current assumptions about these effects by means of a comprehensive review of the available literature. For inclusion in this review studies needed to meet stringent criteria and the effects reported were compared to the results of the other studies to substantiate the validity of the conclusions.

## ***Materials and methods***

### *Structured evaluation of the literature*

We performed a literature search via PubMed using the following keywords: MDMA OR ecstasy OR XTC OR 3,4-methylenedioxymethamphetamine, and human OR volunteer. This yielded a total of 1446 articles, which were subsequently manually scanned for:

- a. Administration of MDMA in healthy volunteers
- b. A placebo-controlled design
- c. Measurement of acute-effect parameters
- d. Administration of a known and verified dose
- e. Being an original investigation.

Only articles that met all the abovementioned criteria were included and their references were also scanned for relevant articles. The test results mentioned in the selected articles were all recorded onto a datasheet, together with dose information and number of participants.

### *Grouping of individual test results*

A structured procedure (de Visser, van der Post et al. 2001; de Visser, van der Post et al. 2003; Dumont, de Visser et al. 2005) was adopted to obtain an overview of the responses of tests or test variants to MDMA involving a progressive evaluation of all selected tests. The results from tests that were used only once or by one research group could not be generalised, and were therefore not analysed individually. Tests that could be regarded as variants from a basic form were grouped. Subsequently, clusters of tests were grouped further based on their predominant domain (see Table 1). The effects on these domains were also reviewed whenever relevant. The three categories we identified were:

1. “Functional” tests; tests of neuropsychological function; i.e. executive function, attention, visual/visuomotor & auditory function and motor function.

2. “Phenomenological” tests; tests that attempt to quantify personal experiences; i.e. subjective measurements.
3. “Physiological” measures; tests that measure physiological parameters; i.e. neurophysiological, endocrine and other physiological measures.

Because even for comparable methods a large diversity of test parameters was found, we were unable to quantitatively record the individual test results. Instead, if a test yielded a statistically significant difference from placebo or baseline this was scored as + when the effect indicated an improvement or increase; if the effect was not significant it was classified as = or as - when it significantly demonstrated an impairment or decrease. Whether a difference from placebo was scored as improvement (+) or impairment (-) depended on the psychosocial desirability of the response (i.e. an increase in reaction-time scores was interpreted as an impairment). Although, of course, statistical significance is not only determined by the variance and the size of the effect but also by factors like group size, these factors could not be taken into account as the results were too variable for a formal meta-analysis. Nonetheless, our semi-quantitative review did allow an evaluation of the applicability of a test as an effect measure in typical acute-drug-effects studies with limited numbers of participants. No efforts were made to further quantify the level of statistical significance.

#### *Test criteria*

Not all tests are equally valuable. Ideally, a test should meet the following criteria to be considered to be representing the effect of the drug of interest:

- a. Be sensitive to a specific effect of the drug of interest
- b. Show a clear and consistent response across studies
- c. Reflect a clear dose-response relationship
- d. Demonstrate a plausible association between the effect and the pharmacology of the drug of interest

However, only the first criterion is a prerequisite, with the other criteria strengthening the justification for the choice of a particular test.

### *Consistency of responses*

As mentioned above, the response of a test to a drug can be either positive (increased compared to placebo) or negative (decreased compared to placebo), or it can show no change. A useful test is expected to show a consistent response to a drug within a certain dose range. A test is not considered useful if the outcome is as often positive as it is negative, i.e. showing large variations around baseline outcomes, or if a large proportion of studies fails to show significant effects. Tests were therefore arbitrarily considered to produce a consistent response when results were significant and reflecting a similar outcome (either positive or negative) in at least 20% of the reviewed studies. Accordingly, test results were judged as inconsistent when fewer than 20% of the tests showed statistically significant results, or when the directions of the responses were variable (i.e. when more than 20% decreased and more than 20% increased). This arbitrary cut-off value only eliminated tests that hardly ever responded to the drug and could therefore not be considered to be useful tests for this drug.

## Results

Of the total, 29 articles were found to meet all criteria, yielding 150 separate tests that were subdivided into 39 clusters and eight domains (see Table 1). On average, each study included ten subjects (range: 2-16). The average age was 24.7 years, with ages ranging from 18 to 40 years. One of every four participants was female and all participants had completed some form of secondary education. Reported demographics were relatively uniform across studies, with a notable exception of the group of Vollenweider who included MDMA naïve participants whereas most studies had required previous MDMA use.

Test name	Cluster
<b>Executive domain</b>	
Tower of London <sup>(Lamers, Ramaekers et al. 2003)</sup>	Planning
Word fluency <sup>(Lamers, Ramaekers et al. 2003)</sup>	Language
<b>Attention</b>	
DSST <sup>(Cami, Farre et al. 2000; Farre, de la Torre et al. 2004; Hernandez-Lopez, Farre et al. 2002)</sup>	DSST
Stroop test - % errors <sup>(Vollenweider, Gamma et al. 1998)</sup> , Stroop test – reaction time <sup>(Vollenweider, Gamma et al. 1998)</sup>	Selective attention
Continuous Performance Task <sup>(Gamma, Buck et al. 2000)</sup>	Continuous performance
DAT – tracking error <sup>(Lamers, Ramaekers et al. 2003)</sup> , DAT reaction time <sup>(Lamers, Ramaekers et al. 2003)</sup> , MCRT – initiation time <sup>(Lamers, Ramaekers et al. 2003)</sup> , OMEDA- divided attention error <sup>(Lamers, Ramaekers et al. 2003)</sup>	Divided attention
<b>Visual, visuomotor and auditory domain</b>	
OMEDA- time to contact error <sup>(Lamers, Ramaekers et al. 2003)</sup>	Movement estimation
Signal detection task <sup>(Lamers, Ramaekers et al. 2003)</sup>	Visual search
<b>Motor domain</b>	
MCRT – movement time <sup>(Lamers, Ramaekers et al. 2003)</sup> , Critical tracking <sup>(Lamers, Ramaekers et al. 2003)</sup>	Motor control
Vienna apparatus-reaction time <sup>(Cami, Farre et al. 2000; Farre, de la Torre et al. 2004; Hernandez-Lopez, Farre et al. 2002)</sup>	Reaction time
<b>Subjective domain</b>	
VAS closeness to others <sup>(Harris, Baggott et al. 2002)</sup> , EWL emotional excitability - sensitivity <sup>(Liechti, Baumann et al. 2000)</sup> , VAS social <sup>(Tancer and Johanson 2003)</sup> , POMS friendly <sup>(Cami, Farre et al. 2000; Lamers, Ramaekers et al. 2003; Tancer and Johanson 2003; Tancer 2004)</sup> , VAS friendly <sup>(Harris, Baggott et al. 2002; Tancer and Johanson 2003)</sup>	Social interaction

Table 1. Overview of all reported tests and measurements (with the source studies between brackets), clusters and domains (continued on next page)

Test name	Cluster
<b>Subjective domain</b>	
VAS alert <sup>(Tancer and Johanson 2003)</sup> , POMS arousal <sup>(Cami, Farre et al. 2000;Tancer and Johanson 2003;Tancer 2004)</sup> , EWL mood questionnaire- activity <sup>(Frei, Gamma et al. 2001;Gamma, Buck et al. 2000;Liechti, Baumann et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000;Vollenweider, Gamma et al. 1998)</sup> , SDEQ autonomic arousal <sup>(Harris, Baggott et al. 2002)</sup> , SDEQ cognitive improvement <sup>(Harris, Baggott et al. 2002)</sup> , VAS stimulated <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002;Tancer and Johanson 2003;Tancer 2004)</sup> , ARCI A <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002;Tancer and Johanson 2003;Tancer 2004)</sup> , ARCI BG <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002;Tancer and Johanson 2003;Tancer 2004)</sup> , VAS performance <sup>(Cami, Farre et al. 2000)</sup> , VAS concentration <sup>(Cami, Farre et al. 2000)</sup> , POMS vigor <sup>(Cami, Farre et al. 2000;Lamers, Ramaekers et al. 2003;Tancer and Johanson 2003;Tancer 2004)</sup>	Arousal
EWL well-being- heightened mood <sup>(Frei, Gamma et al. 2001;Gamma, Buck et al. 2000;Liechti, Baumann et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000;Vollenweider, Gamma et al. 1998)</sup> , SDEQ mood euphoria <sup>(Harris, Baggott et al. 2002c)</sup> , VAS active <sup>(Cami, Farre et al. 2000)</sup> , VAS passive <sup>(Cami, Farre et al. 2000)</sup> , ARCI MBG <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002;Tancer and Johanson 2003;Tancer 2004)</sup>	Euphoria
OAV Oceanic Boundlessness <sup>(Frei, Gamma et al. 2001;Gamma, Buck et al. 2000;Liechti, Baumann et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000;Vollenweider, Gamma et al. 1998)</sup> , EWL well-being-self-confidence <sup>(Frei, Gamma et al. 2001;Gamma, Buck et al. 2000;Liechti, Baumann et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000;Vollenweider, Gamma et al. 1998)</sup> , VAS confident <sup>(Harris, Baggott et al. 2002;Tancer and Johanson 2003)</sup> , VAS fear <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS miserable <sup>(Tancer and Johanson 2003)</sup> , POMS elation <sup>(Cami, Farre et al. 2000;Tancer 2004)</sup> , VAS calm <sup>(Cami, Farre et al. 2000)</sup> , VAS contentedness <sup>(Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , SDEQ relaxation <sup>(Harris, Baggott et al. 2002)</sup> , EWL emotional excitability <sup>(Frei, Gamma et al. 2001;Gamma, Buck et al. 2000;Liechti, Baumann et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000;Vollenweider, Gamma et al. 1998)</sup> , POMS positive mood <sup>(Cami, Farre et al. 2000;Tancer and Johanson 2003)</sup> , PANSS (positive and negative syndrome scale) <sup>(Harris, Baggott et al. 2002)</sup>	Mood
VAS self conscience <sup>(Tancer and Johanson 2003)</sup> , EWL anxiety – thoughtfulness-contemplativeness <sup>(Liechti, Baumann et al. 2000)</sup> , VAS insightful <sup>(Harris, Baggott et al. 2002)</sup> , EWL – extroversion <sup>(Frei, Gamma et al. 2001;Gamma, Buck et al. 2000;Liechti, Baumann et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000;Vollenweider, Gamma et al. 1998)</sup>	Extroversion
EWL anxiety – depressiveness <sup>(Liechti, Baumann et al. 2000)</sup> , POMS depression <sup>(Cami, Farre et al. 2000;Lamers, Ramaekers et al. 2003;Tancer and Johanson 2003)</sup> , VAS down <sup>(Tancer and Johanson 2003)</sup> , VAS depression or sadness <sup>(Cami, Farre et al. 2000)</sup> , VAS sadness <sup>(Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup>	Depression
EWL – inactivation – dazed state <sup>(Liechti, Baumann et al. 2000)</sup> , POMS confusion <sup>(Cami, Farre et al. 2000;Tancer 2004)</sup> , VAS confusion <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002;Tancer and Johanson 2003)</sup>	Confusion
EWL – inactivation – tiredness <sup>(Liechti, Baumann et al. 2000)</sup> , POMS fatigue <sup>(Cami, Farre et al. 2000;Lamers, Ramaekers et al. 2003;Tancer and Johanson 2003)</sup> , VAS tired <sup>(Tancer and Johanson 2003)</sup> , VAS sedated <sup>(Tancer and Johanson 2003;Tancer 2004)</sup> , EWL – inactivation <sup>(Frei, Gamma et al. 2001;Gamma, Buck et al. 2000;Liechti, Baumann et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000;Vollenweider, Gamma et al. 1998)</sup> , SDEQ cognitive impairment <sup>(Harris, Baggott et al. 2002)</sup> , VAS drowsiness <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , ARCI PCAG <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002;Tancer and Johanson 2003;Tancer 2004)</sup>	Sedation
VAS irritable <sup>(Tancer and Johanson 2003)</sup> , VAS on edge <sup>(Tancer and Johanson 2003)</sup> , POMS anger <sup>(Cami, Farre et al. 2000;Lamers, Ramaekers et al. 2003;Tancer and Johanson 2003)</sup> , EWL emotional excitability – aggression-anger <sup>(Liechti, Baumann et al. 2000)</sup>	Aggression

Table 1. Overview of all reported tests and measurements (with the source studies between brackets), clusters and domains (continued on next page)

<i>Test name</i>	<i>Cluster</i>
<b>Subjective domain</b>	
ARCI LSD <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002;Tancer and Johanson 2003;Tancer 2004)</sup> , EWL anxiety – apprehension anxiety <sup>(Liechti, Baumann et al. 2000)</sup> , State-Trait Anxiety Inventory (STAI) <sup>(Liechti, Saur et al. 2000;Liechti and Vollenweider 2000)</sup> , POMS anxiety <sup>(Cami, Farre et al. 2000;Lamers, Ramaekers et al. 2003;Tancer and Johanson 2003;Tancer 2004)</sup> , OAV Anxious Ego Dissolution <sup>(Frei, Gamma et al. 2001;Gamma, Buck et al. 2000;Liechti, Baumann et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000;Vollenweider, Gamma et al. 1998)</sup> , EWL anxiety <sup>(Gamma, Buck et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000a;Vollenweider, Gamma et al. 1998)</sup> , VAS nervous <sup>(Cami, Farre et al. 2000)</sup> , VAS anxious <sup>(Tancer and Johanson 2003;Tancer 2004)</sup> , SDEQ tension <sup>(Harris, Baggott et al. 2002)</sup>	Anxiety
SDEQ ambivalence <sup>(Harris, Baggott et al. 2002)</sup> , HRS somaestasia <sup>(Tancer and Johanson 2003;Tancer 2004)</sup> , HRS affect <sup>(Tancer and Johanson 2003;Tancer 2004)</sup> , HRS perception <sup>(Tancer and Johanson 2003;Tancer 2004)</sup> , HRS cognition <sup>(Tancer and Johanson 2003;Tancer 2004)</sup> , HRS intensity <sup>(Tancer and Johanson 2003;Tancer 2004)</sup> , OAV Visionary restructuralisation <sup>(Frei, Gamma et al. 2001;Gamma, Buck et al. 2000;Liechti, Baumann et al. 2000;Liechti, Saur et al. 2000;Liechti and Vollenweider 2000a;Vollenweider, Gamma et al. 1998)</sup> , VAS different surrounding <sup>(Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS changes in colors <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS changes in shapes <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS changes in lights <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS changes in hearing <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , SDEQ LSD <sup>(Harris, Baggott et al. 2002)</sup> , VAS hallucinations-auditory <sup>(Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS hallucinations-visual <sup>(Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS hallucinations- seeing lights or spots <sup>(Cami, Farre et al. 2000)</sup> , VAS hallucinations- hearing sound or voices <sup>(Cami, Farre et al. 2000)</sup> , VAS hallucinations- seeing animals, things, insects, or people <sup>(Cami, Farre et al. 2000)</sup> , VAS different, changed, or unreal body feeling <sup>(Cami, Farre et al. 2000)</sup> , VAS different or unreal surroundings <sup>(Cami, Farre et al. 2000)</sup> , VAS changes in distances <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup>	Hallucination
VAS high <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Harris, Baggott et al. 2002;Hernandez-Lopez, Farre et al. 2002;Tancer and Johanson 2003;Tancer 2004)</sup> , VAS hungry <sup>(Tancer and Johanson 2003;Tancer 2004)</sup> , VAS drunken <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS diziness <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS any effect <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Harris, Baggott et al. 2002;Hernandez-Lopez, Farre et al. 2002)</sup>	Drug effect
VAS bad drug effect <sup>(Tancer and Johanson 2003)</sup> , VAS good drug effect <sup>(Cami, Farre et al. 2000;Tancer and Johanson 2003)</sup> , Drug liking questionnaire <sup>(Tancer and Johanson 2003)</sup> , VAS drug liking <sup>(Harris, Baggott et al. 2002)</sup> , VAS good effects <sup>(Farre, de la Torre et al. 2004;Harris, Baggott et al. 2002;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS liking <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup> , VAS bad effects <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Harris, Baggott et al. 2002;Hernandez-Lopez, Farre et al. 2002)</sup>	Drug liking
<b>Neurophysiological domain</b>	
EEG <sup>(Frei, Gamma et al. 2001)</sup> , LORETA <sup>(Frei, Gamma et al. 2001)</sup>	EEG
Prepulse inhibition-acoustic startle <sup>(Liechti, Geyer et al. 2001;Vollenweider, Remensberger et al. 1999)</sup>	Inhibition
rCBF change <sup>(Gamma, Buck et al. 2000)</sup>	Cerebral blood flow
Maddox wing <sup>(Cami, Farre et al. 2000;Farre, de la Torre et al. 2004;Hernandez-Lopez, Farre et al. 2002)</sup>	Extraocular muscle tension

Table 1. Overview of all reported tests and measurements (with the source studies between brackets), clusters and domains (continued on next page)

<i>Test name</i>	<i>Cluster</i>
<b>Endocrine domain</b>	
ACTH <sup>(Grob, Poland et al. 1995)</sup>	ACTH
DHEA <sup>(Harris, Baggott et al. 2002)</sup>	DHEA
GH <sup>(Mas, Farre et al. 1999)</sup>	GH
LH <sup>(Harris, Baggott et al. 2002)</sup>	LH
Progesterone <sup>(Harris, Baggott et al. 2002)</sup>	Progesterone
FSH <sup>(Harris, Baggott et al. 2002)</sup>	FSH
Estradiol <sup>(Harris, Baggott et al. 2002)</sup>	Estradiol
Prolactin <sup>(Grob, Poland et al. 1995; Harris, Baggott et al. 2002; Mas, Farre et al. 1999; Pacifici, Pichini et al. 2004)</sup>	Prolactin
ADH <sup>(Forsling, Fallon et al. 2001)</sup>	ADH
Cortisol <sup>(Farre, de la Torre et al. 2004; Forsling, Fallon et al. 2001; Harris, Baggott et al. 2002; Lamers, Ramaekers et al. 2003; Mas, Farre et al. 1999; Pacifici, Pichini et al. 2004; Pacifici, Zuccaro et al. 1999; Pacifici, Zuccaro et al. 2001; Tancer and Johanson 2003)</sup>	Cortisol
<b>Physiological domain</b>	
IL-1 $\beta$ <sup>(Pacifici, Zuccaro et al. 2001)</sup> , IL-4 <sup>(Pacifici, Zuccaro et al. 2001)</sup> , IL-6 <sup>(Pacifici, Zuccaro et al. 2001)</sup> , TNF $\alpha$ <sup>(Pacifici, Zuccaro et al. 2001)</sup> , IFN $\gamma$ <sup>(Pacifici, Zuccaro et al. 2001)</sup> IL-2 <sup>(Pacifici, Pichini et al. 2004; Pacifici, Zuccaro et al. 2001)</sup> , IL-10 <sup>(Pacifici, Pichini et al. 2004; Pacifici, Zuccaro et al. 2001)</sup> TGF- $\beta$ 1 <sup>(Pacifici, Pichini et al. 2004; Pacifici, Zuccaro et al. 2001)</sup> , CD3 <sup>(Pacifici, Zuccaro et al. 1999)</sup> , CD19 <sup>(Pacifici, Pichini et al. 2004; Pacifici, Zuccaro et al. 1999; Pacifici, Zuccaro et al. 2001)</sup> , NK cells <sup>(Pacifici, Pichini et al. 2004; Pacifici, Zuccaro et al. 1999; Pacifici, Zuccaro et al. 2001)</sup> , CD8 <sup>(Pacifici, Pichini et al. 2004; Pacifici, Zuccaro et al. 1999; Pacifici, Zuccaro et al. 2001)</sup> CD4/CD8 ratio <sup>(Pacifici, Pichini et al. 2004; Pacifici, Zuccaro et al. 1999; Pacifici, Zuccaro et al. 2001)</sup> , CD4 <sup>(Pacifici, Pichini et al. 2004; Pacifici, Zuccaro et al. 1999; Pacifici, Zuccaro et al. 2001)</sup>	Immune function
[Na <sup>+</sup> ] <sup>(Forsling, Fallon et al. 2001)</sup>	Ions
Respiratory Rate <sup>(Harris, Baggott et al. 2002)</sup>	Respiratory rate
Osmolality <sup>(Forsling, Fallon et al. 2001)</sup>	Osmolality
Temperature <sup>(Farre, de la Torre et al. 2004; Grob, Poland et al. 1995; Harris, Baggott et al. 2002; Lamers, Ramaekers et al. 2003; Liechti, Baumann et al. 2000; Liechti, Saur et al. 2000; Liechti and Vollenweider 2000; Mas, Farre et al. 1999; Tancer and Johanson 2003; Vollenweider, Gamma et al. 1998)</sup>	Temperature
Pupil-diameter <sup>(Farre, de la Torre et al. 2004; Harris, Baggott et al. 2002; Lamers, Ramaekers et al. 2003; Mas, Farre et al. 1999)</sup>	Pupil-diameter
Systolic Blood Pressure <sup>(Farre, de la Torre et al. 2004; Gamma, Buck et al. 2000; Grob, Poland et al. 1995; Harris, Baggott et al. 2002; Lamers, Ramaekers et al. 2003; Lester, Baggott et al. 2000; Liechti, Saur et al. 2000; Liechti and Vollenweider 2000; Liechti and Vollenweider 2000; Mas, Farre et al. 1999; Tancer and Johanson 2003; Tancer 2004; Vollenweider, Gamma et al. 1998)</sup> , Diastolic Blood Pressure <sup>(Farre, de la Torre et al. 2004; Gamma, Buck et al. 2000; Grob, Poland et al. 1995; Harris, Baggott et al. 2002; Lamers, Ramaekers et al. 2003; Lester, Baggott et al. 2000; Liechti, Saur et al. 2000; Liechti and Vollenweider 2000; Mas, Farre et al. 1999; Tancer and Johanson 2003; Tancer 2004; Vollenweider, Gamma et al. 1998)</sup> , Heart Rate <sup>(Farre, de la Torre et al. 2004; Harris, Baggott et al. 2002; Lamers, Ramaekers et al. 2003; Lester, Baggott et al. 2000; Liechti, Saur et al. 2000; Liechti and Vollenweider 2000; Mas, Farre et al. 1999; Tancer and Johanson 2003; Tancer 2004)</sup>	Cardiovascular

Table 1. Overview of all reported tests and measurements (with the source studies between brackets), clusters and domains

Results are presented as overall domain scores whenever appropriate, subdivided into cluster scores. Separate (i.e. non-grouped) tests that were performed more than four times and by more than one research group are reported per cluster. All the tests that were evaluated are listed in Table 2 together with their corresponding domains and clusters. The effects reflect significant MDMA-induced increases (+) or decreases (-) compared to placebo. The most striking result was the finding that, relative to the other groups, the group of neuropsychological tests yielded very few results. In the next paragraphs the various test results are discussed per domain and cluster.

### *Cognitive effects*

The domains *Executive function* and *Visual, visuomotor and auditory function* both yielded only two test results and were therefore not further evaluated. None of the eleven tests in the *Attention* domain had generated a significant response. No studies were found that had employed tests assessing *Memory*. Of the six tests measuring *Motor function*, two showed significant improvement.

<i>Domain</i>	<i>Cluster</i>	<i>Test</i>	<i>Response (%)</i>	<i>Dose range effect (mg/kg)</i>	<i>Dose range no effect (mg/kg)</i>	<i>n</i>
Executive						2
Attention			=		1.0-1.7	11
Memory						0
Visual, visuomotor & auditory						2
Motor			33	1.1	1.1-1.7	6
Subjective						
	Social interaction		36	1.1-2.0	0.5-2.1	14
		POMS friendly	29	1.0-1.1	1.1-2.1	7
	Arousal		38	1.1-2.1	0.5-2.1	58
		POMS arousal	=		1.1-2.1	5
		POMS vigor	=		1.0-2.1	7
		ARCI A	88	1.1-2.1	1.6	8
		ARCI BG	38	1.3-2.0	1.1-2.1	8
		VAS stimulated	100	1.1-2.1		8
	Euphoria		88	0.5-2.1	1.1-1.6	16
		ARCI MBG	75	1.3-2.1	1.1-1.6	8
	Mood		62	0.5-2.0	0.5-2.1	42
		POMS elation	60	1.1-1.7	1.6-2.1	5
	Extroversion		70	1.5-1.7	0.5-2.0	10
	Depression		=		1.0-2.0	10
	Confusion		58	1.0-2.1	1.1-2.0	12
		POMS confusion	40	1.7-2.1	1.1-2.1	5
		VAS confusion	40	1.3-1.7	1.0-2.0	5
	Sedation		=	1.5-2.0	0.5-2.1	32
		ARCI PCAG	=		1.1-2.1	8
	Aggression		=		1.0-2.0	7
	Anxiety		67	0.5-2.1	1.1-2.0	36
		ARCI LSD	100	1.1-2.1		8
		POMS anxiety	57	1.0-2.1	1.1	7
	Hallucination		46	1.1-2.1	0.5-1.6	57
	Drug effect		53	1.1-2.1	0.5-2.1	30
		VAS any effect	84	1.1-1.7	0.5	6
		VAS high	90	1.1-2.1	0.5	10
	Drug liking		57	1.1-2.0	0.5-2.0	21
		VAS good effect	84	1.1-1.7	0.5	6
		VAS bad effect	16	1.5	0.5-1.7	6
Neurophysiological			89	1.3-1.7	1.1	9
Endocrine						
	Cortisol	Cortisol	92	0.5-2.0	0.5	12
	Prolactin	Prolactin	56	0.75-1.5	0.25-1.0	9
Physiological						
	Temperature	Temperature	21	1.0-1.5	0.25-1.7	14
	Pupil diameter	Pupil diameter	83	1.0-1.5	0.5	6
	Cardiovascular		69	1.0-2.1	0.25-1.0	61
		Heart rate	68	1.0-2.1	0.25-1.0	19
		Systolic blood pressure	71	1.0-2.1	0.25-1.0	21
		Diastolic blood pressure	71	1.0-2.1	0.25-1.0	21

Table 2. Domain, cluster and dose-related test responses (in percentages) for all tests analysed. Legend: Response (%)= percentage of results that showed an increase relative to placebo; Dose range effect (mg/kg) = Dose range for responsive results; Dose range no effect (mg/kg)= Dose range for non-responsive results; n = total number of reported test results.

### Subjective effects

A large majority of the studies we reviewed included some type of subjective test. For the subjective assessments, individual scales were grouped into the following 13 scale clusters: social interaction, arousal, euphoria, mood, extroversion, depression, confusion, sedation, aggression, anxiety, hallucination, drug effect, and drug liking. The overall subjective effects are depicted in Figure 1.

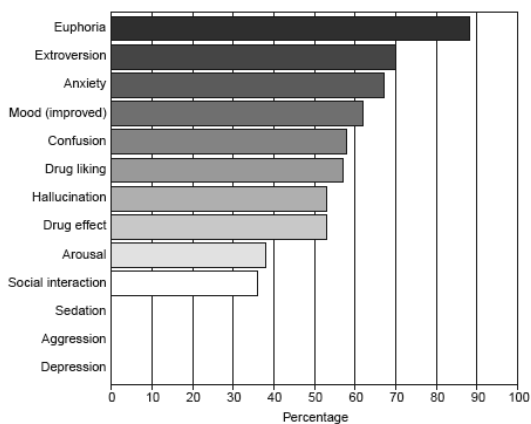


Figure 1. The subjective mood rating scales (clustered) with their reported increases (in percentages) after MDMA administration relative to placebo.

In the scale cluster *Social interaction*, reflecting the entactogenic effects of MDMA, five of the 14 test results proved to be elevated.

- POMS *Friendly* was evaluated separately; two out of seven scores showed increase.

In the cluster *Arousal*, comprising scales

measuring arousing, activating effects, a total of 58 outcomes were analysed of which 22 were increased.

- POMS *Vigor* did not show a response.
- ARCI *A* was increased in seven of the eight test results.
- ARCI *BG* was reported eight times, of which three test results were increased.
- VAS *Stimulated* was increased in all eight test results.
- POMS *Arousal* did not show a response.

For the scale cluster *Euphoria* 16 test results were reported of which 14 showed an increase.

- ARCI *MBG* was increased in six out of eight test results.

The scale cluster *Mood* comprised a large group of subjective mood scales. MDMA induced robust effects here: 26 of the total of 42 test results were increased. Negative mood scales (fear, miserable) did not respond.

- POMS *Elation* was increased in two out of five test results.

Scores for the scale cluster *Extroversion* were increased in seven of ten test results.

The scale cluster *Depression* did not show a response.

Seven of the 12 test results in the cluster *Confusion* were increased.

- POMS *Confusion* was increased in two out of five test results.
- VAS *Confusion* was also increased in two out of five test results.

The scale cluster *Sedation* did not show a response.

- One of seven ARCI *PCAG* scores was decreased.

The scale cluster *Aggression* did not show a response.

The scale cluster *Anxiety* showed increases in 24 of the 36 test results.

- ARCI *LSD* was reported eight times and all test results were increased.
- POMS *Anxiety* showed increase in four of seven test results.

The scale cluster *Hallucination* showed an effect profile similar to that of *Anxiety* scores with 35 of 66 test results being increased after MDMA. Note that the dose range inducing elevated values (1.1-2.1 mg/kg) was slightly higher than the range in the tests that failed to show an effect (0.5-1.6 mg/kg).

In the scale cluster *Drug effect*, reflecting side-effects attributed to the drug, 16 out of 30 test results were increased.

- VAS *Any effect* was increased in five out of six test results, the unresponsive dose being 0.5 mg/kg.
- VAS *High* showed no change in one test result (dose; 0.5 mg/kg) out of a total of ten, all other test results were increased.

The scale cluster *Drug liking* was increased in eight of the 15 test results. Most unresponsive test results were observed for negative drug-liking scales, most clearly illustrated by the two separately evaluated VAS scales:

- VAS *Good effects* was increased in five of six test results.
- VAS *Bad effects* was increased in only one of six test results.

### *Neurophysiological measurements*

Neurophysiological measures were both few and diverse, although all but one test showed a significant response. No specific measure was reported often enough to justify a separate evaluation.

### *Endocrine measurements*

The most frequently assessed hormones were cortisol and prolactin. Cortisol levels were increased in eleven of the twelve studies. The one study that failed to show a significant increase used the lower MDMA dose of 0.5 mg/kg. In five of the nine studies prolactin levels were elevated with the unresponsive results on average being associated with a lower dose.

### *Physiological effects*

*Temperature* was increased in three of 14 test results and *Pupil diameter* was measured six times and increased in all studies using a dose range of 1.0-1.5 mg/kg; the one test using a dose of 0.5 mg/kg failed to demonstrate an effect.

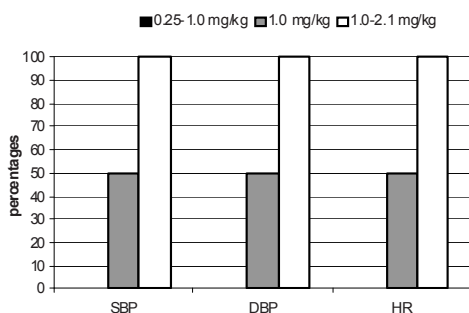


Figure 2. Dose-response relationship for the MDMA-induced cardiovascular effects (in percentages) relative to placebo. SBP= systolic blood pressure; DBP= diastolic blood pressure; HR= heart rate; %= percentage of studies reporting an increase

depicted in Figure 2. *Systolic blood pressure* was reported 21 times with a dose range of 0.25-1.0 mg/kg (n=6) failing to induce a change whereas all tests using a dose range of 1.0 to 2.1 mg/kg (n=15), were increased. The results reported for

The cluster *Cardiovascular* effects comprised 61 test results of which 18 did not show any change (dose range 0.25-1.0 mg/kg). Of the six trials that used 1.0 mg/kg, three reported increases. Studies administering a dose of 1.0-2.1 mg/kg MDMA all reported increase.

This remarkable dose-response association was also seen in the separately analysed tests within this group, whose outcomes are

*Diastolic blood pressure* were identical to the values reported for systolic pressure. Finally, of the total of 19 heart-rate measurements six showed no change, all within the dose range 0.25-1.0 mg/kg. The remaining 13 tests using doses between 1.0 to 2.1 mg/kg yielded increases.

## ***Discussion***

To our knowledge this review comprises all placebo-controlled studies published to date that administered MDMA to healthy humans. The tests reported in the selected studies were reviewed, and the dose-related results were recorded in a database. Most studies that were discarded did not measure acute effects in humans, while a small percentage of the remaining studies performed the research in a “naturalistic” setting thus not conforming to our selection criteria.

The majority of the tests were performed infrequently, and, rather than rejecting tests on the basis of their limited application - by which we would have ignored possibly valuable information - we opted for an evaluation of tests grouped according to the effect or function they measured. Arguably, as a result of methodological or other differences, such group appraisals may compromise the comparative value of or even devalue the effects reported. Any manipulation of data might obscure information: tests that in fact represent the ‘ideal’ measure (i.e. represent an MDMA effect) could be masked by other non-responsive tests in the same group. However, rejection of these tests on the basis of limited experience would have ignored possibly valuable information. Our evaluation effectively yielded three categories of tests measuring functional, phenomenological or physiological aspects. Next a summary and the implications of our findings will be discussed for the various domains of each category.

The studies that performed clinical research into the acute effects of MDMA in healthy volunteers almost without exception employed subjective and physiological tests, but assessments of *neuropsychological functioning* were not as frequent. Most surprising was the complete lack of studies reporting on memory even though retrospective studies into the long-term effects of MDMA most consistently indicate this area to be affected (Verbaten 2003;Verkes, Gijsman et al. 2001). The domains ‘executive’ and ‘visual, visuomotor and auditory’ also suffered from a low number of reported results, which prohibited their evaluation (see Table 2). The domain ‘attention’ comprised eleven results but showed no change, while the ‘motor’ domain, with a total of six results, showed modest increase. The limited

number of tests in these domains clearly hinders any meaningful conclusion to be drawn from their evaluation, although this category is in potential most valuable.

Although several research groups have performed detailed work on MDMA effects, with Vollenweider *et al.* (Vollenweider, Liechti *et al.* 2002) extensively researching acute MDMA effects, De la Torre *et al.* (Pacifici, Zuccaro *et al.* 2000) focussing on immune function and Lamers *et al.* (Lamers, Ramaekers *et al.* 2003) on driving-related behaviour, we feel that reproduction and extension of their work is warranted to allow the results obtained by these research groups to be generalised.

In contrast to the functional studies, our search yielded an abundance of *phenomenological* data (see Fig. 1). The entactogenic profile of MDMA was represented in that, relative to placebo, increases were reported for the pleasurable subjective effects, as reflected by the scale clusters ‘euphoria’, ‘extroversion’ and ‘social interaction’ scores, whereas the scores for the negative clusters ‘aggression’, ‘sedation’ and ‘depression’ failed to show change.

Most *neurophysiological* measurements that were evaluated responded to MDMA administration, but none of the tests were performed often enough to warrant separate evaluation. However, since neurophysiological assessments are designed to detect changes in physiological parameters of the CNS rather than to measure a specific cognitive function, these tests will respond to almost any psychoactive drug, not just MDMA.

Endocrine effects were limited to the evaluation of plasma cortisol and prolactin measurements. Results were as may be expected from a primary serotonin-releasing agent: the increase in cortisol levels was more robust than that of prolactin.

Of the physiological effects that were evaluated, only temperature showed a very weak effect. The implications of this divergent outcome will be discussed later in this section.

A remarkable dose-response association was observed in the cardiovascular measurements, where 1.0 mg/kg showed to be a clear cut-off dose for MDMA to have cardiovascular effects; below 1.0 mg/kg MDMA failed to induce any changes while all the studies that administered more than 1.0 mg/kg MDMA reported significant increases compared to placebo (Fig. 2).

Pupil diameter measurements all proved very sensitive to MDMA. All but one result, linked to a low dose (0.5 mg/kg), were increased after MDMA administration. This measure, although very sensitive, is not specific for MDMA, however, as many agents that interact with the autonomic nervous system cause pupil dilation (Dumont, de Visser et al. 2005). This limitation holds for all physiological measures, which, as discussed in the methods section, devaluates the relevance of these tests. Nevertheless, physiological data are, of course, crucial when formulating safety guidelines.

Clinical research into the actions of psychoactive compounds has a drawback in that the outcomes may not reflect the effects the same drug would induce in 'normal' situations. Clearly, the drug's effects are dependent upon the user's surroundings and mood, factors that are nearly impossible to fully reconstruct in the laboratory. This holds for the current weak temperature-related findings, for example, where robust increases after MDMA administration have been reported: some reports even mentioned ecstasy induced hyperthermia and fatal complications (Garcia-Repetto, Moreno et al. 2003; Ravina, Quiroga et al. 2004). It should be noted that animal studies have shown that the effects of MDMA on body temperature are dependent on ambient temperature (Green, O'Shea et al. 2004), where normal room temperature (20-22°C) proved not to induce any changes in body temperature. As the temperature in clinical laboratories is most likely to be around the 20°C mark, the studies conducted in this setting will inherently not show a significant effect of MDMA on body temperature.

On a related note, the scale cluster *Anxiety* showed an increase, which seems to conflict with the drug's overall subjective profile of pleasurable, desired effects. However, this unexpected effect may be associated with the unusual circumstances the participants found themselves in, which may have caused considerable psychological stress. Also, the cluster contains data of studies with MDMA-naïve volunteers in whom the unfamiliar experience with the drug might have translated into anxiety. Yet, an analysis of the anxiety scores of the subgroup of MDMA-familiar volunteers still yielded a 58%-increase. Similarly, the social-interaction scores did not increase as much as we had expected from this

entactogenic drug. Again, for social interactions to increase, circumstances and surroundings are crucial, making it plausible that the laboratory conditions also depressed this characteristic property of MDMA.

Psychopharmacological research into the acute effects of drugs in humans is heavily dependent on the tests that are employed. With this in mind it is important that validated test batteries are used that can detect alterations in the broad range of CNS functions. This would vastly improve the transparency of experimental findings and facilitate the comparison and generalisation of results obtained in clinical trials with psychoactive compounds. Although advances have been made, to date no such generally approved compendium of tests that is both sensitive to stimulation and sedation of the CNS has been developed. MDMA research of course also suffers from this lack of standardisation. On the other hand, in this review firm conclusions were even more hindered by the generally limited number of neuropsychological tests the selected studies employed. Future studies should avoid these shortcomings. Moreover, the authors welcome a broad debate to identify which tests are most sensitive or best suited for detecting improvement or impairment in the several specific areas of cognitive functioning featuring in this report.

The assembled data showed that typical MDMA effects are fully expressed at doses above 1.0 mg/kg, at which level the drug's adverse effects will also manifest themselves. These side effects are addressed in a comprehensive report in which Vollenweider and team review their own work into MDMA (Liechti, Gamma et al. 2001). The most prevalent adverse drug reactions were difficulty in concentrating, jaw clenching, lack of appetite, dry mouth/thirst and impaired balance. The effects of MDMA on the body's hydration balance (induction of ADH release) are significant and it is a potent stimulant of the sympathetic nervous system, causing increases in blood pressure, heart rate and perspiration. Since these effects can potentially set off serious complications in susceptible participants, researchers intending to mount clinical trials should be aware of these hazards.

In conclusion, MDMA displays all its prominent features at doses of 1.0 mg/kg and above, which is in line with the desirable doses reported by recreational

users (Croft, Klugman et al. 2001; Soar, Parrott et al. 2004). The potentially hazardous adverse effects are also fully expressed at this level. In the relevant studies generated by our search of the literature, findings reflecting the subjective (the entactogenic profile), physiological (cardiovascular, pupil diameter) and endocrine effects (cortisol, prolactin) were the most prominent and abundant. MDMA effects on neuropsychological functioning were reported infrequently, thus rendering firm conclusions impossible and supporting our recommendation for more intensive research into the acute cognitive effects of MDMA.



***Acute neuropsychological effects of  
MDMA and ethanol (co-)  
administration in healthy volunteers***

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### *Abstract*

In Western societies, a considerable percentage of young people expose themselves to 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”). Commonly, ecstasy is used in combination with other substances, in particular alcohol (ethanol). MDMA induces both arousing as well as hallucinogenic effects, whereas ethanol is a general central nervous system depressant.

The aim of the present study is to assess the acute effects of single and co-administration of MDMA and ethanol on executive, memory, psychomotor, visuomotor and visuospatial, and attention function, as well as on subjective experience.

We performed a four-way, double blind, randomized, crossover, placebo-controlled study in 16 healthy volunteers (9 male, 7 female) between the ages of 18-29. MDMA was given orally (100 mg) and blood alcohol concentration (BAC) was maintained at 0.6 ‰ by an ethanol infusion regime.

Co-administration of MDMA and ethanol was well tolerated and did not show greater impairment of performance compared to the single drug conditions. Impaired memory function was consistently observed after all drug conditions, whereas impairment of psychomotor function and attention was less consistent across drug conditions.

In conclusion, co-administration of MDMA and ethanol did not exacerbate the effects of either drug alone. Although the impairment of performance by all drug conditions was relatively moderate, all induced significant impairment of cognitive function.

## ***Introduction***

In Western societies, a considerable proportion of young people expose themselves to 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”) (Gross 2002;Parrott 2001;Tancer and Johanson 2007). Ecstasy has gained widespread use in the ‘club’ scene, typically all-night parties with loud music and intense lights (Winstock, Griffiths et al. 2001). The average dose of ecstasy used recreationally is reported to be around 80-90 mg of MDMA with considerable individual variation (Tanner-Smith 2006). Ecstasy users are generally multidrug users, who have experience with various recreational drugs and use these in combination with ecstasy (Gouzoulis-Mayfrank and Daumann 2006). Probably due to its availability, alcohol remains one of the most co-used substances (Barrett, Gross et al. 2005). As the use of alcohol is known to induce impairment of cognitive function and decrease the awareness of this impairment, this can lead to dangerous behaviour like driving under influence (Lamers and Ramaekers 2001;Riley, James et al. 2001).

MDMA acts primarily by releasing serotonin (5-HT) from presynaptic 5-HT terminals. It reverses the direction of the reuptake transporter and increases 5-HT levels at the postsynaptic receptors (Liechti and Vollenweider 2000b;Mlinar and Corradetti 2003;Pifl, Drobny et al. 1995). MDMA is also a potent releaser of dopamine and (nor)adrenaline (Colado, O'Shea et al. 2004;Liechti and Vollenweider 2001).

MDMA is rapidly absorbed following oral administration. Within 30 minutes MDMA is detectable in the blood. Plasma levels peak at 1-2 hr after drug administration, and maximum behavioural and subjective effects occur around 1-2 hr and have declined by 4 hr in spite of persisting plasma levels (de la Torre, Farre et al. 2004;Green, Mechan et al. 2003). Increasing the dose does not result in a proportional rise in plasma concentrations, which is indicative for non-linear pharmacokinetics (de la Torre, Farre et al. 2000a).

The behavioural effects of MDMA resemble, but are not restricted to, effects of psychostimulants (e.g. amphetamines or ‘speed’) as well as hallucinogenics (e.g. lysergic acid or ‘LSD’), although MDMA's most characteristic

effects are described as an increase in empathy and friendliness (Vollenweider et al. 2002). This led to MDMA being categorized as an ‘entactogen’, as coined by Nichols and Oberlender (Nichols and Oberlender 1990).

Most research into the cognitive effects of MDMA in humans has focused on the long term effects, where only memory was consistently found to be impaired (Verbaten 2003;Verkes, Gijsman et al. 2001). Our review of the acute effects of MDMA in humans showed that cognitive effects were assessed only in a limited number of studies, using diverse tests and generally addressing only certain aspects of neuropsychological function. As such, no consensus on MDMA’s cognitive effects could be reached (Dumont and Verkes 2006). Since then, reports on the effects of MDMA generally confirmed previous findings (Kuypers, Samyn et al. 2006;Kuypers, Wingen et al. 2007;Ramaekers, Kuypers et al. 2006B;Tancer and Johanson 2007). Interestingly, two studies reported effects of MDMA on memory, which had not been assessed previously. These reports showed acute impairment of immediate and delayed recall of words as well as spatial memory by MDMA (Kuypers and Ramaekers 2005;Kuypers and Ramaekers 2007).

Drinks containing ethanol, commonly referred to as alcohol, are widely available and regularly used in Western society. Ethanol is chiefly a central nervous system (CNS) depressant. It inhibits both excitatory and inhibitory postsynaptic potentials by potentiating the action of GABA at its receptor (Suzdak, Schwartz et al. 1988). Reports of the cognitive effects of combined use of MDMA and ethanol in humans have been sparse in the literature. Studies that were performed assessed psychomotor function, attentional performance and subjective effects (Hernandez-Lopez, Farre et al. 2002;Kuypers, Samyn et al. 2006;Ramaekers, Kuypers et al. 2006b). In general, MDMA and ethanol had no or opposite effects on effect measures, and as such co-administration did not exacerbate single drug effects.

In the current study, we employed a series of tests sensitive to changes in all common neuropsychological domains induced by several pharmacological compounds, including amphetamines (Wezenberg, Hulstijn et al. 2004).

It is generally acknowledged that the combined use of alcohol with other CNS-depressant drugs may enhance the effects of ethanol or of the other drugs.

MDMA, however, has stimulant effects while ethanol is a sedative agent, suggesting that the effects of co-administration are diminished rather than augmented compared to the effects following single administration. This hypothesis was investigated during acute co-administration of MDMA and ethanol in healthy volunteers.

## ***Materials and methods***

### *Study Design*

This study utilized a four-way, double blind, randomized, crossover, placebo-controlled design. Sixteen volunteers were randomly assigned to one of four treatment sequences. Each volunteer received a capsule containing either MDMA 100 mg or placebo and an ethanol/placebo infusion (target BAC of 0.6<sup>0</sup>/<sub>00</sub>) with a wash-out of 7 days between each treatment.

### *Study outline*

Subjects arrived in the morning and were admitted to the study after a negative urine drug screen (opiates, cocaine, benzodiazepines, amphetamines, methamphetamines and delta-9-tetrahydrocannabinol), as well as a negative alcohol breath test and recording of signs and symptoms of possible health problems. A light breakfast was offered. Drug administration was scheduled at 10:30h and the alcohol infusion was started at 11:00h for a duration of three hours. At 11:30h subjects performed the psychological test battery as described below. Specific test times are reported in Table 1. Subjects received lunch at 14:00h and were sent home at 17:00h after a medical check. Adverse events were recorded throughout the study day. Vital signs were monitored using a Datascope<sup>®</sup> Accutorr Plus<sup>™</sup> cardiovascular monitor and Braun<sup>®</sup> type 6021 ThermoScan during the study day. The data presented in this report are a subset of a larger data set, which will be reported elsewhere.

### *Subjects*

Sixteen healthy volunteers (9 male, 7 female), regular users of ecstasy and alcohol, aged 18-29 years and within 80-130% of their ideal bodyweight were recruited through advertisement on the internet and at local drug testing services. They were all in good physical and mental health as determined by assessment of medical history, a medical-, ECG- and clinical-, haematological- and chemical blood examination. Previous drug use was assessed using a structured interview. Fifteen

volunteers were right handed and one was left handed. The study was approved by the local Medical Ethics Committee. All subjects gave their written informed consent before participating in the study and were compensated for their participation. Subject demographics and drug history are reported in Table 2.

One subject had a mild adverse reaction (local vascular reaction) to the alcohol infusion and one subject did not refrain from drug use, both (1 male, 1 female) were excluded from further participation and results obtained were not included in the data-analysis.

#### *Drugs and Dosages*

MDMA (or matched placebo) was given as a capsule in a single dose of 100 mg via oral administration (dose range: 1.1-2.2 mg/kg). MDMA was obtained from Lipomed AG, Arlesheim, Switzerland and encapsuled according to Good Manufacturing Practice (GMP) by the Department of Clinical Pharmacy UMC St Radboud, Nijmegen, the Netherlands. MDMA 100 mg orally is a relevant dose in the range of normal single recreational dosages. Previous experiments in humans used doses up to 150 mg without serious adverse events.

Ethanol (or matched placebo) was administered continuously by IV infusion of 10% ethanol in glucose solution resulting in an ethanol blood concentration of 0.6 ‰ with a duration of three hours as described below.

#### *Alcohol clamping*

To standardize alcohol delivery and maintain a constant alcohol blood concentration over time, an intravenous ethanol clamp was used. Ethanol was administered by infusion of a 10% ethanol in glucose solution for a duration of three hours. The infusion rate was calculated using frequent breath alcohol concentrations measurements, according to a previously designed algorithm (Amatsaleh, Dumont et al. 2006). Breath alcohol concentration was assessed using a HONAC AlcoSensor IV<sup>®</sup> Intoximeter.

An intravenous administration route was chosen, ensuring standardization of the rate and bioequivalence of ethanol administration. This is an important

prerequisite for predictable pharmacokinetics of ethanol. The process was semi-automated using a computer spreadsheet programme, which uses measured breath alcohol concentrations to calculate the infusion rate needed to maintain the ethanol level at 0.6 ‰. This is a relevant dose equivalent to peak levels of approximately 2-3 units of alcoholic beverages. In many European countries driving is prohibited at blood alcohol concentrations (BAC) above 0.5 ‰. This limit has been confirmed by a report that shows that at an average BAC of 0.6 ‰ psychomotor performance is significantly impaired (Amatsaleh, Schoemaker et al. 2006). A BAC of 0.6 ‰ is equivalent to approximately 2-3 alcoholic beverages commonly used in social settings in Western society, which is considered to be a safe and relatively moderate dose, despite its significant CNS effects.

#### *MDMA blood analysis*

For the assessment of serum levels of MDMA blood samples were collected 90 minutes after drug administration from each subject on each study day. Venous blood samples (10 ml) were collected into heparinised tubes, centrifuged immediately at 4 °C for 15 minutes. Plasma was split into aliquots of 2mL (to prevent overfreeze/thawing) and frozen rapidly using liquid nitrogen, stored at -80 °C. Samples were analyzed for MDMA and MDA concentration by the Toxicology unit of the Leyenburg hospital, the Hague, the Netherlands.

#### *Neuropsychological tests, apparatus and procedure*

The performance on all neuropsychological tests was recorded by means of a digitizing tablet (WACOM UD-1218-RE), a laptop computer, a pressure sensitive pen (which could also be used as a cursor) and test-forms. The x and y coordinates of the pen tip on and up to 5 mm above the digitizer were sampled with a frequency of 200 Hz and a spatial accuracy of 0.2 mm. The time schedule of the tests is summarized in Table 1.

To familiarize the subjects with the tests and procedures, they were invited to the hospital to perform a practice session within one week before the actual study

days. All tests had 5 equivalent versions for 4 test-days and one practice day, test-versions were counterbalanced over test-days.

<i>Neuropsychological tests</i>	<i>Description</i>	<i>Time (h:m)</i>
Drug administration		0:00
18 Words list immediate recall	<i>Immediate recall of 18 word list</i>	1:00
SDST	<i>Translate symbols to digits with key present in 90 s.</i>	1:05
SDRT	<i>Translate symbols to digits from memory</i>	1:08
Pursuit task	<i>Keep dot within moving circle</i>	1:10
Tangles task	<i>Tangled line leads to which target?</i>	1:13
Switch task	<i>Follow, possibly conflicting, instructions (choice between left or right)</i>	1:17
18 Words list delayed recall	<i>Delayed recall of 18 word list</i>	1:22
18 Words list delayed recognition	<i>Recognize words of 18 words list memorized earlier among 18 distractors</i>	1:23
Point task	<i>Keep pen steady in air, measures tremor</i>	1:25
Visual Analog Scales	<i>16 100mm scales for subjective experiences</i>	1:30

Table 1. *Timeline. Times are relative to drug administration.*

### Executive function

*Switch task* This test is a reaction time task measuring simple as well as complex reaction time, assessing executive performance (Baker and Letz 1986). After a random period of 0.75 to 1.75 seconds two rectangular fields appeared on both sides of a circle in the centre of the screen. Only one of the two fields provided the subjects with information, either a color, an arrow or both. The other non-informative field always had a neutral grey color. Five conditions were subsequently presented to subjects. If only green fields appeared, subjects had to move as fast as possible into the green field. If green and red fields appeared, subjects had to move into the green field and away from the red field as soon as they appeared. If green fields with a left or right arrow were presented, subjects were to move into the direction of the arrow. Green and red fields with a left or right arrow indicated that subjects were to follow the direction of the arrows in the green field, but the opposite direction of the arrows in the red field. Finally, the first condition was repeated. All conditions contained 20 trials except condition four in which there were 40 trials (total = 120 trials). The outcome measures were the mean reaction

times per condition. The last condition is a repetition of the first to check for possible changes in attention.

### Memory

*Eighteen words list* A verbal memory test based on the classic Auditory Verbal Learning Test (Vakil and Blachstein 1993). A variant was made consisting of a list of eighteen words. The classic test uses 15 words. A longer wordlist was chosen, however, to prevent ceiling effects. The list was presented verbally three times. Under normal circumstances subjects are supposed to remember an increasing number of words after each trial. Directly after each presentation, and after an interval of 20 minutes, subjects were asked to recall as many words as possible. After the delayed recall trial a list of thirty-six words was presented from which they were asked to recognize the eighteen words previously presented. The incorrect words were distracters and resembled the correct words in a semantic or phonologic manner. Responses were either correct positive (when a word that was recognized was indeed part of the list presented during immediate recall) or false positive (when a word was recognized but was not part of the list presented during immediate recall, e.g. the word was a distracter). The outcome measure was the number of correctly recalled/recognized words for the average of the three immediate recall trials, the delayed recall trial and the delayed recognition trial.

*Symbol Digit Recall Test (SDRT)* The SDRT followed directly after the Symbol Digit Substitution Test (SDST), which is discussed in the last paragraph of this section. After subjects had finished the SDST, they were shown the symbols of the SDST without the translation key, one at a time, and asked to produce the corresponding numbers. This test is based on an extended procedure of the SDST to measure incidental learning (Kaplan, Fein et al. 1991). The outcome measure was the number of correctly translated symbols.

### Psychomotor function

*Pursuit task* To measure implicit procedural learning a computerized version of the rotor pursuit task was used. This test is based on the classic rotary

pursuit task (Ammons 1951). It is a continuous motor task. Subjects had to follow the movement of a large target stimulus on the computer screen with a cursor by moving the pen over the XY-tablet. The speed of the target gradually increased when the cursor was contained within the target but decreased considerably when it was not. The target followed a spatially predictable circular path over the screen. The outcome measure for this test was the total number of rotations within two minutes.

*Point task* The point task, a measure for tremor, required subjects to try to keep the cursor inside a very small circle for one minute, while avoiding contact between the pen and the test form. The outcome measure for this test was the deviation from the target.

#### Visuospatial and visuomotor function

*Tangle task* The Tangle task required the subject to visually track a particular line winding through two to four other lines. On subsequent trials the tangles increased in complexity; they got longer and made more 90-degree turns. The paper form had a start area and five target areas, numbered 1 to 5, which reflect the maximum target areas on the screen, starting with only three target areas.

This test is modelled after the visualisation test from the “kit for factor referenced cognitive tests”. It was selected by the US NAVY to study environmental and other time-course effects and has good task stability and reliability (Bittner, Jr., Carter et al. 1986). The outcome measures are the reaction time per trial and the number of correct trials in two minutes.

#### Attention

*Symbol Digit Substitution Test (SDST)* This test is a version of the subtest from the WAIS (Wechsler Adult Intelligence Scale) (Wechsler 1981). Subjects had to substitute the nine symbols for the digits 1-9 on the basis of a given translation key. The outcome measure was the total number of digits completed in 90 seconds. According to Hege et al. (Hege, Ellinwood, Jr. et al. 1997) this task measures many cognitive components, e.g. visuospatial scanning, intermediate memory, perceptual

motor speed, and speed of cognitive processing. Therefore, subsequent analyses were performed in order to attempt and disentangle these cognitive processes. Based on pen pressure, movement trajectories were defined as either pen-up periods or pen-down periods. This allowed for subsequent analysis of matching times and movement (writing) times in the symbol digit substitution test. For the motor component, the mean writing times were computed. For the more cognitive component, the mean matching times were computed. These analyses have been previously performed (Sabbe, Hulstijn et al. 1999; Wezenberg, Verkes et al. 2005).

### Subjective

Subjective effects were recorded using the Bond and Lader (Visual Analogue) Mood Rating Scale (BLMRS). This inventory was completed at the end of each neuropsychological test battery on each study day.

The BLMRS scale consisted of 16 lines, each 10 cm in length, with opposite terms at each end of the line (alert/drowsy, calm/excited, strong/feeble, muzzy/clear-headed, well-coordinated/clumsy, lethargic/energetic, contented/discontented, troubled/tranquil, mentally slow/quick witted, tense/relaxed, attentive/dreamy, incompetent/proficient, happy/sad, antagonistic/amicable, interested/bored, withdrawn/gregarious). Subjects were asked to indicate which item was more appropriate by marking the line. The outcome measure was the distance to the marker on each scale. These scale scores were then aggregated to scores for 'calmness', 'alertness' and 'contentedness' as described by Bond and Lader (Bond, James et al. 1974).

### *Statistical Analyses*

Statistical evaluation (using SPSS 11.5 for Windows) was performed with GLM Repeated Measures Analysis of Variance (ANOVA). Main and interaction effects were tested using a two factor ('ethanol' and 'MDMA'), two level (absent versus present) multivariate model.

The analysis of the data was based on Maxwell and Delaney (2004) and Kirk (1995). First the presence of interaction (non-additivity) was tested with  $\alpha =$

.05. When the interaction was not statistically significant we proceeded by testing the main effects, each at  $\alpha = .05$ . In the case of a significant interaction we proceeded by testing simple main effects of each drug, i.e. MDMA vs. placebo and ethanol vs. placebo.

## Results

Subject demographics are summarized in Table 2. 14 out of 16 subjects completed the study procedure. One subject had a mild adverse reaction (local vascular reaction which subsided with infusion stop) to the alcohol infusion and one subject did not refrain from drug use, both were discontinued from study participation and data already obtained was not included in statistical analysis. Only significant results are mentioned in this section, unless stated otherwise.

	<i>Mean</i>	<i>SD</i>	<i>min</i>	<i>max</i>
Age (years)	22.1	2.9	18	29
Education (years)	16.5	1.6	12	18
Height (cm)	174.7	12.3	147.0	189.1
Weight (kg)	67.5	12.4	45.7	88.4
Opiates	0.1	0.3	0	1
LSD	2.5	6.6	0	25
Amphetamines	37.3	81.1	0	250
Ecstasy	94.6	138.4	14	431
Cannabis	1174.3	1665.5	20	5840
Cocaine	33.7	105.7	0	400
Alcohol	2367.9	1981.6	50	5200
Solvents	3.6	13.3	0	50
Barbiturates	0	0	0	0
Benzodiazepines	18.6	57.3	0	216
Psilocybin	6.9	10.4	0	30

Table 2; Volunteer demographics/drug history. Drug quantities mentioned are lifetime drug exposures, not further specified.

MDMA blood concentration 90 minutes after administration did not differ for MDMA single vs. MDMA and ethanol co-administration and was on average 196 µg/L (SD=83 µg/L). Blood alcohol concentration was maintained at an average of 0.54 ‰ (SD=0.07 ‰).

### *Executive function*

Executive function (Switch task) did not show any significant main or interaction effects.

### *Memory function*

Memory function was assessed by the 18 words list (outcome measures were 'immediate recall', 'delayed recall' and 'recognition', see Figure 1) as well as the

Symbol Digit Recall Task (SDRT). Immediate recall was impaired only by ethanol ( $F(1,12)=8.71$ ,  $p=0.011$ ).

Delayed recall as assessed by the 18 words list was impaired by MDMA ( $F(1,12)=10.447$ ,  $p=0.007$ ) as well as by ethanol ( $F(1,12)=16.031$ ,  $p=0.002$ ). The SDRT, also a test for delayed recall, showed a similar pattern of impairment by MDMA ( $F(1,12)=5.300$ ,  $p=0.038$ ) as well as by ethanol ( $F(1,12)=7.654$ ,  $p=0.016$ ).

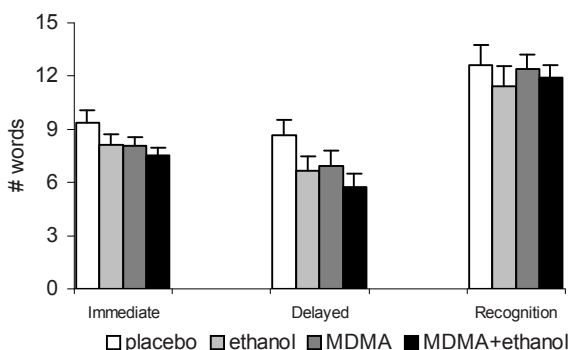


Figure 1. *Memory effects (18 words list (mean, s.e.m.)), Immediate: immediate recall, average score of three trials of correctly recalled verbally presented words, Delayed: correctly recalled verbally presented words 20 minutes after presentation, Recognition: correctly recognized verbally*

### *Psychomotor function*

Psychomotor function was assessed with tests for tremor (Point task), accuracy (Pursuit task) and speed (Symbol Digit Substitution Task (SDST) writing

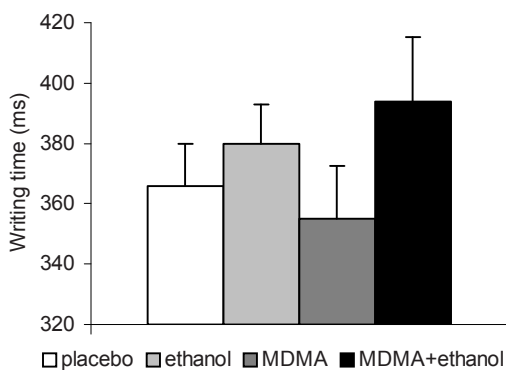


Figure 2. *Psychomotor effects: SDST writing time (mean, s.e.m.).*

time, see Figure 2), other SDST results are reported in the section 'Attention'. Ethanol impaired psychomotor speed as reflected in the increase in SDST writing time ( $F(1,12)=9.295$ ,  $p=0.009$ ).

### *Visuospatial and visuomotor function*

Visuospatial and visuomotor function were measured with the Tangle task, subdivided into 'total number correctly solved' and 'reaction time', and did not show any significant effects, although a trend of impairment by MDMA ( $F(1,12)=3.966$ ,  $p=0.068$ ) was observed.

### *Attention*

Attention was assessed with the SDST task; the outcome measures were 'motor time' (see 'Psychomotor function'), 'matching time' (figure 3) and 'total number correctly substituted'.

The time required to match symbols to the corresponding numbers showed a significant MDMA and ethanol interaction ( $F(1,12)=6.214$ ,  $p=0.027$ ). Tests for simple main effects revealed that both single drug conditions reduced attention compared to placebo (ethanol  $F=6.248$ ,  $p=0.027$ ; MDMA  $F=6.822$ ,  $p=0.022$ , see Figure 3).

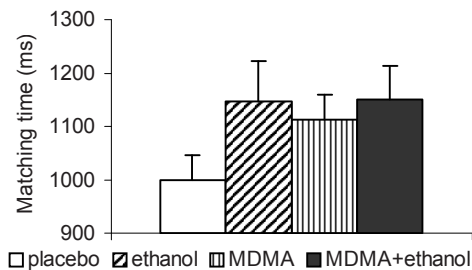


Figure 3. Attention: SDST matching time, i.e., time needed for translation (mean,s.e.m.).

### *Subjective effects*

Subjective effects are depicted in Figure 4. Feelings of 'Contentedness' were increased significantly by MDMA only ( $F(1,12)=4.710$ ,  $p=0.049$ ).

A significant interaction effect ( $F(1,12)=7.358$ ,  $p=0.018$ ) was found for feelings of 'Alertness'. Tests for simple main effects revealed that ethanol, but not MDMA, significantly decreased feelings of alertness compared to placebo ( $F=50.613$ ,  $p<0.001$ ).

Feelings of 'Calmness' were reduced only by MDMA ( $F(1,12)=20.259$ ,  $p=0.001$ ).

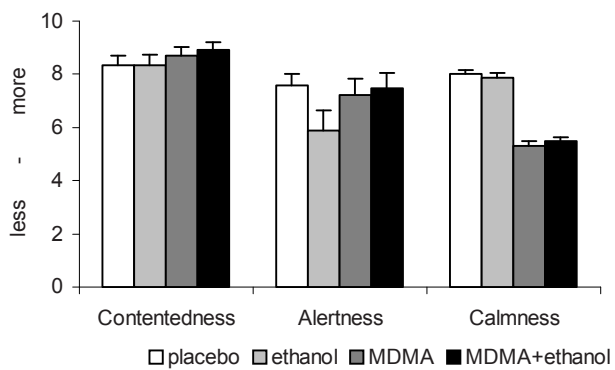


Figure 4. *Subjective effects (Aggregated Bond and Lader MRS scores (mean, s.e.m)).*

## *Discussion*

This study demonstrates that the effects of 100 mg MDMA, commonly known as ecstasy, on cognitive function are no greater than the effects of a relatively low dose of ethanol. This is remarkable as these results suggest that the effects of 100 mg MDMA are comparable to the peak effects of 2-3 alcoholic beverages. Co-administration of these compounds did not result in any significant cognitive impairments beyond those observed after administration of only ethanol. The use of moderate amounts of alcohol is common in Western societies and, although impairing cognitive function, socially accepted, while ecstasy use remains very controversial. Of course, our findings only relate to the acute neuropsychological implications of ecstasy use and not to the physiological and long-term effects, which rightfully remain topics of discussion (Gouzoulis-Mayfrank and Daumann 2006a; Nutt 2006; Parrott 2007a).

Drug effects observed in this placebo controlled crossover study were moderate. Co-administration was well-tolerated as indicated by the subjective scores, which were comparable to those found after single administration of MDMA. An interaction of MDMA and ethanol was found for subjective alertness scores. Ethanol, as expected, reduced subjective alertness, while MDMA co-administration reversed the reduction of subjective alertness by ethanol. In the present study MDMA by itself did not significantly affect subjective alertness, although this effect has been consistently reported in other studies and is a well-known effect of amphetamines. However, MDMA did significantly reduce subjective calmness, i.e., subjects felt more excited after MDMA. Probably, the Bond and Lader mood rating scale is not well suited for the assessment of subjective effects of psychoactive drug effects and future studies should employ more appropriate subjective drug effect measures such as the Profile Of Mood States (POMS) (de Wit, Enggasser et al. 2002).

When considering the results for each neuropsychological domain executive function was not affected by any drug condition. A previous study showed impairment of executive function by ethanol but not MDMA, although ethanol

impaired performance in only one out of three tests of executive function (Lamers, Ramaekers et al. 2003). The BAC in this study was 0.3 promille at the time of testing compared to 0.56 promille in our current study, suggesting a lack of sensitivity of the test employed in the current study.

The abovementioned previous study also reported visuospatial and visuomotor impairment by MDMA but not by ethanol. Although not significantly, our current results show a similar pattern where MDMA showed a trend of impairment of visuospatial and visuomotor function, whereas ethanol did not.

Psychomotor function was impaired only after ethanol administration (SDST writing time, see Figure 2). The majority of studies addressed in our review of acute effects of MDMA in humans (Dumont and Verkes 2006) did not report any change in psychomotor function after MDMA either. However, increased psychomotor function after MDMA has also been found (Lamers, Ramaekers et al. 2003; Ramaekers, Kuypers et al. 2006a). These studies administered 75 mg instead of 100 mg. Possibly, the effects of MDMA are biphasic, with a low dose of MDMA exhibiting more amphetamine-like effects, e.g. arousal, increasing performance, whereas higher doses may elicit more hallucinogenic effects and impair performance (Liechti, Gamma et al. 2001; Solowij, Hall et al. 1992).

As mentioned above, MDMA co-administration reversed the ethanol induced feelings of sedation, although MDMA was unable to reverse the psychomotor impairment induced by ethanol. This dissociation between subjective and objective sedation confirms previous findings by Hernandez-Lopez et al. (2002).

Several studies assessed MDMA's effect on attention using the Digit Symbol Substitution Task (DSST), although no significant effects were found (Cami, Farre et al. 2000; Farre, de la Torre et al. 2004; Kuypers and Ramaekers 2005). One study reported decreased DSST performance after ethanol as well as after ethanol and MDMA co-administration, but no effect of MDMA (Hernandez-Lopez, Farre et al. 2002). Our findings confirm these findings to a large extent. We found no main effects of MDMA or ethanol on attention, although an interaction of ethanol and MDMA for 'matching time' (time required to match the number to the corresponding symbol) was found. Co-administration of MDMA and ethanol

increased 'matching time' comparable to the increase observed after both MDMA and ethanol single administration, compliant with our hypothesis of competitive mechanisms of action of both drugs (see Figure 3).

Studies investigating the long term effects of MDMA consistently found memory to be affected (Verbaten 2003). In the present report, almost all memory measures showed quantitatively comparable impairment for each drug condition (see Figure 1), although the effect of MDMA on immediate recall did not reach statistical significance. Only delayed recognition was not impaired in any drug condition. These findings suggest a deficit in the retrieval of verbal information encoded in memory, rather than impairment in the storage of information. Our findings are similar to the results of a previous study on MDMA induced effects on memory (Kuypers and Ramaekers 2005). In this previous study no memory impairment was observed after methylphenidate administration, a pronounced dopamine and norepinephrine releaser, suggesting the involvement of serotonin in memory impairment. Several other studies also have shown serotonin mediated modulation of memory function through interaction with the cholinergic neurotransmitter system, although the details of this complicated interaction remain elusive (Cassel and Jeltsch 1995; Garcia-Alloza, Zaldua et al. 2006; Meneses 2007). Generally, subjects stated that they were well aware of their impaired memory after MDMA.

Blood alcohol concentration (BAC) was on average 0.56 promille. At this level, driving is prohibited by law in many European countries, because of its interference with normal functioning. Although the effects were moderate, ethanol impaired cognitive performance in various tests. Similar moderate effects were observed with MDMA 100 mg, considered to be slightly above the average recreational dose (Tanner-Smith 2006). This might be considered surprising for a drug with reported robust subjective stimulating and hallucinogenic properties. However, since the effects caused by a single dose of 100 mg MDMA were comparable to the effects of a BAC of 0.56 promille, this dose should by inference be considered unacceptable in motorized traffic.

Arguably, the moderate drug effects as found in this study could be explained by 'missing' the time of the maximal drug effects. Although the average MDMA blood concentration reported here (196 µg/L) is comparable to the C<sub>max</sub> of 100 mg MDMA (199,8 µg/L) as reported by de la Torre et al. (2000), MDMA concentration was assessed at the end of the testing procedure. However, Hernandez Lopez et al. (Hernandez-Lopez, Farre et al. 2002), found significant effects at 60 as well as 90 minutes after drug administration arguing against the suggestion of 'missing' peak drug effects.

The circumstances in which these substances are normally used cannot be fully recreated in the laboratory and this may have suppressed the effects of both substances. It is not unlikely that these substances show enhanced effects when tested under typical circumstances and surroundings. Recently, Parrott et al. (Parrott, Rodgers et al. 2006) concluded that the increase in physical activity and body temperature typically experienced when using MDMA, enhance MDMA effects. Ball et al. (Ball, Budreau et al. 2006) demonstrated that a familiar surrounding increased MDMA induced locomotor response as well as single neuron activity in rats, compared to unfamiliar surroundings. Therefore, the psychosocial context in which MDMA is used, along with the different expectations and behaviour, probably influences its effects (Sumnall, Cole et al. 2006). It is unlikely however, that this affects the quality of the interactions of MDMA and ethanol.

In conclusion, co-administration of MDMA and ethanol did not impair cognitive function significantly more than MDMA or ethanol administration alone. The most prominent effect of (co-)administration of MDMA and ethanol was an impairment of memory. Ethanol also impaired psychomotor function. Although the impairment of performance by each drug condition was relatively moderate, this significant impairment of cognitive function should be considered intolerable in motorized traffic and other cognitively demanding situations as confirmed by previous research and as defined by law. However, the effects of these drugs in the concentrations used in the present study on established neuropsychological tests appear to be smaller than one would assume based on their reputation.



# ***Acute psychomotor effects of MDMA and ethanol (co-) administration over time in healthy volunteers***

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### *Abstract*

In Western societies a considerable percentage of young people use 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”). The use of alcohol (ethanol) in combination with ecstasy is common. The aim of the present study was to assess the acute psychomotor and subjective effects of (co-) administration of MDMA and ethanol over time and in relation to the pharmacokinetics.

We performed a four-way, double blind, randomized, crossover, placebo-controlled study in 16 healthy volunteers (9 male, 7 female) between the ages of 18 and 29. MDMA (100 mg) was given orally, while blood alcohol concentration was maintained at pseudo-steady state levels of approximately 0.6 ‰ for three hours by a 10% intravenous ethanol clamp.

MDMA significantly increased psychomotor speed but did not affect psychomotor accuracy and induced subjective arousal. Ethanol impaired both psychomotor speed and accuracy, and induced sedation. Co-administration of ethanol and MDMA improved psychomotor speed but impaired psychomotor accuracy compared to placebo, and reversed ethanol induced sedation. Pharmacokinetics and pharmacodynamics showed maximal effects at 90-150 minutes after MDMA administration after which drug effects declined in spite of persisting MDMA plasma concentration, with the exception of ethanol induced sedation, which manifested itself fully only after the infusion was stopped.

In conclusion, results show that subjects were more aroused when intoxicated with both substances combined compared to placebo, but psychomotor accuracy was significantly impaired. These findings may have implications for general neuropsychological functioning as this may provide a sense of adequate performance that does not agree with a significant reduction in psychomotor accuracy.

## ***Introduction***

In Western societies a significant proportion of young people expose themselves to 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”) (Parrott 2001). Ecstasy has gained widespread use in the ‘club’ scene, typically at all-night parties with loud, intense music and lights (Winstock, Griffiths et al. 2001). The average dose of ecstasy used recreationally is reported to contain around 80-90 mg of MDMA with considerable individual variation (Tanner-Smith 2006). Ecstasy users are generally polydrug users, having experience with different psychoactive substances and combining them with ecstasy (Gouzoulis-Mayfrank and Daumann 2006). Alcohol (ethanol) is frequently used with ecstasy (Barrett, Gross et al. 2005; Izco, Orio et al. 2007).

MDMA releases serotonin (5-HT) from presynaptic 5-HT terminals by reversal of the reuptake transporter and thus increases 5-HT levels at the postsynaptic receptors (Liechti and Vollenweider 2000; Mlinar and Corradetti 2003; Pifl, Drobny et al. 1995). MDMA is also a potent releaser of dopamine and (nor) adrenaline (Colado, O'Shea et al. 2004; Liechti and Vollenweider 2001; Sprague, Brucher et al. 2004).

MDMA is rapidly absorbed following oral administration. Within 30 minutes MDMA is detectable in the blood. Plasma levels peak 1-2 hours after drug intake. Maximal behavioural and subjective effects also occur around 1-2 hours and decline after 4 hours (de la Torre, Farre et al. 2004; Green, Mehan et al. 2003). A moderate increase of plasma MDMA levels when ethanol is co-administered with MDMA has been reported (Hernandez-Lopez, Farre et al. 2002), but also disputed (Kuypers, Samyn et al. 2006).

Ethanol depresses both excitatory and inhibitory postsynaptic potentials by allosterically potentiating the action of GABA at its receptor-complex (Suzdak, Schwartz et al. 1988). Consequently, alcohol has both arousing and sedating effects that are dose-dependent with high inter-individual variability (Gulick and Gould 2007).

Reports on the effects of co-administration of MDMA and ethanol in humans are relatively sparse (Hernandez-Lopez, Farre et al. 2002; Kuypers, Samyn et al. 2006; Pacifici, Zuccaro et al. 2001; Ramaekers and Kuypers 2006b). Of these, two studies reported on psychomotor performance. Kuypers et al. (Kuypers, Samyn et al. 2006) assessed the effects of 75 and 100 mg MDMA in combination with orally administered ethanol (mean peak blood alcohol concentration (BAC) reached was 0.6<sup>0</sup>/<sub>00</sub>). The authors reported that certain aspects of psychomotor performance, assessed by actual driving tests (mean BAC below the legal limit (0.5<sup>0</sup>/<sub>00</sub>) during driving tests), were impaired by ethanol (standard deviation of lateral position (SDLP)) and improved by MDMA (SDLP and standard deviation of speed). Co-administration of 100 but not 75 mg MDMA reversed ethanol induced impairment of SDLP. Ethanol also increased brake reaction time. Psychomotor performance assessed using the Critical Tracking Task (CTT) was impaired by ethanol and unaffected by MDMA. Another study assessed the effects of 100 mg MDMA and 0.8 g/kg ethanol (peak BAC of 1.25<sup>0</sup>/<sub>00</sub>) co-administration on psychomotor function over time (Hernandez-Lopez, Farre et al. 2002). In this study, ethanol impaired psychomotor performance using the Digit Symbol Substitution Task (DSST), an effect which was counteracted by MDMA co-administration. MDMA alone did not affect psychomotor performance. MDMA showed stimulant effects assessed with the Maddox wing test, an effect which was counteracted by ethanol co-administration. Maximal effects occurred 90 minutes after MDMA administration and declined thereafter.

We recently reported on the peak effects of MDMA and ethanol co-administration on neuropsychological function (Dumont, Wezenberg et al. 2008). Only moderate effects were observed, although the timing of tests relative to drug administration remained an issue. Although the time when C<sub>max</sub> is reached (T<sub>max</sub>) provides a guideline to assume peak effects, the dynamic effects do not necessarily follow the kinetic time profile of a compound. This study aimed to assess the acute psychomotor and subjective effects of MDMA and ethanol (co-) administration over time controlling for the pharmacokinetics. This study extends previous studies by employing an ethanol clamp, effectively eliminating the variations in BAC of orally

administered alcohol. As previous studies showed a robust neuroendocrine response after MDMA administration (de la Torre, Farre et al. 2000b; Harris, Baggott et al. 2002; Mas, Farre et al. 1999), the current study also assessed neuroendocrine response to assess possible moderating effects of ethanol co-administration on MDMA induced neuroendocrine response.

We hypothesize that the stimulating effects of MDMA will moderate ethanol's subjective as well as objective sedation. As MDMA also induces mild hallucinogenic effects which are expected to impair cognitive function, we hypothesize that tests not reliant on psychomotor speed would not benefit from MDMA co-administration compared to ethanol (Dumont, Wezenberg et al. 2008). Peak drug effects were hypothesized to co-incide with MDMA T<sub>max</sub>, although effects were expected to decline in spite of persisting plasma levels as previously reported (Hernandez-Lopez, Farre et al. 2002).

## ***Materials and methods***

### *Study Design*

This study utilized a four-way, double blind, randomized, crossover, and placebo-controlled design and was conducted according to the principles of the Declaration of Helsinki. Each volunteer received a capsule containing either MDMA 100 mg or placebo and an ethanol/placebo infusion (target blood alcohol concentration (BAC) of  $0.6^{0}_{00}$ ) with a washout of 7 days between each treatment.

### *Study outline*

Subjects were admitted to each study day after a urinary drug check (opiates, cocaine, benzodiazepines, amphetamines, methamphetamines and delta-9-tetrahydrocannabinol, AccuSign<sup>®</sup>, Princeton BioMeditech, Princeton, USA) (drug use was not allowed 14 days prior to the first study day until study completion) and the recording of possible signs and symptoms of health problems. A light breakfast was offered two hours prior to drug administration. Drug administration was scheduled at 10:30h and the ethanol infusion was started at 11:00h. Subjects received a standardized lunch at 14:00h and were sent home around 17:00h.

Outcome measures were assessed repeatedly, i.e. before MDMA administration and at 30, 90, 150, 240, 300, and 360 minutes post drug administration, and consisted of pharmacokinetic samples of breath (for ethanol) or blood (for MDMA and MDA) and pharmacodynamic assessments as specified below. Parts of these data were presented at a meeting of the Dutch Society for Clinical Pharmacology, the abstract of which has been published (Dumont, Valkenberg et al. 2007).

### *Subjects*

Sixteen healthy volunteers (9 male, 7 female), regular users of ecstasy (at least eight exposures in the last two years) and alcohol (at least one exposure per week),  $22.1 \pm 2.9$  (mean  $\pm$  SD) years of age (range 18-29) and within 80-130% of their ideal body weight were recruited through advertisement on the internet and at

local drug testing services. Lifetime ecstasy exposure was on average 95 doses (SD=138; range 14-431). More detailed demographic data have been reported elsewhere (Dumont, Wezenberg et al. 2008). Exclusion criteria included pregnancy, (history of) psychiatric illness (assessed using the Structured Clinical Interview for DSM-IV axis 1 disorders, non-patient version (First, Frances et al. 1994), Axis II disorders were excluded using the Temperament and Character Inventory (Svrakic, Whitehead et al. 1993), use of over-the-counter medication within 2 months prior to the study start, (history of) treatment for addiction problems, excessive smoking (>10 cigarettes/day) and orthostatic dysregulation. Physical and mental health was determined by assessment of medical history, a physical- and ECG examination as well as standard haematological and chemical blood examinations. The local Medical Ethics Committee approved the study. All subjects gave their written informed consent before participating in the study, and were paid for their participation. Subjects were aware that the active drug conditions would be ethanol, MDMA, and ethanol and MDMA co-administration, but that the order in which they received the treatments was randomized.

One subject had a mild adverse reaction (local vascular reaction) to the ethanol infusion and one subject did not refrain from drug use, both (1 male, 1 female) were excluded from further participation and results obtained from these subjects were not included in the final data analysis.

#### *Ethanol clamping*

Ethanol (or glucose 5% as its placebo) was administered continuously by IV infusion of 10% ethanol in 5% glucose solution, aimed to maintain a blood ethanol concentration of 0.6<sup>0</sup>/<sub>00</sub> for three hours. The infusion rate was calculated using frequent breath alcohol concentration measurements, according to a previously designed algorithm (Amatsaleh, Dumont et al. 2006). Breath alcohol concentration was assessed using a HONAC Alco Sensor IV<sup>®</sup> Intoximeter. The process was semi-automated using a computer spreadsheet programme, which used measured breath alcohol concentrations to calculate the infusion rate needed to maintain the ethanol level at 0.6<sup>0</sup>/<sub>00</sub>. The operator of the breath alcoholmeter and the ethanol infusion

pump was unblinded for alcohol-treatment, but did not communicate with the study team or the subjects about the results at any stage of the trial. A sham-procedure was used during ethanol placebo occasions.

The alcohol clamp was targeted at 0.6<sup>0</sup>/<sub>00</sub>, since in many European countries driving is prohibited at blood alcohol concentrations (BAC) at or around this level. Consequently, the psychomotor effects of an ethanol concentration equal to or exceeding 0.6<sup>0</sup>/<sub>00</sub> are likely to affect an individual's ability to drive a car. Recent studies showed that a BAC of 0.6<sup>0</sup>/<sub>00</sub> induces significant effects on eye movements (Amatsaleh, Schoemaker et al. 2006; Nyberg, Wahlstrom et al. 2004). Moreover, despite significant CNS effects, this dose is considered to be a safe and relatively moderate dose. A BAC of 0.6<sup>0</sup>/<sub>00</sub> is equivalent to approximately 2-3 alcoholic beverages, reflecting normal social drinking.

#### *MDMA*

MDMA (or matched placebo) was given as a capsule in a single oral dose of 100 mg. MDMA 100 mg orally is a relevant dose in the range of normal single recreational dosages. Previous experiments in humans used doses up to 150 mg without serious adverse events. MDMA was obtained from Lipomed AG, Arlesheim, Switzerland and encapsulated according to Good Manufacturing Practice (GMP) by the Department of Clinical Pharmacy of Radboud University Nijmegen Medical Centre.

#### *Analytical Methods*

All reagents were of analytical grade. A high-performance liquid chromatography–diode array detection (HPLC-DAD) method was employed to measure plasma MDMA and MDA concentrations. In brief: 100 µl internal standard solution (1.27 µg MDEA in 1.0 ml water) was added to 1.0 ml plasma, 500 µl borate buffer (0.05 M, pH 9.3) was added, the solution was mixed, and 5 ml dichloromethane added. The tube was stoppered and shaken for 10 min. Thereafter, the tube was centrifuged for 5 min at 2500 g. The organic layer was separated from the water layer and transferred into a clean tube and 500 µl mobile phase (880 ml

0.015 M phosphate buffer, pH 3.3 plus 120 ml acetonitrile) was added. The tube was shaken for 10 min and centrifuged for 5 min. An aliquot of 60 µl from the upper layer was injected onto the HPLC-DAD system consisting of a model 1100 solvent delivery system (Agilent Technologies, Amstelveen, the Netherlands), column oven (Agilent Technologies, Amstelveen, the Netherlands) with a Symmetry C18 column (Waters, Middelburg, the Netherlands), model 1100 DAD (Agilent Technologies, Amstelveen, the Netherlands). Flow was 1 ml/min and separation took place at 50°C. Peaks were recorded from 199-400 nm. Recovery was >90% for MDMA, MDA and MDEA with a coefficient of variation (CV) of <2%. The lower limit of detection for both MDMA and MDA was 3 µg/l with a CV of 20%. The upper limit of detection was 500 µg/l for MDMA and 165 µg/l for MDA. Peak purity was >0.995 for positive identification of MDMA, MDA and MDEA. MDMA, MDA and MDEA were kindly obtained from the Dutch Forensic laboratory. Dichloromethane was obtained from Biosolve (Valkenswaard, the Netherlands) and acetonitrile was obtained from Rathburn (Walkerburn, United Kingdom).

#### *Neuroendocrine assays*

Cortisol and prolactin were determined as measures of neuroendocrine activity, as previous research has shown that the serum concentrations of these neuropeptides increases after MDMA administration (de la Torre, Farre et al. 2000b; Harris, Baggott et al. 2002; Mas, Farre et al. 1999b). Serum total cortisol was measured by Fluorescence Polarization Immunoassay (FPIA) on a TDX batch analyzer (Abbott, Hoofddorp, the Netherlands). Serum prolactin was measured by Fluorescence Immuno Enzymometric Assay (FIEMA) on an AxSym automated immunoanalyzer (Abbott, Hoofddorp, the Netherlands).

#### *Saccadic and smooth pursuit eye movements*

Saccadic eye movements are a measure for psychomotor speed and sedation. Eye movements were quantified by recordings of field potential changes due to eye rotations. Similar to EEG patterns and the architecture of evoked

potentials in rats (Meeren, Van Luitelaar et al. 1998), saccadic motion is dependent on the state of alertness (van Steveninck, van Berckel et al. 1999).

For the saccadic test, which lasted 1.5 minutes, the subject was presented with sudden changes of target position at random intervals. The target consisted of an array of light emitting diodes on a bar fixed at 50 cm in front of the head support. Each recording session consisted of 15 saccades of 15 degrees stimulus amplitudes. The outcome measures are peak saccadic velocity and reaction time.

For smooth pursuit eye movements, a measure for psychomotor accuracy, the target moved sinusoidal at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz during 60 s. The amplitude of target displacement corresponded to 20 degrees eyeball rotation to both sides. The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage.

Saccadic- and smooth pursuit eye movements were recorded using Nihon-Kohden® and Cambridge Electronics Design (CED®) hardware, and CED Spike2® software for sampling and analysis of eye movements. Effects on the saccadic eye movements, the Saccadic Eye Velocity (PV), were analysed according to published rules (Meeren, Van Luitelaar et al. 1998; Sundstrom and Backstrom 1998). Head movements were restrained using a fixed head support. Eye movements are used to locate objects and predict the path of moving objects, and as such can be expected to be relevant for driving related abilities (Orban de Xivry and Lefevre 2007). Moreover, they are sensitive to the effects of serotonergic challenges (Gijsman, van Gerven et al. 2002), as well as to the effects of ethanol at the currently employed concentration (Amatsaleh, Dumont et al. 2006).

### *Body sway*

Subjects were asked to close their eyes while in upright position and were attached to the body sway apparatus that records cumulative horizontal body movement (in mm) for two minutes. The test is a measure for postural stability (Wright 1971).

### *Subjective effects*

Subjective effects were assessed using the short version of the Addiction Research Centre Inventory (ARCI). The ARCI is a 49 item self-report questionnaire that consists of five subscales, each representing the characteristics of a specific drug group; i.e. pentobarbital-chlorpromazine-alcohol group (PCAG, a measure for sedation), morphine-benzedrine group (MBG, a measure for euphoria), amphetamine scale (A, an empirically derived scale sensitive to D-amphetamine effects), benzedrine group (BG, a measure for stimulant effects) and lysergic acid diethyl amide scale (LSD, a measure for dysphoria and psychomimetic changes) (Lamas, Farre et al. 1994). The ARCI questionnaire was performed at baseline, and 90 and 300 minutes after drug administration.

### *Statistical Analyses*

The pharmacodynamic parameters were analyzed by mixed model analyses of variance (using SAS PROC MIXED) with treatment, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline was defined as the average of the available values obtained prior to dosing. Treatment effects are reported as the contrasts between the 4 treatments where the average of the measurements up to the last time point was calculated within the statistical model. Contrasts are reported along with 95% confidence intervals and analyses are two-sided with a significance level of 0.05. Graphical representation shows mean and standard error of the mean of data.

Statistical evaluation of the differences in MDMA kinetics between MDMA only and MDMA and ethanol (co-) administration (using SPSS 11.5 for Windows) was performed with GLM Repeated Measures Analysis of Variance (ANOVA).

## Results

### Pharmacokinetics

MDMA and MDA kinetics did not differ between MDMA single and MDMA and ethanol conditions. The maximal plasma MDMA concentration (C<sub>max</sub>)

was on average 202.5

µg/l (SD=74.1 µg/l)

150 minutes after

drug administration

(Figure 1). Plasma

MDA concentration

on average increased

to 9.4 µg/l (SD=3.6

µg/l) 360 minutes

after drug

administration.

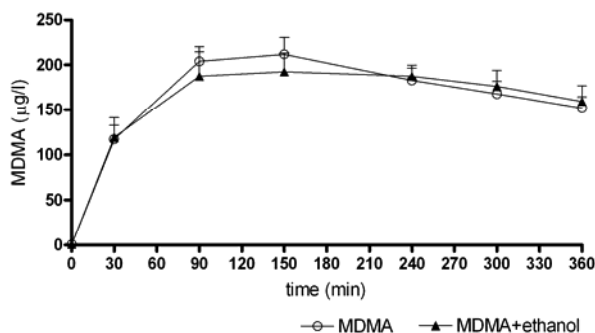


Figure 1. Plasma MDMA concentrations (mean, s.e.m.) for the MDMA, and the MDMA and ethanol condition.

Ethanol kinetics are shown in Figure 2, during the pseudo-steady state

phase blood alcohol

concentration was on

average 0.56<sup>0</sup>/<sub>00</sub>

(SD=0.057<sup>0</sup>/<sub>00</sub>).

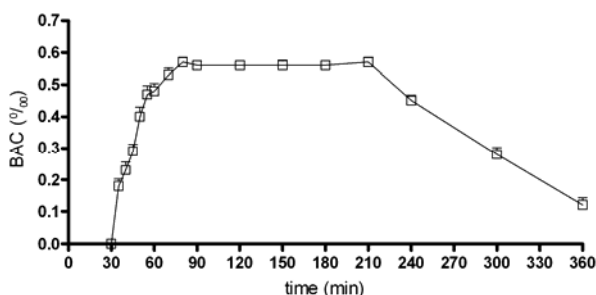


Figure 2. Blood alcohol concentrations (BAC, (mean, s.e.m.)).

### Pharmacodynamics

Only

significant results are

mentioned in this

section unless noted

otherwise. Statistically significant main effects of treatment, time and treatment by time as well as drug condition comparisons are summarized in Table 1. For the drug condition comparisons, mean change, 95% confidence interval (95% CI) and corresponding p-values are reported.

### Neuroendocrine measurements

Serum cortisol concentrations showed a pronounced increase after MDMA as well as after MDMA and ethanol (co-) administration compared to the placebo and ethanol condition (Figure 3A). The cortisol response did not differ between the MDMA and the MDMA and ethanol condition. Serum cortisol concentrations peaked 90 minutes after drug administration and decreased to baseline levels 360 minutes after drug administration in spite of persisting plasma MDMA levels.

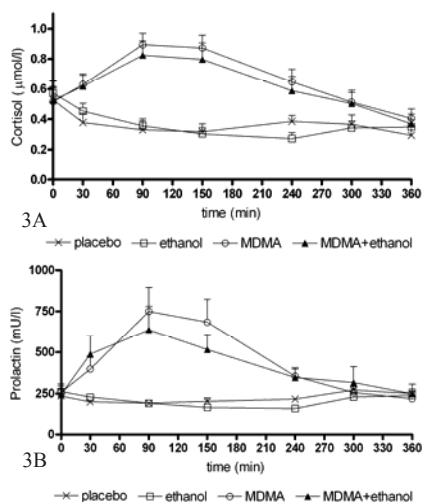


Figure 3. Neuroendocrine measures. Figure 3A: Serum cortisol concentration (mean, s.e.m.). Figure 3B: Serum prolactin concentration (mean, s.e.m.)

Serum prolactin concentrations (Figure 3B) showed a profile similar to that of cortisol. Peak prolactin concentrations were observed 90 minutes after MDMA and MDMA and ethanol (co-) administration. Prolactin levels after MDMA as well as after MDMA and ethanol (co-) administration returned to baseline levels 300 minutes after administration of the drug(s). There was no significant difference between the MDMA and the MDMA and ethanol condition.

<i>Test</i>	<i>Treatment effect</i>	<i>Time Effect</i>	<i>Treatment by Time</i>	<i>Ethanol vs. Plac</i>	<i>MDMA vs. Plac</i>	<i>MDMA+Eth vs. Plac</i>	<i>MDMA+Eth vs. MDMA</i>	<i>MDMA+Eth vs. Ethanol</i>	<i>MDMA vs. Ethanol</i>
Neuroendocrine									
Cortisol (µmol/l)	<0001	<0001	<0001		0.32 (0.25, 0.39) p=<.0001	0.28 (0.21, 0.36) p=<.0001		0.28 (0.24, 0.35) p=<.0001	0.31 (0.24, 0.39) p=<.0001
Prolactin (mIU/l)	<0001	<0001	<0001		213 (105, 322) p=0.0003	196 (85, 307) p=0.0009		223 (111, 335) p=0.0003	240 (130, 351) p=<.0001
Psychomotor									
Body Sway (mm)	NS	<0001	NS						
Smooth Pursuit (%)	<0001	<0001	<0001	-7.6 (-11.0, -4.1) p=<.0001		-7.5 (-10.8, -4.2) p=<.0001	-9.2 (-12.6, -5.9) p=<.0001		9.3 (5.9, 12.7) p=<.0001
Peak Velocity (deg/s)	<0001	0.4030	<0001	-18.0 (-33.8, -2.2) p=0.0269	35.6 (19.7, 51.5) p=<.0001	16.7 (0.9, 32.5) p=0.0393	-18.9 (-34.7, -3.1) p=0.0206	34.7 (19.0, 50.5) p=0.0001	53.7 (37.9, 69.4) p=<.0001
Reaction time (ms)	NS	0.0001	NS						
Subjective									
ARCI PCAG	0.0010	0.0075	NS	2.29 (0.80, 3.77) p=0.0035				-2.90 (-4.37, -1.42) p=0.0003	-2.64 (-4.11, -1.16) p=0.0008
ARCI MBG	<0001	<0001	<0001		2.93 (1.49, 4.37) p=0.0002	3.72 (2.28, 5.16) p=<.0001		3.19 (1.75, 4.63) p=<.0001	2.40 (0.96, 3.84) p=0.0017
ARCI A	<0001	0.0006	0.0009		1.64 (0.84, 2.44) p=0.0002	2.15 (1.36, 2.94) p=<.0001		1.69 (0.89, 2.48) p=0.0001	1.18 (0.36, 2.00) p=0.0060
ARCI BG	<0001	0.0018	0.0189	-1.00 (-1.93, -0.07) p=0.0351		1.30 (0.37, 2.24) p=0.0073		2.30 (1.38, 3.23) p=<.0001	1.86 (0.93, 2.79) p=0.0002
ARCI LSD	0.0007	0.0085	0.0023	1.32 (0.30, 2.34) p=0.0122	2.00 (1.00, 3.00) p=0.0002	1.93 (0.92, 2.94) p=0.0004			

Table 1: Results, main effects, and drug comparisons (reported are mean difference, 95% confidence interval, and p-value). Plac= placebo, Eth= ethanol, MDMA+Eth= MDMA and ethanol co-administration.

### Body sway

There were no significant treatment or treatment by time effects for the body sway measurements.

### Eye movements

Smooth pursuit eye movements (psychomotor accuracy) were significantly impaired after ethanol as well as after ethanol and MDMA (co-) administration compared to the placebo and MDMA condition (Figure 4).

Psychomotor speed and sedation/arousal were assessed by saccadic eye movements. Outcome measures are peak saccadic velocity (PV, see Figure 5A) and reaction time (RT, see Figure 5B) respectively. Subjects showed increased PV after MDMA administration compared to the placebo, ethanol and MDMA-ethanol condition. Ethanol decreased PV compared to the placebo condition. Co-administration of MDMA with ethanol increased PV compared to the ethanol as well as to the placebo condition.

A trend for a significant treatment by time interaction for RT was observed ( $p=0.0571$ ). Drug comparisons showed that reaction time of saccadic eye movements was significantly increased after ethanol compared to placebo ( $p=0.023$ ), although the effect was moderate.

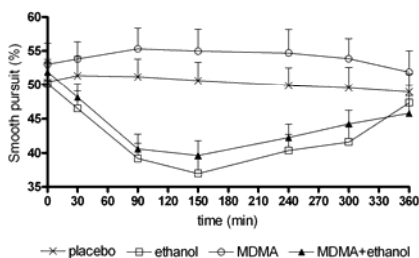


Figure 4. Psychomotor accuracy; smooth pursuit eye movements (mean, s.e.m.).

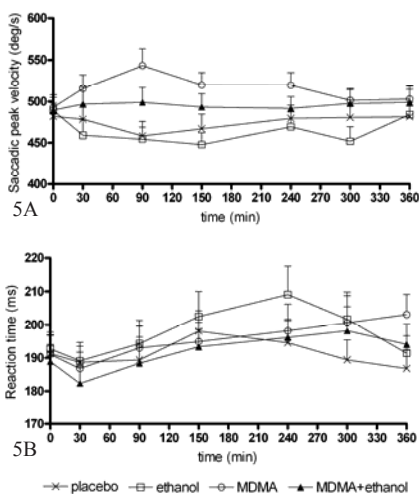


Figure 5. Psychomotor speed measures. Figure 5A: Saccadic peak velocity (mean, s.e.m.). Figure 5B: Reaction time of saccadic eye movements (mean, s.e.m.).

### *Subjective effects*

All ARCI sub-groups showed comparable time profiles. Maximal effects were observed 90 minutes after drug administration and effects had returned to baseline values 300 minutes after drug administration. The exception to this time profile were the ARCI pentobarbital-chlorpromazine-alcohol (PCAG) and benzedrine (BG) group-scores after ethanol administration, which showed a linear relation in time (positive for PCAG, negative for BG) with maximal effects 300 minutes post drug administration, i.e. subjects felt increasingly sedated after ethanol compared to the placebo, MDMA and MDMA-ethanol conditions.

Drug induced euphoria (ARCI morphine-benzedrine group-scores (MBG)) was reported after MDMA and MDMA-ethanol (co-) administration compared to the placebo and ethanol condition.

Subjects reported amphetamine-like effects (ARCI amphetamine (A) group) in the MDMA and MDMA-ethanol condition compared to the placebo and ethanol condition.

Arousal was assessed by the ARCI BG group. MDMA administration showed a trend for increased arousal compared to the placebo condition ( $p=0.069$ ). MDMA and ethanol co-administration increased arousal significantly compared to the placebo condition. Ethanol decreased subjective arousal compared to the placebo, MDMA and MDMA-ethanol condition.

Psychomimetic changes (ARCI lysergic acid (LSD) group) were experienced during all active drug conditions compared to placebo; these effects did not differ between drug conditions.

## *Discussion*

Here we report the acute pharmacokinetic and pharmacodynamic effects of MDMA and ethanol co-administration over time. The results suggest distinct effects of MDMA and ethanol on psychomotor function as well as on subjective experience. Co-administration effects were either averages of or comparable to single drug effects, but there was no reinforcement of the effects. We hypothesized that (1) the stimulating effects of MDMA would moderate ethanol's sedating effects, that (2) tests not reliant on psychomotor speed would not benefit from MDMA co-administration compared to ethanol and that (3) peak drug effects would co-incide with MDMA T<sub>max</sub>, although effects were expected to decline in spite of persisting plasma levels.

Our results support these hypotheses as (1) co-administration of ethanol and MDMA reversed ethanol induced subjective as well as objective sedation, and (2) co-administration of ethanol and MDMA improved psychomotor speed but impaired psychomotor accuracy compared to placebo. Third, MDMA's peak dynamic effects coincided with the time of maximal plasma MDMA concentrations (C<sub>max</sub>), although effects diminished thereafter in spite of persisting plasma MDMA levels. The time profile of MDMA induced subjective and performance effects was congruent with neuroendocrine response, and MDMA and ethanol co-administration did not affect the neuroendocrine response compared to MDMA alone. The time course of ethanol induced effects also correlated with its kinetic profile, with the exception of ethanol-induced sedation, which had peak effects that were delayed compared to the kinetic profile.

MDMA improved psychomotor speed but did not affect psychomotor accuracy. Lamers et al (Lamers, Ramaekers et al. 2003) also reported increased psychomotor speed after 75 mg MDMA in the Motor Choice Reaction Task (MCRT). While MDMA's most characteristic effect is the release of serotonin, it also releases dopamine (Colado, O'Shea et al. 2004; Liechti and Vollenweider 2001). Psychomotor speed is likely to benefit from the increased dopamine availability and the resulting increase in arousal (Mehta and Riedel 2006). However, tasks not solely

depending on speed, like psychomotor accuracy, are less likely to benefit from the increase in arousal. Ethanol on the other hand generally impaired psychomotor function, in line with previous reports (Hernandez-Lopez, Farre et al. 2002; Kuypers, Samyn et al. 2006; Dumont, Wezenberg et al. 2008).

Co-administration of MDMA and ethanol caused a significant increase in psychomotor speed compared to placebo (although slightly less than MDMA alone). Hernandez-Lopez et al. (2002) also reported that MDMA co-administration reduced ethanol induced impairment of psychomotor function as measured with the DSST task, although this did not reach the level of significance. This discrepancy may be due to the fact that the DSST task is not a pure psychomotor speed task, but also assesses other cognitive functions such as memory, which is impaired by MDMA (Dumont, Wezenberg et al. 2008).

Psychomotor accuracy on the other hand was decreased after the combination (comparable to the effects of ethanol alone). These findings are similar to those of a previous study that reported that ethanol administration decreased performance on the critical tracking task (CTT, a laboratory task) as well as measures of actual driving tests (increase of standard deviation of speed (SD(speed)) and standard deviation from lateral position (SDLP)) (Kuypers, Samyn et al. 2006). In line with our hypothesis that MDMA may overcome ethanol induced impairment of psychomotor speed but not accuracy, MDMA did not affect CTT scores, i.e. MDMA co-administration could not overcome ethanol induced impairment. Co-administration of 100 mg MDMA did however reverse ethanol induced impairment of driving performance (ethanol increased SDLP, an effect which was counteracted by MDMA). Our finding of reversal of ethanol induced sedation by MDMA co-administration (as measured by the reaction time of saccadic eye movements) is congruent with these results and support our hypothesis that MDMA may overcome ethanol induced sedation.

Subjective effects were as expected; MDMA was experienced as arousing and induced mild euphoria. Co-administration of ethanol with MDMA showed a profile similar to MDMA induced subjective effects. Subjective effects returned to baseline values five hours after drug administration, with the exception of ethanol

induced sedation, which increased with time and showed maximal effects five hours after drug administration. Co-administration of MDMA with ethanol reversed ethanol induced subjective sedation. This may have important implications, for instance when a subject who has used both MDMA and ethanol decides to drive. MDMA may cause him or her to feel fit enough to drive, while actual performance may be profoundly impaired by alcohol.

Remarkably, sedation as assessed by the reaction time of saccadic eye movements was also delayed relative to ethanol kinetics. Sedation was most pronounced 30 minutes after the ethanol infusion was stopped and declined thereafter, in line with reports that show that ethanol induces sedation mainly in the descending limb of the kinetic profile of ethanol (Pohorecky 1988). As already mentioned, subjective assessments also reflected delayed increase in sedation compared to ethanol administration, whereas other subjective measures had a more direct relationship with blood alcohol concentration. The results reported here show that alcohol may increase sedation even after the intake has stopped. This is particularly relevant if an ethanol-intoxicated subject decides to drive home after a tiresome social event.

The C<sub>max</sub> after 100 mg orally administered MDMA (202.5 µg/l) was comparable to data reported elsewhere (de la Torre, Farre et al. 2000a). MDMA kinetics did not differ between MDMA single and MDMA-ethanol co-administration. Two studies investigated the effects of MDMA and ethanol co-administration on MDMA kinetics in humans, where ethanol co-administration increased plasma MDMA concentration significantly in one (Hernandez-Lopez, Farre et al. 2002) but not the other study (Kuypers, Samyn et al. 2006). A possible explanation for this discrepancy might lie in the fact that the study by Hernandez Lopez et al. (2002) achieved a peak BAC of 1.25<sup>0</sup>/<sub>00</sub>, whereas Kuypers et al. (2006) achieved a peak BAC of 0.59<sup>0</sup>/<sub>00</sub> comparable to our steady state BAC of 0.56<sup>0</sup>/<sub>00</sub>. Future studies should address this issue by assessing the effects of co-administration of different doses of ethanol with MDMA.

Serum cortisol and prolactin concentrations increased significantly after MDMA administration, although both concentrations returned to baseline values

before a decrease in MDMA levels was observed. In other words, the neuro-endocrine response diminished in spite of persisting plasma MDMA concentrations, a pattern observed in most other outcome measures as well. As MDMA is a serotonin releaser, both by reversing the direction of the reuptake transporter as well as by releasing neurotransmitter from the vesicles (Mlinar and Corradetti 2003), it is likely that the available neurotransmitter pool is rapidly depleted, and as a result the neuroendocrine effects of MDMA diminish in spite of persisting plasma MDMA concentration (Green, Mechan et al. 2003).

A short-term reduction of sensitivity to MDMA may also explain why users often take multiple doses of this drug during the night, so-called 'drug-binging' (Gross, Barrett et al. 2002). Several anecdotal reports as well as personal communication with subjects indicate that the purpose of 'binging' is to prolong rather than to increase the effects (de la Torre, Farre et al. 2000b; Parrott 2006; Riley, James et al. 2001).

Several limitations of our study should be mentioned. First, although eye movements play a significant role in everyday psychomotor performance (Orban de Xivry and Lefevre 2007), and the results of this measure are in agreement with those of actual driving tests, the relevance of the effects measured using laboratory tests for actual driving performance remains arguable. Second, subjective effects were assessed only at baseline and 90 and 300 minutes after drug administration, which limits the conclusions regarding the time profile of subjective effects. However, Hernandez-Lopez et al (Hernandez-Lopez, Farre et al. 2002), who assessed subjective effects more frequently over time, also reported that peak effects occurred at 90 minutes after drug administration after which effects declined. Third, although the ARCI measures several aspects of subjective performance, we did not directly assess subjective driving performance. We would recommend such a measure in future studies as it would provide a simple yet effective method of relating objective driving abilities to subjective perception of driving performance (Kuypers, Samyn et al. 2006). Last, our conclusions are based on a BAC of  $0.56^{0}/_{00}$ . As suggested in the discussion of the effects of ethanol on MDMA kinetics, ethanol effects might be dose-dependent, an issue which warrants further research.

In conclusion, we have shown that MDMA significantly increased psychomotor speed but not accuracy and induced significant subjective arousal, effects which were maximal around MDMA C<sub>max</sub>, and declined thereafter. Ethanol on the other hand impaired both psychomotor speed and accuracy, and induced sedation. Only the latter effect did not correspond with ethanol kinetics, sedation was observed during the descending limb of the BAC profile only. Co-administration of MDMA with ethanol reversed ethanol induced sedation and improved psychomotor speed to above placebo levels, although psychomotor accuracy remained impaired. These findings may have implications for general functioning and when driving. Individuals will be more aroused when intoxicated with both substances, which may provide a false sense of better performance, although the accuracy of their performance is actually significantly impaired.



# ***Ethanol co-administration moderates MDMA effects on human physiology***

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### ***Abstract***

Alcohol is frequently used in combination with MDMA. Both drugs affect cardiovascular function, hydration and temperature regulation, but may have partly opposing effects. The present study aims to assess the acute physiologic effects of (co-) administration of MDMA and ethanol over time.

A four-way, double blind, randomized, crossover, placebo-controlled study in 16 healthy volunteers (9 male, 7 female) between the ages of 18 and 29. MDMA (100 mg) was given orally and blood ethanol concentration was maintained at pseudo-steady state levels of 0.6‰ by a three-hour 10% intravenous ethanol clamp. Cardiovascular function, temperature and hydration measures were recorded throughout the study days.

Ethanol did not significantly affect physiologic function, with the exception of a short lasting increase in heart rate. MDMA potently increased heart rate and blood pressure and induced fluid retention as well as an increase in temperature. Co-administration of ethanol with MDMA did not affect cardiovascular function compared to the MDMA alone condition, but attenuated the effects of MDMA on fluid retention and showed a trend for attenuation of MDMA induced temperature increase.

In conclusion, co-administration of ethanol and MDMA did not exacerbate physiologic effects compared to all other drug conditions, and moderated some effects of MDMA alone.

## ***Introduction***

3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”) is a frequently used club-drug in Western societies (Gross 2002;Parrott 2001). Apart from its desired effects on mood and perception, ecstasy has powerful physiologic side effects. Moreover, ecstasy users are generally multi-drug users and alcoholic beverages are commonly combined with MDMA (Barrett, Gross et al. 2005;Izco, Orio et al. 2007). However, the physiologic effects of this common combination, with the exception of effects on immune function (Pacifici, Zuccaro et al. 2001), have not been assessed previously.

MDMA is a potent stimulant of cardiovascular action, increasing heart rate and blood pressure (Dumont and Verkes 2006;Green, Mechan et al. 2003). Disturbances in fluid homeostasis due to MDMA consumption, i.e. an increase of anti-diuretic hormone concentration (ADH or vasopressin, which promotes water retention) after MDMA consumption has been reported (Henry, Fallon et al. 1998;Wolff, Tsapakis et al. 2006). This, in turn, can lead to hyponatraemia and serious health risks (Hall and Henry 2006;Rosenson, Smollin et al. 2007). MDMA also affects temperature regulation, generally increasing body temperature. Although this has received considerable attention in the literature, the mechanism of action is controversial (Colado, O'Shea et al. 2004;Colado, Williams et al. 1995;Green, O'Shea et al. 2004;Mechan, Esteban et al. 2002;Saadat, O'Shea et al. 2005). Recent reports suggest the involvement of the sympathetic nervous system in MDMA induced hyperthermia (Mills, Banks et al. 2003;Sprague, Banks et al. 2003;Sprague, Moze et al. 2005). The pharmacology of MDMA induced hyperthermia is of special interest as prevention of hyperthermia has been shown to diminish or even prevent MDMA induced neurotoxicity (Malberg and Seiden 1998;O'Shea, Easton et al. 2002).

Case reports of severe, sometimes fatal, physiologic disturbances after MDMA use, which are often facilitated by unfavorable behavior and/or circumstances, illustrate the relevance of these side effects of MDMA use (Connolly

and O'Callaghan 1999; Kalantar-Zadeh, Nguyen et al. 2006), although the incidence is low relative to the large population at risk (Nutt 2006).

Drinks containing ethanol, commonly referred to as alcohol, are regularly used in social settings. Ethanol is an allosteric modulator of many transmembrane receptors (Pohorecky and Brick 1988), but functionally it acts foremost as a CNS depressant, depressing both excitatory and inhibitory postsynaptic potentials by potentiating the action of GABA at the GABA<sub>A</sub> receptor (Suzdak, Schwartz et al. 1988). Although the chronic effects of ethanol on physiologic function have been assessed frequently, reports of acute physiological effects of ethanol are less frequent. Contrary to the effects of chronic ethanol exposure, which increases blood pressure (Kodavali and Townsend 2006), acute ethanol administration moderately lowers blood pressure and increases heart rate (Pohorecky and Brick 1988; Silva, Silveira et al. 2004; Tawakol, Omland et al. 2004). Ethanol also affects hydration regulation (e.g. promotes diuresis). Although some reports suggested that ethanol attenuates blood ADH concentration (Madeira and Paula-Barbosa 1999), others did not find an effect of ethanol administration on ADH levels (Rivier and Lee 1996; Silva, Silveira et al. 2004). Effects of ethanol on body temperature also remain poorly understood. Generally, ethanol has been found to lower body temperature, tentatively explained by its vasodilatory effects. Recent studies suggest that the effects of ethanol on thermoregulatory behavior are major contributors to the hypothermic effect of ethanol in humans (Turek and Ryabinin 2005; Yoda, Crawshaw et al. 2005).

As both substances have distinct and possibly opposite actions on physiologic function, we hypothesized that moderate ethanol intake may ameliorate the effects of MDMA. Co-administration of ethanol with MDMA may ameliorate MDMA induced water retention by promoting diuresis, and as such protect against hyponatraemia and its consequences. Cardiovascular distress after MDMA may be enhanced by co-administration of ethanol as ethanol acutely increases heart rate. On the other hand, MDMA induced cardiovascular distress may also be attenuated via ethanol's central depressant effects which may attenuate sympathetic drive of cardiovascular function. The hypothermic effect of ethanol may offset MDMA

induced temperature increase when co-administered, which in turn may diminish MDMA's neurotoxic potential.

## ***Materials and methods***

### *Study Design*

This study utilized a four-way, double blind, randomized, crossover, and placebo-controlled design. Sixteen volunteers were randomly assigned to one of four treatment sequences. Each volunteer received a capsule containing either 100 mg MDMA or placebo and an ethanol or placebo infusion (target blood alcohol concentration of 0.6<sup>0</sup>/<sub>00</sub>) with a washout of 7 days between each treatment.

### *Study outline*

Subjects were admitted to each study day after a urinary drug check (opiates, cocaine, benzodiazepines, amphetamines, methamphetamines and delta-9-tetrahydrocannabinol, AccuSign<sup>®</sup>, Princeton BioMeditech, Princeton, USA) (drug use was not allowed 14 days prior to the first study day until study completion) and the recording of possible signs and symptoms of health problems. A light breakfast was offered. Drug administration was scheduled at 10:30h and the ethanol infusion was started at 11:00h. A 30 minute lunch break was scheduled 210 minutes after drug administration. Outcome measures were assessed before MDMA administration and at 30, 90, 150, 240, 300, and 360 minutes post drug administration and consisted of cardiovascular function assessed by heart rate, systolic- and diastolic blood pressure measurements using a Datascope<sup>®</sup> Accutorr Plus<sup>™</sup> cardiovascular monitor and temperature measurements using a Braun<sup>®</sup> type 6021 ThermoScan. Room temperature was kept at 22 degrees Celsius. Blood was collected for measurement of MDMA, antidiuretic hormone (ADH), sodium, norepinephrine and epinephrine plasma concentration. The latter two were collected at baseline, 60 and 150 minutes after drug administration. Subjects received lunch at 14:00h and were sent home at 17:00h after a medical check.

### *Subjects*

Sixteen healthy volunteers (9 male, 7 female), regular users of ecstasy (at least eight exposures in the last two years) and alcohol (at least one exposure per

week),  $22.1 \pm 2.9$  (mean  $\pm$  SD) years of age (range 18-29) and within 80-130% of their ideal body weight were recruited through advertisement on the internet and at local drug testing services. Lifetime ecstasy exposure was on average 95 (SD=138; range 14-431). More detailed demographic data have been reported elsewhere (Dumont, Wezenberg et al. 2008). Exclusion criteria included pregnancy, (history of) psychiatric illness, use of over-the-counter medication within 2 months prior to the study start, (history of) treatment for addiction problems, (familial or personal history of) schizophrenia, excessive smoking (>10 cigarettes/day) and orthostatic dysregulation. Physical and mental health was determined by assessment of medical history, a physical- and ECG examination as well as standard haematological and chemical blood examinations. None of the subjects screened for study participation showed signs of cardiovascular disturbances or (a history of) psychiatric illness. The local Medical Ethics Committee approved the study. All subjects gave their written informed consent before participating in the study, and were paid for their participation. One subject had a mild adverse reaction (local vascular reaction) to the ethanol infusion and one subject did not refrain from drug use, both (1 male, 1 female) were excluded from further participation and results obtained from these subjects were not included in the final data analysis.

### *Ethanol clamping*

Ethanol (or glucose 5% as its placebo) was administered continuously by IV infusion of 10% ethanol in 5% glucose solution, aimed to maintain an ethanol blood concentration of  $0.6\text{‰}$  for three hours. The alcohol clamp was targeted at  $0.6\text{‰}$ , the equivalent of approximately 2-3 alcoholic beverages. This concentration is just above the legal limit for traffic participation in many Western countries and commonly used in social settings, as it is considered to be a safe and relatively moderate level, despite significant CNS effects (Amatsaleh, Schoemaker et al. 2006). The infusion rate was calculated using frequent breath alcohol concentration measurements, according to a previously designed algorithm (Amatsaleh, Dumont et al. 2006). Breath alcohol concentration was assessed using a HONAC AlcoSensor IV<sup>®</sup> Intoximeter. The process was semi-automated using a computer spreadsheet

program, which uses changes in the measured breath alcohol concentrations to calculate the infusion rate that is needed to maintain the ethanol level at 0.6<sup>0</sup>/<sub>00</sub>. The operator of the breath alcoholmeter and the ethanol infusion pump was unblinded for alcohol-treatment, but did not communicate with the study team or the subject about the results at any stage during the trial. A sham-procedure including a mock-spreadsheet was used on ethanol-placebo-occasions.

### *MDMA*

MDMA (or matched placebo) was given orally as a capsule in a single dose of 100 mg. MDMA was obtained from Lipomed AG, Arlesheim, Switzerland and encapsulated according to Good Manufacturing Practice (GMP) by the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre. 100 mg MDMA orally is a relevant dose in the range of normal single recreational dosages. Previous experiments in humans used doses up to 150 mg without serious adverse events.

### *Analytical Methods*

All reagents were of analytical grade.

MDMA analysis: Plasma samples were stored frozen at -70 °C until the time of analysis. An HPLC–diode array detection (HPLC-DAD) method was employed to measure MDMA plasma concentration (Dumont, Wezenberg et al. 2008).

Hormone analysis: ADH was measured by an in-house radioimmunoassay RIA employing 125I-labelled ADH and an antibody raised against arginine-vasopressine, performed after prepurification of ADH by means of Sep-Pak C18 columns. The average recovery was 75±8%. Within- and between-assay CVs were: 7.1 and 14.0% at 2.6 pmol/l, 3.4 and 8.5% at 5.0 pmol/l and 4.0, and 9.4% at 8.9 pmol/l.

Plasma norepinephrine and epinephrine concentration was measured by a sensitive and specific HPLC with fluorometric detection as described previously (Willemsen, Ross et al. 1995). Blood samples were collected after the subject had

remained in seated position for at least 15 minutes and were processed within 30 minutes after collection.

### *Statistical Analyses*

Pharmacodynamic parameters were analyzed by mixed model analyses of variance (using SAS PROC MIXED; SAS 9.1.3 for Windows, SAS Institute, Inc., Cary, NC) with treatment, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline was defined as the average of the available values obtained prior to dosing. Treatment effects are reported as the contrasts between the 4 treatments where the average of the measurements up to last time point was calculated within the statistical model. Contrasts are reported along with 95% confidence intervals and analyses are two-sided with a significance level of 0.05.

Temperature measurements were also converted to a composite measure (TAUC) as described by Miller et al. (Miller and O'Callaghan 2003). This composite measure represents the area under the curve of a plot of temperature (°C) and time (min) and has units of °C min. Statistical evaluation of TAUC was performed using the GLM Repeated Measures Analysis of Variance (ANOVA) in SPSS 12 for windows. The relationships between MDMA dose corrected for body weight and MDMA maximal plasma concentration and between MDMA dose corrected for body weight and heart rate were statistically evaluated using a linear regression analysis in SPSS 12 for Windows.

## Results

Only significant results are mentioned in this section unless noted otherwise. Main effects of treatment, time and treatment by time as well as drug condition comparisons are summarized in Table 1. For the drug condition comparisons, (percentual) change, 95% confidence interval (95% CI) and corresponding p-values are reported.

### Pharmacokinetics

Mean MDMA maximal plasma concentration ( $C_{max}$ ) was 202.5 ng/ml ( $SD=74.1$  ng/ml) 150 minutes after drug administration, mean ethanol steady state concentration was 0.56 ‰ ( $SD=0.06$  ‰). A positive linear relationship was found for MDMA dose corrected for body weight and MDMA  $C_{max}$  ( $R=0.85$ ,  $p<0.001$ , see Figure 1). Ethanol co-administration did not affect this relationship. MDMA and ethanol kinetics in time are reported elsewhere (Dumont et al, in press).

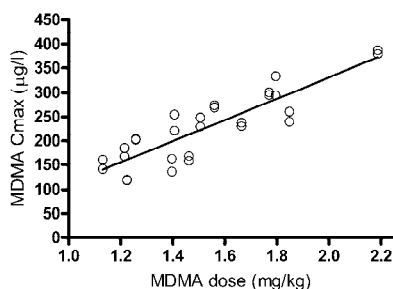


Figure 1. Effect of MDMA dose corrected for body weight on MDMA maximal plasma concentration ( $C_{max}$ ).

### Cardiovascular function

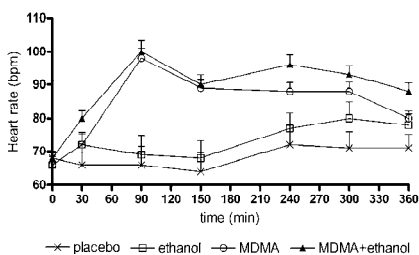


Figure 2A: Heart rate (in bpm) per drug condition (mean, s.e.m.).

Heart rate was increased in all drug conditions compared to placebo as shown in figure 2A. Co-administration of MDMA and ethanol did not increase heart rate compared to the MDMA alone condition. Maximal heart rate increase was correlated with MDMA dose corrected for body weight and showed a

positive linear dose response curve ( $R= 0.69$ ,  $p<0.001$ ).

Systolic and diastolic blood pressure showed a similar response to drug administration in time and were increased after MDMA as well as MDMA and ethanol (co-) administration compared to the placebo and ethanol condition, Figure 2B shows the mean arterial pressure (MAP).

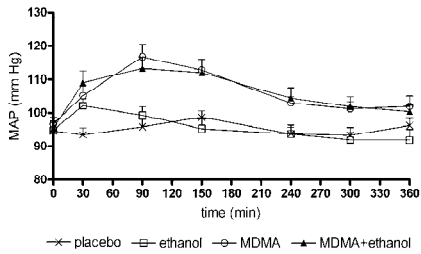


Figure 2B: Mean arterial pressure (MAP, in mm Hg) per drug condition (mean, s.e.m.).

*Norepinephrine and epinephrine concentrations*

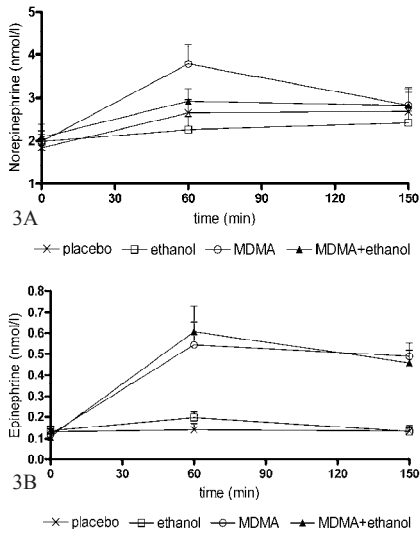


Figure 3. Norepinephrine and epinephrine plasma concentrations. Figure 3A: Norepinephrine plasma concentration (in nmol/l) per drug condition (mean, s.e.m.). Figure 3B: Epinephrine plasma concentration (in nmol/l) per drug condition (mean, s.e.m.).

and MDMA increased norepinephrine concentrations.

Norepinephrine levels, shown in Figure 3A, were increased after MDMA administration compared to the placebo and ethanol condition. Epinephrine levels, shown in Figure 3B, were increased after MDMA as well as MDMA and ethanol (co-) administration compared to the ethanol and placebo condition. Co-administration of ethanol with MDMA did not increase norepinephrine levels compared to placebo. Ethanol alone did not affect norepinephrine concentrations compared to placebo either. Compared to the ethanol condition, co-administration of ethanol

Measure	Treatment effect	Time Effect	Treatment by Time	Ethanol vs. Plac	MDMA vs. Plac	MDMA+Eth vs. Plac	MDMA+Eth vs. Ethanol	MDMA+Eth vs. MDMA	MDMA vs. Ethanol
Epinephrine and norepinephrine concentration									
E (nmol/l)	<.0001				306.6% (197.1%, 456.4%) p=<.0001	293.0% (187.1%, 438.1%) p=<.0001	212.8% (128.5%, 328.2%) p=<.0001		223.6% (136.5%, 342.6%) p=<.0001
NE (nmol/l)	0.0014		0.0153		22.5% (4.2%, 44.1%) p=0.0155		25.2% (6.6%, 47.2%) p=0.0077		40.3% (19.4%, 65.0%) p=0.0002
Cardiovascular parameters									
HR (bpm)	<.0001	<.0001	<.0001	9.0% (2.4%, 16.1%) p=0.0085	25.7% (18.0%, 33.9%) p=<.0001	31.7% (23.7%, 40.2%) p=<.0001	20.8% (13.5%, 28.7%) p=<.0001		15.3% (8.3%, 22.7%) p=<.0001
SBP (mm Hg)	<.0001	0.0002			11.2% (7.2%, 15.4%) p=<.0001	12.0% (7.9%, 16.2%) p=<.0001	10.9% (6.8%, 15.0%) p=<.0001		10.1% (6.1%, 14.3%) p=<.0001
DBP (mm Hg)	<.0001	<.0001			10.9% (5.9%, 16.2%) p=<.0001	10.9% (5.9%, 16.2%) p=<.0001	12.3% (7.3%, 17.6%) p=<.0001		12.3% (7.2%, 17.5%) p=<.0001
Hydration parameters									
ADH (pmol/l)	0.0286				22.2% (2.5%, 45.7%) p=0.0269		-23.1% (-35.5%, -8.4%) p=0.0044		
Na (mmol/l)	0.0132	<.0001	<.0001		-0.9% (-1.6%, -0.2%) p=0.0128				-1.1% (-1.8%, -0.4%) p=0.0023
Temperature									
Temperature (°C)	0.0029		0.0034		0.4 °C (0.2, 0.7) p=0.0021				0.5 °C (0.2, 0.8) p=0.0008

Table 1. Results: p-values for treatment, time and treatment by time effects (N= 14). Comparisons of drug conditions show (percentual) change, 95% confidence intervals (between brackets) and p-values. E=epinephrine, NE=norepinephrine, ADH= antidiuretic hormone, Plac= placebo and Eth= ethanol.

### Hydration

Antidiuretic hormone (ADH or vasopressin) plasma concentrations are shown in Figure 4 and were increased after MDMA administration compared to placebo. Ethanol administration alone did not affect ADH levels compared to placebo. Co-administration of ethanol with MDMA reversed the MDMA induced increase of ADH concentrations to levels comparable to placebo.

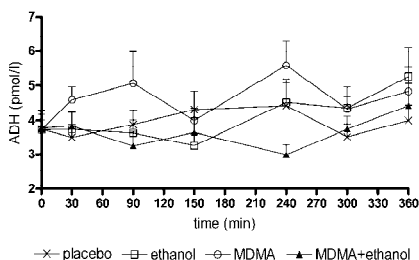


Figure 4. Antidiuretic hormone plasma concentrations (ADH, in pmol/l) per drug condition (mean, s.e.m.).

Sodium plasma concentrations were decreased after MDMA administration compared to all other conditions. Co-administration of ethanol with MDMA did not significantly affect sodium plasma concentrations compared to the placebo condition. Ethanol alone also did not have an effect on sodium plasma concentrations.

### Temperature

Temperature, shown in Figure 5A, increased significantly after MDMA administration compared to the placebo (by 0.4 °C on average) as well as compared to the ethanol condition. Ethanol did not affect temperature significantly compared to any drug condition, although there was a trend ( $p=0.09$ ) for attenuation of the MDMA effect on temperature during co-administration of MDMA and ethanol. Temperature data was also converted to AUC data (TAUC,

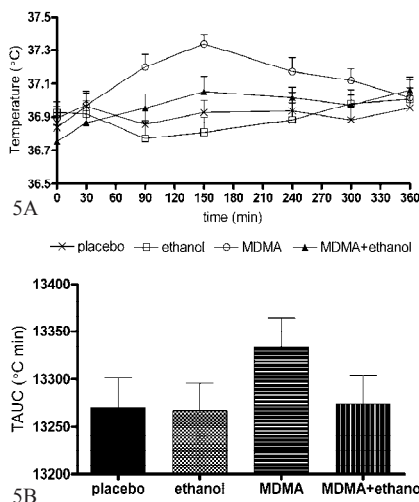


Figure 5. Temperature effects. Figure 5A: Temperature (in °C) per drug condition (mean, s.e.m.). Figure 5B: Temperature AUC (TAUC in °C min) per drug condition (mean, s.e.m.).

Figure 5B). Following a significant main effect of drug condition ( $F(3,11)=4.049$ ,  $p=0.036$ ), subsequent pairwise comparisons showed a significant increase in TAUC after MDMA administration compared to placebo ( $p=0.015$ ), ethanol ( $p=0.018$ ), and MDMA and ethanol co-administration ( $p=0.016$ ). Other comparisons did not reveal significant differences between drug conditions. In other words, co-administration of ethanol with MDMA reversed the MDMA induced increase of TAUC to levels comparable to placebo.

## ***Discussion***

This study confirms previous findings that MDMA exerts potent stimulatory effects on the human cardiovascular system, induces an increase in temperature and a disturbance of water homeostasis. Ethanol single administration did not affect the physiological parameters investigated in this study, with the exception of a mild increase in heart rate. Co-administration of ethanol with MDMA did not exacerbate MDMA induced stimulation of cardiovascular measures, and moderated the effect of MDMA on temperature and water homeostasis. Thus, co-administration of low-dose ethanol, i.e. the equivalent of 2-3 alcoholic beverages, with MDMA attenuates some and does not exacerbate any of MDMA's physiologic effects.

A relatively wide range (1.1-2.2 mg/kg) of MDMA dose corrected for body weight was administered in the current study. Our current findings show a significant positive linear relationship between MDMA dose corrected for body weight and C<sub>max</sub>, where an increase in dose of 0.1 mg/kg elevated MDMA C<sub>max</sub> with approximately 20 µg/l (Figure 1). Ethanol co-administration did not affect this relationship. A previous study reported a non-linear dose response relationship for MDMA dose vs. MDMA peak blood concentrations (de la Torre, Farre et al. 2000a). Although this study assessed the C<sub>max</sub> of separate doses (50, 100 and 150mg), the sample size was relatively small (N=6, two subjects per dose tested) and doses were not corrected for body weight (reported weight range was 66–83 kg).

A significant, positive linear dose-response relationship was found for the effect of MDMA dose (corrected for body weight) on heart rate, where an increase of 0.1 mg/kg induced an average increase in heart rate of 4.2 bpm. Ethanol co-administration did not affect this relationship. Although ethanol single administration led to a modest increase in heart rate, co-administration of ethanol and MDMA did not show an additive effect on heart rate.

Blood pressure was increased after MDMA as well as after MDMA and ethanol co-administration (see Figure 2B). The lack of ethanol effects on blood pressure is in contrast with previous findings, which reported a moderate decrease of

blood pressure after ethanol administration (Pohorecky and Brick 1988;Silva, Silveira et al. 2004;Tawakol, Omland et al. 2004). The ethanol administration route (infusion of 10% ethanol solution over three hours) may have counteracted the effects of ethanol on blood pressure.

Hyponatraemia has been suggested to be a, potentially fatal, side effect of MDMA use, likely due to MDMA induced increase in anti-diuretic hormone (ADH) concentration (Hartung, Schofield et al. 2002;Henry, Fallon et al. 1998). In line with this suggestion, we report a decreased sodium plasma concentration and increased ADH concentration after MDMA administration. Co-administration of ethanol with MDMA attenuated the MDMA induced increase in ADH concentration (Figure 4). After co-administration, sodium concentration did not differ from placebo or from the MDMA condition. Although the effects reported here are relatively small and are unlikely to be of clinical relevance, the circumstances in which MDMA is typically used may exacerbate these effects (Kalantar-Zadeh, Nguyen et al. 2006) (crowded, high ambient temperature clubs in combination with vigorous dancing as well as excessive water intake stimulated by public education). Ethanol single administration did not affect these measures. Previous reports have shown that ethanol can attenuate ADH release (Madeira and Paula-Barbosa 1999), although others did not find an effect of ethanol administration on ADH levels (Rivier and Lee 1996;Silva, Silveira et al. 2004). Possibly, the continuous administration of low levels of ethanol is insufficient to disturb ADH regulation *per se*, whereas the increased ADH concentration after MDMA allowed ethanol to demonstrate its attenuating effect on ADH release.

Body temperature (Figure 5) has been shown to robustly increase after MDMA administration in animals (Green, O'Shea et al. 2005). In the current (human) study, MDMA increased body temperature slightly but significantly with an average maximal increase of 0.4 degrees Celsius. Although this effect is very moderate in comparison to the animal data, the effects on temperature regulation may be of significance in recreational MDMA use settings. These environments are assumed to be crowded and to have a high ambient temperature. Combined with vigorous dancing these factors may facilitate the MDMA induced increase of body

temperature beyond the effect size observed in the current, strictly controlled laboratory study (Freedman, Johanson et al. 2005; Parrott, Rodgers et al. 2006; Williams, Dratcu et al. 1998). Two naturalistic studies assessed the effects of (among others) MDMA on body temperature during a 'club night' (Cole, Sumnall et al. 2005; Irvine, Keane et al. 2006). Both studies did not find a significant rise in body temperature in users of psychostimulants (among which MDMA). However, all psychostimulant users in the study of Cole et al. (2005) reported the co-use of alcohol, thus confirming our current findings. The study by Irvine et al. (2006), which showed a trend for increased body temperature, did not assess alcohol co-use, although the authors did note that all participants were regular users of alcohol.

In rats, MDMA induced hyperthermia has been shown to be mediated by the sympathetic nervous system, more specifically via  $\alpha_1$  mediated vasoconstriction and  $\beta_3$  mediated thermogenesis in rats (Blessing 2005; Mills, Banks et al. 2003; Mills, Weaver et al. 2007; Sprague, Moze et al. 2005). In the current study, MDMA indeed potently increased epinephrine (E) and norepinephrine (NE) plasma concentrations (Figure 3). Ethanol co-administration attenuated the MDMA induced NE (but not E) increase as well as MDMA induced temperature increase. Moreover, the above mentioned report by Sprague et al. (2005) showed that the blockade of either heat generation (mediated by the  $\beta_3$  receptor) or blockade of vasoconstriction (mediated by the  $\alpha_1$  receptor) could only reduce hyperthermia by approximately 50%. In the current study, temperature after MDMA and ethanol co-administration did not differ from placebo and showed a trend for attenuation of MDMA induced temperature increase. This is in line with the abovementioned report, as ethanol co-administration attenuated NE concentration, effectively diminishing heat generation (the  $\beta_3$  receptor shows a higher affinity for NE over E), although ethanol co-administration did not reduce the elevated E plasma concentrations. As the  $\alpha_1$  receptor, mediating vasoconstriction, has equal affinity for NE and E, the increased E concentration is likely to powerfully maintain vasoconstriction, and thus impair heat dissipation, confirming the findings of Sprague et al. (2005) in humans. The attenuation of NE output did not affect cardiovascular measures, although the reduced NE concentration should reduce vasoconstriction (mediated by NE) and

thus lower blood pressure. The potent stimulatory effects on heart rate (mainly mediated by E) have probably counteracted any effect of the reduced NE concentration on blood pressure (Figure 2).

Although MDMA's neurotoxic potential in humans is still a matter of debate (Gouzoulis-Mayfrank and Daumann 2006a), MDMA induced temperature increase is of particular interest as its prevention has been shown to be an effective way of reducing or even preventing MDMA induced neurotoxicity in animal studies (Goni-Allo, Mathuna et al. 2008; Malberg and Seiden 1998; O'Shea, Easton et al. 2002). However, our findings suggest that low-dose ethanol co-administration, by attenuating sympathetic output, may moderate MDMA induced neurotoxicity. Although this provides interesting avenues for future studies regarding MDMA induced neurotoxicity, the current study did not assess these effects under the circumstances where temperature increase may become clinically relevant. In fact, a recent study in rats reported that ethanol did not attenuate MDMA induced temperature increase in high ambient temperature (Cassel, Ben et al. 2007). Moreover, as ethanol is able to increase hydroxyl radical formation, higher doses of ethanol may potentiate MDMA induced oxidative stress, and thus neurotoxicity, as suggested by a recent study that showed that repeated pre-exposure to high doses of ethanol exhausted CNS anti-oxidant resources and potentiated the neurotoxic effects of a subsequent dose of MDMA (Izco, Orio et al. 2007).

Our results should be considered explorative due to some limitations of our study design. Firstly, the attenuating effects of ethanol co-administration on temperature and hydration did not reach statistical significance when directly compared to the single MDMA condition (with the exception of ADH and TAUC effects). It is likely that our study did not have sufficient power to statistically distinguish between these relatively small effects, although as discussed these effects may be enhanced and become relevant under more naturalistic conditions. Secondly, we assessed the effects of a single dose of MDMA and subsequent ethanol administration, and effects may differ depending on the dose assessed and the sequence of drug administration. Moreover, effects were assessed at a single ambient temperature of 22°C, and different ambient temperatures may induce

different effects. In general, the circumstances in which these substances are normally used cannot be fully recreated in the laboratory. These circumstances, along with the different expectations and behaviour, likely influence the effects of MDMA (Sumnall, Cole et al. 2006). Thus, further research should investigate the effects of the surroundings that ecstasy users are exposed to while being intoxicated, although such studies face considerable issues regarding feasibility (Irvine, Keane et al. 2006). Such studies should also assess the effects of different doses of ethanol and MDMA on physiologic function to corroborate our suggestions. Lastly, the difference in ethanol administration and resulting kinetics may have influenced our results, as it has been shown that some ethanol effects (i.e. sedation) manifest only during ascending or descending BAC (Pohorecky and Brick 1988).

As studies investigating the therapeutic potential of MDMA are emerging (Parrott 2007b; Sessa 2007; Sessa and Nutt 2007), information on how to treat and possibly even prevent side effects of MDMA in clinical studies appears vital. Our results suggest that antagonism of the sympathetic nervous system during MDMA use may diminish temperature increase, and, although speculative, thereby may diminish the possibility of neurotoxicity. Moreover, the cardiovascular distress induced by MDMA may also be effectively treated or prevented via this intervention. Last, careful management of fluid intake may effectively manage the symptoms of inappropriate increase in ADH concentration under controlled circumstances.

In conclusion, co-administration of ethanol and MDMA did not exacerbate physiologic effects compared to all other drug conditions, and moderated some effects of MDMA alone. It should be stressed that these findings are only valid for the relatively low dose of ethanol (0.6 ‰ or 2-3 alcoholic beverages) as employed in the current study. Although the effects observed in this study are considered subtle, they demonstrate that MDMA and ethanol dysregulate physiological systems that are particularly important during the typical circumstances in which MDMA is used (Cassel, Ben et al. 2007; Cornish, Shahnawaz et al. 2003; Green, Sanchez et al. 2004; Hargreaves, Hunt et al. 2007).



***Acute psychomotor, memory and  
subjective effects of MDMA and THC  
(co-) administration over time in healthy  
volunteers***

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### *Abstract*

In Western societies a considerable percentage of young people expose themselves to the combination of 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”) and cannabis.

The aim of the present study was to assess the acute effects of (co-) administration of MDMA and THC (the main psychoactive compound of cannabis) on pharmacokinetics, psychomotor performance, memory and subjective experience over time.

We performed a four-way, double blind, randomized, crossover, placebo-controlled study in 16 healthy volunteers (12 male, 4 female) between the ages of 18 and 27. MDMA (100 mg) was given orally, THC (4, 6, and 6 mg, interval of 90 minutes) was vaporized and inhaled.

THC induced more robust cognitive impairment compared to MDMA, and co-administration did not exacerbate single drug effects on cognitive function. However, co-administration of THC with MDMA increased desired subjective drug effects and drug strength compared to the MDMA condition, which may explain the widespread use of this combination.

## ***Introduction***

In Western societies a significant proportion of young people expose themselves to 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”) (Parrott 2001). Ecstasy users are generally multidrug users, having experience with different psychoactive substances and combining them with ecstasy (Gouzoulis-Mayfrank and Daumann 2006b). Cannabis (main active compound  $\Delta^9$ -tetrahydrocannabinol or THC) is frequently co-used with ecstasy (Parrott, Milani et al. 2007). Despite the prevalence of co-administration of MDMA and THC, the effects of combined use of these substances in humans have so far not investigated.

MDMA releases serotonin (5-HT) from presynaptic 5-HT terminals by reversal of the reuptake transporter and thus increases 5-HT levels at the postsynaptic receptors (Liechti and Vollenweider 2000;Mlinar and Corradetti 2003;Pifl, Drobny et al. 1995). MDMA is also a potent releaser of dopamine and (nor) adrenaline (Colado, O'Shea et al. 2004;Liechti and Vollenweider 2001;Sprague, Brucher et al. 2004). In a previous study by our group, MDMA was found to increase psychomotor speed without affecting psychomotor accuracy. MDMA impaired the delayed recall of words, whereas word recognition was unaffected. MDMA increased subjective arousal and decreased subjective calmness (Dumont, Wezenberg et al. 2008). These effects generally co-incided with maximal MDMA plasma concentration but declined to baseline values in spite of persisting MDMA plasma concentration, which is generally consistent with the literature (Dumont and Verkes 2006). MDMA is rapidly absorbed following oral administration, and within 30 minutes detectable in the blood. MDMA plasma levels peak 1-2 hours after drug intake.

THC, the major psychoactive compound in cannabis (Ilan, Gevins et al. 2005;Wachtel, ElSohly et al. 2002), is an agonist for the CB<sub>1</sub> and CB<sub>2</sub> receptors of the endocannabinoid system (ECS). The CB<sub>1</sub> receptor is abundantly expressed in the central nervous system whereas the CB<sub>2</sub> receptor is expressed predominantly in the periphery (Ameri 1999). The central effects of THC have received abundant attention in the scientific literature and generally include, but are not restricted to,

impairment of memory and psychomotor function and subjective relaxation. A recent review revealed that cannabis affects most functional CNS-domains, but due to great variations in study methodology only increases of heart rate and subjective feelings (feeling 'high') were found to be reliable biomarkers of cannabis effects (Zuurman, Ippel et al. 2009). THC, a highly lipophilic compound, is rapidly distributed into fatty tissue (among which the CNS), and after inhalation peak plasma concentration are reached within minutes and show a rapid decline, although cognitive effects and subjective effects are maximal after 15 to 60 minutes and last for several hours (Curran, Brignell et al. 2002; Strougo, Zuurman et al. 2008).

As combined use of MDMA and THC is common (Parrott, Gouzoulis-Meyfrank et al. 2004; Parrott, Milani et al. 2007), and these substances both affect memory as well as psychomotor function, we aimed to assess the cognitive and subjective effects of co-administration of these substances over time under controlled laboratory conditions in experienced users. Previous research regarding the cognitive effects of co-administration of MDMA and THC is limited to a study in rats and showed that co-administration induced a synergistic impairment of working memory (Young, McGregor et al. 2005). Thus, co-administration was expected to show additive impairment of memory, whereas effects on psychomotor performance were expected to be attenuated due to the opposing actions of the stimulant MDMA and the relaxant THC.

## ***Materials and methods***

### *Study Design*

This study utilized a four-way, double blind, randomized, crossover, and placebo-controlled design and was conducted according to the principles of the Declaration of Helsinki. Each volunteer received a capsule containing either MDMA 100 mg or placebo and inhaled a vapor containing consecutively 4, 6, and 6 mg of THC (dosing intervals of 90 minutes) or placebo vapor containing vehicle with a washout of 7 days between each condition.

### *Study outline*

Subjects were admitted to each study day after a urinary drug check (opiates, cocaine, benzodiazepines, amphetamines, methamphetamines and delta-9-tetrahydrocannabinol, AccuSign<sup>®</sup>, Princeton BioMeditech, Princeton, USA: drug use was not allowed 14 days prior to the first study day until study completion) and the recording of possible signs and symptoms of health problems. As THC was administered during study days, urine positive for THC led to exclusion only on study day 1. A light breakfast was offered two hours prior to drug administration. MDMA administration was scheduled at 10:30h and THC was administered at 0, 90, and 180 minutes after MDMA administration. Subjects received a standardized lunch at 14:00h and were sent home around 17:00h.

Outcome measures were assessed repeatedly, i.e. before MDMA administration and at 15, 60, 105, 150, 240 and 300 minutes post drug administration, with the exception of the 18 word list memory task, which was performed 120 minutes after drug administration. Repeated measures consisted of blood sampling for analysis of study drug kinetics and assessments of postural stability, psychomotor function, memory, and subjective effects as specified below. To familiarize the subjects with the tests and procedures, they were invited to the hospital to perform a practice session one week before the actual study days.

## Subjects

Sixteen healthy volunteers (12 male, 4 female), regular users of ecstasy (at least 8 exposures in the last two years) and THC (on average two exposures per week in the last year), between the ages of 18 and 27, were recruited through advertisement on the internet and at local drug testing services. Detailed demographic data are shown in Table 1. Exclusion criteria included pregnancy, (history of) psychiatric illness (assessed using the Structured Clinical Interview for DSM-IV axis I disorders, non-patient version (First, Frances et al. 1994), Axis II disorders were excluded using the Temperament and Character Inventory (Svrakic, Whitehead et al. 1993)), use of over-the-counter medication within 2 months prior to the study start, (history of) treatment for addiction problems, excessive smoking (>10 cigarettes/day) and orthostatic dysregulation. Physical and mental health was determined by assessment of medical history, a physical- and ECG examination as well as standard haematological and chemical blood examinations. The local Medical Ethics Committee approved the study. All subjects gave their written informed consent before participating in the study, and were paid for their participation.

One subject did not refrain from drug use, after which further study participation was denied. Two subjects experienced an adverse event that was judged to be likely related to study drug administration (one subject experienced a short lasting (55 seconds) heart rate increase of >180 bpm and another subject experienced mild hallucinations, the latter subsiding along with other drug effects). These subjects were excluded from further participation, data of completed study days obtained prior to these adverse events were analysed as described.

	<i>Mean</i>	<i>s.e.m.</i>	<i>Min</i>	<i>Max</i>
Age (years)	21	0.5	18	27
Education (years)	16	0.3	12	18
Height (cm)	178	1.7	165	189
Weight (kg)	71	2.1	60	86
Opiates	26	9	1	50
LSD	33	13	2	108
Ecstasy	143	53	10	702
Amphetamines	96	50	1	624
Cannabis	1716	429	364	6570
Cocaine	46	19	2	234
Alcohol	6071	1221	144	15600
Solvents	122	70	1	834
Benzodiazepines	7	3	1	25
Psilocybin	19	6	1	60
GHB	33	19	1	208
Ketamine	211	116	1	1040

Table 1: *Demographic data of study participants, drug use is quantified as the cumulative number of lifetime drug exposures (not further specified).*

### *Study drugs*

THC was purified according to Good Manufacturing Practise (GMP)-compliant procedures (Farmalyse BV, Zaandam, The Netherlands) from the flowers of *Cannabis sativa* grown under Good Agricultural Practice (Bedrocan BV Medicinal Cannabis, Veendam, The Netherlands) (Choi, Hazekamp et al. 2004; Hazekamp, Choi et al. 2004). Each dose (4, 6 and 6 mg) of THC (>98% purity by HPLC/GC) was dissolved in 200 µl 100 vol% alcohol. THC was stored in the dark at -20°C in 1 ml amber glass vials containing a teflon screw-cap secured with Para film to minimize evaporation. The solvent was used as placebo.

On each study day, THC (4, 6 and 6 mg) or placebo were administered by inhalation at 90-minute intervals using a Volcano<sup>®</sup> vaporizer (Storz-Bickel GmbH, Tuttlingen, Germany), a validated method of intrapulmonary THC administration (Abrams, Vizoso et al. 2007; Hazekamp, Ruhaak et al. 2006). Concurrent with MDMA administration, THC (4 mg) was administered to ensure tolerability. 90 and 180 minutes after drug administration, 6 mg of THC was administered. Within five minutes before administration THC was vaporized at a temperature of about 225°C and the vapour was stored in a polythene bag equipped with a valved mouthpiece, preventing the loss of THC in between inhalations. The transparent bag was covered with a black plastic bag to prevent unblinding. Subjects were not allowed to speak, and were instructed to inhale deeply and hold their breath for 10 seconds after each inhalation. Within 2-3 minutes the bag was to be fully emptied. The inhalation procedure was practiced at screening using the vehicle only.

The inhalation schedule was predicted to cause THC plasma concentrations and effects which roughly correspond to those of one marijuana cigarette. The decision to proceed to the next THC dose was made by a physician, based on adverse events and physical signs.

MDMA (or matched placebo) was given as a capsule in a single oral dose of 100 mg. MDMA was obtained from Lipomed AG, Arlesheim, Switzerland and encapsulated according to GMP by the Department of Clinical Pharmacy of Radboud University Nijmegen Medical Centre.

### *Pharmacokinetic measurements*

#### *THC*

For determination of the concentration of plasma THC and its two most important metabolites (11-OH-THC and 11-nor-9-carboxy-THC), venous blood was collected in EDTA tubes of 4.5 ml blinded with aluminium foil. Blood samples were taken 5 and 20 minutes after each THC administration and immediately put on ice and were processed (spun at 1500 g for 10 minutes at 4 °C ) within 30 minutes after collection. THC blood samples were handled sheltered from light. Plasma samples were stored at a temperature of -80°C for less than 3 months before laboratory analysis. Concentrations of THC and the metabolites were shown to be stable over this period (Hazekamp, Choi et al. 2004).

Determination of THC, 11-OH-THC and 11-nor-9-carboxy-THC content was performed using a validated high performance liquid chromatography with tandem mass spectrometric detection. Calibration range was 1.00 – 500 ng/ml for all compounds. Over this range the intra-assay coefficient of variation was between 4.0 and 6.5%. The inter-assay coefficient of variation was between 1.4 and 9.4%.

#### *MDMA*

An HPLC–diode array detection (HPLC-DAD) method was employed to assess MDMA and MDA plasma concentration, which has been described in detail previously (Dumont, Schoemaker et al, in press).

### *Pharmacodynamic measurements*

#### *Eye movements*

Saccadic eye movements are a measure for psychomotor speed and sedation. Eye movements were quantified by recordings of field potential changes due to eye rotations. Similar to EEG patterns and the architecture of evoked potentials in rats (Meeren, Van Luijtelaa et al. 1998), saccadic motion is dependent on the state of alertness (van Steveninck, van Berckel et al. 1999). For the saccadic

test, which lasted 1.5 minutes, the subject was instructed to look at a target that suddenly changed position at random intervals. The target consisted of an array of light emitting diodes on a bar fixed at 50 cm in front of the head support. Each recording session consisted of 15 saccades of 15 degrees stimulus amplitudes. The outcome measures are peak saccadic velocity and reaction time.

For smooth pursuit eye movements, a measure for psychomotor accuracy, the target moved sinusoidal at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz during 60 s. The amplitude of target displacement corresponded to 20 degrees eyeball rotation to both sides. The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage.

Saccadic- and smooth pursuit eye movements were recorded using Nihon-Kohden® and Cambridge Electronics Design (CED®) hardware, and CED Spike2® software for sampling and analysis of eye movements. Effects on the saccadic eye movements, the Saccadic Eye Velocity (PV), were analysed according to published rules (Meeren, Van Luitelaar et al. 1998; Sundstrom and Backstrom 1998). Head movements were restrained using a fixed head support. Eye movements are used to locate objects and predict the path of moving objects, and as such can be expected to be relevant for driving related abilities (Orban de Xivry and Lefevre 2007). Moreover, they are sensitive to the effects of serotonergic challenges, MDMA and cannabis (Dumont, Valkenberg et al. 2007; Gijsman, van Gerven et al. 2002; Zuurman, Roy et al. 2008).

### *Body sway*

Subjects were asked to close their eyes while in upright position and were attached to the body sway apparatus that records cumulative horizontal body movement (in mm) for two minutes. The test is a measure for postural stability (Wright 1971).

### *Pursuit task*

To measure implicit procedural learning a computerized version of the rotor pursuit task was used. This test is based on the classic rotary pursuit task (Ammons 1951). It is a continuous motor task. Subjects had to follow the movement of a large target stimulus on the computer screen with a cursor by moving the pen over a XY-tablet. The speed of the target gradually increased when the cursor was contained within the target but decreased considerably when it was not. The target followed a spatially predictable circular path over the screen. The outcome measure for this test was the total number of rotations within two minutes.

### *Eighteen words list*

The eighteen words list is a verbal memory test based on the classic Auditory Verbal Learning Test (Vakil and Blachstein 1993). A variant was made consisting of a list of eighteen words. The classic test uses fifteen words. A longer wordlist was chosen to prevent ceiling effects. The list was presented verbally three times 120 minutes after MDMA administration (30 minutes after the second THC administration). Under normal circumstances subjects are supposed to remember an increasing number of words after each trial. Directly after each presentation, and after an interval of 20 minutes, subjects were asked to recall as many words as possible. After the delayed recall trial a list of thirty-six words was presented from which they were asked to recognize the eighteen words previously presented. The incorrect words were distracters and resembled the correct words in a semantic or phonologic manner. Responses were either correct positive (when a word that was recognized was indeed part of the list presented during immediate recall) or false positive (when a word was recognized but was not part of the list presented during immediate recall, e.g. the word was a distracter). The outcome measure was the number of correctly recalled/recognized words for the average of the three immediate recall trials, the delayed recall trial and the delayed recognition trial.

### *N-back task*

The N-back task, a test of working memory, is widely used for the detection of working memory deficits (Meyer-Lindenberg, Poline et al. 2001). Subjects were presented with a starting circle and six possible target circles surrounding the starting circle on the screen, reflecting the same positions as on the paper form. In the 1-back condition, subjects had to respond to the stimulus that was presented in the previous trial. In the 2-back condition, subjects had to respond to the stimulus presented two trials before. In the 3-back condition, subjects had to respond to the stimulus presented three trials before. The outcome measure was the time needed until completion of 25 correct trials.

### *Bond and Lader (Visual Analogue) Mood Rating Scale (BLMRS).*

The BLMRS scale consists of 16 lines, each 10 cm in length, with opposite terms at each end of the line (Bond, James et al. 1974). Subjects were asked to indicate which item was more appropriate by marking the line. The outcome measure of these visual analogue scales was the distance to the marker on each scale. These scale scores were aggregated to scores for 'calmness', 'alertness' and 'contentedness' as described by Bond and Lader (1974).

### *Subjective drug experience visual analogue scales*

To assess subjective drug experience and motivation, three visual analogue scales were constructed (drug liking, drug strength and motivation). Similar to the BLMRS, these were each 10 cm in length, and subjects were asked to quantify these terms by marking the line. The outcome measure was the distance to the marker on each scale.

### *Bowdle visual analogue scales*

Psychedelic effects were monitored by an adapted version of the visual analogue scales (13 items, each 10 cm in length), originally described by Bowdle et al (Bowdle, Radant et al. 1998). Individual scales were aggregated to scores for 'feeling high', 'drowsy', 'internal perception' (reflecting inner feelings not

corresponding to reality) and 'external perception' (reflecting a misperception of an external stimulus or a change in the awareness of the subject's surroundings) (Zuurman, Roy et al. 2008).

#### *Statistical Analyses*

The pharmacodynamic parameters were analyzed by mixed model analyses of variance (using SAS PROC MIXED, SAS 9.1.3 for Windows, SAS Institute, Inc., Cary, NC) with treatment, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline was defined as the average of the available values obtained prior to dosing. Treatment effects are reported as the contrasts between the 4 treatments where the average of the measurements up to the last time point was calculated within the statistical model. Contrasts are reported along with 95% confidence intervals and analyses are two-sided with a significance level of 0.05.

## Results

### Pharmacokinetics

MDMA and MDA kinetics did not differ between MDMA alone and MDMA plus THC conditions. Mean MDMA maximal plasma concentrations (C<sub>max</sub>) were on average 213.3 µg/l (s.e.m.=7.9 µg/l) 105 minutes after drug administration and showed minimal decline during the sampling period (on average 168.3 µg/l (s.e.m.=5.4 µg/l) 300 minutes after drug administration). Mean MDA plasma concentrations on average rose to 12.0 µg/l (s.e.m.=0.5 µg/l) 300 minutes after drug administration.

Plasma THC concentrations and its two most important metabolites (11-OH-THC and 11-nor-9-carboxy-THC) did not differ between the THC alone and MDMA plus THC conditions (see Table 2). THC and 11-OH-THC consistently showed peak concentrations directly after administration and declined thereafter, whereas 11-nor-9-carboxy-THC concentrations inclined throughout the sampling period.

Condition	Dose	THC	11-THC	11-9-THC
THC	4 mg	59.7 (5.6)	2.8 (0.9)	8.4 (0.8)
MDMA+THC		53.8 (6.9)	2.9 (0.8)	9.2 (1.2)
THC	6 mg (1st)	84.5 (9.0)	3.7 (1.0)	16.0 (1.9)
MDMA+THC		84.6 (8.6)	4.7 (1.0)	18.5 (1.7)
THC	6 mg (2nd)	74.8 (6.9)	4.8 (1.2)	20.6 (1.5)
MDMA+THC		73.3 (7.1)	6.9 (1.3)	21.7 (2.6)

Table 2: Peak THC, 11-OH-THC (11-THC) and 11-nor-9-carboxy-THC (11-9-THC) plasma concentrations (in ng/ml; mean, (s.e.m.)). 1<sup>st</sup>= administered at 90 min. and 2<sup>nd</sup>= administered at 180 min after MDMA administration.

### Pharmacodynamics

Only significant results are mentioned in this section unless noted otherwise. Main effects of treatment, time and treatment by time as well as drug condition comparisons are summarized in Table 3. For the drug condition comparisons, reported are mean change, 95% confidence interval (95% CI) and corresponding p-values.

### *Body sway*

Body sway was increased, i.e. postural position was impaired, in all drug conditions compared to placebo. THC alone as well as co-administration of THC plus MDMA increased body sway compared to the MDMA alone condition.

### *Eye movements*

Although smooth pursuit eye movements (psychomotor accuracy) were not significantly impaired in any drug condition compared to placebo, MDMA and THC showed opposite effects on this measure: (co-)administration of MDMA increased smooth pursuit eye movements compared to the THC administration.

Psychomotor speed and sedation/arousal were assessed by saccadic eye movements (respectively peak saccadic velocity (PV) and reaction time). PV was increased in the MDMA condition as well as in the MDMA plus THC condition compared to the placebo and the THC condition. THC did not affect PV. Saccadic reaction time did not show a significant main effect of drug administration.

### *Rotor pursuit task*

The Rotor Pursuit task (see Figure 1) performance was significantly impaired in the THC condition and the MDMA plus THC condition compared to the placebo and MDMA condition. MDMA alone did not affect the Rotor Pursuit task.

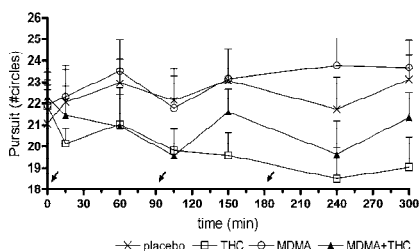


Figure 1. Rotor pursuit task scores per drug condition (mean, s.e.m.). Arrows indicate THC administration ( $t=0$  (4 mg),  $t=90$  (6 mg), and  $t=180$  (6 mg) minutes).

<i>Test</i>	<i>Treatment effect</i>	<i>Time Effect</i>	<i>Treatment by Time</i>	<i>THC vs. Plac</i>	<i>MDMA vs. Plac</i>	<i>MDMA+THC vs. Plac</i>	<i>MDMA+THC vs. MDMA</i>	<i>MDMA+THC vs. THC</i>	<i>MDMA vs. THC</i>
<b>Psychomotor</b>									
Body Sway (mm)	<.0001	0.0468	<.0001	70.7% (43.1%, 103.7%) <.0001	27.8% ( 7.7%, 51.6%) 0.0063	58.2% (32.3%, 89.0%) <.0001	23.8% ( 3.9%, 47.5%) 0.0185	-25.2% (-37.1%, -11.0%) 0.0017	
Smooth Pursuit (%)		0.0068	0.0475				3.3 ( 0.4, 6.3) 0.0274	3.3 ( 0.4, 6.2) 0.0262	
Peak Velocity (deg/s)	<.0001	0.0374	<.0001		51.3 (35.7, 66.9) <.0001	46.3 (30.6, 62.0) <.0001	36.6 (20.6, 52.5) <.0001	41.5 (25.6, 57.5) <.0001	
Reaction time (ms)									
Rotor pursuit (# of circles)	<.0001			-3.2 (-4.6, -1.8) <.0001		-2.6 (-4.0, -1.2) 0.0007	-2.5 (-3.9, -1.1) 0.0010	3.1 ( 1.7, 4.5) <.0001	
<b>Memory</b>									
Immediate recall (# of words)	F(3, 36.2)= 6.94, 0.0008			-2.17 (-3.4, -0.9) 0.0011	-1.41 (-2.6, -0.2) 0.0213	-2.48 (-3.7, -1.3) 0.0002			
Delayed recall (# of words)									
Delayed recognition (# of words)									
1-back task (s)	0.0073	0.0212		1.9 ( 0.6, 3.2) 0.0048		1.9 ( 0.7, 3.2) 0.0042	1.3 ( 0.0, 2.6) 0.0434	-1.3 (-2.5, -0.0) 0.0488	
2-back task (s)		0.0041							
3-back task (s)	0.0164					5.0 ( 2.0, 8.0) p=0.0018			

Table 3: Results: main effects, and drug comparisons (reported are mean difference, 95% confidence interval, and p-value). Plac= placebo, MDMA+THC= MDMA and THC co-administration (continued on next page).

<i>Test</i>	<i>Treatment effect</i>	<i>Time Effect</i>	<i>Treatment by Time</i>	<i>THC vs. Plac</i>	<i>MDMA vs. Plac</i>	<i>MDMA+THC vs. Plac</i>	<i>MDMA+THC vs. MDMA</i>	<i>MDMA+THC vs. THC</i>	<i>MDMA vs. THC</i>
<b>Subjective (cm)</b>									
Bond and Lader									
Alertness	<.0001	0.0016	0.0002	-2.86 (-3.75, -1.96) <.0001	-1.38 (-2.28, -.489) 0.0033	1.473 (0.571, 2.375) 0.0020	2.346 (1.453, 3.238) <.0001		
Contentedness	<.0001	0.0312		-1.09 (-1.54, -.65) <.0001		0.91 (0.45, 1.36) 0.0003	0.99 (0.55, 1.43) <.0001		
Calmness	<.0001	<.0001	<.0001		-1.78 (-2.75, -.82) 0.0006	-2.58 (-3.53, -1.63) <.0001	-2.02 (-2.98, -1.07) 0.0001	-1.23 (-2.19, -.027) 0.0133	
Bowdle									
Internal perception	0.0004	<.0001	0.0016	0.4 (0.2, 0.7) p=0.0037	0.4 (0.1, 0.7) p=0.0159	0.7 (0.4, 1.0) p=<.0001	0.3 (0.0, 0.6) p=0.0365		
External perception	<.0001	<.0001	<.0001	1.0 (0.6, 1.4) <.0001	0.8 (0.4, 1.2) 0.0002	1.6 (1.2, 2.0) <.0001	0.8 (0.4, 1.2) 0.0005	0.6 (0.2, 1.0) 0.0058	
Feeling high	<.0001	<.0001	<.0001	4.6 (3.4, 5.9) <.0001	1.7 (0.4, 2.9) 0.0002	4.8 (3.4, 6.1) <.0001	3.1 (1.8, 4.4) <.0001	-2.9 (-4.2, -1.7) <.0001	
Drowsy	0.0020			2.2 (0.9, 3.4) 0.0011		2.2 (1.0, 3.5) 0.0008			
Drug experience									
Drug liking	<.0001	0.0020	0.0127		2.4 (1.4, 3.3) <.0001	2.1 (1.1, 3.1) <.0001	1.7 (0.7, 2.7) 0.0016	1.9 (0.9, 2.9) 0.0003	
Drug strength	<.0001	<.0001	<.0001	2.0 (1.4, 2.6) <.0001	1.5 (0.9, 2.1) <.0001	2.4 (1.7, 3.0) <.0001	0.9 (0.3, 1.5) 0.0049		
Motivation	0.0038	0.0025	0.0263	-1.2 (-1.9, -.5) 0.0020			1.0 (0.2, 1.7) 0.0121	1.3 (0.6, 2.0) 0.0010	

Table 3: Results: main effects, and drug comparisons (reported are mean difference, 95% confidence interval, and p-value). Plac= placebo, MDMA+THC= MDMA and THC co-administration.

### 18 Word list

Immediate recall of words was impaired in all drug conditions compared to placebo. Delayed recall and delayed recognition did not show a significant main effect of drug administration.

### N-back task

Performance on the 1-back task was impaired in the THC condition and MDMA plus THC condition compared to the placebo and MDMA condition.

2-back performance did not show a significant main effect of drug condition, although drug condition comparisons revealed a trend for impairment of 2-back performance in the THC condition compared to the MDMA condition ( $p=0.053$ ).

Co-administration of MDMA plus THC impaired 3-back performance compared to placebo. THC administration showed a trend for impairment of 3-back performance compared to placebo ( $p=0.055$ ).

### Bond and Lader Mood Rating Scale

Subjective alertness was reduced in the THC condition compared to the placebo and the MDMA condition. Although co-administration of MDMA plus THC attenuated this reduction in alertness compared to the THC condition, subjective alertness was still reduced in the MDMA plus THC condition compared to placebo. Subjective contentedness was reduced in the THC condition compared to

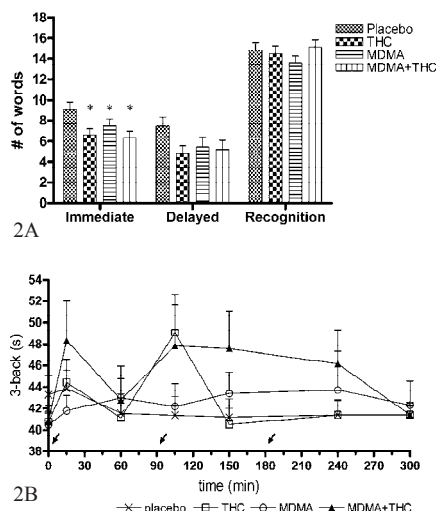


Figure 2. Memory effects. Figure 2A: 18 word list results per drug condition (mean, s.e.m.,  $*=p<0.05$ ). Arrows indicate THC administration ( $t=0$  (4 mg),  $t=90$  (6 mg), and  $t=180$  (6 mg) minutes). Immediate: average number of words immediately recalled over three consecutive immediate recall trials, Delayed: words recalled after a delay of 20 minutes, Recognition: number of words recognized among 18 distractor words. Figure 2B: Working memory: 3-back results per drug condition (mean, s.e.m.). Arrows indicate THC administration ( $t=0$  (4 mg),  $t=90$  (6 mg), and  $t=180$  (6 mg) minutes).

the placebo as well as the MDMA condition. Co-administration of MDMA plus THC abolished this effect: contentedness after co-administration did not differ compared to the placebo or the MDMA condition. Subjective calmness was reduced in the MDMA condition and the MDMA plus THC condition compared to the placebo and THC condition. THC did not affect calmness ratings.

### Drug liking and Drug strength scale

'Drug liking' ratings were increased in the MDMA condition and MDMA plus THC condition compared to the placebo and THC condition. 'Drug strength' ratings were increased after all drug conditions compared to placebo. Co-administration of THC plus MDMA further increased ratings of drug strength compared to the MDMA condition.

Motivation was decreased in the THC condition compared to the placebo, MDMA, and MDMA plus THC condition. In other words, co-administration of MDMA with THC reversed the THC induced reduction of motivation.

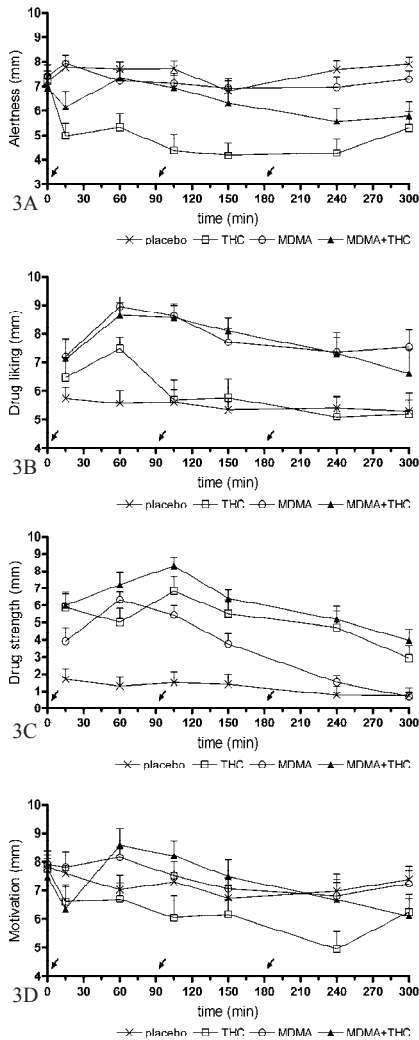


Figure 3. Subjective effects. Arrows indicate THC administration ( $t=0$  (4 mg),  $t=90$  (6 mg), and  $t=180$  (6 mg) minutes). Figure 3A: Subjective alertness (mean, s.e.m.). Figure 3B: Subjective drug liking (mean, s.e.m.). Figure 3C: Subjective drug strength (mean, s.e.m.). Figure 3D: Subjective motivation (mean, s.e.m.).

### *Bowdle scale*

All drug conditions increased ratings of internal and external perception compared to placebo. Co-administration of THC plus MDMA increased both internal and external perception compared to the placebo as well as MDMA condition, and external perception also increased compared to the THC condition. Ratings of 'feeling high' were increased in all drug conditions compared to placebo. 'Feeling high' ratings showed a more robust increase in the THC condition compared to the MDMA condition, and co-administration of THC plus MDMA further increased subjective 'feeling high' compared to the MDMA condition (but not compared to the THC condition). Feeling 'drowsy' scores were increased in the THC as well as the MDMA plus THC condition compared to placebo.

## ***Discussion***

This study assessed the cognitive and subjective effects of co-administration of MDMA and THC in humans, a frequent recreational drug combination. As MDMA is a psychostimulant, while THC generally impairs psychomotor function, psychomotor effects of these substances separately were expected to be attenuated after co-administration. However, results show that MDMA generally could not attenuate THC's impairment of psychomotor function.

Rotor pursuit performance was impaired by THC administration. This is in agreement with previous findings, where THC moderately impaired driving related performance (Weinstein, Brickner et al. 2008), and actual driving behavior (Ramaekers, Robbe et al. 2000). THC also robustly impaired postural stability, an effect that has been reported previously (Zuurman, Roy et al. 2008). MDMA had no effect on rotor pursuit performance, but it increased body sway, albeit to a lesser extent than THC. In a previous study with different treatment combinations, we did not find an overall effect of MDMA on postural stability (Dumont, Schoemaker et al. in press), but a *post hoc* direct comparison of drug conditions did show a significant postural effect of MDMA compared to placebo (unpublished data). Co-administration of MDMA and THC further impaired rotor pursuit performance and postural stability compared to MDMA, but not compared to THC, indicating that the detrimental effects of THC prevailed. Direct comparison of the MDMA condition with the THC condition also showed that the effect of THC on rotor pursuit performance and postural stability was more robust.

Although psychomotor performance was impaired by THC, THC did not affect eye movements, which confirms previous reports (Ploner, Tschirch et al. 2002; Zuurman, Roy et al. 2008), and is congruent with cannabinoid receptor distribution patterns: eye movements are primarily driven by brain stem areas, which show little CB<sub>1</sub> receptor expression (Zuurman, Roy et al. 2008). MDMA on the other hand increased saccadic peak velocity but not accuracy, which is also in line with a previous study (Dumont, Schoemaker et al. in press ). Effects of co-

administration of MDMA and THC were similar to those observed in the MDMA only condition .

The effects of THC and MDMA on memory were complex. Both THC and MDMA impaired word recall: immediate recall of words was significantly reduced in both single drug conditions. Delayed recall and recognition were unaffected by drug administration. Previous results regarding THC effects on memory generally are congruent with our results, where THC impaired immediate (Curran, Brignell et al. 2002; Hart, van et al. 2001; Heishman, Arasteh et al. 1997) but also delayed recall (Curran, Brignell et al. 2002) of a word list. MDMA's impairment of word list performance in the current study was comparable in size to the effects reported earlier. However, in a previous study the reduction of immediate recall failed to reach significance, whereas impaired delayed recall did (Dumont, Wezenberg et al. 2008). Co-administration of MDMA and THC did not exacerbate impairment of word list recall compared to either drug alone.

As previous (animal) research showed that co-administration of MDMA and THC induced a synergistic impairment of working memory (Young, McGregor et al. 2005), co-administration was expected to show additive impairment on tests of working memory compared to single drug effects. However, the effects of these substances on the N-back task, a test of working memory, were subtle and did not appear to be additive, although the complexity of THC induced impairment warrants further research regarding this topic. The effects of THC on the N-back working memory task were time- and dose dependent, where THC generally induced a robust but short-lived impairment of working memory. The 2-back condition did not show an effect of drug administration. THC impaired performance in the 1-back condition and showed a trend of impairment ( $p=0.055$ ) in the 3-back condition, congruent with previous reports where THC impaired N-back performance (Ilan, Smith et al. 2004), although Curran et al. (2002) found no effect of THC on working memory using the serial sevens task. The discrepancy of THC effects on 2- and 3-back performance versus the 1-back performance may be explained by the fact that the 1-back condition may assess psychomotor function rather than working memory as subjects only have to locate the dot that lit-up, i.e. performance will primarily be determined

by the time the subject needs to reach the target, rather than correctly memorizing which dot lit up  $n$  times before. In this sense, these results may reflect THC induced impairment of psychomotor function rather than working memory. A recent systematic literature review also showed complex effects of THC/cannabis on working memory, with possible indications for an inverse dose response relationship (Zuurman, Ippel et al. 2009).

N-back performance was unaffected in the MDMA condition. Co-administration of MDMA and THC impaired 1-back and 3-back performance. Although THC alone did not significantly impair 3-back performance, the observed trend suggests that the impairment of n-back performance after co-administration was driven primarily by THC, and co-administration of MDMA and THC did not, contrary to our hypothesis, exacerbate single drug induced memory impairment.

These results suggest that THC may exert much of its cognitive impairment via a common mechanism of reduced alertness. This is in line with its classification as a relaxant/sedative drug, and with reports that show that subjects are able to compensate for these impairments at the cost of greater effort (Curran, Brignell et al. 2002). The stimulant effects of MDMA may attenuate this effect, but could not overcome THC induced impairments in the current study. Subjective ratings show that the subjects were aware of these impairments: THC increased subjective ratings of feeling 'drowsy', and reduced ratings of 'motivation' and 'alertness'. Co-administration of MDMA reversed the THC induced reduction of subjective motivation, and attenuated the reduction of alertness by THC, although the latter was still significantly decreased after co-administration compared to placebo. The fact that subjects appeared aware of the THC induced cognitive impairment may be of significance when participating in traffic while intoxicated. Subjects who are aware of their reduced alertness are likely to adapt their behavior, thus reducing the risk of traffic accidents (Ronen, Gershon et al. 2008).

Subjective effects further suggest that the combination may be popular because it enhances the pleasurable subjective effects of each drug alone. Both THC and MDMA induced robust subjective drug effects and increased subjective ratings of 'feeling high', internal perception (reflecting inner feelings not corresponding to

reality) and external perception (reflecting a misperception of an external stimulus or a change in the awareness of the subject's surroundings), and both were comparable in 'drug strength'. MDMA increased subjective 'drug liking', whereas in the THC condition 'drug liking' ratings appeared inversely dose-related: 'drug liking' was robustly decreased after the high THC dose (6 mg) compared to the lower dose (4 mg). Congruent with drug liking ratings, subjective contentedness was dose-dependently reduced in the THC condition. This apparent inverse dose response relationship is in line with an overall assessment of the literature on the effects of cannabis/THC (Zuurman, Ippel et al. 2009). Co-administration of THC and MDMA enhanced subjective drug effects: ratings of 'drug strength', 'internal and external perception', and 'feeling high' were increased compared to the MDMA condition, whereas ratings of 'contentedness', 'external perception', and 'drug liking' were increased compared to the THC condition. The perceived increase of drug strength, combined with enhanced sensory drug effects, without an unacceptable decrease of cognitive function, offers a plausible incentive for combining cannabis with ecstasy in recreational settings.

Some limitations should be addressed. In the current study some effects of THC on memory failed to reach significance (although trends were observed). Likely, this may be related to the short lived effects of THC on memory. As can be seen in Figure 2, the effects of THC on memory were robust around 15 minutes but were diminished 60 minutes after drug administration, a pattern which could be observed after all three doses, although memory was assessed 60 minutes after the third dose only. This suggestion is congruent with previous studies showing that THC impaired N-back task performance 20 but not 60 minutes after THC administration (Ilan, Smith et al. 2004), and that THC induced impairment of immediate recall was the strongest in the period immediately after drug administration (Heishman, Arasteh et al. 1997). Future studies with more frequent test intervals relative to drug administration are recommended to elucidate the time profile and possible dose dependency of THC induced memory impairment. This also points to another limitation of our study. To maintain a stable effect level of THC during co-administration of MDMA, we assessed the effects of a single dose

of MDMA and three consecutive THC doses. Effects may differ depending on the dose assessed and the timing of drug administration, and our approach cannot be considered to be fully representative of all modes of combined drug use in practice. In general, the circumstances in which these substances are normally used cannot be fully recreated in the laboratory, although they may influence the effects of MDMA (Sumnall, Cole et al. 2006). However, the doses of each drug used in this study were similar to normal recreational use. In this sense, the current study sets a relevant benchmark for future evaluations of other dose combinations.

In conclusion, our study shows that co-administration of MDMA and THC did not exacerbate single drug induced cognitive impairment. Compared to MDMA (100 mg), THC (4, 6 and 6 mg) induced more robust impairment of cognitive function. Subjective effects show that subjects were aware of these impairments, and that the combination of THC with MDMA enhanced the perceived drug strength and desired drug effects compared to the MDMA condition. These results suggest that cannabis increases the desired effects of ecstasy without an unpredictable increase in cognitive impairment, which may explain the wide-spread recreational use of this combination.

# ***Cannabis co-administration potentiates ecstasy effects on heart rate and temperature in humans***

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### *Abstract*

The present study assessed the acute physiologic effects of (co-)administration of  $\Delta^9$ -THC (the main psychoactive compound of cannabis) and MDMA over time in 16 healthy volunteers. Pharmacokinetics, and cardiovascular, temperature, and catecholamine responses were assessed over time.

Both single drug conditions increased heart rate robustly, and co-administration showed additive effects. MDMA increased epinephrine and norepinephrine concentrations, THC did not affect the catecholamine response. Co-administration of MDMA and THC attenuated the increase of norepinephrine concentrations compared to the MDMA condition.

These results show that THC mediates its heart rate increase independent of sympathetic (catecholaminergic) activity and likely via direct CB1 agonism in cardiac tissue. Furthermore, THC co-administration did not prevent MDMA induced temperature increase, but delayed the onset and prolonged the duration of temperature elevation. These effects may be of particular relevance for the cardiovascular safety of ecstasy users in nightclubs with high ambient temperature and intensive dancing.

## ***Introduction***

3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”) is a frequently used club-drug in Western societies (Gross 2002;Parrott 2001). Next to its desired effects on mood and perception, ecstasy has powerful effects on human physiology. Moreover, ecstasy users generally are multi-drug users, and cannabis is commonly combined with MDMA (Parrott, Milani et al. 2007).

MDMA is a potent stimulant of cardiovascular action, increasing heart rate and blood pressure. MDMA also affects temperature regulation, generally increasing body temperature (Dumont and Verkes 2006;Freedman, Johanson et al. 2005;Green, Mechan et al. 2003). Although the relationship between body and brain temperature in humans is as yet unclear (Kiyatkin 2007), the pharmacology of MDMA induced temperature increase is of special interest as the prevention of hyperthermia has been shown to diminish or even prevent MDMA induced neurotoxicity (Malberg and Seiden 1998b;O'Shea, Easton et al. 2002a). Although MDMA induced temperature increase has received abundant attention in the literature, the mechanism is as yet unclear (Colado, O'Shea et al. 2004;Colado, Williams et al. 1995;Green, O'Shea et al. 2004;Mechan, Esteban et al. 2002;Saadat, O'Shea et al. 2005). In a previous report we suggested that the increase in both cardiovascular measures and temperature after MDMA administration is mediated by increases in both norepinephrine and epinephrine blood concentrations (Dumont, Kramers et al. 2009). These findings in humans corroborate previous findings in animals (Mills, Banks et al. 2003;Sprague, Banks et al. 2003;Sprague, Moze et al. 2005). Case reports of severe, sometimes fatal, physiologic disturbances after MDMA use, which are often facilitated by unfavorable behavior such as vigorous dancing and/or circumstances such as high ambient temperatures, illustrate the relevance of these side effects of MDMA use (Connolly and O'Callaghan 1999;Kalantar-Zadeh, Nguyen et al. 2006). However, the incidence of these adverse events after ecstasy use is low relative to the large population at risk ((Nutt 2006), but see also (Parrott 2007)).

A recent review showed that one of the most reliable markers of THC use is a concentration related increase of heart rate (Zuurman, Ippel et al. 2009). Although it is assumed that the cardiovascular effects of THC are mediated by sympathetic stimulation, studies suggest that THC may induce these effects (partly) via direct stimulation of peripheral CB<sub>1</sub> receptors (Sidney 2002). In spite of the increased heart rate, THC does not markedly affect blood pressure. This is most probably related to concomitant vasodilatory effects (Hall and Solowij 1998;Zuurman, Roy et al. 2008b). THC induced vasodilatation may, in theory, also lead to a decrease in body temperature, although most clinical studies did not report significant temperature effects of THC (Zuurman, Ippel et al. 2009).

Although studies into the effects of co-administration of MDMA and THC in humans are absent, several reports suggest that THC co-use may protect against MDMA induced temperature increase and resulting neurotoxicity (Fisk, Montgomery et al. 2006;Morley, Li et al. 2004;Parrott, Milani et al. 2007). Both substances are potent stimulators of heart rate, while MDMA increases blood pressure which is unaffected by THC. MDMA leads to these effects via sympathetic stimulation, but the mechanism of THC (increase of sympathetic function or direct peripheral CB<sub>1</sub> stimulation) is unclear. We hypothesize that 1) MDMA and THC co-administration may show additive effects on cardiovascular function as these substances may induce their effects through different mechanisms, and that 2) the vasodilatory effect of THC may attenuate MDMA induced vasoconstriction and resulting temperature increase when co-administered. To address these issues, this study assessed the effects of MDMA and THC co-administration on cardiovascular function, temperature, pharmacokinetics and plasma levels of norepinephrine and epinephrine levels over time.

## ***Materials and methods***

### *Study Design*

This study utilized a four-way, double blind, randomized, crossover, and placebo-controlled design and was conducted according to the principles of the Declaration of Helsinki. Each volunteer received a capsule containing either MDMA 100 mg or placebo, and inhaled three consecutive vapors containing 4, 6, and 6 mg of THC or placebo with dosing intervals of 90 minutes with washout periods of 7 days.

### *Study outline*

Subjects were admitted to each study day after the recording of possible signs and symptoms of health problems, and after a urinary drug check for opiates, cocaine, benzodiazepines, amphetamines, methamphetamines and tetrahydrocannabinol (AccuSign®, Princeton BioMeditech, Princeton, USA). Drug use was not allowed 14 days prior to the first study day until study completion. A light breakfast was offered two hours prior to drug administration. MDMA administration was scheduled at 10:30h and THC was administered at 0, 90, and 180 minutes after MDMA administration. Subjects received a standardized lunch at 14:00h and were sent home around 17:00h.

Outcome measures were assessed repeatedly, i.e. before MDMA administration and at 15, 60, 105, 150, 240 and 300 minutes post drug administration, and consisted of blood sampling (for analysis of study drug kinetics), cardiovascular function assessed by heart rate, systolic- and diastolic blood pressure measurements using a Datascope® Accutorr Plustm cardiovascular monitor, and tympanic temperature measurements using a Braun® type 6021 ThermoScan. Room temperature was kept at 22 degrees Celsius. Heart rate was also monitored continuously using a POLAR® Vantage NV watch, set to sample the average heart rate per five seconds and analysed using POLAR® Precision Performance 2.0 software. Blood samples for analysis of norepinephrine and epinephrine concentration were taken at baseline, and 50, 95, 140, and 195 minutes after drug

administration. To familiarize the subjects with the tests and procedures, they were invited to the hospital to perform a practice session within one week before the first study day.

### *Subjects*

Sixteen healthy volunteers (12 male, 4 female), regular users of ecstasy (lifetime exposure of  $143 \pm 212$  units (mean $\pm$ SD)) and THC (lifetime exposure of  $1716 \pm 1717$  units (mean $\pm$ SD)), 18 - 27 years of age ( $21.4 \pm 2.2$  mean $\pm$ SD) were recruited through advertisements on the internet and at local drug testing services. Detailed demographic data will be reported elsewhere. Exclusion criteria included pregnancy, (history of) psychiatric illness (assessed using the Structured Clinical Interview for DSM-IV Axis I disorders, non-patient version (First, Frances et al. 1994), Axis II disorders were excluded using the Temperament and Character Inventory (Svrakic, Whitehead et al. 1993), use of over-the-counter medication within 2 months prior to the study start, (history of) treatment for addiction problems, excessive smoking ( $>10$  cigarettes/day), unable to refrain from smoking during the study days, and orthostatic hypotension. Physical and mental health was determined by assessment of medical history, a physical- and ECG examination as well as by standard haematological and chemical blood examinations. The local Medical Ethics Committee approved the study. All subjects gave their written informed consent before participating in the study, and were paid for their participation.

One subject did not refrain from drug use, after which further study participation was denied. Two subjects were withdrawn at some point in the study because of side effects. Data of completed study days obtained prior to withdrawal were analysed as described.

### *Study drugs*

#### THC

THC was purified according to Good Manufacturing Practise (GMP)-compliant procedures (Farmalyse BV, Zaandam, The Netherlands) from the flowers of *Cannabis sativa* grown under Good Agricultural Practice (Bedrocan BV Medicinal Cannabis, Veendam, The Netherlands) (Choi, Hazekamp et al. 2004; Hazekamp, Choi et al. 2004). Each dose (4, 6 and 6 mg) of THC (>98% purity by HPLC/GC) was dissolved in 200 µl 100 vol% ethanol. THC was stored in the dark at -20°C in 1 ml amber glass vials containing a teflon screw-cap secured with Para film to minimize evaporation. The solvent was used as placebo.

On each study day, THC (4, 6 and 6 mg) or placebo were administered by inhalation using a Volcano® vaporizer (Storz-Bickel GmbH, Tuttlingen, Germany), a validated method of intrapulmonary THC administration (Abrams, Vizoso et al. 2007; Hazekamp, Ruhaak et al. 2006; Zuurman, Roy et al. 2008). Concurrent with MDMA administration, 4 mg THC was administered to ensure tolerability. Two subsequent doses of 6 mg of THC were administered 90 and 180 minutes after MDMA administration. Within five minutes before administration THC was vaporized at a temperature of about 225°C and the vapour was stored in a polythene bag equipped with a valved mouthpiece, preventing the loss of THC between inhalations. The transparent bag was covered with a black plastic bag to prevent unblinding. Personnel responsible for drug preparation was not involved in any other part of the study. Subjects were not allowed to speak, were instructed to inhale deeply and hold their breath for 10 seconds after each inhalation. Within 2-3 minutes the bag was to be fully emptied. The inhalation procedure was practiced at screening using the solvent only. The inhalation schedule was predicted to cause THC plasma concentrations and effects corresponding to the THC-contents in roughly one marijuana cigarette. The decision to proceed to the next highest THC dose was made by a physician, based on adverse events and physical signs.

## MDMA

MDMA (or matched placebo) was given as a capsule in a single oral dose of 100 mg. MDMA was obtained from Lipomed AG, Arlesheim, Switzerland and encapsulated according to GMP by the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre.

### *Pharmacokinetic measurements*

## THC

For determination of the concentration of plasma THC and its two most important metabolites (11-OH-THC and 11-nor-9-carboxy-THC), venous blood was collected in EDTA tubes (wrapped in aluminium foil) of 4.5 ml. Blood samples were taken 5 and 20 minutes after each THC administration and immediately put on ice and were processed (spun at 1500 g for 10 minutes at 4 °C ) within 30 minutes after collection. THC blood samples were handled sheltered from light. Plasma samples were stored at a temperature of -80°C for less than 3 months before laboratory analysis.

Determination of THC, 11-OH-THC and 11-nor-9-carboxy-THC content was performed using a validated high performance liquid chromatography with tandem mass spectrometric detection. Calibration range was 1.00 – 500 ng/ml for all compounds. Over this range the intra-assay coefficient of variation was between 4.0 and 6.5%. The inter-assay coefficient of variation was between 1.4 and 9.4%. Stability of THC levels in plasma was shown for at least six months.

## MDMA

A validated HPLC–diode array detection (HPLC-DAD) method was employed to measure MDMA and MDA plasma concentration, which has been described in detail previously (Dumont, Kramers et al. 2009).

### Norepinephrine and epinephrine

Plasma (nor)epinephrine concentration was measured by sensitive and specific HPLC with fluorometric detection as described previously (Willemsen, Ross et al. 1995). Blood samples were collected after the subject had remained in a sitting position for at least 15 minutes and were processed within 30 minutes after collection.

### *Statistical Analyses*

The pharmacodynamic parameters were analyzed by mixed model analyses of variance (using SAS PROC MIXED) with treatment, time and treatment by time as fixed effects, with subject, subject by time and subject by treatment as random effects, and with the baseline value as covariate, where baseline was defined as the average of the available values obtained prior to dosing. Treatment effects are reported as the contrasts between the four treatments where the average of the measurements up to the last time point was calculated within the statistical model. Contrasts are reported along with 95% confidence intervals and analyses are two-sided with a significance level of 0.05. Post-hoc evaluation of the specific treatment by time interaction of the MDMA and MDMA+THC treatments regarding temperature and epinephrine data was performed using the same mixed model analyses of variance.

Pharmacokinetic modelling was performed using nonlinear mixed effect modelling as implemented in the NONMEM software package (Version VI, NONMEM Project Group, University of California, San Francisco, CA). Previous assessment of THC pharmacokinetics indicated the requirement of a two-compartment model with a bolus administration in the central compartment (Strougo, Zuurman et al. 2008).

## Results

Only significant results are mentioned in this section unless noted otherwise. Main effects of treatment, time and treatment by time as well as drug condition comparisons are summarized in Table 1. For the drug condition comparisons (percentual) differences, 95% confidence interval (95% CI) and corresponding p-values are reported.

### Pharmacokinetics

MDMA and MDA kinetics did not differ significantly between MDMA single and MDMA and THC conditions. Mean MDMA maximal plasma concentrations ( $C_{max}$ ) were 213.3  $\mu\text{g/l}$  (s.e.m.=7.9  $\mu\text{g/l}$ ) 105 minutes after drug administration (see Figure 1A). Mean MDA plasma concentrations rose to 12.0  $\mu\text{g/l}$  (s.e.m.=0.5  $\mu\text{g/l}$ ) 300 minutes after drug administration.

Mean observed THC plasma concentrations as well as modelled THC concentrations are presented in Figure 1B. THC and 11-OH-THC consistently showed peak concentrations five minutes after administration and declined thereafter, whereas 11-nor-9-carboxy-THC concentrations inclined throughout the sampling period (data not shown).

Plasma THC and metabolite concentrations did not differ significantly between THC single and MDMA and THC conditions.

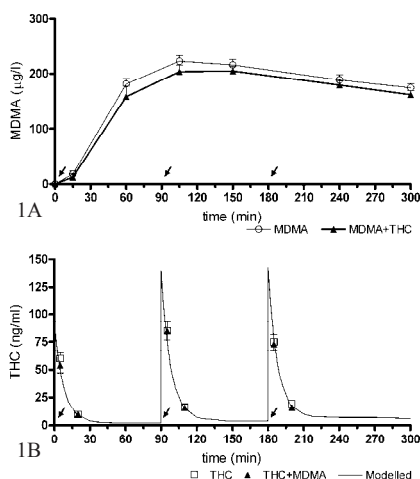


Figure 1. Pharmacokinetics. Arrows indicate THC administration ( $t=0$  (4 mg),  $t=90$  (6 mg), and  $t=180$  (6 mg) minutes). Figure 1A: MDMA kinetics per drug condition (mean, s.e.m.). Figure 1B: THC kinetics per drug condition (mean, s.e.m.). Shown are observed and modelled THC concentrations.

### Adverse events

Five subjects reported side effects (nausea, profuse sweating and paleness) associated with a vasovagal reaction that occurred exclusively in the THC alone condition with the 6 mg THC dose with an onset of 5-15 minutes after THC administration and a duration of 5-30 minutes. Furthermore, one subject reported feeling unwell without any overt physical signs, which resolved within 60 minutes both in the MDMA (onset 30 minutes post drug administration) and in the MDMA and THC condition (onset 60 minutes post drug administration). All of these subjects showed no particular signs during a short medical examination, and continued study participation after symptoms fully subsided. Two subjects also experienced an adverse event in the MDMA plus THC condition: one subject showed a short lasting (55 seconds) heart rate of >180 bpm and another subject reported mild hallucinations, the latter subsiding along with other drug effects. These two subjects were excluded from further study participation.

### Cardiovascular function

Heart rate was increased in all drug conditions compared to placebo (THC: 14.2 bpm, MDMA: 20.4 bpm, MDMA+THC: 29.9 bpm (mean increase over time),

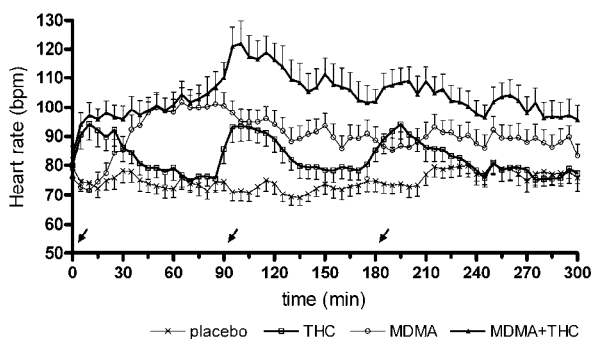


Figure 2. Heart rate averages per five minutes per drug condition, (mean, s.e.m.). Arrows indicate THC administration ( $t=0$  (4 mg),  $t=90$  (6 mg), and  $t=180$  (6 mg) minutes).

see Figure 2). Heart rate was also increased in the MDMA plus THC condition compared to the MDMA alone and the THC alone condition.

### Systolic

blood pressure and diastolic blood

pressure showed similar profiles: both were increased in the MDMA alone condition and the MDMA plus THC condition compared to the placebo (MDMA: 14.4 mm

Hg, and 11.9 mm Hg, MDMA+THC: 13.5 mm Hg, and 9.8 mm Hg respectively (mean increase over time)) and THC alone condition.

### Temperature

Temperature (see Figure 3) was significantly increased in the MDMA alone condition and the MDMA plus THC condition compared to the placebo (MDMA: 0.3°C, MDMA+THC: 0.2°C (mean increase over time)) and THC alone condition. Post-hoc analyses of the MDMA versus the MDMA+THC condition showed a significant treatment by time interaction ( $p < 0.0001$ ).

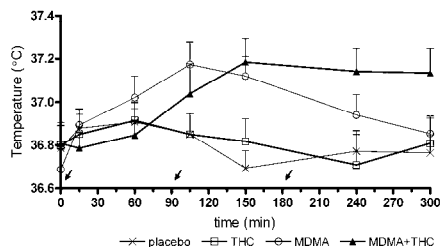


Figure 3. Temperature per drug condition (mean, s.e.m.). Arrows indicate THC administration ( $t=0$  (4 mg),  $t=90$  (6 mg), and  $t=180$  (6 mg) minutes).

### Norepinephrine and epinephrine concentrations

Norepinephrine levels, shown in Figure 4A, were increased after MDMA alone compared to all other drug conditions. Co-administration of THC with MDMA also increased norepinephrine levels compared to the placebo and THC alone condition, but decreased norepinephrine levels compared to MDMA alone. Relative to placebo, norepinephrine levels were unaffected by THC alone.

Epinephrine levels, shown in Figure 4B, were increased during MDMA alone compared to the placebo and the THC alone conditions. Average epinephrine levels in the MDMA plus

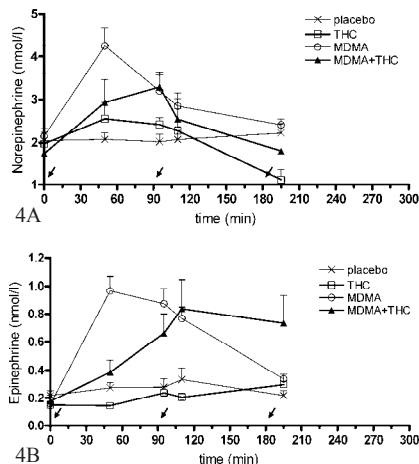


Figure 4. (Nor)Epinephrine plasma concentrations. Arrows indicate THC administration ( $t=0$  (4 mg),  $t=90$  (6 mg), and  $t=180$  (6 mg) minutes). Figure 4A: Norepinephrine plasma concentration per drug condition (mean, s.e.m.). Figure 4B: Epinephrine plasma concentration per drug condition (mean, s.e.m.).

THC condition were increased compared to THC alone, and did not differ significantly compared to placebo or MDMA alone, although compared to the latter, co-administration delayed the onset and prolonged the duration of the increased epinefrine levels. Post-hoc analyses of the latter effect (MDMA condition versus the MDMA+THC condition) showed a significant treatment by time interaction ( $p=0.0077$ ).

Measure	Treatment effect	Time Effect	Treatment by Time	THC vs. Plac	MDMA vs. Plac	MDMA + THC vs. Plac	MDMA + THC vs. MDMA	MDMA + THC vs. THC	MDMA vs. THC
(Nor)Epinephrine concentrations									
E (nmol/l)	0.0034	<.0001	0.0020		26.3 % (3.1, 54.7) p=0.0250			31.5 % (6.9, 61.8) p=0.0110	47.4 % (19.9, 81.1) p=0.0005
NE (nmol/l)	<.0001	0.0013	<.0001		256.2 % (148.8, 409.9) p=<.0001	140.4 % (66.7, 246.6) p=<.0001	-32.5 % (-53.1, -2.9) p=0.0350	148.8 % (71.8, 260.4) p=<.0001	268.8 % (156.2, 430.8) p=<.0001
Cardiovascular parameters									
SBP (mm Hg)	<.0001	<.0001	<.0001		14.4 (9.2, 19.7) p=<.0001	13.5 (8.2, 18.7) p=<.0001		12.3 (6.9, 17.6) p=<.0001	13.3 (8.0, 18.6) p=<.0001
DBP (mm Hg)	<.0001	<.0001	<.0001		11.9 (8.3, 15.4) p=<.0001	9.8 (6.2, 13.5) p=<.0001		7.9 (4.2, 11.5) p=0.0001	9.9 (6.3, 13.5) p=<.0001
HR (bpm)	<.0001	<.0001	<.0001	14.2 (7.5, 21.0) p=0.0001	20.4 (13.5, 27.3) p=<.0001	29.9 (23.2, 36.7) p=<.0001	9.6 (2.7, 16.4) p=0.0074	15.7 (8.9, 22.5) p=<.0001	
Temperature									
Temperature (°C)	0.0009	<.0001	<.0001		0.3 (0.1, 0.4) p=0.0005	0.2 (0.1, 0.3) p=0.0065		0.2 (0.0, 0.3) p=0.0211	0.2 (0.1, 0.4) p=0.0021

Table 1. Results: p-values for treatment, time and treatment by time effects. Comparisons of drug conditions show (percentual) change, 95% confidence intervals (between brackets) and p-values. E=epinephrine, NE=norepinephrine and Plac= placebo

## *Discussion*

The current placebo controlled, double-blind, and randomized trial in healthy volunteers clearly shows that the effects of THC and MDMA on heart rate are additive: both drugs alone induced an average peak increase in heart rate of approximately 30 bpm, and co-administration of the same dosages induced an average peak increase in heart rate of approximately 60 bpm. As expected, MDMA also increased blood pressure (mean increase over time SBP: 14.4 mm Hg, DBP: 11.9 mm Hg), body temperature (mean increase over time 0.3°C) and both epinephrine and norepinephrine plasma concentrations. THC single administration did not affect these measures. Co-administration of THC with MDMA did not affect blood pressure, and attenuated the increase of norepinephrine concentrations due to MDMA alone. The onset of increase in epinephrine concentrations was delayed and the duration of this elevation was prolonged compared to MDMA alone. As a result, the effects were truncated by the end of observation period, and the apparent elevation of epinephrine concentrations by co-administration of THC at T=180min (Figure 4B) was not enough for an average statistically significant increase. Congruent with these findings, THC co-administration modulated the temperature time profile compared to the MDMA alone condition: the onset of the temperature increase was delayed and the duration of temperature elevation was prolonged (Figure 3), although the mean temperature increase over time (0.2°C) was comparable to that observed in the MDMA condition. These findings confirm our hypothesis that MDMA and THC co-administration induces additive effects on heart rate. On the other hand, the hypothesis that THC co-administration may attenuate MDMA induced temperature increase by concomitant vasodilatation was not supported: THC co-administration delayed the onset of the temperature increase but prolonged the duration of temperature elevation.

Despite the prevalent combined use of cannabis and ecstasy, the acute physiologic effects of MDMA and THC co-administration in recreational users have not been investigated before. MDMA increased heart rate for several hours, and THC induced a robust but shortlasting increase in heart rate approximately 15

minutes after administration, findings that are in line with previous reports (Dumont and Verkes 2006; Sidney 2002; Strougo, Zuurman et al. 2008; Zuurman, Roy et al. 2008). The increase of both norepinephrine and epinephrine plasma concentrations after MDMA administration compared to placebo have been described previously (Dumont, Kramers et al. 2009). As norepinephrine is an important mediator of the cardiovascular response to MDMA, it is remarkable that heart rate showed a rapid additive increase after co-administration of THC and MDMA relative to single drug effects, while co-administration of THC attenuated norepinephrine elevation. Similarly, THC single administration did not affect norepinephrine concentrations but robustly increased heart rate. These data suggest that THC exerts a direct and potent stimulatory effect on cardiac CB<sub>1</sub> receptors (Bonz, Laser et al. 2003), instead of increasing heart rate via sympathetic stimulation. These results extend the findings of a recent publication where the time profile of THC induced heart rate effects also suggested a direct stimulatory effect of plasma THC on cardiac CB<sub>1</sub> receptors (Strougo, Zuurman et al. 2008). The involvement of CB<sub>1</sub> receptors is confirmed by another study that showed that the effects of THC on heart rate can be reversed by a selective CB<sub>1</sub> antagonist, which did not have any direct cardiovascular effects of its own (Zuurman, Roy et al. 2008a). Despite the increase in heart rate, THC did not induce an increase in blood pressure. This is in line with the suggestion that THC reduces vascular resistance via peripheral CB<sub>1</sub> receptors (Sidney 2002), which may compensate for the increased heart rate. Several subjects showed a vasovagal reaction to the high (6 mg) THC dose in the THC alone condition, although these effects generally subsided within several minutes. Vasodepressive reactions induced by cannabis (Ghuran and Nolan 2000) as well as other vasodilatory compounds (van Eijk, Pickkers et al. 2004) have been previously described in the literature. Subjects who experienced a vagal reaction in the current study indicated that they had experienced these effects before and more severely after recreational cannabis consumption.

MDMA induced a relatively small but significant increase in body temperature, a finding which confirms previous results (Brown and Kiyatkin 2004; Colado, Williams et al. 1995; Parrott, Rodgers et al. 2006; Williams, Dratcu et

al. 1998). THC alone did not affect temperature. Co-administration of THC with MDMA also increased temperature compared to placebo, although the onset of the temperature increase was delayed and the duration of this increase was longer compared to the MDMA condition. Congruent with earlier reports suggesting the involvement of epinephrine and norepinephrine in MDMA induced temperature elevation (Dumont, Kramers et al. 2009; Sprague, Brutter et al. 2004), the modulation of the temperature time profile corresponds with the modulation of the catecholamine profiles by THC co-administration compared to MDMA alone. After co-administration, norepinephrine and epinephrine concentrations appeared to be reduced at 45 minutes compared to MDMA alone. At later time points epinephrine concentrations were increased in the MDMA plus THC condition compared to MDMA alone. Since increased epinephrine concentrations induce cutaneous vasoconstriction effectively impairing heat dissipation, this is congruent with the delayed and prolonged duration of increased temperature that is seen after co-administration. Thus, although THC may cause a rapid decrease in vascular resistance, the duration of this effect may be too short to attenuate MDMA induced vasoconstriction and resulting impairment of heat dissipation, congruent with the time profile of THC effects on heart rate (Sidney 2002; Strougo, Zuurman et al. 2008).

These findings contradict earlier reports hypothesizing that THC co-administration may diminish MDMA induced temperature increase (Fisk, Montgomery et al. 2006; Parrott, Milani et al. 2007). Our current design only assessed temperature up to 300 minutes after drug administration. At this point in time, temperature had returned to placebo levels after MDMA alone, but in the MDMA plus THC condition temperature was still elevated compared to placebo. The prolongation of the MDMA induced temperature increase by THC co-administration may be of clinical relevance as the prevention of temperature increase has been shown to be an effective way of reducing or even preventing MDMA induced neurotoxicity in animal studies (Goni-Allo, Mathuna et al. 2007; Malberg and Seiden 1998; O'Shea, Easton et al. 2002). Although MDMA's neurotoxic potential in humans is still a matter of debate (Gouzoulis-Mayfrank and Daumann

2006), future studies should assess the full duration of this temperature increase considering that this may potentiate MDMA induced neurotoxicity.

In conclusion, MDMA and THC co-administration induced a potent and additive effect on heart rate, which may lead to significant harm in vulnerable individuals, especially in combination with intense physical exercise during dance parties (Parrott, Rodgers et al. 2006). Our results show that THC mediates its heart rate increase independent of sympathetic (catecholaminergic) activity and likely via direct CB<sub>1</sub> agonism in cardiac tissue. Furthermore, THC co-administration did not prevent MDMA induced temperature increase, but delayed the onset and prolonged the duration of temperature elevation. As the temperature rise was small, it remains to be established whether this THC-effect has an impact on MDMA's putative neurotoxicity. At any rate, recreational drug users that choose to expose themselves to these compounds should be aware that the combined use of THC and MDMA may have serious cardiovascular side-effects.

# ***Increased oxytocin concentrations and prosocial feelings in humans after ecstasy (3,4-methylenedioxy-methamphetamine) administration***

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### *Abstract*

MDMA (3,4-methylenedioxymethamphetamine or ‘ecstasy’) is a recreationally used drug with remarkable and characteristic prosocial effects. In spite of abundant attention in the scientific literature, the mechanism of its prosocial effects has not been elucidated in humans. Recently, research in animals has suggested that the neuropeptide oxytocin may induce these effects.

In a double blind, randomized, crossover, and placebo-controlled study in fifteen healthy volunteers we assessed blood oxytocin and MDMA concentrations and subjective prosocial effects after oral administration of 100 mg MDMA or placebo.

MDMA induced a robust increase of blood oxytocin concentrations and an increase of subjective prosocial feelings. Within subjects, the variations in these feelings were significantly and positively correlated with variation in oxytocin levels, and the correlations between these feelings and oxytocin were significantly stronger than those between these feelings and blood MDMA levels.

In conclusion, MDMA induces oxytocin release in humans, which may be involved in the characteristic prosocial effects of ecstasy.

## ***Introduction***

Ecstasy (3,4-methylenedioxymethamphetamine (MDMA)) is a street drug, which gained widespread use in the 'club' scene (Winstock, Griffiths et al. 2001). MDMA causes characteristic behavioral effects of increased empathy and friendliness (Vollenweider, Liechti et al. 2002). These unique prosocial effects led to MDMA being categorized as a separate drug class called 'entactogens' (Nichols and Oberlender 1990), as well as to (calls for) clinical trials investigating the potential for therapeutic use of MDMA in psychiatric disorders (Parrott 2007b; Sessa 2007; Sessa and Nutt 2007). Although appropriate social behavior is vital for human health and well-being, as exemplified by many disorders that feature impaired social functioning (such as social phobia, psychopathy and autism), the neurobiological mechanisms that mediate social behavior remain poorly understood.

A plausible mediator of MDMA's subjective effects is oxytocin, a neurohypophysial nonapeptide, which is synthesized in the supra-optic and the paraventricular nuclei of the hypothalamus (Gimpl and Fahrenholz 2001). Oxytocin has, next to its peripheral effects (i.e. induction of parturition and lactation), also received abundant attention for its role in social behavior. Previous research showed that oxytocin induces prosocial and affiliative behavior in animals as well as in humans (Baumgartner, Heinrichs et al. 2008; Campbell 2008; Domes, Heinrichs et al. 2007; Young 2002; Zak, Stanton et al. 2007). A recent study showed that MDMA induced oxytocin release in rats, an effect which was blocked by 5-HT<sub>1a</sub> antagonism. MDMA's prosocial effects were attenuated by co-administration of the oxytocin receptor antagonist tocinoic acid that had no effect on social behavior when given alone (Thompson, Callaghan et al. 2007). Other studies reported that high ambient temperature increased both the prosocial effects of MDMA, and Fos expression (a marker of gene activation) of oxytocinergic cells in rats, further suggesting a role for oxytocin in the prosocial effects of MDMA (Cornish, Shah Nawaz et al. 2003; Hargreaves, Hunt et al. 2007).

One study assessed whether MDMA induced oxytocin release in humans (Wolff, Tsapakis et al. 2006). The authors reported a trend for a small increase of

plasma oxytocin concentration in volunteers with positive urine drug screens for MDMA. The results are arguable however, because of the naturalistic design of this observational study, where subjects were assessed 'pre- and post clubbing', without actual control over drug intake or timing of blood sampling.

The aim of the present, randomized, placebo controlled, crossover study was to investigate whether MDMA induces oxytocin release in humans.

## ***Materials and methods***

### *Study Design*

This study utilized a double blind, randomized, crossover, and placebo-controlled design and was conducted according to the principles of the Declaration of Helsinki and approved by the local ethics committee. Each volunteer received a capsule containing either MDMA 100 mg or a matched placebo with a washout period of 7 days.

### *Study outline*

Subjects were admitted to each study day after a urinary drug check (opiates, cocaine, benzodiazepines, amphetamines, methamphetamines and delta-9-tetrahydrocannabinol; AccuSign<sup>®</sup>, Princeton BioMeditech, Princeton, USA; drug use was not allowed 14 days prior to the first study day until study completion) and the recording of possible signs and symptoms of health problems. A light breakfast was offered. MDMA administration was scheduled at 10:30h. Subjects received a standardized lunch at 14:00h and were sent home at 17:00h. Outcome measures were assessed repeatedly and consisted of blood sampling for MDMA and oxytocin concentration and assessments of subjective effects as specified below. Subjects also performed an extensive cognitive test battery that will be reported elsewhere. To familiarize the subjects with the tests and procedures, subjects performed a practice session within one week before the first study day.

### *Subjects*

Fifteen healthy volunteers (12 male, 3 female), regular users of ecstasy (lifetime drug exposure of  $110.5 \text{ doses} \pm 175.3 \text{ mean} \pm \text{SD}$ , range 10-702), 18-24 years of age ( $21.1 \pm 1.7 \text{ mean} \pm \text{SD}$ ) and a body weight of  $71.1 \text{ kg} \pm 8.5 \text{ mean} \pm \text{SD}$  (range 60-86) were recruited through advertisement on the internet and at local drug testing services. Physical and mental health was determined by assessment of medical history, a physical- and ECG examination as well as standard haematological- and chemical blood examination. Exclusion criteria included a

diagnosis of psychiatric illness (assessed using the Structured Clinical Interview for DSM-IV axis 1 disorders, non-patient version (First, Frances et al. 1994), Axis II disorders were excluded using the Temperament and Character Inventory (Svrakic, Whitehead et al. 1993) or substance dependence and pregnancy. The study was approved by the local Medical Ethics Committee. All subjects gave their written informed consent before participating in the study, and were paid for their participation. One subject did not refrain from drug use after the first studyday and further study participation was denied. The data obtained during this day (MDMA condition) were included in the data analysis. Two subjects experienced mild psychological discomfort (mild anxiety resolving within 60 minutes) after MDMA administration that resulted in partially missing data.

#### *Study drug*

MDMA (or matched placebo) was given as a capsule in a single oral dose of 100 mg. MDMA was obtained from Lipomed AG, Arlesheim, Switzerland and encapsulated according to Good Manufacturing Practice by the Department of Clinical Pharmacy of Radboud University Nijmegen Medical Centre.

#### *Blood sampling*

Blood samples were obtained using an indwelling catheter. Blood samples for analysis of oxytocin content were taken at baseline, i.e. before MDMA administration, and 5, 20, 95, 110, 185, 200, 240 and 300 min post drug administration. Blood samples were immediately put on ice and were processed (spun at 1500 g for 10 minutes at 4 °C ) within 30 minutes after collection. Blood samples for analysis of MDMA content were taken at baseline and at 15, 60, 105, 150, 240 and 300 minutes post drug administration. All plasma samples were stored frozen at –80 °C until the time of analysis.

#### *Analytical Methods*

MDMA plasma concentration was assessed by HPLC–diode array detection (HPLC-DAD) (Dumont, Schoemaker et al., in press).

Blood oxytocin analysis was performed in serum after prepurification of oxytocin by means of Sep-Pak C18 columns by an in-house radioimmunoassay (RIA) employing  $^{125}\text{I}$ -labelled oxytocin and an antibody raised in rabbits, with sheep anti-rabbit antibodies to separate bound and free radioactivity. The average recovery was  $78 \pm 6\%$ . Within- and between-assay CVs were 2.2 and 6.6% at 7.2 pmol/l. The analytical range was 1-90 pmol/l with a sensitivity of 1 pmol/l. All reagents were of analytical grade.

### *Subjective effects*

Subjective prosocial effects were assessed at baseline, and 15, 60, 105, 150, 240 and 300 minutes post drug administration using two items of the Bond and Lader (Visual Analogue) Mood Rating Scale (BLMRS) that specifically assess prosocial effects (antagonistic/amicable and withdrawn/gregarious) (Bond, James et al. 1974).

### *Statistical Analyses*

Statistical evaluation (two-sided alpha of 0.05) of drug effects on subjective measures (using SPSS 14 for Windows) was performed with a mixed model analysis of variance with drug and time as fixed factors and subject as random factor (with variance components structure). Given the limited number of subjects and the large differences in variation found at different timepoints it was not possible to formulate adequate mixed effect models for analysis of drug effects on oxytocin levels. Therefore the area under the curve (determined using the trapezoid rule:  $\Sigma_n = (Y_{(n)} + Y_{(n+1)})/2 * t$ . Y being oxytocin concentration per time point, and t the time in minutes per interval) was used to estimate the total amount of oxytocin and this was compared for the different conditions using a paired t-test. The relationship between subjective feelings and oxytocin or MDMA concentrations was analyzed using a summary-statistics approach. Correlations between each of the subjective parameters and oxytocin or MDMA levels (using individual time points) were determined for each subject. In order to perform the correlation analysis in an equal amounts of samples, subjective measures were correlated with all MDMA time

points, while the correlation with oxytocin was assessed using the time points closest to the MDMA sampling times. Next, using the Wilcoxon signed rank tests with exact p-values, we analysed whether these correlations were symmetrical around 0 (indicating no relationship between a subjective feeling and oxytocin or MDMA), and whether the correlations between each subjective parameter and oxytocin or MDMA were equally strong.

## Results

### MDMA kinetics

The mean maximum plasma MDMA concentrations ( $C_{max}$ ) were 222.7  $\mu\text{g/l}$  (s.e.m.=9.8  $\mu\text{g/l}$ ) 105

minutes after drug administration.

Plasma MDMA concentrations showed a minimal decline and were 174.6  $\mu\text{g/l}$  (s.e.m.=10.3  $\mu\text{g/l}$ ) on average at 300 minutes after drug administration (see

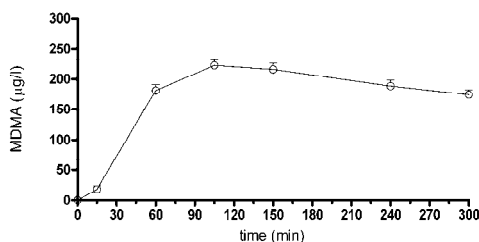


Figure 1. MDMA concentrations in time (mean, SEM).

Figure 1).

### Oxytocin kinetics

Plasma oxytocin concentrations (transformed to AUC data) were significantly increased in the MDMA condition compared to placebo ( $t(12) = 4.27$ ,  $MSE = 1125.78$ ,  $p = 0.001$ ). Mean plasma oxytocin concentrations increased from

0.8 pmol/l (s.e.m.= 0.3

pmol/l) at baseline to an

average maximum

concentration of 34.3

pmol/l (s.e.m.= 7.2

pmol/l) at 110 minutes

after drug

administration, and

declined thereafter to an

average of 4.0 pmol/l

(s.e.m. = 0.8 pmol/l) at 300 min after drug administration (Figure 2). No treatment

order effect was found.

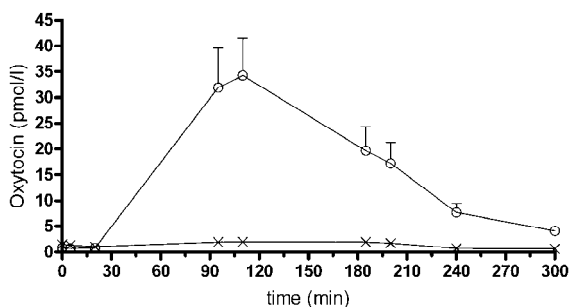


Figure 2. Oxytocin concentrations per condition in time (mean, SEM). Legend: x= placebo, o= MDMA.

### Subjective prosocial effects

Subjective amicability showed a significant treatment effect ( $F(1, 165) = 9.7, p = 0.002$ ). Subjective gregariousness showed a significant time effect ( $F(6, 162) = 2.6, p = 0.018$ ).

Both subjective amicability and subjective gregariousness showed a significant treatment by time interaction ( $F(6, 164) = 3.5, p = 0.003$ , and  $F(6, 162) = 4.0, p = 0.001$ , respectively, see Figure 3).

Both subjective amicability and subjective gregariousness showed a significant positive correlation with oxytocin concentrations (median correlation obtained over subjects = 0.37,  $p = 0.001$

and 0.29,  $p = 0.049$  respectively). Subjective amicability was also significantly correlated with MDMA concentrations (median correlation obtained over subjects = 0.23,  $p = 0.049$ ), but subjective gregariousness was not correlated with MDMA concentrations (median correlation obtained over subjects = 0.23,  $p = 0.46$ ). Further analysis using the Wilcoxon signed rank tests with exact p-values showed that both subjective amicability and subjective gregariousness were correlated significantly stronger with oxytocin than with MDMA ( $p = 0.013$  and  $p = 0.030$  respectively).

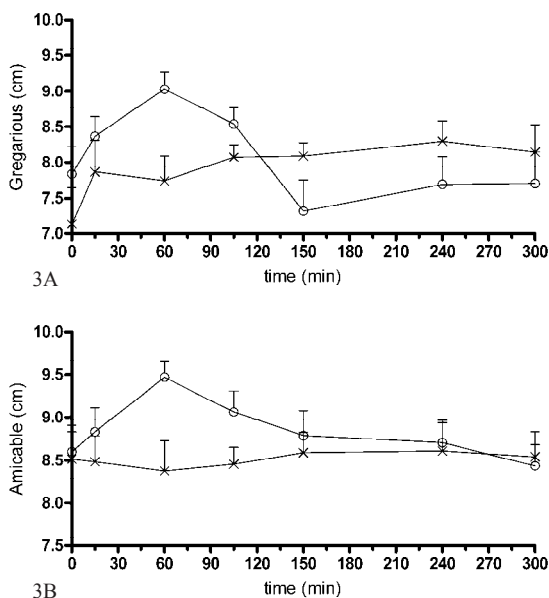


Figure 3. Subjective responses. Figure 3A: Subjective amicability per condition (mean, SEM). Figure 3B: Subjective gregariousness per condition (mean, SEM). Legend: x= placebo, o= MDMA.

## *Discussion*

We here show that MDMA robustly increased oxytocin concentrations as well as subjective prosocial effects, and that the increase in prosocial effects correlated stronger with blood oxytocin concentrations than with blood MDMA concentrations. These findings tentatively suggest that oxytocin may be involved in the characteristic prosocial effects of MDMA.

A previous study reported a non-significant increase of plasma oxytocin (0.41 pmol/l) in a clubbing population that had positive urine MDMA tests post clubbing (Wolff, Tsapakis et al. 2006). Our results show a much stronger effect of MDMA on plasma oxytocin concentration with an average increase of 34.3 pmol/l with peak levels of 90 pmol/l. The naturalistic basis of the previous study is a likely cause of this discrepancy: timelines between drug intake and blood sampling were not reported and it is likely that the robust increase of oxytocin concentrations were 'missed' due to this study design.

Animal research has previously shown a role for oxytocin in social cognition and affiliative behavior (Campbell 2008; Lim and Young 2006). Thompson et al. (2007) confirmed a role for oxytocin in MDMA's prosocial effects in an elegant study where they showed that MDMA administration increased social interaction as well as oxytocin plasma concentrations in male rats. MDMA's prosocial effects were attenuated by co-administration of the oxytocin receptor antagonist tocinoic acid, which had no effect on social behavior when given alone, thus confirming that oxytocin mediated MDMA induced prosocial behavior. MDMA induced oxytocin release was shown to be mediated by the 5-HT1A receptor, since oxytocin concentrations did not increase if administration of MDMA was preceded by administration of a 5-HT1A antagonist (Thompson, Callaghan et al. 2007).

A plausible mechanism of action for oxytocin mediated prosocial effects was reported in a study that showed that oxytocin attenuates the amygdala response to novel social encounters (Baumgartner, Heinrichs et al. 2008). In addition, a recent report demonstrated that attenuation of the amygdala inhibits excitatory flow from

the amygdala to brain stem sites mediating peripheral fear response (Huber, Veinante et al. 2005). For the case of MDMA, oxytocin may thus reduce anxiety related to social interaction, effectively promoting social behavior (Amaral, Bauman et al. 2003; Rosen and Donley 2006). Combined with its stimulating effects and mild enhancement of sensory input, it is not surprising that MDMA has become such a popular 'club-drug' (Dumont and Verkes 2006; Vollenweider, Liechti et al. 2002).

Although the results of animal research strongly support our conclusions, the findings of the present study should be considered explorative and some limitations should be addressed. Firstly, we measured oxytocin concentrations in blood, whereas cerebral spinal fluid oxytocin concentrations are expected to provide a more direct relation to the central effects. Indeed, a delay between maximal subjective effects ( $t=60\text{min}$ ) and measured peak plasma oxytocin concentration ( $t=110\text{min}$ ) was observed. Congruent with this finding, several reports have suggested that the release of oxytocin from the posterior pituitary gland into the peripheral circulation is preceded and driven by central, auto-stimulatory oxytocin release in the paraventricular nucleus and supra-optic nucleus (Amico, Tenicela et al. 1983; Armstrong 2007; Ludwig and Leng 2006). However, this remains speculative as the relationship between peripheral and central oxytocin release has not yet been defined (Landgraf and Neumann 2004).

Secondly, we assessed subjective prosocial effects. Future studies should employ objective measures of social interaction such as the Trust Game or Dictator Game (Sanfey 2007) to verify that subjects not only perceive themselves as being friendlier but in fact show increased social behavior.

Thirdly, to reduce the variance in observed oxytocin concentrations, future studies should also consider dosing MDMA according to body weight, rather than administering a fixed dose. Moreover, oxytocin concentrations should be assessed concurrently with MDMA and subjective assessments and between 20 and 95 minutes, where the current study did not assess oxytocin concentrations but did find the most pronounced subjective prosocial effects, to assess the onset of peripheral oxytocin levels elevation and its relation to prosocial effects.

Lastly, although our results suggest that oxytocin is involved in MDMA's prosocial effects in humans, these results remain tentative as the current design cannot determine whether oxytocin really mediated MDMA's prosocial effects. This should be verified in a MDMA interaction study using an oxytocin receptor antagonist such as Atosiban (Uvnas-Moberg, Bruzelius et al. 1993), although several issues regarding oxytocin receptor antagonism remain (Chini and Manning 2007).

In summary, we showed that MDMA, a drug with characteristic prosocial effects, robustly induces oxytocin release. The current results tentatively suggest that oxytocin may be involved in the characteristic prosocial effects of MDMA, congruent with previous reports of prosocial effects of oxytocin (Baumgartner, Heinrichs et al. 2008; Domes, Heinrichs et al. 2007; Guastella, Mitchell et al. 2008; Kirsch, Esslinger et al. 2005; Zak, Stanton et al. 2007), and may have implications for diseases that are characterised by impaired social functioning, such as social phobia, psychopathy and autism. Indeed several reports showed that there may be a link between these diseases and altered oxytocin function (Adolphs 2003; Guastella, Mitchell et al. 2008; Hammock and Young 2006; Lerer, Levi et al. 2008; McNamara, Borella et al. 2008; Talarovicova, Krskova et al. 2007). Although many issues and questions regarding oxytocin and its effects need to be addressed, this neuropeptide may provide a promising insight into the neurobiology of human social behavior.



## ***Summary and discussion***

## ***Summary of results***

### *Chapter 2. A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers*

All studies that reported acute effects of MDMA administered to humans were collected, and effects were summarized. Findings reflecting the subjective (the entactogenic profile), physiological (cardiovascular, pupil diameter) and endocrine effects (cortisol, prolactin) were the most prominent. MDMA effects on neuropsychological functioning were reported infrequently, thus rendering firm conclusions impossible and supporting our recommendation for more intensive research into the acute cognitive effects of MDMA. However, MDMA displayed all its prominent and desirable features at doses of 1.0 mg/kg and above, which is in line with the desirable doses reported by recreational users (Croft, Klugman et al. 2001; Soar, Parrott et al. 2004). The potentially hazardous adverse effects were also fully expressed at this level, illustrated by the dose response effect of MDMA on heart rate with a cut-off of 1.0 mg/kg.

### *Chapter 3. Acute neuropsychological effects of MDMA and ethanol (co-) administration in healthy volunteers*

This study assessed the peak cognitive effects of 100 mg orally administered MDMA and a three hours intravenous infusion of ethanol (resulting in a steady blood alcohol concentration of 0.6 promille), alone and in combination, in 16 healthy volunteers. Co-administration of MDMA and ethanol did not impair cognitive function significantly more than MDMA or ethanol administration alone. The most prominent effect of (co-)administration of MDMA and ethanol was an impairment of memory. Ethanol also impaired psychomotor function. Although the impairment of performance by each drug condition was relatively moderate, this significant impairment of cognitive function should be considered unacceptable in motorized traffic and other cognitively demanding situations as confirmed by previous research and as defined by law. However, the effects of these drugs in the

concentrations used in the present study on established neuropsychological tests appear to be smaller than one would assume based on their reputation.

*Chapter 4. Acute psychomotor effects of MDMA and ethanol (co-) administration over time in healthy volunteers*

This chapter reports psychomotor performance in relation to subjective performance after MDMA and ethanol (co-)administration over time, and shows that MDMA significantly increased psychomotor speed but not accuracy and induced significant subjective arousal, effects which were maximal around maximal MDMA blood concentrations (C<sub>max</sub>), and declined thereafter. Ethanol on the other hand impaired both psychomotor speed and accuracy, and induced sedation. Only the latter effect did not correspond with ethanol kinetics, sedation was only observed during the descending limb of the blood alcohol concentration, ie. after the infusion was stopped. Co-administration of MDMA with ethanol reversed ethanol induced sedation and improved psychomotor speed to above placebo levels, although psychomotor accuracy remained impaired. These findings may have implications for general performance when driving. Individuals will be more aroused when intoxicated with both substances, which may provide a false sense of better performance, although the accuracy of their performance is actually significantly impaired.

*Chapter 5. Ethanol co-administration moderates MDMA effects on human physiology*

In this chapter we report the physiologic effects of MDMA and ethanol (co-) administration over time. Co-administration of ethanol and MDMA did not exacerbate physiologic effects compared to other drug conditions, and moderated some effects of MDMA alone: Ethanol plus MDMA co-administration decreased fluid retention as well as temperature increase compared to MDMA alone. Although the effects observed in this study are considered to be subtle, they demonstrate that MDMA dysregulates physiological systems that are particularly important during

the typical circumstances in which MDMA is used, and that ethanol attenuates some of MDMA's deleterious effects on physiology.

*Chapter 6. Acute psychomotor, memory and subjective effects of MDMA and THC (co-) administration over time in healthy volunteers*

This report regarding the subjective and objective effects on cognitive performance shows that co-administration of MDMA and THC did not exacerbate single drug induced cognitive impairment. Compared to MDMA (100 mg per os), THC (inhalation of vapor containing 4, 6 and 6 mg, dosing interval of 90 minutes) induced more robust impairment of cognitive function, congruent with its classification as a sedative/relaxant. MDMA's stimulant properties could not overcome THC's reduction of performance when co-administered. Subjective effects show that subjects were aware of these impairments, and that the combination of THC with MDMA enhanced the perceived drug strength and desired drug effects compared with the MDMA alone condition. These results suggest that cannabis increases the sensory effects and perceived drug strength of ecstasy without an unpredictable increase in cognitive impairment, which may explain the wide-spread recreational use of this combination.

*Chapter 7. Cannabis co-administration potentiates ecstasy effects on heart rate and temperature in humans*

In this chapter we report the physiologic effects of MDMA and THC (co-) administration over time. MDMA and THC co-administration induced a potent and additive effect on heart rate, which may lead to significant harm in vulnerable individuals, especially in combination with intense physical exercise during dance parties. Our results further show that THC mediates its heart rate increase independent of sympathetic (catecholaminergic) activity and likely via direct CB<sub>1</sub> agonism in cardiac tissue. Furthermore, THC co-administration did not prevent MDMA induced temperature increase, but delayed the onset and prolonged the duration of temperature elevation. As the temperature rise was small, it remains to be established whether this THC-effect has an impact on MDMA's putative

neurotoxicity. At any rate, recreational drug users that choose to expose themselves to these compounds should be aware that the combined use of THC and MDMA may have serious cardiovascular side-effects.

*Chapter 8. Increased oxytocin concentrations and prosocial feelings in humans after ecstasy (3,4-methylenedioxymethamphetamine) administration*

In this chapter, we investigated the mechanism of action of MDMA's characteristic prosocial effects in healthy volunteers. We show that MDMA robustly induces oxytocin release, and that oxytocin concentrations correlated stronger with subjective prosocial effects than MDMA concentrations. Although tentative, the current results suggest that oxytocin may be involved in the characteristic prosocial effects of MDMA, congruent with previous reports of prosocial effects of oxytocin in humans (Baumgartner, Heinrichs et al. 2008; Domes, Heinrichs et al. 2007; Guastella, Mitchell et al. 2008; Kirsch, Esslinger et al. 2005; Zak, Stanton et al. 2007). These findings may have implications for diseases that are characterised by impaired social functioning, such as social phobia, psychopathy and autism, as several reports showed that there may be a link between these diseases and altered oxytocin function (Adolphs 2003; Guastella, Mitchell et al. 2008; Hammock and Young 2006; Lerer, Levi et al. 2008; McNamara, Borella et al. 2008; Talarovicova, Krskova et al. 2007).

***Limitations of the study design***

The following limitations of our study design should be taken into consideration:

Our findings relate only to the employed doses, i.e. a blood alcohol concentration (BAC) of 0.56 promille, 4, 6, and 6 mg of inhaled THC vapor and 100 mg of MDMA administered orally. Different doses may induce different effects, and research in animals such as that reported by Cassel et al. (2005) suggest that higher doses of ethanol for example may induce more dramatic interactions with MDMA.

At the same time, it is unfeasable to examine every possible combination of ethanol or THC and MDMA doses. Although our design is inevitably a model, we believe that it is appropriate for the estimation of the effects of recreational drug use by humans for the following reasons:

The ethanol clamp provides a continuous steady-state BAC of approximately 0.6 promille. Although this target BAC reflects the peak achieved after 2-3 alcoholic drinks, due to the continuous infusion of alcohol over three hours to maintain this BAC the total amount of alcohol administered represents a much larger intake of alcoholic drinks corresponding to almost one bottle of wine, a relevant dose of ethanol in relation to recreational drug use. As recreational alcohol users are expected to spread their alcohol use over the night, our design thus represents continuous moderate use of alcohol. A single high dose of oral alcohol (based on total drinks consumed during or around MDMA exposure) as typically employed in previous research regarding MDMA and ethanol interactions (Hernandez-Lopez, Farre et al. 2002; Kuypers, Samyn et al. 2006; Ramaekers and Kuypers 2006a) will show a rapid increase and a steady decline in BAC, resembling binge drinking. This will induce larger peak effects compared to the effects of our more steady infusion of alcohol. It is unknown whether binge drinking or moderate continuous drinking is more prevalent in MDMA-users, and our study represents only the latter pattern of alcohol use (Cassel, Hamida et al. 2008; Dumont, Verkes et al. 2008). A similar line of reasoning pertains to our THC administration method: we administered 4, 6, and 6 mg of THC, which resulted in peak THC blood concentrations that approximate THC levels observed after smoking roughly one joint, a normal dose used recreationally. There is a lack of evidence regarding the real world drinking and smoking behavior and resulting BAC and THC blood concentrations by recreational drug users combining MDMA and ethanol or THC, and further research regarding these uncertainties may elucidate these issues.

As mentioned, the THC doses employed resulted in THC effects achieved after the use of roughly one joint, thus resembling recreational cannabis use. However, a joint typically contains a mixture of tobacco and cannabis, and the addition of tobacco may influence THC kinetics and/or dynamics. However, as the

route of administration closely resembles that of smoking THC with tobacco, effects of different THC kinetics as seen after for example oral administration of THC are minimized. Moreover, cannabis is a mixture of many psychoactive compounds, although THC is the main psychoactive ingredient (Ameri 1999), and it is again unfeasible to test all combinations and dosages. The alternative, smoking a joint with a known THC content or a known cannabis content, presents the issue of the absence of or an unknown ratio of other psychoactive compounds and THC. Moreover, as cannabis users are reportedly excellent self-titrators, *ie.* adapt the amount of smoke inhaled and the duration the smoke is contained in the lungs to achieve the desired level of cannabis intoxication (and hence THC blood concentration), cigarettes will typically yield greater variation of THC levels (Hazekamp, Ruhaak et al. 2006). As the currently employed vaporizing method standardizes the amount of THC inhaled as well as the duration the vapor is kept in the lungs, variation in THC blood concentrations is greatly decreased.

MDMA was given orally as a single, fixed dose of 100 mg. While this is a dose that closely resembles the average content of MDMA in ecstasy pills, dosing according to body weight would reduce pharmacokinetic variance between individuals and is recommended for future studies (Parrott 2004). On the other hand, with the single fixed dose, we were able to assess the effect of bodyweight upon MDMA kinetics, and construct dose (100mg MDMA/body weight) response curves for several measures. The use of a single dose of MDMA itself is another abstraction: ecstasy is typically used repeatedly throughout the night, as its desired effects diminish after 2-3 hours after which more ecstasy is used to prolong its effects. Thus, several pills may be taken throughout the night, which was not reflected in our study design. Although the effects of MDMA wear out relatively quickly, MDMA plasma levels do not diminish at the same rate. The repeated administration of MDMA thus causes increasing MDMA plasma levels which likely increases the risk of physiologic adverse events and neurotoxicity, thus, for safety reasons we administered a single dose of MDMA.

To conclude, we are of the opinion that our methods and dosages used represent normal, non-excessive, recreational drug use that users would expect to be

safe, and interactions between these compounds at these dosages would hence be very relevant. No research model can capture all possible real-world behaviors of combined drug use and our design is only a crude estimation of real world drug use, which may involve higher and/or multiple doses that may affect cognitive and physiologic function differently. We welcome future research regarding these issues, although tolerability, safety and ethical issues may hamper such studies in humans.

Another limitation is the fact that these reportedly potent mind altering drugs display relatively minor cognitive deficits. This may be related to test sensitivity, and as shown in chapter 2, a review of the scientific literature regarding acute MDMA effects, cognitive testing shows great variation in test methods used. A selection of the most sensitive and appropriate tests for cognitive function may increase the effect sizes found as well as the comparability of different reports. An alternative explanation for the relatively modest drug effects found in the current studies may lie in the fact that the circumstances in which these substances are normally used cannot be fully recreated in the laboratory and this may have suppressed the effects of these substances. It is not unlikely that these substances show enhanced effects when tested under typical circumstances and surroundings. Recently, Parrott et al. (Parrott, Rodgers et al. 2006) concluded that the increase in physical activity and body temperature typically experienced when using MDMA, enhances MDMA effects, a finding which was corroborated by research in animals (Hargreaves, Hunt et al. 2007). Ball et al. (Ball, Budreau et al. 2006) also demonstrated that, compared to unfamiliar surroundings, a familiar surrounding increased MDMA induced locomotor response as well as single neuron activity in rats. Therefore, the psychosocial context in which MDMA is used, along with the different expectations and behaviour, probably influences the effects (Sumnall, Cole et al. 2006).

## ***Discussion***

*Pharmacology of the streets: facilitating drug (ab)use or preventing drug misuse?*

The effects of recreationally used doses of ecstasy, (combined with) cannabis or ethanol at first hand appear relatively mild, and with the exception of heart rate after (the combination of) MDMA and THC, clinically irrelevant. These results suggest that these substances can be used without acute threats to general health and well-being, and whenever robust effects do occur, such as is the case for cardiovascular stress, findings described in this thesis provide an evidence based rationale for treatment, although most often simply retreating to a relaxing, cool area (so-called chill-out rooms) will be sufficient. Thus, our findings provide a rationale for minimizing recreational drug harm and provide information regarding (pre)cautionary behavior such as not drinking excessive amounts of fluid, taking regular breaks from intensive exercise such as dancing and retreating regularly from hot environmental temperatures into rooms with low ambient temperatures. However, one may argue that this information may provide a basis that facilitates drug use, ie. encourages people to use these drugs. Although this argument is valid, it also takes away the ability of the individual that decides to expose his- or herself to such compounds to minimize its adverse effects by informing him or herself regarding these issues. Moreover, information on the adverse effects of these drugs may actually stop an individual from experimenting, ie. MDMA has long been regarded by users as a 'safe' drug, whereas recent research has clearly shown negative effects of MDMA of which users now can be made aware. Although this may not stop most users from taking this drug, they currently at least can take into account these risks and possibly take precautionary measures to avoid or diminish these adverse events. A similar line of reasoning applies to for example the research regarding cigarette smoking, which bares far greater long-term health risks than MDMA.

### *MDMA as a therapeutic agent?*

A second argument for performing these studies was the fact that these compounds provide powerful tools to examine the basic mechanisms of the central nervous system. MDMA is already under investigation as a therapeutic agent in post-traumatic stress disorder as well as as a palliative agent in terminal cancer patients (Sessa and Nutt 2007). However, and specifically for the case of MDMA's entactogenic effects, the neurobiologic mechanisms behind the characteristic and robust drug effects observed may provide new insights in the way the brain functions and is organized. In chapter 8 we suggest that oxytocin may be responsible for MDMA's pro-social effects, a finding which is supported by several studies that show that oxytocin induces robust pro-social effects in humans (Baumgartner, Heinrichs et al. 2008; Campbell 2008; Domes, Heinrichs et al. 2007). These findings provide a rationale for new therapeutic strategies for for example post-traumatic stress disorder, and other anxiety disorders, and possibly even disorders that feature social disfunction such as autism and psychopathy (Adolphs 2003). As oxytocin nose-spray (Syntocinon) is a registered therapeutic drug to induce labor, with virtually no serious side-effects reported, this option may prove highly valuable in these disorders without MDMA's other effects. Thus, while recreational drugs have side-effects and long-term consequences which limit the applicability of their use in therapeutic settings, studying the pharmacology of such compounds can provide crucial data to develop new therapies by isolating therapeutic effects from (desired and adverse) drug effects.

### *Effects of MDMA put in perspective: acute vs. long term and beyond*

Despite the relative lack of robust effects of MDMA on cognitive function, and manageable physiologic effects, one should not assume that MDMA is a safe drug. Although not explicitly part of this thesis, many studies suggest that MDMA may induce serotonergic neuronal damage via the generation of oxidative compounds (Hall and Henry 2006; O'Shea, Orio et al. 2006). Theories regarding the mechanism of action of MDMA's neurotoxicity are discussed in more detail elsewhere (Gouzoulis-Mayfrank and Daumann 2006a). Several studies in animals

show that MDMA metabolites are oxidative, which may damage neural structures, particularly axon terminals. Although these damaged axons regenerate, they are unable to restore themselves to their original appearance. This effect is called 'pruning': the axon is drastically shortened and shows extensive branching (Green, Mechan et al. 2003). This may induce different serotonergic innervation patterns, as shortened axons may innervate different brain areas. In humans, indications of serotonergic axonal damage after MDMA use have been found, most notably a reduction of SERT itself (McCann, Szabo et al. 2008). This effect appears reversible, and alternatively may be a negative feedback response to the overstimulation of SERT by MDMA itself, and thus does not unequivocally show neurotoxicity. In fact, long-term cognitive consequences of ecstasy use are a matter of debate with inconsistent or contradictory findings throughout the literature. Most studies do show a mild impairment of memory in ecstasy users (Verbaten 2003; Verkes, Gijssman et al. 2001), but as most ecstasy users are multidrug users (a notion supported by our current study population who all were multi-drug users) these effects may also be related to other drugs, most notably cannabis (Parrott, Gouzoulis-Meyfrank et al. 2004). Also, pre-morbid conditions may be a causative factor in the initiation of drug use rather than a consequence of drug use. These and other confounding factors are discussed in greater detail in an excellent review by Gouzoulis-Mayfrank et al (2006). On the other hand, the absence of notable impairments in young healthy ecstasy users does not indicate that ecstasy does not induce long-term cognitive deficits either. Much alike its acute induction of severe cardiovascular stress that may have long-term consequence that only become apparent in subjects with other risk factors or with increased age (Droogmans, Cosyns et al. 2007), ecstasy induced impairments may manifest itself as a premature onset of decreased cognitive function normally associated with advanced age. In other words, ecstasy may reduce the cognitive reserve of these young healthy individuals and impairments will only present themselves when this reserve is called upon, such as in the elderly or after brain trauma. However, and as mentioned, conclusive words regarding this matter have not been said, although the aging of the generation using ecstasy recreationally may elucidate this issue.

## ***Conclusion***

This thesis describes the interactions between MDMA plus ethanol and MDMA plus THC, two frequently used drug combinations, in humans. Results show that the combined use of these drugs generally does not exacerbate single drug effects on cognitive function. Physiologic (side-) effects of MDMA were attenuated by ethanol co-administration but potentiated by THC co-administration. MDMA's characteristic entactogenic effects were shown to be likely mediated by oxytocin, a neuropeptide released by MDMA, and this finding may provide interesting leads for future pharmacotherapy of social disorders such as anxiety, psychopathy and autism. However, the neurobiology of social behavior is as complex as social behavior is vital for human health and well-being, and future research should attempt to describe and elucidate the interactions between the many substrates that play a role in the neurobiology of social behavior, although such an attempt will be challenging.

It is important to note that considering the large number of people that expose themselves to these (and other, possibly even more harmful) combinations, only very few ecstasy induced adverse events are reported. However, the acute harmful effects of such combinations, particularly of MDMA and THC on cardiovascular function, should be communicated to the public as these present the most robust and acute dangers of using these drugs recreationally.

*Nederlandse introductie en  
samenvatting*

## *Ecstasy*

Ecstasy (XTC) is de straatnaam voor de stof 3,4-methylenedioxymethamphetamine (MDMA), een methamphetamine dat zich onderscheidt van andere (meth)amphetaminen doordat het niet alleen de stimulerende eigenschappen van amphetaminen (straatnaam 'speed') en methamphetaminen (straatnaam 'crystal meth') bezit, maar ook mild hallucinerende effecten geeft zoals bekend van bijvoorbeeld het veel potentere hallucinogeen lyserginezuurdiethylamine (LSD). De meest karakteristieke effecten van ecstasy zijn echter de gevoelens van openheid, genegenheid en vriendschap naar anderen toe, wat MDMA de bijnaam 'love-drug' bezorgde. Waarschijnlijk door de combinatie van deze eigenschappen is ecstasy erg populair in het uitgaanscircuit waar het zowel het uithoudingsvermogen, de zintuigen als de sociale interactie verhoogt. Alhoewel er vaak wordt gesproken over 'ecstasy gebruikers', is deze term enigszins misleidend: zelden gebruikt iemand alleen ecstasy en men kan dan ook beter spreken over recreatieve drugsgebruikers: naast ecstasy worden allerlei andere psychoactieve stoffen (drugs) gebruikt, zoals alcohol en cannabis (Hoofdstuk 3 en 6, dit proefschrift).

Momenteel kent Nederland ongeveer 40000 actuele gebruikers van ecstasy (Trimbos Instituut 2008). Ondanks deze grote blootstelling zijn er, in verhouding tot andere recreatieve drugs, weinig rapporten van ernstige intoxicaties met ecstasy bekend, alhoewel er sterfgevallen bekend zijn van individuen die gevoelig zijn voor complicaties van ecstasy gebruik (Hall and Henry 2006; Hartung, Schofield et al. 2002; Kalantar-Zadeh, Nguyen et al. 2006).

## *Neurobiochemische effecten*

Op farmacologisch nivo grijpt MDMA aan op de monoamine heropname pomp in de synaptische spleet, als mede op de opname pomp van de pre-synaptische neurotransmitter opslag blaasjes (Vmat-2). Hoewel aanvankelijk werd verondersteld dat MDMA de heropname pomp blokkeert, is recentelijk aangetoond dat MDMA neurotransmitters uitstoot via dit aangrijpingspunt. MDMA keert de richting waarin

deze pompen werken om: de neurotransmitter wordt zo niet terug naar het presynaptisch gedeelte van het neuron (of het opslag blaasje) gepompt maar vanuit het pre-synaptisch neuron naar de synaptische spleet. Via interferentie met Vmat-2 verhoogt MDMA dus ook de intracellulaire concentratie van de neurotransmitter in de pre-synaps. Dit resulteert in een verhoogde beschikbaarheid van de monoamine neurotransmitters in de synaptische spleet en uiteindelijk in een sterk verhoogde neurotransmissie (Mlinar and Corradetti 2003; Pifl, Drobny et al. 1995).

#### *Betrokken neurotransmitters en neuronale circuits*

De karakteristieke effecten van MDMA (verhoogde empathie en milde hallucinaties) zijn het gevolg van de interferentie met de serotonerge neurotransmissie. Daarnaast heeft MDMA ook een sterk dopaminerge en, in mindere mate, noradrenerge affiniteit welke verantwoordelijk zijn voor de stimulerende effecten (Green, Mechan et al. 2003; Liechti and Vollenweider 2001). MDMA verhoogt tevens de activiteit van het sympathisch zenuwstelsel, hetgeen leidt tot temperatuursstijging en verhoging van de hartslag en bloeddruk (Hoofdstuk 5 & 7, dit proefschrift).

#### *Kinetiek en dynamiek*

MDMA heeft een halfwaarde tijd van 6-8 uur en word gemetaboliseerd via CYP2D6 en CYP2B6, tot deels actieve maar vermoedelijk ook neurotoxische componenten (de la Torre, Farre et al. 2004). Polymorphismen in deze genen zorgen voor een veranderde MDMA kinetiek, hoewel de functionele relevantie hiervan onbekend is (mogelijk spelen ze een rol bij acute toxische reacties). De uiteindelijke bloed concentratie van een orale dosis MDMA is mede afhankelijk van het lichaamsgewicht, waarbij er een negatief, lineair verband lijkt te zijn (Hoofdstuk 5, dit proefschrift). Doordat MDMA tevens de enzymen die MDMA afbreken zwak remt kan er, bij herhaalde of hogere doseringen, mogelijk een onevenredige stijging van de MDMA bloed concentratie optreden (de la Torre, Farre et al. 2004).

De effecten van MDMA zijn kortdurend (2-4 uur) in vergelijking met de bloedconcentratie, waarschijnlijk door downregulatie van de heropname pomp en

uitputting van beschikbare serotonine (Hoofdstuk 4, dit proefschrift). Typisch recreationeel gebruik omvat dan ook het herhaald toedienen van MDMA (omstreeks 1 x per 2-3 uur) om het effect gedurende langere tijd te behouden. Hierbij dient opgemerkt te worden dat de lange halfwaardetijd van MDMA ervoor zorgt dat herhaalde toediening resulteert in een sterk verhoogde MDMA bloedconcentratie, hetgeen het risico op toxische effecten verhoogt.

#### *Acute effecten*

De acute effecten van MDMA zijn relatief mild van aard. Het cognitief functioneren is grotendeels intact en het gedrag van personen onder invloed van MDMA kenmerkt zich door een grote mate van vriendelijkheid en medewerking (Hoofdstuk 2, 3 en 8, dit proefschrift). Indicatoren van MDMA gebruik zijn vergrote pupillen, abnormale kaakspanning (tandenknarsen) en algemene onrust.

Het meest karakteristieke effect van MDMA is de verhoogde empathie: MDMA stelt de gebruiker in staat makkelijker contact te maken met - en zich beter in te leven in - anderen. De klinische relevantie van deze effecten wordt momenteel onderzocht (met name bij posttraumatische stress (Sessa 2007)). Het mechanisme achter deze effecten is weliswaar nog niet volledig opgehelderd, maar recent dieren onderzoek suggereert dat MDMA, via 5-HT1a receptoren, de neuronen van de parvo-ventriculaire (PVN) en supra optische kernen (SON) activeert. Deze neuronen stoten oxytocine en vasopressine uit. Deze neuropeptiden hebben sterke effecten op het sociaal gedrag (Baumgartner, Heinrichs et al. 2008; Domes, Heinrichs et al. 2007; Guastella, Mitchell et al. 2008). Oxytocine remt de angst respons van de amygdala op nieuwe omstandigheden, die op zijn beurt de basale hersenkernen aanstuurt die de perifere symptomen van angst bewerkstelligen. Door de werking van de amygdala te remmen, zal dus ook de angstreactie op onbekende, mogelijk bedreigende, sociale interacties gedempt worden (Baumgartner, Heinrichs et al. 2008; Huber, Veinante et al. 2005). Daarnaast sturen bovengenoemde neuronen ook projecties naar de hypofyse, die oxytocine en vasopressine uitstoot in het bloed. In Hoofdstuk 8 van dit proefschrift wordt aangetoond dat MDMA de bloedconcentratie

van oxytocine sterk verhoogd en dat dit samenhangt met de effecten van MDMA op het sociaal gedrag.

MDMA verhoogt op soortgelijke wijze de bloed concentratie van vasopressine (Anti-Diuretisch Hormoon, ADH), waardoor water retentie optreedt, een effect dat in combinatie met de al eerder genoemde stijging van hartslag en bloeddruk mogelijk tot cerebro- en cardiovasculaire accidenten kan leiden (Hoofdstuk 5, dit proefschrift).

De hallucinerende werking van MDMA is ook serotonerg gemedieerd en verloopt via 5-HT<sub>2</sub> receptoren (Liechti and Vollenweider 2001). Alhoewel andere hallucinerende middelen ook via deze receptoren werken, heeft MDMA in vergelijking met andere hallucinerende middelen een relatief zwak hallucinerend effect aangezien het weliswaar de beschikbare hoeveelheid serotonine sterk verhoogt, maar geen directe agonist is van serotonine receptoren, zoals de meeste andere hallucinogenen (Nichols and Oberlander 1990).

De stimulerende effecten van MDMA zijn, analoog aan die van amphetaminen, dopaminerg en noradrenerg gemedieerd. MDMA verhoogt dan ook het uithoudingsvermogen en de snelheid van bewegen. De nauwkeurigheid van bewegingen verandert echter nauwelijks, hetgeen kan leiden tot een overschatting van de eigen prestaties ten opzichte van het objectief functioneren (Hoofdstuk 4, dit proefschrift).

De symptomimetische effecten van MDMA werden al eerder genoemd en induceren, naast de genoemde potente verhoging van hartslag en bloeddruk, de meest bekende bijwerking van MDMA: temperatuur stijging (Mills, Banks et al. 2003). Alhoewel deze stijging onder laboratorium condities klinisch niet relevant is (een gemiddelde stijging van 0.4 graden Celsius bij 100mg MDMA, Hoofdstuk 5 en 7, dit proefschrift), kunnen individuen die gevoelig zijn voor deze effecten onder ongunstige omstandigheden, zoals vaak voorkomend in uitgaansgelegenheden (hoge omgevingstemperatuur, drukte en intensieve beweging in de vorm van dansen) een lichaamstemperatuur van meer dan 40 graden Celsius bereiken die kan resulteren in spierafbraak, nierfalen en de dood (Brown and Kiyatkin 2004). Hierbij dient echter te worden opgemerkt dat ondanks het grote aantal ecstasy gebruikers deze

bijwerkingen slechts zelden voorkomen. Daarnaast is aangetoond dat MDMA acuut de immuniteit verminderd, al is de klinische relevantie van dit gegeven onbekend (Pacifici, Zuccaro et al. 2001).

#### *Subacute effecten*

Subacuut is er sprake van de 'drie dagen dip' na ecstasy gebruik, waarbij de gebruiker een (kortdurende) toestand ervaart met kenmerken van depressie. Dit is mogelijk gerelateerd aan een verlaagd functioneren van het serotonine systeem. Hierbij dient echter te worden opgemerkt dat de verschillen in omstandigheden (normaal gesproken gebruikt men ecstasy in een uitgaanssituatie in het weekend en valt de drie dagen dip dus in de werkweek, waarbij de uitgaanssituatie meestal als veel plezieriger wordt ervaren, ook wanneer er geen sprake is van drugsgebruik) mogelijk ook (een gedeelte van deze) symptomen kunnen verklaren (Parrott and Lasky 1998; Sumnall, Cole et al. 2006).

#### *Lange termijn effecten*

Doordat het effect van MDMA kortdurend is en sterk afneemt met regelmatig gebruik, waarschijnlijk gerelateerd aan neuro-adaptieve processen zoals down regulatie, heeft deze stof slechts een zeer beperkt verslavend effect. De meeste verslavende stoffen veroorzaken bovendien een veel sterkere afgifte van dopamine dan ecstasy, een belangrijk kenmerk voor verslavingspotentie (Adinoff 2004).

In dierenonderzoek is aangetoond dat MDMA gebruik de axonen van serotonerge neuronen kan beschadigen, deze vertonen dan een 'pruning-effect': de normale lange axonen met weinig vertakkingen worden vervangen door sterk verkorte en vertakte uitlopers. In mensen is aangetoond dat na recent MDMA gebruik een down-regulatie van de serotonerge re-uptake pomp optreedt, mogelijk gerelateerd aan dit 'pruning-effect'. Dit laatste effect is reversibel (McCann, Szabo et al. 2008). De functionele relevantie van deze veranderingen is vooralsnog onbekend en levert nog steeds discussie op. Slechts bij hoge en/of chronische blootstelling uit zich een cognitief disfunctioneren (Gouzoulis-Mayfrank and

Daumann 2006a; Sprague and Nichols 2005) en cardiovasculaire afwijkingen (Droogmans, Cosyns et al. 2007).

### *Alcohol*

Alcoholische (ethanol bevattende) dranken worden vaak gebruikt in de westerse samenleving (meer dan 4 miljoen huidige gebruikers in Nederland). Ethanol is een sedatief middel, en remt de werking van de hersenen door de werking van de GABA<sub>A</sub> receptor te versterken middels allosterische modulatie (Suzdak, Schwartz et al. 1988). Aangezien deze receptor in vrijwel alle delen van het centraal zenuw stelsel voorkomt, heeft alcohol zeer diverse effecten die tevens ook afhankelijk zijn van de dosering. De meest bekende effecten van alcohol zijn een verminderd geheugen en verstoorde bewegingsfunctie (bijvoorbeeld wankel lopen en onduidelijk praten). De dempende effecten kunnen het algemeen functioneren drastisch beperken en in extreme gevallen ernstige bijwerkingen veroorzaken zoals ademdepressie. Omdat alcohol zo vaak gebruik wordt, komen ernstige bijwerkingen vaak voor. Er zijn in 2006 dan ook 1742 fatale accidenten met als hoofdoorzaak alcohol intoxicatie gemeld (Trimbos Instituut 2008). Naast de sedatieve effecten heeft ethanol relatief geringe effecten op het cardiovasculair systeem: ethanol veroorzaakt vaatverwijding en verhoogt de hartslag. De vaatverwijding in de huid kan leiden tot een daling van de lichaamstemperatuur en kan onder ongunstige omstandigheden leiden tot onderkoeling (Pohorecky and Brick 1988). De kinetiek van alcohol verschilt sterk tussen personen onderling en hangt ondermeer af van ras, geslacht, gewicht en gebruiksfrequentie. Gemiddeld zullen twee tot drie alcoholische dranken leiden tot een bloed concentratie van 0,6 promille, een concentratie waarbij men wettelijk gezien geen voertuigen meer mag besturen.

## *Cannabis*

THC (tetrahydrocannabinol), het meest actieve bestanddeel van cannabis, is een sederend/relaxerend middel met een zwak hallucinogene werking. In hogere concentraties kan THC angst opwekken (Block, Erwin et al. 1998). THC vermindert het geheugen en vertraagt de bewegingssnelheid. Daarnaast verhoogt THC de hartslag kortdurend maar zeer robuust en vermindert het de perifere vaatweerstand (Sidney 2002). THC is niet toxisch aangezien in de hersenstam, waar de vitale functies worden aangestuurd, vrijwel geen receptoren voor THC aanwezig zijn. THC is een agonist voor de CB<sub>1</sub> en CB<sub>2</sub> receptoren van het endocannabinoïd systeem (ECS). CB<sub>1</sub> receptoren komen voornamelijk voor in het centraal zenuwstelsel, terwijl CB<sub>2</sub> receptoren zich voornamelijk buiten het centraal zenuwstelsel bevinden (Ameri 1999). Het ECS wijkt af van klassieke neurotransmitter systemen aangezien de receptoren zich meestal pre-synaptisch bevinden terwijl de neurotransmitter zelf (bijvoorbeeld anandamide, een endogeen analoog van THC) post-synaptisch geproduceerd wordt. Het ECS bewerkstelligt op deze manier onder andere negatieve feedback van de neurotransmissie in de synaps. De endocannabinoïd productie wordt voornamelijk gestart na prikkeling van het post-synaptisch membraan door reguliere synaptische neurotransmissie, waarbij het gevormde endocannabinoïd teruggediffundeert naar de pre-synaptisch gelocaliseerde receptor. Prikkeling van deze endocannabinoïd receptor remt vervolgens de afgifte van neurotransmitter.

THC is een lipofiel molecuul en wordt dan ook snel vanuit het bloed opgenomen in vet weefsel (waaronder het centraal zenuwstelsel). Slechts enkele minuten na inhalatie bereikt de THC bloed concentratie maximale waarden waarna deze zeer snel daalt. De cognitieve en subjectieve effecten zijn maximaal rond 15 tot 30 minuten na inhalatie en duren enkele uren (Curran, Brignell et al. 2002; Strougo, Zuurman et al. 2008).

### ***Dit proefschrift***

Zoals al eerder genoemd zijn ecstasy gebruikers vaak recreatieve drugs gebruikers die allerlei psychoactieve middelen gebruiken, waaronder ecstasy, alcohol en cannabis. In de praktijk worden deze drugs ook gecombineerd gebruikt om gewenste effecten te versterken en/of ongewenste effecten af te zwakken. Dit proefschrift beschrijft de effecten van ecstasy in combinatie met alcohol of cannabis, de meest gebruikte drugscombinaties met ecstasy. Om deze effecten in kaart te brengen hebben wij twee studies, waarin telkens zestien gezonde vrijwilligers getest werden, uitgevoerd met een dubbel blind, placebo gecontroleerd en gerandomiseerd crossover design. Studie 1 betrof de interactie tussen MDMA en alcohol. MDMA werd in capsule vorm toegediend in een dosering van 100 mg, wat bij benadering overeenkomt met de gemiddeld gebruikte dosering bij recreatief ecstasy gebruik (Tanner-Smith 2006). Aangezien oraal toegediende alcohol een grote kinetische variatie kent, is de alcohol intraveneus toegediend, waarbij de infusiesnelheden dusdanig werden aangepast dat gedurende drie uur een stabiele bloed alcohol concentratie (BAC) van 0,6 promille (vergelijkbaar met de BAC na 2-3 alcoholische consumpties) bereikt werd (Zoethout, van Gerven et al. 2008). De resultaten van studie 1 worden besproken in de Hoofdstukken 3 tot en met 5. Studie 2 betrof de interactie tussen MDMA (wederom 100 mg oraal) en THC (4, 6 en 6 mg, waarbij vergelijkbare effecten worden bereikt als na het roken van een joint). THC werd toegediend middels een verdamper, waarbij de THC, opgelost in ethanol, op een gecontroleerde manier werd geïnhaleerd door de proefpersoon (Zuurman, Roy et al. 2008). De resultaten van studie 2 worden beschreven in de Hoofdstukken 6 en 7. Deze studie leverde tevens het mogelijk mechanisme achter MDMA's karakteristieke pro-sociale effecten op, deze resultaten worden in Hoofdstuk 8 besproken.

## ***Samenvatting van de Hoofdstukken***

*Hoofdstuk 2 A review of acute effects of 3,4 - methylenedioxymethamphetamine in healthy volunteers* beschrijft de kennis omtrent de acute effecten van MDMA in gezonde vrijwilligers. Alle literatuur binnen dit thema is systematisch gecategoriseerd en samengevat. Hieruit blijkt dat er een grote verscheidenheid aan cognitieve testen is gebruikt waardoor er geen definitieve uitspraken over specifieke cognitieve effecten van MDMA gedaan kunnen worden. Deze studies rapporteerden in het algemeen milde cognitieve beperkingen, sterke subjectieve effecten en een (dosisgerelateerde) verhoging van de hartslag.

*Hoofdstuk 3 Acute neuropsychological effects of MDMA and ethanol (co-) administration in healthy volunteers* beschrijft de piek effecten van (de interactie tussen) MDMA en alcohol. De resultaten tonen aan dat de combinatie van 100 mg MDMA met een bloed alcohol concentratie van 0,6 promille geen versterking van de effecten van deze stoffen apart geeft. Tevens toont deze studie aan dat de cognitieve beperkingen van een gemiddelde dosis ecstasy vergelijkbaar zijn met de beperkingen na twee tot drie glazen alcohol.

*Hoofdstuk 4 Acute psychomotor effects of MDMA and ethanol (co-) administration over time in healthy volunteers* beschrijft de effecten van bovenstaande combinatie op het psychomotore functioneren over de tijd. Uit de resultaten blijkt dat MDMA de snelheid, maar niet de nauwkeurigheid, van het psychomotore functioneren verbetert, terwijl alcohol beide aspecten verslechtert. De combinatie van deze stoffen liet additieve effecten zien. Alcohol verminderde nog steeds de nauwkeurigheid van bewegingen, maar het stimulerende effect van MDMA nam de sedatie door alcohol weg. Dit kan leiden tot overmoedig gedrag van mensen die onder invloed zijn van deze combinatie, aangezien zij geen beperking in hun bewegingssnelheid ervaren, terwijl zij onverminderd beperkt zijn in de nauwkeurigheid van diezelfde bewegingen. Aangezien een substantieel percentage van deelnemers aan feesten onder invloed van deze combinatie naar huis rijdt, kan dit tot gevaarlijke situaties leiden.

*Hoofdstuk 5 Ethanol co-administration moderates MDMA effects on human physiology* beschrijft de fysiologische effecten van MDMA in combinatie met alcohol. De resultaten tonen aan dat MDMA water retentie en verhoging van de temperatuur en de hartslag induceert. Alcohol, dat zelf nauwelijks effecten liet zien op deze maten, verminderde deze effecten van MDMA, met uitzondering van de hartslagverhoging. Gelijktijdig gebruik van een lage dosis (2-3 glazen) alcohol en MDMA kan dus de bijwerkingen van ecstasy beperken.

*Hoofdstuk 6 Acute psychomotor, memory and subjective effects of MDMA and THC (co-) administration over time in healthy volunteers* beschrijft de cognitieve effecten van (de combinatie van) MDMA en THC, het actieve bestanddeel van cannabis. Deze studie toont aan dat THC het cognitief functioneren sterker beperkt dan MDMA. De combinatie van MDMA en THC verergerde deze effecten echter niet. De combinatie van cannabis met ecstasy versterkte tevens de subjectieve gewenste effecten van ecstasy, wat de populariteit van deze drug combinatie kan verklaren.

*Hoofdstuk 7 Cannabis co-administration potentiates ecstasy effects on heart rate and temperature in humans* beschrijft de effecten van MDMA en THC op de fysiologie en laat zien dat sommige schadelijke effecten van MDMA, nl. temperatuurstijging en een verhoogde hartslag, versterkt worden door toevoeging van THC. De combinatie van deze stoffen kan leiden tot een gevaarlijke stijging van de hartslag, wat in combinatie met de ongunstige omstandigheden (intensieve lichaamsbeweging), mogelijk tot acute en/of langdurige gezondheidsproblemen kan leiden.

*Hoofdstuk 8 Increased oxytocin concentrations and prosocial feelings in humans after ecstasy (3,4-methylenedioxymethamphetamine) administration* beschrijft een plausibel mechanisme van de typische pro-sociale effecten van MDMA. Deze studie laat zien dat MDMA de bloedconcentratie van oxytocine sterk verhoogt, en dat deze stijging samenhangt met de stijging in gevoelens van vriendelijkheid en gezelligheid. In andere studies is aangetoond dat exogeen toegediend oxytocine mensen 'socialer' maakt: ze kunnen zich beter inleven in andermans gevoelens en zijn eerder geneigd tot vriendelijk gedrag. Verder

onderzoek naar de sterke toename van de endogene oxytocine afgifte door MDMA kan bijdragen aan de ontwikkeling van nieuwe therapieën bij ziektebeelden met afwijkingen in het sociale gedrag, zoals autisme of angststoornissen.

### ***Conclusie***

De doseringen van MDMA, alcohol en cannabis in deze studies zijn vergelijkbaar met gemiddeld doseringen tijdens recreationeel gebruik. Elke stof afzonderlijk had daarbij verschillende maar beperkte effecten op het cognitieve functioneren. De combinatie van MDMA met alcohol of cannabis had weinig extra invloed op het cognitief functioneren ten opzichte van deze drugs alleen. De combinatie met alcohol verminderde de mate waarin de proefpersonen zich van deze beperkingen bewust waren, terwijl cannabis de gewenste subjectieve effecten van MDMA versterkte. De effecten van de twee verschillende combinaties op de fysiologie waren deels tegengesteld: de combinatie van alcohol met MDMA verminderde de (potentieel gevaarlijke) bijwerkingen van MDMA terwijl de combinatie van THC met MDMA de bijwerkingen soms aanzienlijk versterkte. Daarnaast heeft dit onderzoek aangetoond dat MDMA de bloedconcentratie van oxytocine verhoogt, wat samenging met de entactogene (verhoogd sociaal gedrag) effecten van MDMA. Deze bevindingen dienen gebruikt te worden in de voorlichting van gebruikers van deze combinaties, alsmede bij de rationele aanpak van intoxicaties met deze combinaties. Tevens kan het verder bestuderen van het mechanisme van de unieke sociale effecten van MDMA leiden tot nieuwe inzichten en farmacotherapieën voor psychiatrische stoornissen, zoals bijvoorbeeld angststoornissen, post-traumatische stress stoornis en autisme.

## *References*

- Abrams DI, Vizoso HP, Shade SB, Jay C, Kelly ME, Benowitz NL (2007) Vaporization as a smokeless cannabis delivery system: a pilot study. *Clin. Pharmacol. Ther.* 82: 572-578
- Adinoff B (2004) Neurobiologic processes in drug reward and addiction. *Harv. Rev. Psychiatry* 12: 305-320
- Adolphs R (2003) Cognitive neuroscience of human social behaviour. *Nat. Rev. Neurosci.* 4: 165-178
- Amaral DG, Bauman MD, Capitanio JP, Lavenex P, Mason WA, Mauldin-Jourdain ML, Mendoza SP (2003) The amygdala: is it an essential component of the neural network for social cognition? *Neuropsychologia* 41: 517-522
- Amatsaleh AA, Dumont GJ, Schoemaker RC, van Gerven JM (2006) Optimisation of a novel method for alcohol infusion by clamping of breath alcohol concentration. *Br. J. Clin. Pharm.* 61: 475
- Amatsaleh AA, Schoemaker RC, van Gerven JM (2006) Investigation of the effects of ethanol on central nervous system parameters in healthy volunteers. *Br. J. Clin. Pharm.* 61: 626
- Ameri A (1999) The effects of cannabinoids on the brain. *Prog. Neurobiol.* 58: 315-348
- Amico JA, Tenicela R, Johnston J, Robinson AG (1983) A time-dependent peak of oxytocin exists in cerebrospinal fluid but not in plasma of humans. *J. Clin. Endocrinol. Metab.* 57: 947-951
- Ammons RB (1951) Effect of Distribution of Practice on Rotary Pursuit Hits. *J Exp Psychol* 41: 17-22
- Armstrong WE (2007) The neurophysiology of neurosecretory cells. *J. Physiol.* 585: 645-647
- Baker EL, Letz R (1986) Neurobehavioral testing in monitoring hazardous workplace exposures. *J. Occup. Med.* 28: 987-990
- Ball KT, Budreau D, Rebec GV (2006) Context-dependent behavioural and neuronal sensitization in striatum to MDMA (ecstasy) administration in rats. *Eur. J. Neurosci.* 24: 217-228
- Barrett SP, Gross SR, Garand I, Pihl RO (2005) Patterns of simultaneous polysubstance use in Canadian rave attendees. *Subst. Use Misuse* 40: 1525-1537
- Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E (2008) Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. *Neuron* 58: 639-650
- Bitner AC, Jr., Carter RC, Kennedy RS, Harbeson MM, Krause M (1986) Performance Evaluation Tests for Environmental Research (PETER): evaluation of 114 measures. *Percept Mot Skills* 63: 683-708
- Blessing WW (2005) BAT control shows the way: medullary raphe/parapyramidal neurons and sympathetic regulation of brown adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288: R557-R560
- Block RI, Erwin WJ, Farinpour R, Braverman K (1998) Sedative, stimulant, and other subjective effects of marijuana: relationships to smoking techniques. *Pharm. Biochem. Behav.* 59: 405-412
- Bond AJ, James DC, Lader MH (1974) Physiological and psychological measures in anxious patients. *Psychol. Med* 4: 364-373
- Bonz A, Laser M, Kullmer S, Kniesch S, Babin-Ebell J, Popp V, Ertl G, Wagner JA (2003) Cannabinoids acting on CB1 receptors decrease contractile performance in human atrial muscle. *J. Cardiovasc. Pharmacol.* 41: 657-664
- Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP (1998) Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. *Anesthesiology* 88: 82-88
- Brody S, Krause C, Veit R, Rau H (1998) Cardiovascular autonomic dysregulation in users of MDMA ("Ecstasy"). *Psychopharmacology (Berl)* 136: 390-393
- Brown PL, Kiyatkin EA (2004) Brain hyperthermia induced by MDMA (ecstasy): modulation by environmental conditions. *Eur. J. Neurosci.* 20: 51-58
- Cami J, Farre M, Mas M, Roset PN, Poudevida S, Mas A, San L, de la Torre R (2000) Human pharmacology of 3,4-methylenedioxymethamphetamine ("ecstasy"): Psychomotor performance and subjective effects. *J. Clin. Psychopharm.* 20: 455-466
- Campbell A (2008) Attachment, aggression and affiliation: The role of oxytocin in female social behavior. *Biol. Psychol.* 77: 1-10
- Cassel JC, Ben HS, Jones BC (2007) Attenuation of MDMA-induced hyperthermia by ethanol in rats depends on ambient temperature. *Eur. J. Pharmacol.* 571: 152-155
- Cassel JC, Hamida SB, Jones BC (2008) Ethanol and MDMA: a comment on the paper by Dumont et al. *Psychopharmacology (Berl)* 200: 305-306

- Cassel JC, Jeltsch H (1995) Serotonergic modulation of cholinergic function in the central nervous system: cognitive implications. *Neuroscience* 69: 1-41
- Cassel JC, Riegiert C, Rutz S, Koenig J, Rothmaier K, Cosquer B, Lazarus C, BIRTHELMER A, Jeltsch H, Jones BC, Jackisch R (2005) Ethanol, 3,4-methylenedioxymethamphetamine (ecstasy) and their combination: long-term behavioral, neurochemical and neuropharmacological effects in the rat. *Neuropsychopharmacology* 30: 1870-1882
- Chini B, Manning M (2007) Agonist selectivity in the oxytocin/vasopressin receptor family: new insights and challenges. *Biochem. Soc. Trans.* 35: 737-741
- Choi YH, Hazekamp A, Peltenburg-Looman AM, Frederich M, Erkelens C, Lefeber AW, Verpoorte R (2004) NMR assignments of the major cannabinoids and cannabiflavonoids isolated from flowers of *Cannabis sativa*. *Phytochem. Anal.* 15: 345-354
- Colado MI, O'Shea E, Green AR (2004) Acute and long-term effects of MDMA on cerebral dopamine biochemistry and function. *Psychopharmacology (Berl)* 173: 249-263
- Colado MI, Williams JL, Green AR (1995) The hyperthermic and neurotoxic effects of 'Ecstasy' (MDMA) and 3,4-methylenedioxymethamphetamine (MDA) in the Dark Agouti (DA) rat, a model of the CYP2D6 poor metabolizer phenotype. *Br. J. Pharmacol.* 115: 1281-1289
- Cole JC, Sumnall HR, Smith GW, Rostami-Hodjegan A (2005) Preliminary evidence of the cardiovascular effects of polysubstance misuse in nightclubs. *J. Psychopharmacol.* 19: 67-70
- Connolly E, O'Callaghan G (1999) MDMA toxicity presenting with severe hyperpyrexia: a case report. *Crit. Care Resusc.* 1: 368-370
- Cornish JL, Shahnawaz Z, Thompson MR, Wong S, Morley KC, Hunt GE, McGregor IS (2003) Heat increases 3,4-methylenedioxymethamphetamine self-administration and social effects in rats. *Eur. J. Pharmacol.* 482: 339-341
- Croft RJ, Klugman A, Baldeweg T, Gruzelier JH (2001) Electrophysiological evidence of serotonergic impairment in long-term MDMA ("ecstasy") users. *Am. J. Psychiatry* 158: 1687-1692
- Curran HV (2000) Is MDMA ('ecstasy') neurotoxic in humans? An overview of evidence and of methodological problems in research. *Neuropsychobiol.* 42: 34
- Curran HV, Brignell C, Fletcher S, Middleton P, Henry J (2002) Cognitive and subjective dose-response effects of acute oral Delta 9-tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology (Berl)* 164: 61-70
- Daumann J, Fischermann T, Heekeren K, Henke K, Thron A, Gouzoulis-Mayfrank E (2004) Memory-related hippocampal dysfunction in poly-drug ecstasy (3,4-methylenedioxymethamphetamine) users. *Psychopharmacology (Berl)* 180: 607-611
- de la Torre R, Farre M, Ortuno J, Mas M, Brenneisen R, Roset PN, Segura J, Cami J (2000a) Non-linear pharmacokinetics of MDMA ('ecstasy') in humans. *Br. J. Clin. Pharmacol.* 49: 104-109
- de la Torre R, Farre M, Roset PN, Lopez CH, Mas M, Ortuno J, Menoyo E, Pizarro N, Segura J, Cami J (2000b) Pharmacology of MDMA in humans. *An. N. Y. Acad. Sciences* 914: 225-237
- de la Torre R, Farre M, Roset PN, Pizarro N, Abanades S, Segura M, Segura J, Cami J (2004) Human pharmacology of MDMA - Pharmacokinetics, metabolism, and disposition. *Ther. Drug Mon.* 26: 137-144
- de Visser SJ, van der Post J, Pieters MSM, Cohen AF, van Gerven JMA (2001) Biomarkers for the effects of antipsychotic drugs in healthy volunteers. *Br. J. Clin. Pharm.* 51: 119-132
- de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JMA (2003) Biomarkers for the effects of benzodiazepines in healthy volunteers. *Br. J. Clin. Pharm.* 55: 39-50
- de Wit H, Enggasser JL, Richards JB (2002) Acute administration of d-amphetamine decreases impulsivity in healthy volunteers. *Neuropsychopharmacology* 27: 813-825
- Domes G, Heinrichs M, Michel A, Berger C, Herpertz SC (2007) Oxytocin improves "mind-reading" in humans. *Biol. Psychiatry* 61: 731-733
- Droogmans S, Cosyns B, D'haenen H, Creten E, Weytjens C, Franken PR, Scott B, Schoors D, Kemdem A, Close L, Vandenbosche JL, Bechet S, Van CG (2007) Possible association between 3,4-methylenedioxymethamphetamine abuse and valvular heart disease. *Am. J. Cardiol.* 100: 1442-1445
- Dumont G, Kramers C, Sweep F, Willemsen J, Touw D, Schoemaker R, van GJ, Buitelaar J, Verkes R (2008) Ethanol co-administration moderates 3,4-methylenedioxymethamphetamine effects on human physiology. *J. Psychopharmacol.* Accepted for publication
- Dumont GJ, Valkenberg MM, Schoemaker R, Buitelaar JK, van Gerven JM, Verkes RJ (2007) Acute MDMA and ethanol interaction effects on psychomotor performance. *Br. J. Clin. Pharm.* 63: 503
- Dumont GJ, Verkes RJ (2006) A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers. *J. Psychopharmacol.* 20: 176-187

- Dumont GJ, Verkes RJ, Buitelaar JK, van Gerven JM (2008) Response to the comments by Cassel et al. *Psychopharmacology (Berl)* 200: 451-452
- Dumont GJ, Wezenberg E, Valkenberg MM, de Jong CA, Buitelaar JK, van Gerven JM, Verkes RJ (2008) Acute neuropsychological effects of MDMA and ethanol (co-)administration in healthy volunteers. *Psychopharmacology (Berl)* 197: 465-474
- Dumont GJH, de Visser SJ, Cohen AF, van Gerven JMA (2005) Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects. *Br. J. Clin. Pharm.* 59: 495-510
- Escobedo I, O'Shea E, Orio L, Sanchez V, Segura M, de la Torre R, Farre M, Green AR, Colado MI (2005) A comparative study on the acute and long-term effects of MDMA and 3,4-dihydroxymethamphetamine (HHMA) on brain monoamine levels after i.p. or striatal administration in mice. *Br. J. Pharm.* 144: 231-241
- Farre M, de la Torre R, Mathuna BO, Roset PN, Peiro AM, Torrens M, Ortuno J, Pujadas M, Cami J (2004) Repeated doses administration of MDMA in humans: pharmacological effects and pharmacokinetics. *Psychopharmacology (Berl)* 173: 364-375
- First MB, Frances AJ, Pincus HA, Vettorello N, Davis WW (1994) DSM-IV in progress. *Changes in substance-related, schizophrenic, and other primarily adult disorders. Hosp. Com. Psychiatry* 45: 18-20
- Fisk JE, Montgomery C, Wareing M, Murphy PN (2006) The effects of concurrent cannabis use among ecstasy users: neuroprotective or neurotoxic? *Hum. Psychopharmacol.* 21: 355-366
- Forsling M, Fallon JK, Kicman AT, Hutt AJ, Cowan DA, Henry JA (2001) Arginine vasopressin release in response to the administration of 3,4-methylenedioxymethamphetamine ("ecstasy"): is metabolism a contributory factor? *J. Pharmacy Pharm.* 53: 1357-1363
- Freedman RR, Johanson CE, Tancer ME (2005) Thermoregulatory effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology (Berl)* 183: 248-256
- Frei E, Gamma A, Pascual-Marqui R, Lehmann D, Hell D, Vollenweider FX (2001) Localization of MDMA-induced brain activity in healthy volunteers using low resolution brain electromagnetic tomography (LORETA). *Hum. Brain Map.* 14: 152-165
- Freudenmann RW, Oxler F, Bernschneider-Reif S (2006) The origin of MDMA (ecstasy) revisited: the true story reconstructed from the original documents. *Addiction* 101: 1241-1245
- Gamma A, Buck A, Buck A, Berthold T, Hell D, Vollenweider FX (2000) 3,4-methylenedioxymethamphetamine (MDMA) modulates cortical and limbic brain activity as measured by [(H2O)-O-15]-PET in healthy humans. *Neuropsychopharmacology* 23: 388-395
- Garcia-Alloza M, Zaldúa N, ez-Ariza M, Marcos B, Lasheras B, Javier Gil-Bea F, Ramirez MJ (2006) Effect of selective cholinergic denervation on the serotonergic system: implications for learning and memory. *J. Neuropathol. Exp. Neurol.* 65: 1074-1081
- Garcia-Repetto R, Moreno E, Soriano T, Jurado C, Gimenez MP, Menendez M (2003) Tissue concentrations of MDMA and its metabolite MDA in three fatal cases of overdose. *Forensic Science Int.* 135: 110-114
- Ghuran A, Nolan J (2000) Recreational drug misuse: issues for the cardiologist. *Heart* 83: 627-633
- Gijsman HJ, van Gerven JM, Verkes RJ, Schoemaker RC, Pieters MS, Pennings EJ, Hessing TJ, Cohen AF (2002) Saccadic peak velocity and EEG as end-points for a serotonergic challenge test. *Hum. Psychopharmacol.* 17: 83-89
- Gimpl G, Fahrenholz F (2001) The oxytocin receptor system: structure, function, and regulation. *Physiol. Rev.* 81: 629-683
- Goni-Allo B, Mathuna O, Segura M, Puerta E, Lasheras B, de la TR, Aguirre N (2008) The relationship between core body temperature and 3,4-methylenedioxymethamphetamine metabolism in rats: implications for neurotoxicity. *Psychopharmacology (Berl)* 197: 263-278
- Gouzoulis-Mayfrank E, Daumann J (2006a) Neurotoxicity of methylenedioxyamphetamines (MDMA; ecstasy) in humans: how strong is the evidence for persistent brain damage? *Addiction* 101: 348-361
- Gouzoulis-Mayfrank E, Daumann J (2006b) The confounding problem of polydrug use in recreational ecstasy/MDMA users: a brief overview. *J. Psychopharmacol.* 20: 188-193
- Green AR, Mehan AO, Elliott JM, O'Shea E, Colado MI (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharm. Rev.* 55: 463-508
- Green AR, O'Shea E, Colado MI (2004) A review of the mechanisms involved in the acute MDMA (ecstasy)-induced hyperthermic response. *Eur. J. Pharm.* 500: 3-13

- Green AR, O'Shea E, Saadat KS, Elliott JM, Colado MI (2005) Studies on the effect of MDMA ('ecstasy') on the body temperature of rats housed at different ambient room temperatures. *Br. J. Pharmacol.* 146: 306-312
- Green AR, Sanchez V, O'Shea E, Saadat KS, Elliott JM, Colado MI (2004) Effect of ambient temperature and a prior neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA) on the hyperthermic response of rats to a single or repeated ('binge' ingestion) low dose of MDMA. *Psychopharmacology (Berl)* 173: 264-269
- Grob CS, Poland RE, Chang L, Ernst T (1995) Psychobiologic effects of 3,4-methylenedioxymethamphetamine in humans: Methodological considerations and preliminary observations. *Behav. Brain Res.* 73: 103-107
- Gross SR, Barrett SP, Shestowsky JS, Pihl RO (2002) Ecstasy and drug consumption patterns: a Canadian rave population study. *Can. J. Psychiatry Rev. Can. Psychiatrie* 47: 546-551
- Guastella AJ, Mitchell PB, Dadds MR (2008) Oxytocin Increases Gaze to the Eye Region of Human Faces. *Biol. Psychiatry* 63: 3-5
- Gulick D, Gould TJ (2007) Acute ethanol has biphasic effects on short- and long-term memory in both foreground and background contextual fear conditioning in C57BL/6 mice. *Alc. Clin. Exp. Res.* 31: 1528-1537
- Hall AP, Henry JA (2006) Acute toxic effects of 'Ecstasy' (MDMA) and related compounds: overview of pathophysiology and clinical management. *Br. J. Anaesth.* 96: 678-685
- Hall W, Solowij N (1998) Adverse effects of cannabis. *Lancet* 352: 1611-1616
- Hammock EA, Young LJ (2006) Oxytocin, vasopressin and pair bonding: implications for autism. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 361: 2187-2198
- Hargreaves GA, Hunt GE, Cornish JL, McGregor IS (2007) High ambient temperature increases 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy")-induced Fos expression in a region-specific manner. *Neuroscience* 145: 764-774
- Harris DS, Baggott M, Mendelson JH, Mendelson JE, Jones RT (2002) Subjective and hormonal effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology (Berl)* 162: 396-405
- Hart CL, van GW, Haney M, Foltin RW, Fischman MW (2001) Effects of acute smoked marijuana on complex cognitive performance. *Neuropsychopharmacology* 25: 757-765
- Hartung TK, Schofield E, Short AI, Parr MJ, Henry JA (2002) Hyponatraemic states following 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') ingestion. *QJM.* 95: 431-437
- Hazekamp A, Choi YH, Verpoorte R (2004) Quantitative analysis of cannabinoids from *Cannabis sativa* using <sup>1</sup>H-NMR. *Chem. Pharm. Bull. (Tokyo)* 52: 718-721
- Hazekamp A, Ruhaak R, Zuurman L, van GJ, Verpoorte R (2006) Evaluation of a vaporizing device (Volcano) for the pulmonary administration of tetrahydrocannabinol. *J. Pharm. Sci.* 95: 1308-1317
- Hege SG, Ellinwood EH, Jr., Wilson WH, Helligers CA, Graham SM (1997) Psychomotor effects of the anxiolytic abecarnil: a comparison with lorazepam. *Psychopharmacology (Berl)* 131: 101-7
- Heishman SJ, Arasteh K, Stitzer ML (1997) Comparative effects of alcohol and marijuana on mood, memory, and performance. *Pharmacol. Biochem. Behav.* 58: 93-101
- Henry JA, Fallon JK, Kicman AT, Hutt AJ, Cowan DA, Forsling M (1998) Low-dose MDMA ("ecstasy") induces vasopressin secretion. *Lancet* 351: 1784
- Hernandez-Lopez C, Farre M, Roset PN, Menoyo E, Pizarro N, Ortuno J, Torrens M, Cami J, de la Torre R (2002) 3,4-methylenedioxymethamphetamine (ecstasy) and alcohol interactions in humans: Psychomotor performance, subjective effects, and pharmacokinetics. *J. Pharm. Exp. Ther.* 300: 236-244
- Huber D, Veinante P, Stoop R (2005) Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. *Science* 308: 245-248
- Ilan AB, Gevins A, Coleman M, ElSohly MA, de WH (2005) Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. *Behav. Pharmacol.* 16: 487-496
- Ilan AB, Smith ME, Gevins A (2004) Effects of marijuana on neurophysiological signals of working and episodic memory. *Psychopharmacology (Berl)* 176: 214-222
- Irvine RJ, Keane M, Felgate P, McCann UD, Callaghan PD, White JM (2006) Plasma drug concentrations and physiological measures in 'dance party' participants. *Neuropsychopharmacology* 31: 424-430
- Izco M, Orio L, O'Shea E, Colado MI (2007) Binge ethanol administration enhances the MDMA-induced long-term 5-HT neurotoxicity in rat brain. *Psychopharmacology (Berl)* 189: 459-470

- Johnson EA, O'Callaghan JP, Miller DB (2004) Brain concentrations of d-MDMA are increased after stress. *Psychopharmacology (Berl)* 173: 278-286
- Jones DC, Duvauchelle C, Ikegami A, Olsen CM, Lau SS, de la Torre R, Monks TJ (2005) Serotonergic neurotoxic metabolites of ecstasy identified in rat brain. *J. Pharm. Exp. Ther.* 313: 422-431
- Kalantar-Zadeh K, Nguyen MK, Chang R, Kurtz I (2006) Fatal hyponatremia in a young woman after ecstasy ingestion. *Nat. Clin. Pract. Nephrol.* 2: 283-288
- Kaplan E, Fein D, Morris R, Delis D (1991) Wais-R as a neuropsychological instrument. The psychological corporation, San Antonio, TX
- Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, Gruppe H, Mattay VS, Gallhofer B, Meyer-Lindenberg A (2005) Oxytocin modulates neural circuitry for social cognition and fear in humans. *J. Neurosci.* 25: 11489-11493
- Kiyatkin, E. A. (2007) Physiological and pathological brain hyperthermia. *Prog. Brain. Res.* 162: 219-243
- Kodavali L, Townsend RR (2006) Alcohol and its relationship to blood pressure. *Curr. Hypertens. Rep.* 8: 338-344
- Kuypers KP, Ramaekers JG (2005) Transient memory impairment after acute dose of 75mg 3,4-Methylene-dioxymethamphetamine. *J. Psychopharmacol.* 19: 633-639
- Kuypers KP, Ramaekers JG (2007) Acute dose of MDMA (75 mg) impairs spatial memory for location but leaves contextual processing of visuospatial information unaffected. *Psychopharmacology (Berl)* 189: 557-563
- Kuypers KP, Samyn N, Ramaekers JG (2006) MDMA and alcohol effects, combined and alone, on objective and subjective measures of actual driving performance and psychomotor function. *Psychopharmacology (Berl)* 187: 467-75
- Kuypers KP, Wingen M, Samyn N, Limbert N, Ramaekers JG (2007) Acute effects of nocturnal doses of MDMA on measures of impulsivity and psychomotor performance throughout the night. *Psychopharmacology (Berl)* 192: 111-119
- Lamas X, Farre M, Llorente M, Cami J (1994) Spanish version of the 49-item short form of the Addiction Research Center Inventory (ARCI). *Drug Alc. Depend.* 35: 203-209
- Lamers CTJ, Ramaekers JG (2001) Visual search and urban city driving under the influence of marijuana and alcohol. *Hum. Psychopharmacol. Clin. Exp.* 16: 393-401
- Lamers CTJ, Ramaekers JG, Muntjewerff ND, Sikkema KL, Samyn N, Read NL, Brookhuis KA, Riedel WJ (2003) Dissociable effects of a single dose of ecstasy (MDMA) on psychomotor skills and attentional performance. *J. Psychopharmacol.* 17: 379-387
- Landgraf R, Neumann ID (2004) Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. *Front. Neuroendocrinol.* 25: 150-176
- Lerer E, Levi S, Salomon S, Darvasi A, Yirmiya N, Ebstein RP (2008) Association between the oxytocin receptor (OXTR) gene and autism: relationship to Vineland Adaptive Behavior Scales and cognition. *Mol. Psychiatry* 13: 980-988
- Lester SJ, Baggott M, Welms S, Schiller NB, Jones RT, Foster E, Mendelson J (2000) Cardiovascular effects of 3,4-methylenedioxymethamphetamine - A double-blind, placebo-controlled trial. *Ann. Int. Med.* 133: 969-973
- Liechti ME, Baumann C, Gamma A, Vollenweider FX (2000) Acute psychological effects of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") are attenuated by the serotonin uptake inhibitor citalopram. *Neuropsychopharmacology* 22: 513-521
- Liechti ME, Gamma A, Vollenweider FX (2001) Gender differences in the subjective effects of MDMA. *Psychopharmacology (Berl)* 154: 161-168
- Liechti ME, Geyer MA, Hell D, Vollenweider FX (2001) Effects of MDMA (ecstasy) on prepulse inhibition and habituation of startle in humans after pretreatment with citalopram, haloperidol, or ketanserin. *Neuropsychopharmacology* 24: 240-252
- Liechti ME, Saur MR, Gamma A, Hell D, Vollenweider FX (2000) Psychological and physiological effects of MDMA ("ecstasy") after pretreatment with the 5-HT<sub>2</sub> antagonist ketanserin in healthy humans. *Neuropsychopharmacology* 23: 396-404
- Liechti ME, Vollenweider FX (2000) Acute psychological and physiological effects of MDMA ("Ecstasy") after haloperidol pretreatment in healthy humans. *Eur. Neuropsychopharmacology* 10: 289-295
- Liechti ME, Vollenweider FX (2001) Which neuroreceptors mediate the subjective effects of MDMA in humans? A summary of mechanistic studies. *Hum. Psychopharm. - Clin. Exp.* 16: 589-598

- Lim MM, Young LJ (2006) Neuropeptidergic regulation of affiliative behavior and social bonding in animals. *Horm. Behav.* 50: 506-517
- Ludwig M, Leng G (2006) Dendritic peptide release and peptide-dependent behaviours. *Nat. Rev. Neurosci.* 7: 126-136
- Madeira MD, Paula-Barbosa MM (1999) Effects of alcohol on the synthesis and expression of hypothalamic peptides. *Brain Res. Bull.* 48: 3-22
- Malberg JE, Seiden LS (1998) Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. *J. Neurosci.* 18: 5086-5094
- Mas M, Farre M, de la Torre R, Roset PN, Ortuno J, Segura J, Cami J (1999) Cardiovascular and neuroendocrine effects and pharmacokinetics of 3, 4-methylenedioxymethamphetamine in humans. *J. Pharmacol. Exp. Ther.* 290: 136-145
- Mccann UD, Szabo Z, Seckin E, Rosenblatt P, Mathews WB, Ravert HT, Dannals RF, Ricaurte GA (2005) Quantitative PET Studies of the Serotonin Transporter in MDMA Users and Controls Using [(11)C]McN5652 and [(11)C]DASB. *Neuropsychopharmacology* 30: 1741-1750
- McCann UD, Szabo Z, Vranesic M, Palermo M, Mathews WB, Ravert HT, Dannals RF, Ricaurte GA (2008) Positron emission tomographic studies of brain dopamine and serotonin transporters in abstinent (+/-)3,4-methylenedioxymethamphetamine ("ecstasy") users: relationship to cognitive performance. *Psychopharmacology (Berl)* 200: 439-450
- McNamara IM, Borella AW, Bialowas LA, Whitaker-Azmitia PM (2008) Further studies in the developmental hyperserotonemia model (DHS) of autism: Social, behavioral and peptide changes. *Brain Res.* 16: 203-214
- Mechan AO, Esteban B, O'Shea E, Elliott JM, Colado MI, Green AR (2002) The pharmacology of the acute hyperthermic response that follows administration of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') to rats. *Br. J. Pharmacol.* 135: 170-180
- Meeren HK, Van Luijckelaar EL, Coenen AM (1998) Cortical and thalamic visual evoked potentials during sleep-wake states and spike-wave discharges in the rat. *Electroencephalogr. Clin. Neurophysiol.* 108: 306-319
- Mehta MA, Riedel WJ (2006) Dopaminergic enhancement of cognitive function. *Curr. Pharm. Des* 12: 2487-2500
- Meneses A (2007) Do serotonin(1-7) receptors modulate short and long-term memory? *Neurobiol. Learn Mem.* 87: 561-572
- Meyer-Lindenberg A, Poline JB, Kohn PD, Holt JL, Egan MF, Weinberger DR, Berman KF (2001) Evidence for abnormal cortical functional connectivity during working memory in schizophrenia. *Am. J. Psychiatry* 158: 1809-1817
- Miller DB, O'Callaghan JP (2003) Elevated environmental temperature and methamphetamine neurotoxicity. *Environ. Res.* 92: 48-53
- Mills EM, Banks ML, Sprague JE, Finkel T (2003) Pharmacology: uncoupling the agony from ecstasy. *Nature* 426: 403-404
- Mills EM, Rusyniak DE, Sprague JE (2004) The role of the sympathetic nervous system and uncoupling proteins in the thermogenesis induced by 3,4-methylenedioxymethamphetamine. *J. Mol. Med.* 82: 787-799
- Mills EM, Weaver KL, Abramson E, Pfeiffer M, Sprague JE (2007) Influence of dietary fats on ecstasy-induced hyperthermia. *Br. J. Pharmacol.* 151: 1103-1108
- Mlinar B, Corradetti R (2003) Endogenous 5-HT, released by MDMA through serotonin transporter- and secretory vesicle-dependent mechanisms, reduces hippocampal excitatory synaptic transmission by preferential activation of 5-HT1B receptors located on CA1 pyramidal neurons. *Eur. J. Neuroscience* 18: 1559-1571
- Morley KC, Li KM, Hunt GE, Mallet PE, McGregor IS (2004) Cannabinoids prevent the acute hyperthermia and partially protect against the 5-HT depleting effects of MDMA ("Ecstasy") in rats. *Neuropharmacology* 46: 954-965
- Nichols DE, Oberlander R (1990) Structure-activity relationships of MDMA and related compounds: a new class of psychoactive drugs? *Ann. N. Y. Acad. Sci.* 600: 613-623
- Nutt DJ (2006) A tale of two Es. *J. Psychopharmacol.* 20: 315-317
- Nyberg S, Wahlstrom G, Backstrom T, Poromaa IS (2004) No difference in responsiveness to a low dose of alcohol between healthy women and men. *Pharmacol. Biochem. Behav.* 78: 603-610

- O'Shea E, Easton N, Fry JR, Green AR, Marsden CA (2002) Protection against 3,4-methylenedioxymethamphetamine-induced neurodegeneration produced by glutathione depletion in rats is mediated by attenuation of hyperthermia. *J. Neurochem.* 81: 686-695
- O'Shea E, Orio L, Escobedo I, Sanchez V, Camarero J, Green AR, Colado MI (2006) MDMA-induced neurotoxicity: long-term effects on 5-HT biosynthesis and the influence of ambient temperature. *Br. J. Pharmacol.* 148: 778-785
- Orban de Xivry JJ, Lefevre P (2007) Saccades and pursuit: two outcomes of a single sensorimotor process. *J. Physiol.* 584: 11-23
- Pacher P, Batkai S, Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. *Pharm. Rev.* 58: 389-462
- Pacifici R, Pichini S, Zuccaro P, Farre M, Segura M, Ortuno J, Di Carlo S, Bacosi A, Roset PN, Segura J, de la Torre R (2004) Paroxetine inhibits acute effects of 3,4-methylenedioxymethamphetamine on the immune system in humans. *J. Pharm. Exp. Ther.* 309: 285-292
- Pacifici R, Zuccaro P, Farre M, Pichini S, Di Carlo S, Roset PN, Lopez CH, Ortuno J, Segura J, Cami J, de la Torre R (2000) Immunomodulating activity of MDMA. *Ann. N. Y. Acad. Sci.* 914: 215-224
- Pacifici R, Zuccaro P, Farre M, Pichini S, Di Carlo S, Roset PN, Ortuno J, Segura J, de La TR (1999) Immunomodulating properties of MDMA alone and in combination with alcohol: a pilot study. *Life Sciences* 65: L309-L316
- Pacifici R, Zuccaro P, Lopez CH, Pichini S, Di Carlo S, Farre M, Roset PN, Ortuno J, Segura J, de la Torre R (2001) Acute effects of 3,4-methylenedioxymethamphetamine alone and in combination with ethanol on the immune system in humans. *J. Pharm. Exp. Ther.* 296: 207-215
- Parrott AC (2004) Is ecstasy MDMA? A review of the proportion of ecstasy tablets containing MDMA, their dosage levels, and the changing perceptions of purity. *Psychopharmacology (Berl)* 173: 234-241
- Parrott AC (2006) MDMA in humans: factors which affect the neuropsychobiological profiles of recreational ecstasy users, the integrative role of bioenergetic stress. *J. Psychopharmacol.* 20: 147-163
- Parrott AC (2007a) Ecstasy versus alcohol: Tolstoy and the variations of unhappiness. *J. Psychopharmacol.* 21: 3-6
- Parrott AC (2007b) The psychotherapeutic potential of MDMA (3,4-methylenedioxymethamphetamine): an evidence-based review. *Psychopharmacology (Berl)* 191: 181-193
- Parrott AC, Gouzoulis-Mayfrank E, Rodgers J, Solowij N (2004) Ecstasy/MDMA and cannabis: the complexities of their interactive neuropsychobiological effects. *J. Psychopharmacol.* 18: 572-575
- Parrott AC, Lasky J (1998) Ecstasy (MDMA) effects upon mood and cognition: Before, during and after a Saturday night dance. *Psychopharmacology (Berl)* 139: 261-268
- Parrott AC, Milani RM, Gouzoulis-Mayfrank E, Daumann J (2007) Cannabis and Ecstasy/MDMA (3,4-methylenedioxymethamphetamine): an analysis of their neuropsychobiological interactions in recreational users. *J. Neural Transm.* 114: 959-968
- Parrott AC, Rodgers J, Buchanan T, Ling J, Heffernan T, Scholey AB (2006) Dancing hot on Ecstasy: physical activity and thermal comfort ratings are associated with the memory and other psychobiological problems reported by recreational MDMA users. *Hum. Psychopharmacol.* 21: 285-298
- Parrott AC (2001) Human psychopharmacology of Ecstasy (MDMA): a review of 15 years of empirical research. *Hum. Psychopharmacol.* 16: 557-577
- Pijl C, Drobny H, Reither H, Hornykiewicz O, Singer EA (1995) Mechanism of the Dopamine-Releasing Actions of Amphetamine and Cocaine - Plasmalemmal Dopamine Transporter Versus Vesicular Monoamine Transporter. *Mol. Pharm.* 47: 368-373
- Ploner CJ, Tschirch A, Ostendorf F, Dick S, Gaymard BM, Rivaud-Pechoux S, Sporkert F, Pragst F, Stadelmann AM (2002) Oculomotor effects of delta-9-tetrahydrocannabinol in humans: implications for the functional neuroanatomy of the brain cannabinoid system. *Cereb. Cortex* 12: 1016-1023
- Pohorecky LA, Brick J (1988) Pharmacology of ethanol. *Pharmacology and Therapeutics* 36: 335-427
- Ramaekers JG, Kuypers KP (2006a) Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on behavioral measures of impulsivity: alone and in combination with alcohol. *Neuropsychopharmacology* 31: 1048-1055

- Ramaekers JG, Kuypers KP, Samyn N (2006a) Stimulant effects of 3,4-methylenedioxymethamphetamine (MDMA) 75 mg and methylphenidate 20 mg on actual driving during intoxication and withdrawal. *Addiction* 101: 1614-1621
- Ramaekers JG, Lamers CTJ, Riedel WJ (2002) The effects of mdma on cognitive and psychomotor performance in recreational mdma users. *International Journal of Neuropsychopharmacology* 5: S32
- Ramaekers JG, Robbe HW, O'Hanlon JF (2000) Marijuana, alcohol and actual driving performance. *Hum. Psychopharmacol.* 15: 551-558
- Ravina P, Quiroga JM, Ravina T (2004) Hyperkalemia in fatal MDMA ('ecstasy') toxicity. *Int. J. Card.* 93: 307-308
- Reneman L, Booij J, De BK, Reitsma JB, de Wolff FA, Gunning WB, den Heeten GJ, Van Den BW (2001a) Effects of dose, sex, and long-term abstinence from use on toxic effects of MDMA (ecstasy) on brain serotonin neurons. *Lancet* 358: 1864-1869
- Reneman L, Booij J, Majoie CBLM, van den Brink W, den Heeten GJ (2001b) Investigating the potential neurotoxicity of Ecstasy (MDMA): an imaging approach. *Hum. Psychopharm. – Clin. Exp.* 16: 579-588
- Ricaurte GA, Yuan J, McCann UD (2000) (+/-)3,4-methylenedioxymethamphetamine ('Ecstasy')-induced serotonin neurotoxicity: Studies in animals. *Neuropsychobiology* 42: 5-10
- Riley SC, James C, Gregory D, Dingle H, Cadger M (2001) Patterns of recreational drug use at dance events in Edinburgh, Scotland. *Addiction* 96: 1035-1047
- Rivier C, Lee S (1996) Acute alcohol administration stimulates the activity of hypothalamic neurons that express corticotropin-releasing factor and vasopressin. *Brain Res.* 726: 1-10
- Ronen A, Gershon P, Drobiner H, Rabinovich A, Bar-Hamburger R, Mechoulam R, Cassuto Y, Shinar D (2008) Effects of THC on driving performance, physiological state and subjective feelings relative to alcohol. *Accid. Anal. Prev.* 40: 926-934
- Rosen JB, Donley MP (2006) Animal studies of amygdala function in fear and uncertainty: relevance to human research. *Biol. Psychol.* 73: 49-60
- Rosenson J, Smollin C, Sporer KA, Blanc P, Olson KR (2007) Patterns of ecstasy-associated hyponatremia in California. *Ann. Emerg. Med.* 49: 164-71, 171
- Saadat KS, O'Shea E, Colado MI, Elliott JM, Green AR (2005) The role of 5-HT in the impairment of thermoregulation observed in rats administered MDMA ('ecstasy') when housed at high ambient temperature. *Psychopharmacology (Berl)* 179: 884-890
- Sabbe B, Hulstijn W, van HJ, Tuynman-Qua HG, Zitman F (1999) Retardation in depression: assessment by means of simple motor tasks. *J. Affect. Disord.* 55: 39-44
- Saldana SN, Barker EL (2004) Temperature and 3,4-methylenedioxymethamphetamine alter human serotonin transporter-mediated dopamine uptake. *Neuroscience Letters* 354: 209-212
- Sanchez V, O'Shea E, Saadat KS, Elliott JM, Colado MI, Green AR (2004) Effect of repeated ('binge') dosing of MDMA to rats housed at normal and high temperature on neurotoxic damage to cerebral 5-HT and dopamine. *J. Psychopharm.* 18: 412-416
- Sanfey AG (2007) Social decision-making: insights from game theory and neuroscience. *Science* 318: 598-602
- Schifano F, Di Furia L, Forza C, Minicuci N, Bricolo R (1998) MDMA ('ecstasy') consumption in the context of polydrug abuse: a report on 150 patients. *Drug Alc. Depend.* 52: 85-90
- Sessa B (2007) Is there a case for MDMA-assisted psychotherapy in the UK? *J. Psychopharmacol.* 21: 220-224
- Sessa B, Nutt DJ (2007) MDMA, politics and medical research: have we thrown the baby out with the bathwater? *J. Psychopharmacol.* 21: 787-791
- Sidney S (2002) Cardiovascular consequences of marijuana use. *J. Clin. Pharmacol.* 42: 64S-70S
- Silva TP, Silveira GA, Fior-Chadi DR, Chadi G (2004) Effects of ethanol consumption on vasopressin and neuropeptide Y immunoreactivity and mRNA expression in peripheral and central areas related to cardiovascular regulation. *Alcohol* 32: 213-222
- Soar K, Parrott AC, Fox HC (2004) Persistent neuropsychological problems after 7 years of abstinence from recreational ecstasy (MDMA): A case study. *Psychol. Rep.* 95: 192-196
- Solowij N, Hall W, Lee N (1992) Recreational MDMA use in Sydney: a profile of 'Ecstasy' users and their experiences with the drug. *Br. J. Addict.* 87: 1161-1172

- Sprague JE, Banks ML, Cook VJ, Mills EM (2003) Hypothalamic-pituitary-thyroid axis and sympathetic nervous system involvement in hyperthermia induced by 3,4-methylenedioxymethamphetamine (Ecstasy). *J. Pharm. Exp. Ther.* 305: 159-166
- Sprague JE, Brutter RE, Mills EM, Caden D, Rusyniak DE (2004) Attenuation of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy)-induced rhabdomyolysis with alpha1- plus beta3-adrenoreceptor antagonists. *Br. J. Pharmacol.* 142: 667-670
- Sprague JE, Moze P, Caden D, Rusyniak DE, Holmes C, Goldstein DS, Mills EM (2005b) Carvedilol reverses hyperthermia and attenuates rhabdomyolysis induced by 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) in an animal model. *Crit. Care Med.* 33: 1311-1316
- Sprague JE, Nichols DE (2005) Neurotoxicity of MDMA (ecstasy): beyond metabolism. *Trends Pharmacol. Sci.* 26: 59-60
- Strougo A, Zuurman L, Roy C, Pinquier J, van GJ, Cohen A, Schoemaker R (2008) Modelling of the concentration--effect relationship of THC on central nervous system parameters and heart rate -- insight into its mechanisms of action and a tool for clinical research and development of cannabinoids. *J. Psychopharmacol.* 22: 717-726
- Sumnall HR, Cole JC, Jerome L (2006) The varieties of ecstatic experience: an exploration of the subjective experiences of ecstasy. *J. Psychopharmacol.* 20: 670-682
- Sundstrom I, Backstrom T (1998) Citalopram increases pregnanolone sensitivity in patients with premenstrual syndrome: an open trial. *Psychoneuroendo.* 23: 73-88
- Suzdak PD, Schwartz RD, Skolnick P, Paul SM (1988) Alcohols stimulate gamma-aminobutyric acid receptor-mediated chloride uptake in brain vesicles: correlation with intoxication potency. *Brain Res.* 444: 340-345
- Svrakic DM, Whitehead C, Przybeck TR, Cloninger CR (1993) Differential diagnosis of personality disorders by the seven-factor model of temperament and character. *Arch. Gen. Psychiatry* 50: 991-999
- Talarovicova A, Krskova L, Kiss A (2007) Some assessments of the amygdala role in suprahypothalamic neuroendocrine regulation: a minireview. *Endocr. Regul.* 41: 155-162
- Tancer M, Johanson CE (2003) Reinforcing, subjective, and physiological effects of MDMA in humans: a comparison with d-amphetamine and mCPP. *Drug Alc. Depend.* 72: 33-44
- Tancer M, Johanson CE (2007) The effects of fluoxetine on the subjective and physiological effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology (Berl)* 189: 565-573
- Tancer ME (2001) The subjective effects of MDMA and mCPP in moderate MDMA users. *Drug Alc. Depend.* 65: 97-101
- Tanner-Smith EE (2006) Pharmacological content of tablets sold as "ecstasy": Results from an online testing service. *Drug Alc. Depend* 83: 247-254
- Tawakol A, Omland T, Creager MA (2004) Direct effect of ethanol on human vascular function. *Am. J. Physiol. Heart Circ. Physiol.* 286: H2468-H2473
- Thompson MR, Callaghan PD, Hunt GE, Cornish JL, McGregor IS (2007) A role for oxytocin and 5-HT(1A) receptors in the prosocial effects of 3,4 methylenedioxymethamphetamine ("ecstasy"). *Neuroscience* 146: 509-514
- Trimbos Instituut (2008) Nationale Drug Monitor Utrecht
- Turek VF, Ryabinin AE (2005) Ethanol versus lipopolysaccharide-induced hypothermia: involvement of urocortin. *Neuroscience* 133: 1021-1028
- Uvnas-Moberg K, Bruzelius G, Alster P, Lundeberg T (1993) The antinociceptive effect of non-noxious sensory stimulation is mediated partly through oxytocinergic mechanisms. *Acta. Physiol. Scand.* 149: 199-204
- Vakil E, Blachstein H (1993) Rey Auditory-Verbal Learning Test: structure analysis. *J. Clin. Psychol.* 49: 883-890
- van Eijk LT, Pickkers P, Smits P, Bouw MP, van der Hoeven JG (2004) Severe vagal response after endotoxin administration in humans. *Intensive Care Med.* 30: 2279-2281
- van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF (1999) The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. *J. Psychopharmacol.* 13: 10-17
- Verbaten MN (2003) Specific memory deficits in ecstasy users? The results of a meta-analysis. *Hum. Psychopharm. - Clin. Exp.* 18: 281-290

- Verkes RJ, Gijsman HJ, Pieters MSM, Schoemaker RC, de Visser S, Kuijpers M, Pennings EJM, de Bruin D, Van de Wijngaart G, van Gerven JMA, Cohen AF (2001) Cognitive performance and serotonergic function in users of ecstasy. *Psychopharmacology (Berl)* 153: 196-202
- Vollenweider FX, Gamma AG, Liechti M, Huber T (1998) Psychological and cardiovascular effects and short-term sequelae of MDMA ("Ecstasy") in MDMA-naïve healthy volunteers. *Neuropsychopharmacology* 19: 241-251
- Vollenweider FX, Liechti ME, Gamma A, Greer G, Geyer M (2002) Acute psychological and neurophysiological effects of MDMA in humans. *J. Psychoactive Drugs* 34: 171-184
- Vollenweider FX, Remensberger S, Hell D, Geyer MA (1999) Opposite effects of 3,4-methylenedioxymethamphetamine (MDMA) on sensorimotor gating in rats versus healthy humans. *Psychopharmacology (Berl)* 143: 365-372
- Wachtel SR, ElSohly MA, Ross SA, Ambre J, de WH (2002) Comparison of the subjective effects of Delta(9)-tetrahydrocannabinol and marijuana in humans. *Psychopharmacology (Berl)* 161: 331-339
- Wechsler D (1981) The Psychometric Tradition - Developing the Wechsler Adult Intelligence Scale. *Contemp. Educ. Psychol.* 6: 82-85
- Weinstein A, Brickner O, Lerman H, Greenland M, Bloch M, Lester H, Chisin R, Mechoulam R, Bar-Hamburger R, Freedman N, Even-Sapir E (2008) Brain imaging study of the acute effects of Delta9-tetrahydrocannabinol (THC) on attention and motor coordination in regular users of marijuana. *Psychopharmacology (Berl)* 196: 119-131
- Wezenberg E, Hulstijn W, Sabbe B, Ruigt GS, Verkes RJ (2004) Psychomotor and cognitive effects of lorazepam and D-amphetamine in healthy subjects. Development of sensitive screening tests of psychomotor- and cognitive functions. *Eur. Neuropsychopharmacol.* 14: S362
- Wezenberg E, Verkes RJ, Sabbe BG, Ruigt GS, Hulstijn W (2005) Modulation of memory and visuospatial processes by biperiden and rivastigmine in elderly healthy subjects. *Psychopharmacology (Berl)* 181: 582-594
- Willemsen JJ, Ross HA, Jacobs MC, Lenders JW, Thien T, Swinkels LM, Benraad TJ (1995) Highly sensitive and specific HPLC with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans. *Clin. Chem.* 41: 1455-1460
- Williams H, Dratcu L, Taylor R, Roberts M, Oyefeso A (1998) "Saturday night fever": ecstasy related problems in a London accident and emergency department. *J. Accid. Emerg. Med.* 15: 322-326
- Winstock AR, Griffiths P, Stewart D (2001) Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. *Drug Alc. Depend.* 64: 9-17
- Wolff K, Tsapakis EM, Winstock AR, Hartley D, Holt D, Forsling ML, Aitchison KJ (2006a) Vasopressin and oxytocin secretion in response to the consumption of ecstasy in a clubbing population. *J. Psychopharmacol.* 20: 400-410
- Wright BM (1971) A simple mechanical ataxia-meter. *J. Physiol.* 218: 27P-28P
- Yoda T, Crawshaw LI, Nakamura M, Saito K, Konishi A, Nagashima K, Uchida S, Kanosue K (2005) Effects of alcohol on thermoregulation during mild heat exposure in humans. *Alcohol* 36: 195-200
- Young JM, McGregor IS, Mallet PE (2005) Co-administration of THC and MDMA ('ecstasy') synergistically disrupts memory in rats. *Neuropsychopharmacology* 30: 1475-1482
- Young LJ (2002) The neurobiology of social recognition, approach, and avoidance. *Biol. Psychiatry* 51: 18-26
- Zak PJ, Stanton AA, Ahmadi S (2007) Oxytocin increases generosity in humans. *PLoS ONE* 2: e1128
- Zoethout RW, van Gerven JM, Dumont GJ, Paltansing S, van Burgel ND, van der LM, Dahan A, Cohen AF, Schoemaker RC (2008) A comparative study of two methods for attaining constant alcohol levels. *Br. J. Clin. Pharm.* 66: 674-681
- Zuurman L, Ippel AE, Moin E, van Gerven JM (2009) Biomarkers for the effects of cannabis and THC in healthy volunteers. *Br. J. Clin. Pharm.* 67: 5-21
- Zuurman L, Roy C, Schoemaker R, Asset G, Amatsaleh A, Guimaeres L, Pinquier J, Cohen A, van GJ (2008) Inhibition of THC-induced effects on the central nervous system and heart rate by a novel CB1 receptor antagonist AVE1625. *J. Psychopharmacol.* Accepted for publication



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## *Curriculum vitae*

Glenn Dumont was born on the fifth of May, 1980, in Heerlen, The Netherlands. He obtained his Atheneum diploma from the Bernardinus College Heerlen in 1998. In 2003 he obtained his Master of Science degree in Biomedical Sciences at the University of Leiden, writing his final paper at the Centre for Human Drug Research (CHDR) in Leiden. In 2004 he started working as a Ph.D. student at the department of Psychiatry at the Radboud University Medical Center Nijmegen, under the supervision of co-promotor dr. R.J. Verkes and the promoters prof. dr. J.K. Buitelaar (dept. of Psychiatry, Nijmegen) and prof. dr. J.M.A. van Gerven (CHDR, Leiden). In August 2009 he was registered as a Clinical Pharmacologist. Currently he teaches psychopharmacology and the neurobiology of behavior at the department of Life Sciences, Zuyd University (Hogeschool Zuyd), Heerlen, and works as junior psychopharmacologist at Moleman Psychopharmacology, Amerongen.

## ***Publications***

Publications contained in this thesis:

- G.J.H. Dumont, C. Kramers, F.C.G.J. Sweep, D.J. Touw, J.G. van Hasselt, M. de Kam, J.M.A. van Gerven, J.K. Buitelaar and R.J. Verkes. *Cannabis co-administration potentiates ecstasy effects on heart rate and temperature in humans* Clin. Pharm. & Ther. 2009 Accepted for publication.
- G.J.H. Dumont, F.C.G.J. Sweep, R. van der Steen, R. Hermsen, A.R.T. Donders, D.J. Touw, J.M.A. van Gerven, J.K. Buitelaar and R.J. Verkes. *Increased oxytocin concentrations and prosocial feelings in humans after ecstasy (3,4-methylenedioxymethamphetamine) administration* Soc. Neuroscience 2009 4:359-366.
- G.J.H. Dumont, C. Kramers, F.C.G.J. Sweep, J.J. Willemsen, D.J. Touw, R.C. Schoemaker, J.M.A. van Gerven, J.K. Buitelaar and R.J. Verkes. *Ethanol co-administration moderates MDMA effects on human physiology*. J Psychopharmacol. 2008 Accepted for publication.
- G.J.H. Dumont, R.C. Schoemaker, D.J. Touw, F.C.G.J. Sweep, J.K. Buitelaar, J.M.A. van Gerven and R.J. Verkes. *Acute psychomotor effects of MDMA and ethanol (co-) administration over time in healthy volunteers*. J Psychopharmacol. 2008 Accepted for publication.
- G.J.H. Dumont, E. Wezenberg, M.M.G.J. Valkenberg, C.A.J. de Jong, J.K. Buitelaar, J.M.A. van Gerven and R.J. Verkes. *Acute neuropsychological effects of MDMA and ethanol (co-) administration in healthy volunteers* Psychopharmacology (Berl). 2008 Apr;197(3):465-474.
- G.J.H. Dumont and R.J. Verkes. *A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers*. J Psychopharmacol. 2006 Mar;20(2):176-187.

Other publications:

- G.J.H. Dumont, R.J. Verkes, J.K. Buitelaar, and J.M.A. van Gerven. *A response to Cassel et al.* Psychopharmacology (Berl). 2008 Oct;200(3):451-452.
- R.W. Zoethout, J.M. van Gerven, G.J. Dumont, S. Paltansing, N.D. van Burgel, M. van der Linden, A. Dahan, A.F. Cohen and R.C. Schoemaker. *A comparative study of two methods for attaining constant alcohol levels*. Br J Clin Pharmacol. 2008 Nov;66(5):674-681.
- G.J.H. Dumont, S.J. de Visser, A.F. Cohen, J.M.A. van Gerven. *Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects*. Br J Clin Pharmacol. 2005 May;59(5):495-510.

## **Series Donders Institute for Brain, Cognition and Behaviour**

1. van Aalderen-Smeets, S.I. (2007). Neural dynamics of visual selection. Maastricht University, Maastricht, The Netherlands.
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