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# What is the correlation between saliva levels of dexamphetamine and performance on visual and auditory-visual illusions?

Degree Project in Medicine

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# TABLE OF CONTENTS

**LIST OF ABBREVIATIONS**

DA – Dopamine

DEX – Dexamphetamine

MDMA – 3,4-Methylenedioxymethamphetamine

3-FI – Three flash illusion

SIFI – Sound induced flash illusion

VIFI – Visually induced flash illusion

ISI – Interstimulus interval

HPLC – High performance liquid chromatography

BPRS – Brief psychiatric rating scale

SAPS – Scale for the assessment of positive symptoms

MIS – Magical ideation scale

AMQ – Amphetamine mood questionnaire

AMW – Amphetamine mood withdrawal (subscale of AMQ)

AMP – Amphetamine mood positive (subscale of AMQ)

PCA – Principal components analysis

# ABSTRACT

Degree Project, Programme in Medicine 2017. **Author**: Sofia Henriksson. **Institution**: Pharmacology, Pharmacy & Anaesthesiology Unit, University of Western Australia, Perth.

**Title**: "What is the correlation between saliva levels of dexamphetamine and performance on visual and auditory-visual illusions?"

**Background**: Schizophrenia is a psychiatric disorder associated with dopamine (DA) hyperactivity. Given that dexamphetamine (DEX) elevates levels of DA in the brain, DEX challenges can be used to model schizophrenia in research. Hallucinations and delusions are two characteristic symptoms of schizophrenia and psychosis. Previous research suggests a widened temporal binding window in which sensory stimuli can bind to form one perception as a mechanism of how DA gives rise to hallucinations and delusions. Using illusion tests, sensory stimuli can be manipulated and perception measured in an objective manner. Looking into temporal and spatial windows in the visual-visual and visual-auditory sensory modalities, 26 healthy participants tested the Three Flash Illusion test (3-FI), Sound Induced Flash Illusion test (SIFI), Visually Induced Flash Illusion test (VIFI), Phantom Word Illusion (PWI) and McGurk effect on one day given DEX and one day given placebo. **Aims:** To determine levels of DEX in saliva samples taken during the testing days and correlate those levels closest in time to the illusion tests with performance on illusion test. **Methods:** Liquid-liquid extraction and high performance liquid chromatography was used to determine the levels of DEX in saliva. Raw data was analysed primarily with principal components analysis. **Results:** For those significant differences in performance on illusion tests between DEX and placebo days, levels of DEX in saliva correlated positively only with performance on VIFI and PWI tests and not in the McGurk illusion. **Conclusions:** DEX-levels in saliva showed in our study to be an unreliable predictor of performance on the illusion tests. However, more studies with greater sample sizes are necessary to draw any final conclusions.

**Key words**: Dexamphetamine, saliva, temporal window, McGurk, phantom word, flash illusion

## 1. BACKGROUND

### 1.1 Introduction to schizophrenia and the dopamine hypothesis

Schizophrenia is a psychiatric disorder, according to the diagnostic criteria of the DSM-V, characterized by symptoms including delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behaviour and negative symptoms (2). Negative symptoms are diminished emotional expression, avolition (inability to motivate oneself to meaningful activities), alogia (poverty to speak), asocial behaviour (reduced interest in social activities) and anhedonia (inability to feel pleasure from usually enjoyable activities) (2). Although the exact pathways to schizophrenia are not yet fully understood, the neurotransmitter DA has been shown to play a significant role in the pathogenesis of the disease (3). DA's role in schizophrenia is formulated in the "dopamine hypothesis", updated over the years. The dopamine hypothesis is reviewed in Howes (3) in which a revised dopamine hypothesis is presented, referred to as "version III". According to this version, they state that multiple factors; a combination of genes and environmental influences, contribute to the development of dopamine dysregulation which in turn is the "common pathway to psychosis in schizophrenia". The dopamine dysregulation manifests as elevated pre-synaptic striatal dopamine availability and striatal synaptic dopamine release. Howes argues also that because anti-psychotic drugs blocking dopamine is efficient treatment for any psychosis, regardless of original diagnosis, dopamine hyperactivity is associated with psychosis in general rather than schizophrenia alone. Hallucinations and delusions are major characteristics of psychosis. Hallucinations are perceptions of reality without actual present stimuli (2, 4), for example hearing voices when there are none(2). Delusions are fixed beliefs that persist by the sufferer even though conflicting evidence is present (2). Even though DA has been associated with these symptoms, the mechanisms to how DA gives rise to them is, however, still not fully understood. One suggested mechanism for both delusions and hallucinations is that out of context dopamine-firing causes addition of salience to irrelevant stimuli. Paying attention to irrelevant stimuli over time would further explain development of delusions and hallucinations (5). Another proposed theory is that dopamine leads to impaired integration of prior beliefs and current sensory information (6). Another suggested mechanism through which dopamine acts is widening of temporal and spatial windows for different sensory stimuli to bind (7). While proposed separately, it may well be that all three mechanisms occur and are inter-related.

## 1.2 The amphetamine model

One problem studying individuals with schizophrenia is treatment with antipsychotics, a possibly confounding factor difficult to control for. To avoid this, recent research has been done on healthy individuals, given drugs that elevate DA levels in the brain (7, 8). Giving amphetamine in high doses will induce psychosis similar to that found in schizophrenia (9). Amphetamine challenges can thus be used as a model to investigate the role of dopamine in the pathogenesis of the disease and foremost psychosis. DEX is chemically similar to the neurotransmitters dopamine, noradrenalin and serotonin making it a competitive substrate to their reuptake transporters (DAT, NET and SERT) from the synapse back to the nerve terminal. Within the cell, DEX also prevents the transport of the neurotransmitters into their storage vesicles (through binding to VMAT2). The net result is a reversal of the direction of the catecholamine (and to a lesser extent, the serotonin) transporters, actively pumping monoamines out into the synaptic cleft, independent of the neuron electrical activity (10).

## 1.3 Illusions and its importance in investigation of pathology in psychosis

Illusions are, in contrast to hallucinations, misperceptions of stimuli that are present in the environment and occur in "normal" sensory processing (4). Illusion tests are thus a valuable method to investigate perception of sensory stimuli. Illusion tests have the potential to investigate whether individuals with schizophrenia and healthy individuals administered DEX differ in perceptions of sensory stimuli compared to healthy controls. If both individuals with schizophrenia and participants administered DEX differ from controls in their experiences of illusions in a similar manner, this indicates that it is likely that the patients' experiences are due to the increase in DA release, and not due to the antipsychotic or other treatments or characteristics that they may have.

Temporal window (as introduced previously) is one factor that can be manipulated in illusion tests. Previous research from this lab has particularly been interested in the idea of a widened temporal binding window as a possible mechanism of psychosis. This theory is based on observations from the study by Albrecht et al (2011) (7) on the rubber hand illusion. In the study, DEX increased participants' ratings of a rubber hand as being their own when a paintbrush was stroked *asynchronously* on the rubber hand (in view) and participants' own hand (out of view). In the placebo group, however, the illusion strength was weakened in the asynchronous condition. A widened temporal window as an effect of increased DA firing would allow for stimuli that otherwise would be excluded in perception processing, to

temporally be allowed into the processing. Using this as an explanation of the results, healthy controls would thus be able to tell that the touch from the paintbrush and the visual cue, temporally incoherent, come from two different sources. Individuals with DEX, on the other hand, with the suggested widened temporal window would allow the two temporally incoherent stimuli to be perceived as coming from one single source.

## *1.4 Flash illusions*

Previous studies have shown that at a certain interstimulus interval, ISI, between two flashes, the two flashes can be perceived as three (11). Bowen (11) showed that this occurs at approximately ISI 100 ms in healthy participants. Norton (12) confirmed this finding, and found further that individuals with schizophrenia tend to get the illusion at longer ISI's (130 ms), proposing a widened temporal window for the integration of the visual stimuli. Shams (13) showed that auditory stimuli can also induce a phantom flash in healthy participants. The study showed that as only one target flash is shown, healthy participants perceive more than one flash when two auditory beeps are played in close proximity to the flash. Chatterjee (14) investigated a flash illusion phenomenon with spatially incongruent flashes in healthy participants. The study found that a single target flash in a target position can be perceived as more flashes if there are confusing peripheral flashes, spatially separated from the target flash. The more inducing flashes, the more phantom flashes perceived in the target location. The study also showed that the closer the inducer flash is to the target flash, the more likely it is for participants to perceive a phantom flash. This study thus investigated the spatial binding window.

Suggesting that DA increases the temporal and spatial binding windows it could be expected to see greater illusion strength at greater ISI in individuals administered DEX and when the inducing flashes are further away from the target flash, compared to individuals administered placebo. A study by Jha (15) investigated individuals administered DEX and placebo in a cross-over design in similar flash illusion tests, and found the outer boundary of the temporal window to be around 200 ms, however the same for both DEX and placebo conditions. There were, on the other hand, insufficient ISIs near the peak effect, so it could not be determined if DEX shifted the peak to a longer ISI, as the temporal binding window hypothesis would suggest.

## 1.5 The McGurk effect

The McGurk effect involves the audio-visual sensory modalities. It is a misperception of incongruent visual and auditory stimuli first described by McGurk and MacDonald in 1976 (16). They found that syllables said by an actor cut together with a visual movie by the actor pronouncing an incongruent syllable may be perceived by healthy participants as a fusion between the two syllables, as the syllable heard or, to a lesser extent, the syllable seen. That is, if the actor articulated with the lips "ga", but the audio-recording played "ba", the responses could be either the lip reading "ga", the heard "ba", a chimeric of the two, "da", or (for the visual-auditory combination ba-ga) a combination "bga". The relative frequencies of the different kinds of responses have been reported to be 77.9% (auditory), 18.9% (chimeric), 2.2% (visual) and 1% (combination) (17).

Individuals with schizophrenia who have had electroencephalograms while listening to repeated simple auditory stimuli (beeps) or watching repeated simple visual stimuli (light flashes) have been shown to have consistent reductions in amplitudes and peak latency of auditory P300, but not so consistent of visual P300, an average positive brain potential with an onset of approximately 300 ms after auditory stimulus onset, and assumed to be evoked by the stimulus, as an indicator of attention and working memory towards that stimulus (18). In the study of Albrecht (8), healthy volunteers given 0,45 mg/kg (PO) dexamphetamine showed reduction in amplitude of P300 after auditory stimuli, but no reduction in P300 after visual stimuli. This suggests that dopamine hyperactivity might lead to a decreased attention of auditory stimuli, letting visual cues have a greater influence on perception than auditory cues. Taking this theory in to the context of the McGurk illusion, it can be hypothesised that individuals would increase their responses of "seen" and "chimeric" ("fused") syllables and reduce their responses of "heard" syllables after administration of DEX.

## 1.6 Phantom word illusions

The phantom word illusion engages the auditory-modality in which a word played on repeat makes the listener perceive, after a certain time of repetitions, words other than the one played on repeat. This illusion was originally developed from the Octave Illusion by Deutsch in 1974 (19) in which a high-pitch tone was played in one ear and a low-pitch tone in the other ear simultaneously, but alternating between right and left ear. Right handed people perceived in general only a low tone in the left ear and a high tone in the right ear. In an updated version of the illusion, in which the tones were replaced with the words "high" and "low", an English speaking population reported hearing other words than "high" and "low",

for example "buy-loan" and "long-time" (20). As introduced previously, one hypothesis of hallucinations in schizophrenia is that they are due to impaired attentional salience to the relevant cues and increased salience of irrelevant cues, leading to perceptions of objects that are not there (5). Applying this theory to the phantom word illusion in which novel words are heard that are not spoken, this could be explained as being misperceptions of the actual auditory stimuli present. We suggest that the primary cues the nervous system uses to determine relevance are the temporal and spatial coincidence, along with the stimulus properties that increase salience (e.g. intensity, novelty and past history of prediction of motivationally significant events). Arguing that DA increases the temporal window it is thus reasonable to expect that people treated with DEX will hear more words and hear them sooner (i.e. with a shorter latency) when there is an increase in the interstimulus interval between the sounds in the right and left ears. This prediction was, however, not validated in a pilot study of the phantom word illusion in people given 0,45 mg/kg DEX, PO, with only one ISI between the sounds in each ear (21). Expanding the study to test the illusion with different and greater ISI's would thus allow for investigation of the limits of the temporal window, and whether there is a widened temporal window in individuals administered DEX compared to individuals administered placebo.

### 1.7 Levels of dexamphetamine in saliva

There are previous research on quantification of DEX in plasma (22) and how it correlates to subjective and physiological measures (23-25). How DEX concentrations in plasma correlates to performance on illusion tests has, however, to the author's knowledge not been investigated. Rossini (26) worked out a method of how to detect and quantify DEX in saliva using reversed phase high performance liquid chromatography (HPLC) and correlate to performance on two illusion tests. Rossini worked out the method based on the study by Kristensen et al. (27) in which fluvoxamine was analysed in plasma and breast milk, also with HPLC. Levels of DEX in saliva as a predictor of performance on illusion tests is thus a relatively unexplored area. Further studies are needed to confirm the findings of Rossini; how levels of DEX in saliva varies also with physiological and psychological measures.

### 2. AIMS

In the studies of Hourani (1) and Lloyd (28), different versions of the flash,- Phantom word-, and McGurk illusion tests described previously (12-14, 16, 20) were tested on healthy participants; one day they were administered placebo and one day DEX. Their aims were to

investigate the effect of DEX on strength of illusions and differences in temporal- and spatial windows. Details of the individual illusion tests are described in the methods-section. The setup of this study was firstly to determine levels of DEX in consequtive saliva samples taken during the days of testing. By using the data from the illusion tests with significant differences in illusion scores between DEX and placebo days, this study aimed further to determine:

- The day-course concentrations of DEX in saliva.
- The correlation between:
  - DEX-levels in saliva and physiological measures.
  - DEX-levels in saliva and performance on illusion tests.
  - Rate of change of DEX-levels in saliva and performance on illusion tests.

## 3. ETHICAL CONSIDERATIONS

The study of Hourani (1) and Lloyd (28) was approved by the Human Ethics Office of Research Enterprise, approval # RA/4/1/7557. This author's contribution with analysing DEX-levels in saliva was done with de-identified saliva samples.

## 4. METHODS
### 4.1 General methods

The general procedures of this study was developed, and carried out, by Hourani (1) and Lloyd (28). 26 participants (14 women, 12 men) were recruited to the study through word-by-mouth, posters in the pharmacological building at the University of Western Australia and promotion on lectures held by Prof Mathew Martin-Iverson. Participants were aged 18-59 years with a mean weight of 71 kg (SD 12 kg). All participants provided written, informed consent and had a physical and psychiatric evaluation by a psychiatrist previous to the experiments to determine eligibility to participate. They were not economically compensated, but offered lunch on both testing days. Exclusion criteria were ages >59 and <18 (22.38 ±4.47) years, previous psychiatric, neurologic or cardiovascular disorders and regular prescribed medication. For a more detailed list, see appendix 1. On the day of testing participants were asked not to have taken any drugs within 24 hours, including prescription medications (except oral contraceptives and acne medication), coffee and nicotine. This was a double-blind, placebo -controlled, balanced, crossover study. Participants were pseudo-randomized to an identification number and to their first treatment, placebo or DEX, having the one not previously taken on the next session with at least 7 days apart. Participants were

given 0.45 mg/kg of DEX, mean dose 31.6 mg (SD 5.75 mg) of capsules with dexamphetamine-sulphate or placebo, placebo capsules were of the same number and of identical appearance but contained glucose. The clinical dose for treatment of Attention Deficit Hyperactivity Disorder (ADHD) for an adult ranges between 20-70 mg/24 hours (29).

Triplicate measurements of blood pressure and heart rate and duplicate measurements of body temperature were taken prior to ("0 min"), and at 80, 120, 210 and 270 minutes after administration of treatment. The physical measurements were done by the same investigator (not carrying out the illusion-tests because of the parameters giving away the condition). Saliva samples were collected 5 times prior to treatment and at 70, 130, 180 and 225 minutes post treatment. For detailed, summarised schedule from testing day, see appendix 2. Participants were offered a strawberry-flavoured gum (previously shown not to change saliva pH (30)) to chew and throw away 3 minutes prior to saliva collection, if their mouth was dry. Participants were asked to spit at least 1 ml per sample. Saliva-samples were stored in a freezer kept at -80°C.

Before administrating drug or placebo, participants filled in the Amphetamine Mood Questionnaire (AMQ) to assess subjective amphetamine effects as a baseline. The AMQ was also completed another four times during the day post-treatment approximately at the same times as saliva-collection. Three different psychiatric scales were also used to assess psychosis-proneness during the testing days; the Brief Psychiatric Rating Scale (BPRS) assessed at 90 minutes' post treatment (previously shown to be the peak of subjective experiences (15)), the Scale for the Assessment of Positive Symptoms (SAPS) and Magical Ideation Scale (MIS). The BPRS and SAPS were assessed by the same examiner in all trials.

### *4.2 Illusion tests*

*Flash illusion tests*

Three different flash illusion tests were used by Hourani (1). Common for all three was that participants were instructed to focus on a cross on a screen and told that a number of 1, 2, 3 or more flashes would appear in the sample place as the cross had been after pressing any key (target position). In a Three Flash Illusion test (3-FI), similar to that of Norton (12), the temporal window was investigated. Two spatially coincident flashes were displayed at different interstimulus intervals after each other, participants asked to count the number of flashes perceived. The independent variable was time between the two flashes, also called interstimulus interval (ISI) and the dependent variable was number of perceived flashes, three being the illusion (see figure 1). In a Sound Induced Flash Illusion (SIFI), similar to that of

Shams (13), two auditory beeps were played after a single target flash. Again, the independent variable was the ISI, here time between the two beeps, and the dependent variable number of flashes perceived, investigating temporal window in audio-vision (see figure 2). In a Visually Induced Flash Illusion (VIFI), similar to that of Chatterjee (14), both spatial and temporal windows in vision were investigated. Two irrelevant, "inducing", flashes, spatially non-coincident from a target flash were displayed. There were two independent variables:

- Position of the inducing flashes to investigate the spatial window. Positions varied between 3, 6, 9 and 12° eccentricities from target- to inducer flash.
- Time between inducer flashes, ISI, investigating the temporal window. ISI's varied between 25, 42, 50, 75 and 100 ms.

See figure 3 for illustration.
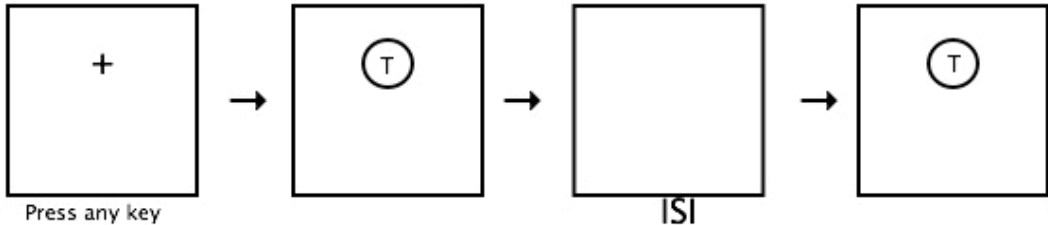


*Figure 1: Illustration of events on screen in the three flash illusion test (3-FI) in study by Hourani (1). + = focus point, T= target flash, ISI = interstimulus interval.*



*Figure 2: Illustration of events on screen in sound induced flash illusion (SIFI) test in study by Hourani (1). + = focus point, T= target flash, speaker = sound, ISI = Interstimulus interval.*
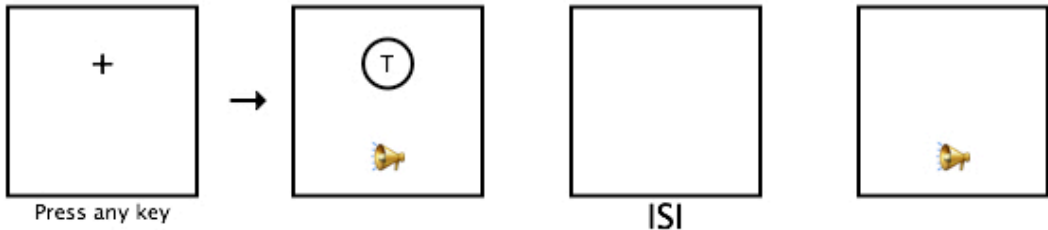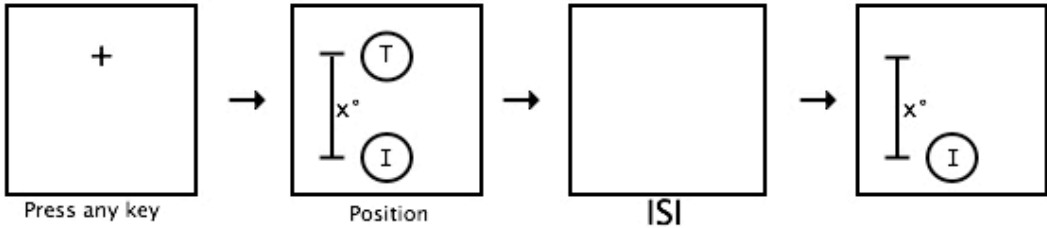


*Figure 3: Illustration of events on screen in visually induced flash illusion (VIFI) in study by Hourani (1). + = focus point and target position, T = target flash, I = irrelevant flash, ISI = Interstimulus interval, X° = distance between target and irrelevant flash.*

*The phantom word illusion*

Lloyd (28) used a version of the phantom word illusion test, similar to that of Deutsch (20). Participants were wearing headphones with an auditory tape playing a two-syllable word on repeat. Two different words were used in separate trials being "harvey" and "highlow". The word was first played in one ear, followed by the same word being played in the next ear, repeating over and over again. The time between the play in the different ears was manipulated with 5 different interstimulus intervals, ISI's, of 220, 440, 660, 880, 1 100 ms. For example, for ISI 220 "highlow" was played in the right ear, followed by silence for 220 ms before "highlow" playing in the left ear, followed by another silence of 220 ms before again playing "highlow" in the right ear. The "harvey"-track was always in the first trial of each new ISI, followed by the "highlow" track with the same ISI-condition, moving from the lowest ISI to the highest. Instructions to participants were to press a button on a joystick when they heard a new word other than the original word, and say it out loud. The independent variable was the interstimulus interval (ISI). The dependent variables were how many new words that were perceived other than "harvey" or "highlow" (count) and the time from start of trial to the perception of the first new word (latency).

*The McGurk illusion*

Lloyd (28) used a version of the McGurk illusion similar to that of McGurk (16) with both a female and a male actor, manipulating also the time between visual stimuli to auditory stimuli (asynchronous conditions), in order to investigate the temporal window in visual-auditory modalities. Videotapes of an actor's face articulating syllables was cut with different auditory stimuli; the same as, or different to, the syllable actually being articulated. The 8 different syllable combinations can be found in table 1. The room was dimly lit and participants had headphones on. Each of the different conditions included a synchronous- (ISI = 0) pair and asynchronous pairs with ISI's of 400, 500, 600, 700, 800, 900 and 1000 ms. Participants watched each tape three times. The dependent variables were syllable perceived, the confidence of the perception and differences depending on sex of actor. See figure 4 for illustration.

*Table 1: Visual and audio stimuli in the McGurk illusion. Control-conditions in italic.*

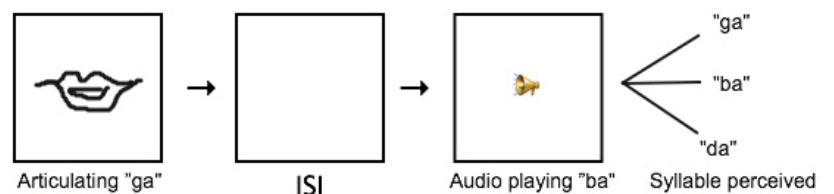| Visual | Audio |
|--------|-------|
| *ba* | *ba* |
| *fa* | *fa* |
| ba | fa |
| ga | ba |
| ba | ga |
| pa | ka |
| fa | ba |
| ka | pa |



*Figure 4: Illustration of events on screen in the McGurk illusion test with examples of the different possible responses: the articulated, the seen or a chimer of the two syllables. ISI = Interstimulus interval.*

### 4.3 Liquid-liquid extraction and reversed phase High Performance Liquid Chromatography (HPLC)

To analyse levels of DEX in saliva, liquid-liquid extraction and reversed phase high performance liquid chromatography (HPLC) was used based on methods from Rossini (26) with modifications especially regarding the HPLC-method.

*Liquid-liquid extraction*

Saliva samples of approximately 1 ml were injected with a known, set, concentration of an internal standard: 3,4-Methylenedioxymethamphetamine (MDMA). Adding NaOH to the sample deprotonated DEX, making it more soluble in organic solvents. Extraction was first done in hexane/1%isoamyl alcohol and then back titration with hydrochloric acid was carried out, concentrating the drugs to a volume of 150 μl, ready to be used in the HPLC-autosampler, a volume seen in Rossini (26) to give peaks of enough size for detection and integration. For detailed extraction-protocol, see appendix 3.

*HPLC*

In contrast to the work of Rossini (26), this study used a Waters 717 plus autosampler, Waters 1525 binary HPLC pump and waters 2487 dual λ absorbance detector. The instrument method settings were, however, the same as used by Rossini (26): The mobile phase was a combination of 90% phosphate buffer and 10% acetonitrile, and a gradient analysis was used changing acetonitrile from 10% to 55% in 10 minutes with a flow of 0.5 ml/min. Gradient analysis was used in order to be able to get enough separation of DEX and MDMA peaks in the chromatogram. A complete run in the HPLC-machine took 18 minutes. The column was also the same: Phenomenex Gemini NX: 150 mm x 4.6 mm with 5 μm particle size and guard column 10x2.1 mm 5μm Hypersil BDS C18. Detection wavelength was set to 216 nm. Integration of peaks of DEX and MDMA was done with Empower® software.

*Identification method*

To be able to identify the peaks of DEX and MDMA, standards of different DEX concentrations and same MDMA concentration in phosphate buffer (see appendix 4) were run. Firstly, we identified the two peaks as either DEX and MDMA based on their relative areas to each other. Next, by running standards of DEX and MDMA before each new HPLC

session of participants' samples, retention times of DEX and MDMA could be identified and compared to the peaks in participants' samples.

### 4.4 Calibration curve

To determine the absolute rather than relative DEX-concentrations in saliva from participants, a calibration curve was necessary. This was done by using saliva samples from healthy volunteers (not taking medication). When running them through the extraction process, in addition to the set concentration of internal standard of MDMA, they were "spiked" with different concentrations of DEX. Because of difficulties measuring volumes of saliva, saliva samples of approximately 1 ml were pre-weighed before being spiked and going through extraction giving the concentrations of DEX and MDMA in saliva in units of ng/g. The different concentrations of DEX were prepared through dissolving 10 mg of solid DEX in 100 ml of phosphate buffer, creating a stock solution (c=100 000 ng/ml). From the stock solution DEX concentrations of 10 000, 5 000, 2 500, 1 250, 625, 400 and 200 ng/ml were prepared. The MDMA solution was prepared from 10 mg of solid MDMA and dissolved in 100 ml of phosphate buffer creating a stock solution (c=100 000 ng/ml). 3 ml of the stock solution was then added to 100 ml of phosphate buffer (c= 3000 ng/ml). Volumes of the different DEX concentrations and MDMA (c=3000ng/ml) to saliva were 100μl. Each of the 7 different concentrations of DEX with the set concentration of MDMA were run through the extraction process twice a day (once in the morning, once in the afternoon) two days in a row. This was done to account for inter- and intra-day variability. Samples were stored in Eppendorf-tubes in a freezer at -80°C before being run through HPLC. When run in the HPLC, samples of 100 μl were injected in to 700 μL Waters polypropylene snap neck total recovery vials and the autosampler was set to two injections (duplicates) of 30 μl volumes each. Average ratios from the different DEX-concentrations (giving an average from 8 values for each concentration) were calculated. The final calibration curve was established by plotting the average ratios of DEX/MDMA vs the respective concentration of DEX (ng/g saliva).

### 4.5 Determination of DEX-levels in saliva from participants

Saliva samples from DEX-days went through the extraction protocol and HPLC procedure as described above. Duplicates were run for each sample. The peaks of DEX and MDMA were identified and integrated. Ratios of DEX/MDMA areas were calculated. Because of

duplicate runs, an average ratio based on two values for each saliva sample were calculated. The absolute DEX concentration was calculated with the equation of best line of fit from the calibration curve.

### *4.6 Statistical Analyses*

Statistical analyses were carried out with R statistics using RStudio Graphical User Interface.

#### *Principal Components Analysis (PCA)*

For most of our data analysis we used principal components analysis (PCA). PCA can only use one value from each variable, a requirement met by subtracting values from DEX days from values from placebo days. The PCA transforms all data to a common scale and then looks for independent sources of covariation. It constructs a number of components that are orthogonal (statistically independent) in which the variables in a component co-vary, called "loadings" on the component. Variables in a component co-vary positively or inversely to each other. Variables with loadings of the same sign (positive or negative) have a positive correlation. A component can be thought of as a subpopulation within the population that share the same covariation of variables loaded onto the component. The number of components depend on the number of variables used in the analysis; the more variables the more components. Component 1 accounts for the greatest variance of the independent components, and the variance falls with the number of components in the same order. Cut-off for significant covariation was set to 0.3 in our study.

#### *Day-course variations of DEX*

To see the variation of DEX-levels in saliva over testing day, average values of DEX-concentrations were calculated from all participants and plotted against the time of saliva-collection (as minutes post treatment). This was done with RStudio .99.903 with the packages plyr 1.84, dply 0.50, methods 3.31, stats 3.31, graphics 3.31, and Rstatistics package 3.31, all for Mac OS 10.10.5.

#### *Physiological measures*

DEX-concentrations form saliva sample 3 (taken 130 min post treatment), systolic- and diastolic blood pressure and heart rate (taken 120 min post treatment) were analysed with PCA.

*Visually induced flash illusion, VIFI*

The results from the study of Hourani (1) showed significant differences in performance on illusion tests between placebo and DEX in the 3-FI or SIFI. In the VIFI there were significant difference in number of perceived flashes between placebo and DEX days at position 6° (all ISI's), and a significant difference between placebo and DEX at ISI 50 ms (all positions). We used PCA to analyse the variables: Difference in individual scores (DEX minus placebo) from only these conditions, DEX-concentrations from the 3<sup>rd</sup> saliva sample (closest in time to the illusion), difference in scores on AMP and AMW and the sum of the difference (DEX minus placebo) in scores of BPRS, MIS and SAPS (abbreviated psychosis). The same variables except changing DEX-levels in saliva with rate of change of DEX in saliva were also analysed with PCA. The rate of change of DEX in saliva was calculated as the individual peak DEX-level divided by the exact time of when that saliva-sample had been collected.

*McGurk illusion*

In Lloyd (28) there was no overall (male- and female actors) significant difference between DEX- and placebo treatment in proportions of audio-, visual- or chimer (of the two) syllables perceived, although there was a trend towards more chimeric and visual responses. On closer examination of the female actor recordings, listening to the sound in the absence of the video, it appeared that she either failed to produce a consonant sound on some words, or else they were so quiet as to not be picked up. We therefore re-analysed the data, using only the data from the male actor using a kernel density permutation test with 1000 permutations (Rstatistics packages sm 2.2-5.4 and boot 1.3-18).

The present study also sought to investigate the effect of DEX saliva levels obtained at 225 min post-treatment (the time closest to the McGurk effect test) and the differences of the average scores from the asynchronous ISIs of 800, 900 and 1000 ms from the DEX treatment day minus those from the placebo treatment day.

*Phantom word illusion*

Lloyd (28) showed that for both count (number of words perceived other than highlow or harvey) and latency (time to the first new word perceived) the differences were greatest with highlow and greater ISI's. Therefore, scores only from highlow and ISI greater than 440 ms were used in the present study. The DEX-levels were from the 2<sup>nd</sup> saliva sample, collected approximately 70 minutes' post treatment, the phantom word-illusion being 75 minutes' post treatment. The scores on the illusion test from placebo days were subtracted from scores from

DEX days. The psychological scores from AMQ, BPRS, MIS and SAPS were treated the same way as in analysis of VIFI. PCA was used to investigate dose-relationships between DEX-levels in saliva and scores on illusion and psychological scales.

## 5. RESULTS

### 5.1 Standard samples

Running samples of the different DEX-concentrations (in buffer) in the HPLC gave two, well-defined peaks in the chromatograms. DEX was identified to be the peak of shortest retention time supported by the change in ratio of the areas of the two peaks looking at different concentrations of DEX and constant concentrations of MDMA. Figures 5 and 6 provide two examples of the chromatograms with standards of DEX concentration 200 ng/ml and MDMA concentration 3000 ng/ml (figure 5) and DEX concentration 5000 ng/ml and MDMA concentration 3000 ng/ml (figure 6).



*Figure 5: Chromatogram from high performance liquid chromatography (detection wavelength 216 nm) with DEX concentration 200 ng/ml and MDMA concentration 3000 ng/ml. Numbers on top of peaks indicates the time of detection. AU=absorbance unit.*
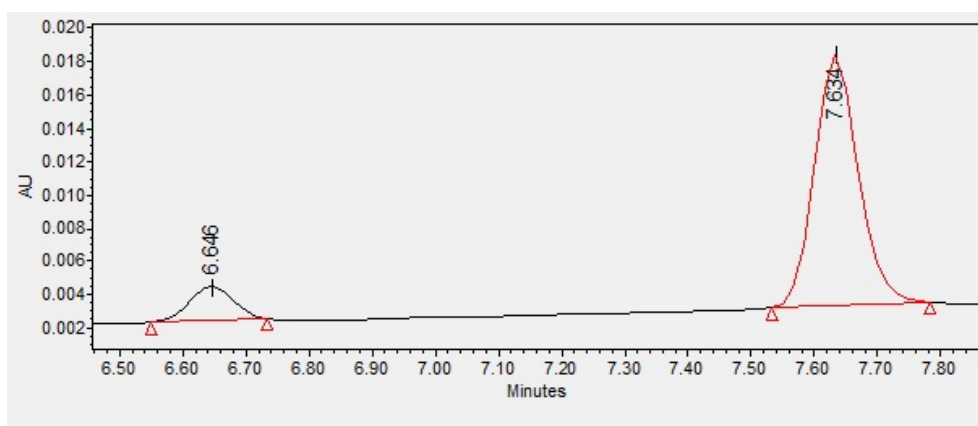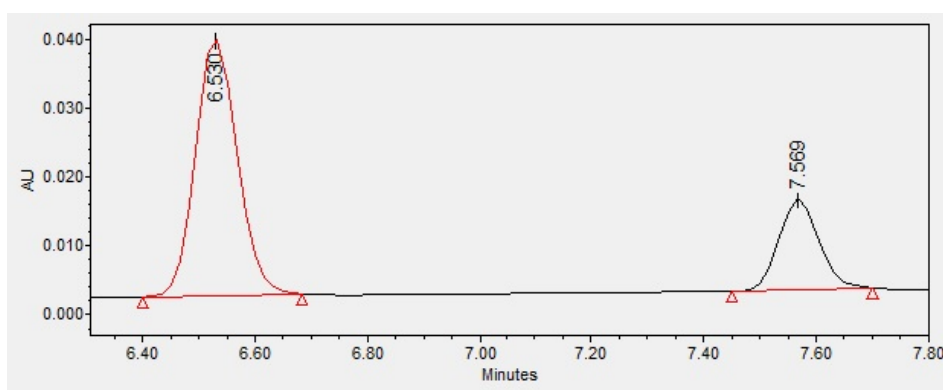


*Figure 6: Chromatogram from high performance liquid chromatography (detection wavelength 216 nm) with DEX concentration of 5000 ng/m and MDMA concentration 3000 ng/ml. Numbers on top of peaks indicates the time of detection. AU=absorbance unit.*

### 5.2 Calibration curve

Some samples were incorrectly handled during the extraction process: Samples with DEX concentrations 1250 ng/ml (2 values) and 2500 ng/ml (2 values) from day 2 and were excluded from analysis. Some samples gave inconclusive chromatograms due to failure of the HPLC-machine, making peaks impossible to integrate: Samples with DEX concentrations 2500 ng/ml (day 1, afternoon sample 2), 10000 ng/ml (day 2, morning sample 2) and 1250 ng/ml (day 2, afternoon sample 2) were also excluded. This resulted in a mean based on 5 values for 2500 and 1250, and 7 values for 10000. All other DEX concentrations were calculated means from 8 values. All different DEX/MDMA-ratios for each DEX-concentration were plotted in a graph with best line of fit. Thus calibration curve is shown in figure 7. The best line of fit has the equation: y=0.005x+0.1654.
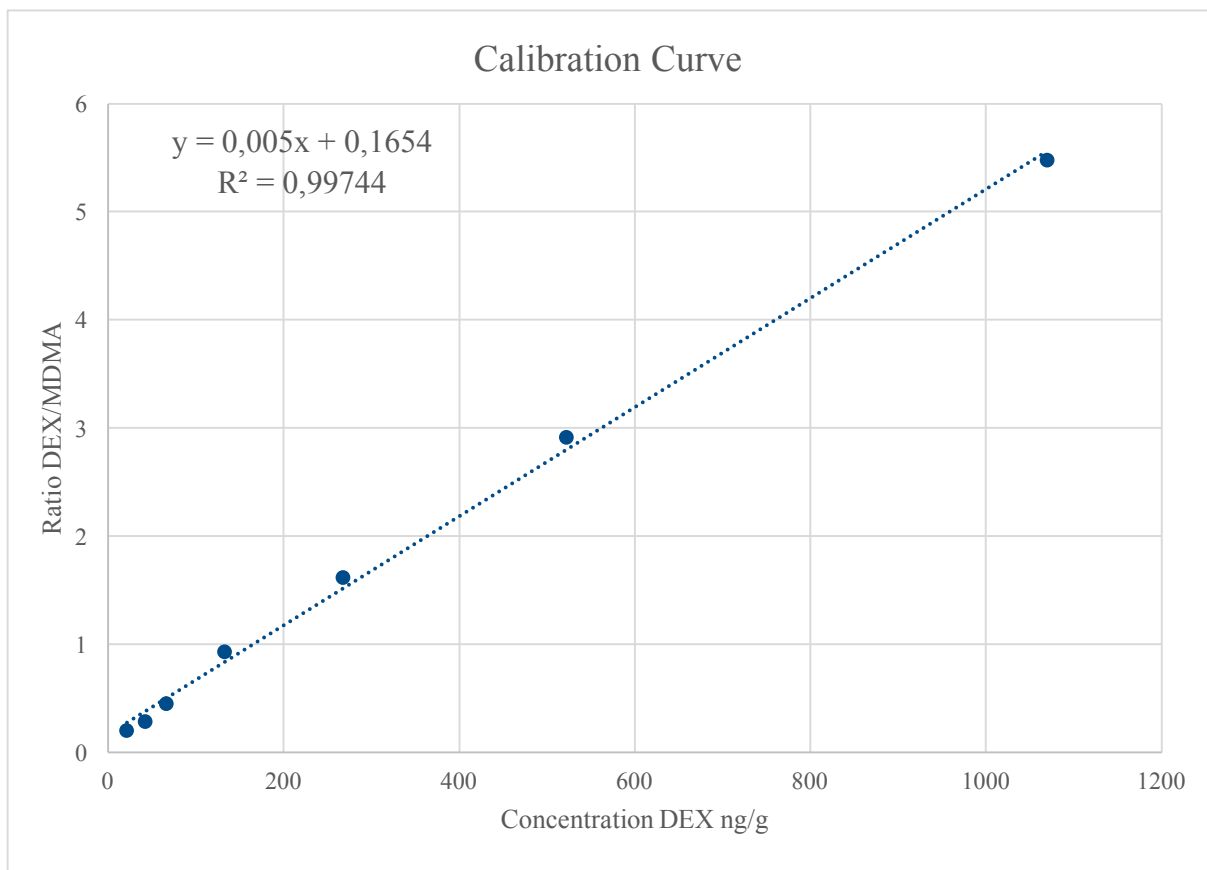


*Figure 7: Calibration curve plotting mean ratios of DEX/MDMA vs concentration of DEX and a best line of fit with the equation y=0.005x+0.1654 and $R^2$=0.99744.*

## 5.3 Variations in DEX concentrations and responses on AMP over day

Data from saliva samples from participant 522 and 519 had to be removed due to failure of the HPLC-machine and inability to read chromatograms giving n=24 for analysis. Mean concentrations of DEX in saliva were plotted with mean scores on the euphoric subscale of AMQ (AMP) (figure 8) and mean difference in scores on the anxiety subscale of AMQ (AMW) (figure 9) over day.
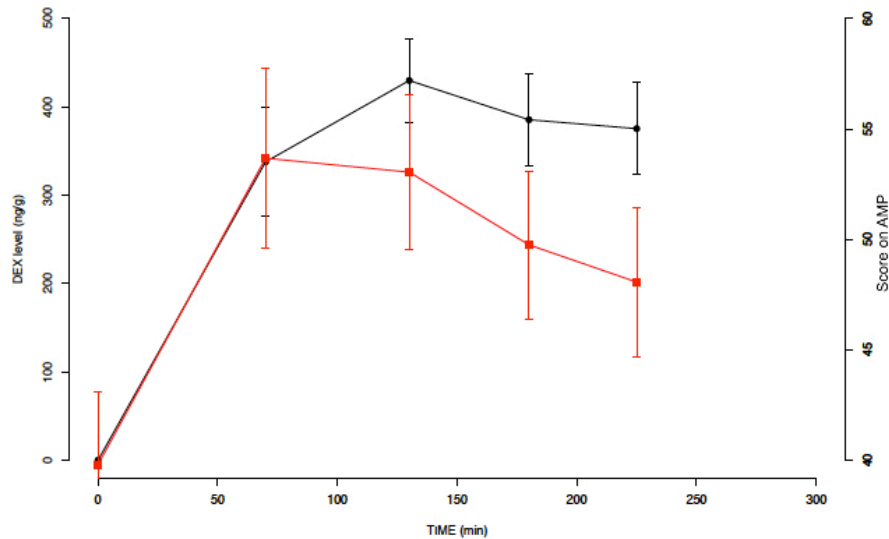


*Figure 8: Mean concentrations of DEX (±SEM) in saliva (left y-axis, black line) and mean scores on AMP-subscale (±SEM) (right y-axis, red line) vs approximate time post treatment. AMP=the euphoric subscale of the amphetamine mood questionnaire. Note that right y-axis starts at 40. Lines are drawn through the points. n=24.*
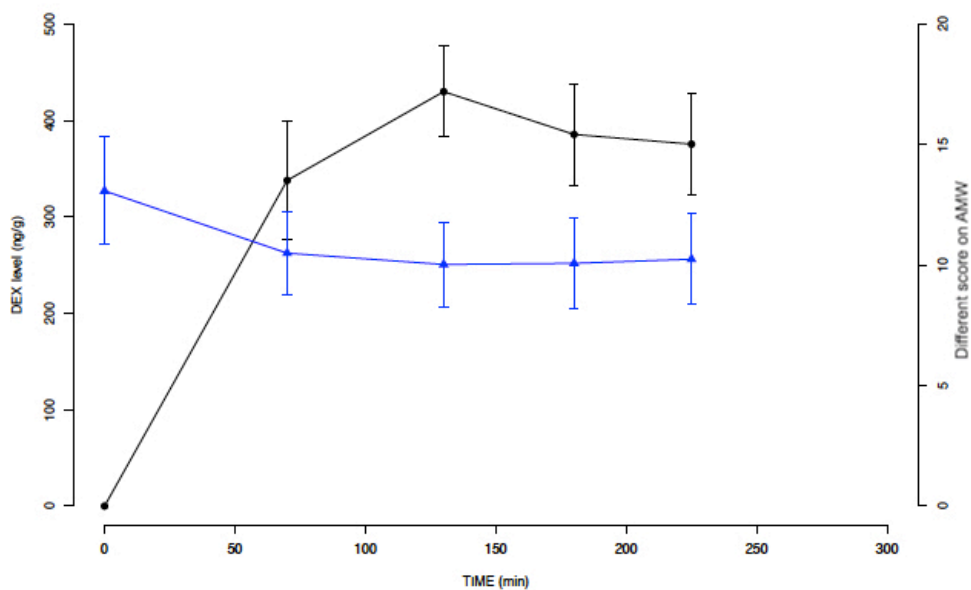


*Figure 9: Mean concentrations of DEX (±SEM) in saliva (left y-axis, black line) and mean difference in score (score from DEX day minus score from placebo day) on AMW-subscale (±SEM) (right y-axis, blue line) vs approximate time post treatment. AMW = the anxiety subscale of the amphetamine mood questionnaire. Lines are drawn through the points. n=24.*

## 5.4 Physiological measures

Lloyd (28) showed no significant main effect of temperature between placebo and DEX days why we decided not to include temperature in our analyses. Again, values from 522 and 519 were excluded giving n=24. PCA of heart rate, diastolic- and systolic blood pressure and concentration of DEX in saliva with cutoff 0.3 gave 4 components with loadings presented in table 2. Importance of components are presented in table 3. Figure 10 shows the PCA plot of components 1 and 2. DEX-level in saliva has a positive relationship with diastolic and systolic blood pressure in component 1 accounting for 37% of the variance. DEX-level in saliva has a positive relationship with heart rate and an inverse relationship with systolic blood pressure in component 2 accounting for 28% of the variance.

*Table 2: Loadings in principal components analysis with variables dias, sys, hr and Conc. Cut-off 0.3. Values from variables were taken approximately 130 min post treatment. n=24.*

|      | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 |
|------|---------|---------|---------|---------|
| Dias | -0.661  |         | 0.307   | 0.634   |
| Sys  | -0.670  | 0.304   |         | -0.656  |
| Hr   |         | -0.819  | 0.393   | -0.401  |
| Conc | -0.315  | -0.412  | -0.851  |         |

*Dias=Diastolic blood pressure, sys=systolic blood pressure, Hr=heart rate, Conc=concentration of DEX in saliva.*

*Table 3: Importance of components in principal components analysis of the variables dias, sys, hr and Conc (loadings can be seen in table 2). Values of the variables were taken approximately 130 min post treatment. Values in the table have been rounded off to three decimal places. n=24.*

|                        | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 |
|------------------------|---------|---------|---------|---------|
| Standard deviation     | 1.219   | 1.067   | 0.952   | 0.686   |
| Proportion of Variance | 0.371   | 0.284   | 0.227   | 0.118   |
| Cumulative proportion  | 0.371   | 0.656   | 0.882   | 1.000   |
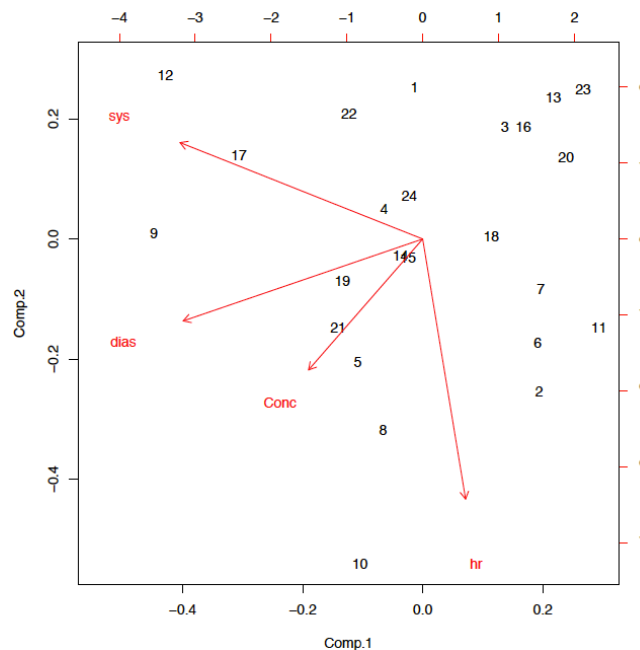


*Figure 10: Principal components analysis of physiological measures (hr=heart rate, dias=diastolic blood pressure, sys=systolic blood pressure) and concentration of DEX in saliva (Conc) approximately 130 min post treatment. n=24. DEX-level has a positive relationship with dias and sys in Comp.1 accounting for 37% of the variance. DEX-level has a positive relationship with hr and an inverse relationship with sys in Comp.2 accounting for 28% of the variance. Cut-off set to 0.3.*

## 5.5 Visually induced flash illusion, VIFI

Again, values from 522 and 519 were excluded (n=24). PCA of AMW, psychosis (sum of difference in scores of SAPS, BPRS and MIS), AMP, difference in response on VIFI (response) and concentration of DEX (Conc) with cutoff 0.3 gave 5 components loadings presented in table 4. Importance of components are presented in table 5. Figure 11 shows the PCA plot of components 1 and 2. DEX-levels in saliva have a positive relationship with psychosis and response and an inverse relationship with AMP in component 1 accounting for 32% of the variance. DEX-levels in saliva have a positive relationship with AMP, and an inverse relationship with response in component 2 accounting for 26% of the variance.

*Table 4: Loadings in principal components analysis with the variables amw, psychosis, amp, response and Conc. Values of all variables were values from DEX-day subtracted from placebo day. Values from the psychological measures were taken approximately 130 min post treatment. VIFI was 115 min post treatment. Cut-off 0.3. n=24.*

|          | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 | Comp. 5 |
|----------|---------|---------|---------|---------|---------|
| amw      |         | 0.748   |         |         | -0.596  |
| psychosis| -0.383  |         | 0.648   | -0.577  |         |
| amp      | 0.382   |         | 0.751   | 0.430   |         |
| Response | -0.463  | 0.455   |         | 0.688   | 0.314   |
| Conc     | -0.647  | -0.313  |         |         | -0.689  |

*Amw = anxiety subscale of amphetamine mood questionnaire (AMQ), amp = euphoric subscale of AMQ. Psychosis = sum of scores on BPRS (brief psychiatric rating scale), SAPS (subjective assessment of positive symptoms) and MIS (magical ideation scale) from DEX-day subtracted from placebo day. Response = score on VIFI (115 min post treatment), Conc=concentration of DEX in saliva from saliva sample taken 130 min post treatment.*

*Table 5: Importance of components in principal components analysis of the variables amw, psychosis, amp, response and Conc (loadings can be seen in table 4). Values of the variables were taken approximately 130 min post treatment. Values in the table have been rounded off to three decimal places. n=24.*

|                       | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 | Comp. 5 |
|-----------------------|---------|---------|---------|---------|---------|
| Standard deviation    | 1.256   | 1.132   | 0.985   | 0.856   | 0.661   |
| Proportion of Variance| 0.316   | 0.256   | 0.194   | 0.147   | 0.088   |
| Cumulative Proportion | 0.316   | 0.572   | 0.766   | 0.912   | 1.000   |
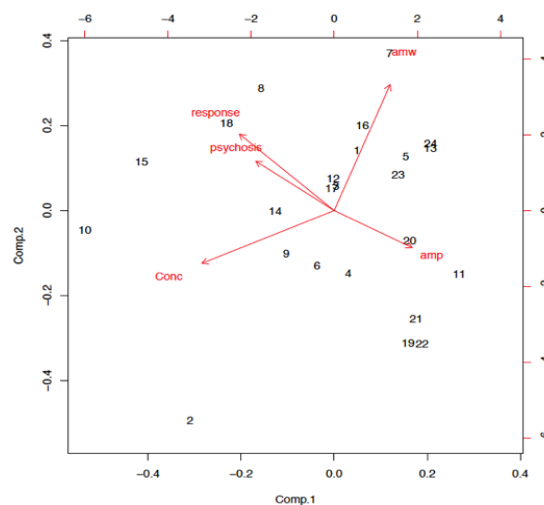


*Figure 11: PCA of psychological measures (amp=positive subscale of AMQ, amw=anxiety subscale of AMQ and psychosis=sum of score from BPRS, MIS and SAPS), concentration of DEX (Conc) approximately 130 min post treatment and scores on VIFI-test (response) at position 6° and ISI 50ms only. n=24. DEX-levels have a positive relationship with psychosis and response and an inverse relationship with amp in Comp.1 accounting for 32% of the variance. DEX-levels have a positive relationship with amp, and an inverse relationship with response in Comp.2 accounting for 26% of the variance. Cut-off set to 0.3. n=24.*

PCA of AMW, psychosis (sum of difference in scores of SAPS, BPRS and MIS), AMP, difference in response on VIFI (response) and rate of change of DEX in saliva (slope) with cutoff 0.3 gave 5 components with loadings presented in table 6. Importance of components are presented in table 7. Figure 12 shows the PCA plot of components 1 and 2. Response, slope and psychosis have a positive relationship in component 1 accounting for 33% of the variance. AMP and psychosis have a positive relationship and an inverse relationship with AMW in component 2 accounting for 20% of the variance.

*Table 6: Loadings in principal components analysis with the variables amw, amp, response, psychosis and slope. Values of variables were values from DEX-day sustracted from placebo day. Values from the psychological measures were taken approximately 130 min post treatment. VIFI was 115 min post treatment. Cut-off 0.3. n=24.*

|          | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 | Comp. 5 |
|----------|---------|---------|---------|---------|---------|
| amw      |         | 0.531   | -0.774  |         |         |
| amp      |         | -0.627  | -0.627  | 0.337   |         |
| response | 0.524   |         |         | 0.836   |         |
| psychosis| 0.507   | -0.498  |         |         | -0.669  |
| slope    | 0.567   |         |         | -0.329  | 0.715   |
| *amw = anxiety subscale of amphetamine mood questionnaire (AMQ), amp = euphoric subscale of AMQ. Psychosis = sum of scores on BPRS (brief psychiatric rating scale), SAPS (subjective assessment of positive symptoms) and MIS (magical ideation scale) from DEX-day subtracted from placebo day. Response = score on VIFI (115 min post treatment), slope = rate of change of DEX in saliva.* | | | | | |

*Table 7: Importance of components in principal components analysis of the variables amw, amp, response, psychosis and slope (loadings can be seen in table 6). Values of the variables were taken approximately 130 min post treatment. Values in the table have been rounded off to three decimal places. n=24.*

|                       | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 | Comp. 5 |
|-----------------------|---------|---------|---------|---------|---------|
| Standard Deviation    | 1.286   | 1.001   | 0.970   | 0.862   | 0.801   |
| Proportion of variance| 0.331   | 0.204   | 0.188   | 0.149   | 0.128   |
| Cumulative Proportion | 0.331   | 0.535   | 0.724   | 0.872   | 1.000   |
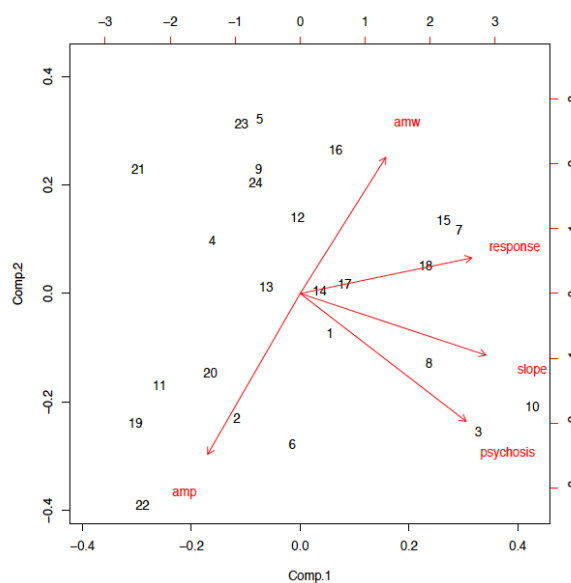


*Figure 12: Principal components analysis of amp (positive subscale of AMQ), amw (anxiety subscale of AMQ), psychosis (sum of score from BPRS, MIS and SAPS), rate of change of DEX-level in saliva (slope) and scores on VIFI-test (response) at position 6° and ISI 50ms only. n=24. Response, slope and psychosis have a positive relationship in Comp.1 accounting for 33% of the variance. Amp and psychosis have a positive relationship and an inverse relationship with amw in Comp.2 accounting for 20% of the variance. Cut-off set to 0.3.*

### 5.6 McGurk

Due to technical problems (7 participants) and failure of the HPLC-machine and integrating peaks (2 participants), only data from 17 participants could be analysed. Figure 13 shows a Kernel density comparison plot. It shows the distribution of auditory, chimeric and visual response-frequencies of DEX and placebo groups with male actor and ISI's >400 ms. Lines within the blue shaded area indicates distributions that do not differ between placebo and DEX days 95% of the times (null hypothesis is true). Lines outside of the blue shaded area, however, indicates a difference in distribution of the different responses between DEX and placebo days 95% of the times. The kernel density estimation seen in figure 13 shows a significant main difference in distribution of responses between DEX and placebo days.
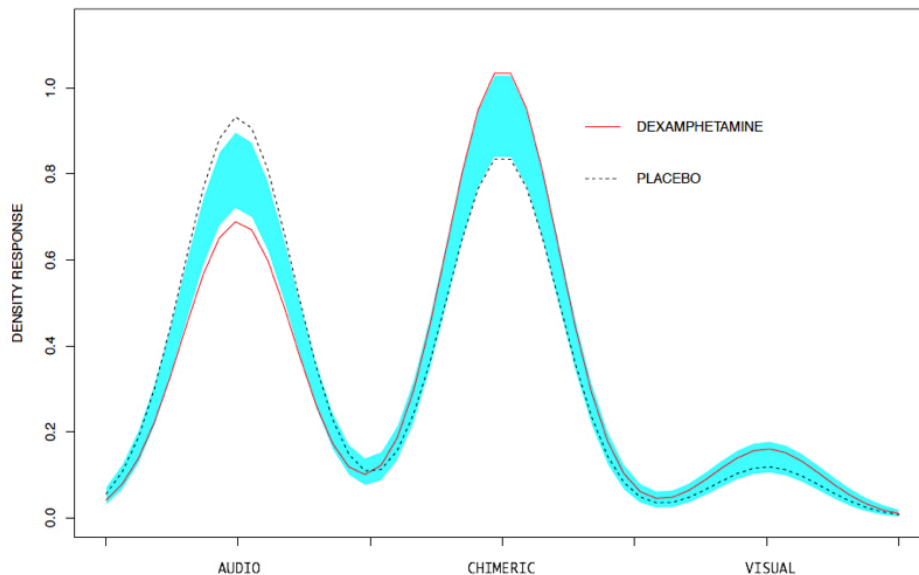


*Figure 13: Kernel density comparison plot of McGurk scores from male actor and ISI's >400 ms. The blue shaded area indicates the 95% confidence interval based on a bootstrap permutation test of equal distribution with 1000 samples. A permutation test indicates that the distribution of responses after DEX is significantly different from the distribution after placebo, p < 0.05, shifting responses from audio-dominated to chimeric responses, relative to placebo. n=17.*

PCA of difference in scores of auditory responses (difa), difference in scores of chimeric responses (difc) and concentration of DEX in saliva with cutoff 0.3 gave 3 components with loadings presented in table 8. Importance of components are presented in table 9. Figure 14 shows the PCA plot of components 1 and 2. DEX-concentration have a positive relationship with difa and an inverse relationship with difc in component 1 accounting for 68% of the variance.

*Table 8: Loadings in principal components analysis with the variables difa, difc and dex.sample5. Concentration of DEX approximately 225 min post treatment. McGurk 240 min post treatment. Cut-off 0.3. n=17.*

|             | Comp. 1 | Comp. 2 | Comp. 3 |
|-------------|---------|---------|---------|
| difa        | 0.668   | -0.219  | -0.711  |
| difc        | -0.661  | 0.265   | -0.702  |
| Dex.sample5 | 0.343   | 0.939   |         |
| *Difa = difference in scores in auditory responses between DEX and placebo day (DEX minus placebo), difc = difference in scores in chimeric responses between DEX and placebo day (DEX minus placebo), dex.sample5 = dex concentration in saliva sample 5, taken 225 minutes post treatment.* | | | |

*Table 9: Importance of components in principal components analysis of the variables difa, difc and dex-sample5 (loadings can be seen in table 8). Values in the table have been rounded off to three decimal places. n=17.*

|                        | Comp. 1 | Comp. 2 | Comp. 3 |
|------------------------|---------|---------|---------|
| Standard Deviation     | 1.427   | 0.929   | 0.316   |
| Proportion of variance | 0.680   | 0.288   | 0.033   |
| Cumulative Proportion  | 0.679   | 0.967   | 1.000   |
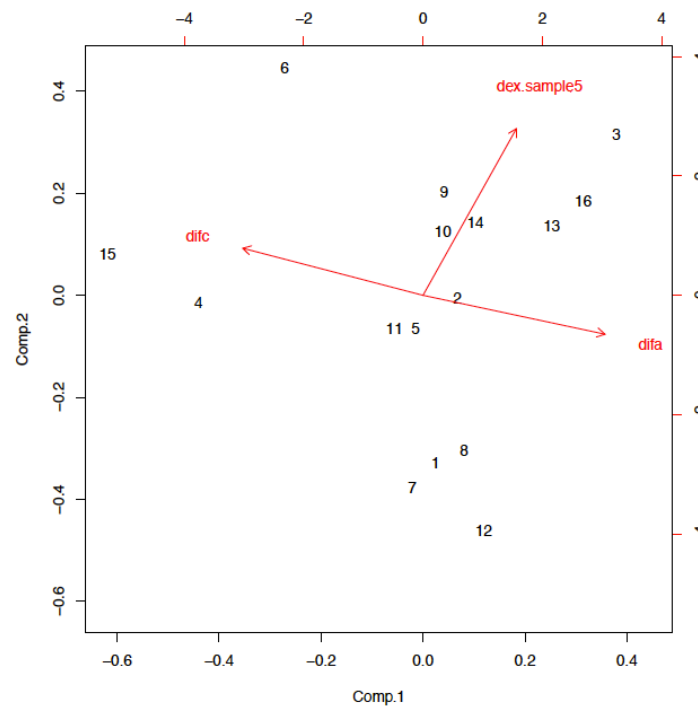


*Figure 14: Principal components analysis of difference in chimeric responses (difc), difference in auditory responses (difa) and DEX-concentration in saliva sample 5 (dex.sample5) closest in time to the illusion (approximately 225 min post treatment) (DEX minus placebo). DEX-concentration have a positive relationship with difa and an inverse relationship with difc in Comp. 1 accounting for 68% of the variance. Cut-off set to 0.3. n=17.*

## 5.7 Phantom word

Due to failure of the HPLC-machine and setup difficulties in the first session days of the phantom word illusion, only 14 participants were included in the data analyses. PCA of concentration of DEX in saliva (Conc), sum of difference in scores of MIS, SAPS and BPRS (Psychosis), number of new words perceived (Count) and time to first new word (Latency) with cutoff 0.3 gave 4 components with loadings presented in table 10. Importance of components are presented in table 11. Figure 15 shows the PCA plot of components 1 and 2. Conc has a positive relationship with psychosis and latency, and an inverse relationship between count in component 1 accounting for 59% of the variance. Conc have a positive relationship with psychosis and count, and an inverse relationship with latency in component 2 accounting for 21% of the variance.

*Table 10: Loadings in principal components analysis with the variables conc, psychosis, count and latency. Phantom word illusion test 75 min post treatment. Cut-off 0.3. n=14.*

|  | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 |
|---|---|---|---|---|
| Conc | -0.438 | 0.590 | -0.653 |  |
| Psychosis | -0.452 | 0.507 | 0.716 |  |
| Count | 0.552 | 0.424 |  | 0.688 |
| Latency | -0.546 | -0.464 |  | 0.683 |

*Conc = concentration of DEX in saliva sample 2 taken 70 min post treatment. Psychosis = sum of scores on BPRS (brief psychiatric rating scale), SAPS (subjective assessment of positive symptoms) and MIS (magical ideation scale) from DEX-day subtracted from placebo day. Count = Number of new words heard other than "highlow". Latency = Time to first new word other than "highlow".*

*Table 11: Importance of components in principal components analysis of the variables conc, psychosis, count and latency (loadings can be seen in table 10). Values in the table have been rounded off to three decimal places. n=14.*

|  | Comp. 1 | Comp. 2 | Comp. 3 | Comp. 4 |
|---|---|---|---|---|
| Standard Deviation | 1.534 | 0.916 | 0.763 | 0.474 |
| Proportion of variance | 0.589 | 0.210 | 0.145 | 0.056 |
| Cumulative Proportion | 0.589 | 0.798 | 0.945 | 1.000 |
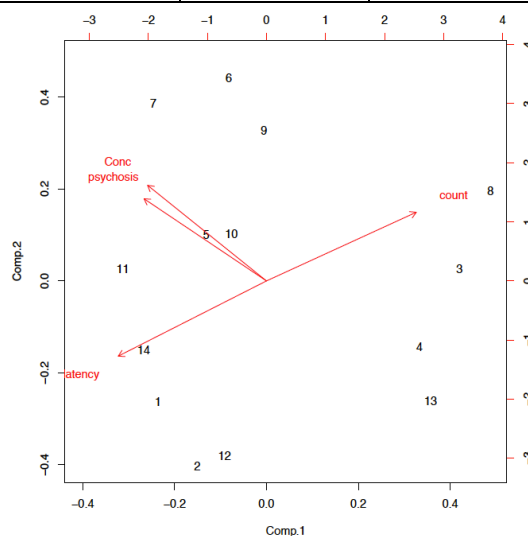


*Figure 15: Principal components analysis of concentration of DEX in saliva (Conc) (saliva sample 70 min post treatment, psychosis (sum of the difference score(DEX-placebo) from MIS, BPRS and SAPS), number of new words heard (count). Conc has a positive relationship with psychosis and latency, and an inverse relationship between count in Comp.1 accounting for 59% of the variance. Conc have a positive relationship with psychosis and count, and an inverse relationship with latency in Comp.2 accounting for 21% of the variance. Cut-off 0.3. n = 14.*

## 6. DISCUSSION

### 6.1 Variations in DEX concentrations in saliva and responses on AMQ over day

DEX-concentrations in saliva peaked on average in the 3$^{rd}$ saliva sample of the day, collected approximately 130 minutes post treatment. In the study of Rossini (26), this peak was found to be in the 4$^{th}$ saliva sample of the day, collected approximately 180 minutes post treatment. However, the error bars of the concentrations in the 3$^{rd}$ and 4$^{th}$ saliva samples in both this study and Rossini's are, however, overlapping, suggesting consistent results. The mean scores of the AMP-subscale increased with DEX (compared to baseline), having the peak approximately 70 minutes' post treatment. These findings are also consistent with previous studies showing that participants have an euphoric experience of DEX (23), and that this experiences is greatest 1-2 hours (23, 25, 31) after DEX-administration. Mean difference in scores (DEX minus placebo) of the AMW-subscale assessing withdrawal symptoms decreased slightly with DEX compared to baseline, and remained relatively constant throughout the test-day. In other words, anxiety was greater in patients on average on DEX-days compared to placebo days, however, the greatest difference was found before DEX had been administered. These findings could be explained by the fact that participants are more nervous at the start of the day, and that a majority of participants get the euphoric rather than the anxious experience from DEX, making the mean difference in scores on AMW-subscale not change (if anything decrease) after DEX-administration.

### 6.2 DEX-concentrations in saliva and physiological measures over day

The results from the PCA shown in figure 10 shows that one subpopulation (component 1) of participants have DEX-concentrations in saliva that co-vary positively with systolic and diastolic blood pressure at approximately 130 min post treatment. This is reasonable, as all these parameters have their peaks around this time. Why heart rate is not loaded onto this component could be explained by the fact that heart rate have a slower rise and has not yet made its deflection. In Ashgar et al. (23) heart rate peaked at 500 minutes post treatment at which the study ended and the time of deflection was never reached. Another subpopulation (figure 10, component 2) of participants have DEX-concentrations that vary positively with heart rate and inversely with systolic blood preassure. This likely corresponds to the subpopulation discussed by Smith (24) as early responders to oral DEX who have an earlier peak for DEX levels and a greater, early rise in heart rate. Systolic pressure tends to drop before diastolic (32), so early responders may have decreasing systolic blood pressure at 130 min while DEX-concentration and heart rate are still rising for this subpopulation.

Taken together, DEX-concentration had a positive relationship with blood preassure in and with heart rate, suggesting that dexamphetamine had a dose-response effect on measures of the sympathetic nervous system.

### 6.3 Visually induced flash illusion, VIFI

DEX-levels in saliva and illusion response loaded positively on to a component that accounted for the greatest variance in the data set (figure 11, component 1) indicating a possible dose-response relationship across individuals. Psychosis proneness also varied in the same direction in this component. In component 2, although accounting for less of the variance, anxiety scores co-varied in the same direction as response, and inversely with levels of DEX in saliva. That anxiety and DEX levels varies in opposite directions can be explained by the fact that most people get euphoric effects of DEX (thus, as DEX levels go up anxiety ratings go down). It is, however, difficult to explain why responses on illusion varies in the opposite direction as DEX concentration in this subpopulation as illusion strength was increased overall with DEX. For this subpopulation, DEX levels in saliva does not predict outcome on VIFI.

Rate of change of DEX-levels in saliva also had a positive relationship with response and psychosis-proneness in one subpopulation (figure 12, component 1). Rate of change of DEX-levels in saliva has previously shown to predict illusory response on another illusion-test (tactile funnelling) in the study by Rossini (26). In component 2 of the same PCA euphoric scores (AMP) logically co-varies positively with psychosis-proneness and inversely with scores on the anxiety subscale (AMW, assessing opposite measures).

Taking the results from the study by Hourani (1) in the context of temporal and spatial windows, illusion-strength increased with DEX, but the highest responses occurred at same ISI as for placebo. This means that there is no widening of the temporal window, as opposed to our predictions or what Norton found in schizophrenia (12). This matches the finding of Jha (15) with a somewhat different VIFI procedure, where no effect of DEX was observed on either the maximal ISI at which the illusion occurred (~200 ms). The increase in illusion-strength could be explained by attentional selectivity (salience of stimuli): The test included relevant and irrelevant stimuli leading to attention competition between the two different locations (attentional selectivity). Since DA has an effect on attentional selectivity, this acute increase in DA causes attention to irrelevant stimuli to increase, providing an explanation to the increase in illusion-strength with DEX. Regarding position (the spatial component) there was an increase in strength of illusion, the effect greatest at the 6 degree position. This may be

indicative of a widening of the spatial window, but cannot be concluded without a larger sample size and investigation of smaller changes in the spatial variable, to determine if there is a peak shift at lower distances. The spatial window has, to the author's knowledge, not yet been investigated on individuals with schizophrenia and it is thus hard to compare the results.

### 6.4 McGurk effect

There was a difference in performance between DEX- and placebo days seen with the male actor at ISI >400 ms: the number of audio-dominated responses decreased and the number of chimeric responses increased significantly after DEX relative to placebo (figure 13). Looking at the actual DEX-levels in saliva samples from the time closest of the illusion there was, however, no correlation between level of DEX in saliva and degree of change in responses of audio-dominated and chimeric responses (figure 14, component 1). That is, no dose-response relationship across individuals could be seen. This may be due to any of the following reasons: (1) Insufficient power (i.e., sample size too small); (2) DEX effect is all-or-none effect; (3) Apparent DEX effect is due to an unknown confounding factor, although known confounding factors were counter-balanced; (4) DEX-levels near the time of illusion is not as reliable a predictor of illusion effects as the slope of the rise in levels; (5) insufficient variation in DEX levels as all people were given the same dose.

Since the McGurk illusion test was carried out late in the day of testing (240 minutes' post testing) we considered it useless to look into rate of change of DEX-levels in saliva as a predictor of illusory response. To the author's knowledge, no previous studies have compared the levels of DEX in saliva with response on the McGurk illusion, which makes it yet harder to draw conclusions from the results.

The McGurk illusion examines visual-auditory stimuli, which have previously been tested on individuals with schizophrenia with conflicting results: de Gelder (33), showed that individuals with schizophrenia reported more visually dominated responses than healthy controls, which is consistent with this study (more chimeric results mean more influence of visual cues). However, a study by Martin (34) showed no differences in illusory responses between individuals with schizophrenia and matched controls. The sound induced flash illusion in the study by Hourani (1) also investigating auditory and visual modalities did not show any significant differences in illusion strength between DEX and placebo days. The conflicting results makes it thus difficult to draw any conclusions about temporal windows and illusion strength in the visual and auditory modalities.

### 6.5 Phantom word illusion

One subpopulation of participants had shorter latency and greater count with DEX compared to placebo, as hypothesised by Lloyd (28) (figure 15, component 2). That is, with higher DEX-levels in saliva, individuals had shorter time to perceive a new word and perceived more new words compared to placebo. Component 1 in the same PCA accounting for most of the variance, however, showed the opposite relationships. That is, with increasing DEX concentration in saliva, the greater latency and less new word perceived by participants while illusion strength actually increased with DEX. Next interesting predictor to look into would be rate of change of DEX-level in saliva. Taken together, the lack of power because of the limited amount of subjects in the phantom word illusion test makes it difficult to draw any conclusions about the relationships between DEX-levels in saliva and illusory outcome. Further studies with greater sample sizes are necessary.

### 6.6 General limitations

One explanation to why DEX-levels in saliva correlated poorly with illusion strength could be explained by subpopulations receiving placebo on the first day. The differences in outcome on illusory effects between DEX and placebo on most of the results are smaller when placebo comes first than when it comes from the second testing day. We ascribe this to the novelty of the testing situation and the initial anxiety regarding participating in a drug study elevating DA levels on the first day of testing. Another explanation could be due to that some individuals are resistant to the illusions (do not get the illusion any of the days). Another aspect is that DEX-levels in saliva may not be a valid representation of brain levels of DEX. For example, individual differences in saliva pH can produce between-subjects' variability in saliva levels without corresponding to variability in brain levels.

One explanation to the limited difference in illusion strength between DEX and placebo days (only significant at certain conditions in VIFI, not significant in SIFI and 3-FI) could be because the dose of DEX is too low. In future studies it would be interesting to administer different doses which would also make dose-response relationships across individuals more reliable (as differences in DEX concentrations across individuals would be greater). To further enhance the psychosis-state, another suggested improvement for future studies would be to have the participants sleep deprived in addition to DEX administration. Sleep deprivation has been shown to increase psychotic-like symptoms in otherwise healthy individuals (35).

Another explanation to why illusion strength was not greater, or why there was no widened temporal binding window with DEX, could be because of acute dopamine adaptation in the brain. This is for example obvious with the subjective effects (peaking at 70 min), while levels of DEX in saliva continues to rise and remain high for a long time. The different physiological measures also vary differently in comparison to DEX levels in saliva. These findings do not only make it hard to draw conclusions about how well DEX levels in saliva mirror DA-levels in the brain, it also gives an indication that a potentially widened window with DEX might be an effect that is only present a short time, perhaps outside of the testing schedule (especially illusion tests carried out late in the day).

As previously mentioned, euphoric subjective effects of DEX peaked at the first testing opportunity of the day set to 70 minutes post treatment. It would be interesting to have a measurement before this, suggesting a change of the time intervals of saliva collection, AMQ assessment and physiological measures to 50, 100, 150, 200 and 250. Alternatively, another control could just be added before 70 minutes (perhaps at 30) which might be more convenient when comparing results with previous studies having the present schedule.

Correlations of DEX-levels in saliva and scores from illusion tests were based on values from the saliva sample closest in time to illusion test, rather than the actual time of the illusion test. Also, looking at the actual time of when saliva samples were taken, this varied a lot between individuals and also from the scheduled times (70, 130, 180, 225 min post treatment) making interpretations of the correlations more uncertain.

Small sample size is another limitation of this study, particularly in the studies involving the McGurk- and Phantom word illusions where a lot of data had to be drawn back because of failure of the illusion test setup.

### 6.7 Limitations of the detection and quantification methods

Regarding the extraction- and HPLC-methods, MDMA as an internal standard was not optimal. Firstly, MDMA and DEX share similar chemical properties which requires the use of gradient analysis, giving poor baselines which in turn makes integration of peaks less reliable. Secondly, preparing the calibration curve by spiking volunteers' saliva with DEX and MDMA, there was, repeatedly, an interfering peak around the time of MDMA further adding to the uncertainty of integrating the MDMA peak.

Furthermore, integration of peaks in saliva samples from participants' testing days was yet harder to integrate, these samples containing more interfering peaks both at the retention time of DEX and MDMA. In some cases, the shoulder of an interfering peak was too close to the

peak of interest that the Empower® software did not allow for integration. In these cases, a print-screen of the chromatogram was printed on paper, the peaks of MDMA and DEX cut out with scissors and weighted. Ratios were obtained from the difference in weight. Inconsistencies in the method of obtaining ratios further increase the uncertainty of the method.

The identification method of standards being run before each new HPLC-session also had its limitations. The retention times of DEX and MDMA were sometimes in between two peaks in the chromatogram, making it difficult to identify the right peak. The interfering peaks were most likely metabolites from DEX.

It might be possible to optimise the HPLC-method in future studies, preferably with an internal standard less like DEX in chemical properties. Although making separation greater between DEX, its metabolites and the internal standard would most likely require a gradient analysis (previously criticised in this paper) and extensive pilot testing. To improve the identification method, standards should at least be run not only in the beginning of an HPLC-session but between one participant's saliva samples and the next.

## 7. CONCLUSION

DEX-levels in saliva showed in our study to be an unreliable predictor of performance on illusion tests. It further raises the question to what extent DEX levels in saliva mirrors actual dopamine levels in the brain. Because of a number of limitations of the study design and small sample size our results are, however, inadequate to draw any well-founded conclusions. The study should be viewed as a pilot study where further studies within the field are necessary.

## 8. POPULÄRVETENSKAPLIG SAMMANFATTNING

Schizofreni är en psykiatrisk sjukdom med många olika symptom, bland annat hallucinationer. Hallucinationer är sensoriska upplevelser som saknar sensorisk stimuli från verkligheten, t ex att höra en röst utan att någon pratar. I dagsläget är det fortfarande oklart exakt vilka mekanismer som ligger bakom schizofreni, men en övertygande faktor är för höga nivåer av signalsubstansen "dopamin" i hjärnan. Ett sätt att studera schizofreni är att ge friska individer dexamfetamin, en substans som ökar dopamin i hjärnan och därmed härmar schizophreni-tillståndet. I två olika studier fann man att individer som tagit dexamfetamin upplevde illusioner starkare än individer som tagit sockerpiller. En illusion är en felaktig upplevelse av verkligheten, men som till skillnad från en hallucination, stimulerats av yttre sensoriskt stimuli. T ex en skugga från ett träd uppfattas som en människa. Syftet med denna studie var att undersöka eventuella samband mellan illusionsstyrka och nivå av dexamfetamin i saliv. Det vill säga, har individer med högre koncentration dexamfetamin i saliv starkare illusioner jämfört med en individ med lägre koncentration?

Det fösta steget i denna studie var att etablera en metod för att mäta koncentrationen av dexamfetamin i saliv. Därefter gjordes jämförelser med hur dexamfetamin-koncentration i saliv varierade över dagen med subjektiva mått av upplevelsen av dexamfetamin, dvs eufori- och ångestkänslor.

Resultaten visade att koncentrationen av dexamfetamin i saliv i snitt var som högst 130 minuter efter administration av dexamfetamin och de subjektiva upplevelserna av drogen som störst 70 minuter efter administration. Eufori dominerade den subjektiva erfarenheten jämfört med ångest. Illusionsstyrka korrelerade med koncentration av dexamfetamin i saliv i vissa illusioner, medan en sådan korrelation inte förekom i andra illusioner. Det vill säga, höga koncentrationer av dexamfetamin i saliv behöver inte nödvändigtvis innebära starkare illusion.

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## APPENDICES

### *Appendix 1 - Inclusion and Exclusion Criteria*
Be >17 and <60 years of age

If female, not be pregnant, and be using contraceptives if sexually active and fertile. This is to exclude you from the experiment in case you have recently become pregnant but do not yet know of it.

Exclusion criterias:

- Heart or severe blood vessel disease,

- High blood pressure

- Glaucoma

- Hyperthyroidism (overactive thyroid)

- Tics (muscle twitching usually in the face or shoulders)

- Sensitivity to dexamphetamine or sympathomimetic amines

- Any degenerative disease of the nervous system

- Epilepsy, or other neurological disorder, including head injury

- Tourette's syndrome or you have a family history of this disorder

- A psychiatric or psychological problem for which you are receiving treatment (schizophrenia, depression, anxiety, etc)

- A serious medical problem for which you are receiving treatment (cardiovascular disorders, respiratory disorders, etc)

- Had or are currently receiving treatment for substance abuse

- A family history of schizophrenia in your first-degree relatives (parents, children or siblings)

- Previously experienced hypersensitivity to dexamphetamine

- Used any drug including alcohol or any illicit drug within 24 hours of each testing session

- Used caffeine on the day of each testing session

- Current prescription medication that you are taking other than oral contraceptives or acne medication

- Used over-the-counter medication in the 48 hours before each testing session (see the last page of this information sheet for a list of medications)

Below is a list of over-the-counter medications that cannot be used in the 48 hours before each testing session:

- Antihistamines,
- Hayfever tablets (e.g. Sudafed),
- Cough syrups,
- Cold and flu tablets,
- Codeine-containing medications,
- Anti-nausea medications,
- Sedatives,
- Herbal supplements, particularly St John's Wort.

*Appendix 2 – Schedule of testing days*

| Time | Minutes after treatment | Task | Duration (minutes) |
|---|---|---|---|
| 9:40 | 0 | Saliva #1 | 5 |
| 9:45 | 0 | Treatment | 60 |
| 10:45 | 60 | 3FI | 10 |
| 10:55 | 70 | Saliva #2 | 5 |
| 11:00 | 75 | Phantom | 30 |
| 11:30 | 105 | Questionnaires: SAPS, BPRS, MIS | 10 |
| 11:40 | 115 | VIFI | 15 |
| 11:55 | 130 | Saliva #3 | 5 |
| 12:00 | 135 | Lunch | 30 |
| 12:30 | 165 | SIFI | 15 |
| 12:45 | 180 | Saliva #4 | 5 |
| 12:50 | 185 | MHI | 40 |
| 13:30 | 225 | Saliva #5 | 5 |
| 13:35 | 230 | TFI | 10 |
| 13:45 | 240 | McGurk | 10 |
| 14:00 | 255 | Home | 5 |

*Appendix 3 - Extraction Protocol*

| Instructions | X |
|---|---|
| Thaw saliva samples and keep on ice. | |
| Pre-weigh 15 ml centrifuge tubes (blue screw cap). Write down the weight. | |
| Pipette approximately 1 ml of saliva into the centrifuge tube. | |
| Weigh tube + saliva and record the new weight. | |
| Add 100 µL of internal standard: 3000 ng/ml of MDMA in phosphate buffer, pH 2.8 to each saliva sample. If calibration curve add 100 µl of the DEX-concentration. | |
| Add 0,1 ml of NaOH in order to make DEX and MDMA less soluble in the saliva. | |
| Add 10 ml of hexane/1%isoamyl alcohol to the alkalinized saliva. | |
| Shake *vigorously* for 5 minutes. An emulsion layer may form. | |
| Centrifuge at 1800 g for 5 minutes at 4°C. | |
| Using a 0,45 µm PVDF filter attached to a syringe, filter the organic phase (the supernatant phase) into a new 10 ml centrifuge tube (yellow cap). | |
| Filter 0,05M HCl with 0,45 PVDF microfilter. | |
| Add 150 µl of the filtered 0,05M HCl to the filtered organic phase.<br>*The HCl back-extracts DEX and MDMA into the aqueous layer.* | |
| Shake tube *vigorously* for 5 minutes and centrifuge at 1800 g for 5 minutes at 4°C. | |
| The big part of the hexane-layer is then aspirated with a vacuum line. | |
| The remaining hexane is finally removed in a centrifugal evaporator SpeedVac at medium heat for 15-30 min (sc100, Savant with a pump vp 190 two solve). | |
| Pipette 150 µl of the HCl into the HPLC-veil. | |
| Set the HPLC system to inject duplicates of 30 µl volumes of each sample. | |

*Appendix 4 - Phosphate buffer*
The phosphate buffer was prepared:

1. 3.12 g of sodium dihydrogenphosphate dehydrate (M=156.01) was introduced in 1 litre of filtered water.
2. 0.68 ml of phosphoric acid solution (85%) was then added to the solution.
3. The pH was adjusted with acid until pH=2.8.