Developmental neurotoxicity of MDMA. A systematic literature review summarized in a putative adverse outcome pathway.

Marta Barenys[1]*, Ingrid Reverte[2,3], Stefan Masjosthusmann[4], Jesús Gómez-Catalán[1], Ellen Fritsche[4,5].

1. GRET, INSA-UB and Toxicology Unit, Pharmacology and Therapeutical Chemistry Department, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.

2. Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy

3. Santa Lucia Foundation (IRCCS Fondazione Santa Lucia), Rome, Italy.

4. IUF - Leibniz Research Institute for Environmental Medicine, 40225 Düsseldorf, Germany.

5. Heinrich-Heine University, 40225 Düsseldorf, Germany.

*Corresponding author: Marta Barenys, Department of Pharmacology, Toxicology and Therapeutic Chemistry, Faculty of Pharmacy and Food Sciences, University of Barcelona, 08028 Barcelona, Spain. mbarenys@ub.edu

Abstract

The increasing use of illegal drugs by pregnant women causes a public health concern because it is associated with health risks for mothers and their developing children. One of such drugs is MDMA (3,4-methylenedioxymethamphetamine) or ecstasy due to its high consumption in relevant age and sex groups and its adverse effects on human and rodent developing brains. To thoroughly review the current knowledge on the developmentally neurotoxic potential of MDMA we systematically collected and summarized articles investigating developmental neurotoxicity (DNT) of MDMA in humans and animals in *in vivo* and *in vitro*. In addition, we summarized the findings in a putative adverse outcome pathway (AOP). From an initial 299 articles retrieved from the bibliographic databases Web of Science, PubMed and DART, we selected 39 articles according to inclusion/exclusion criteria for data collection after title/abstract and full text screening. Of these 3 where epidemiological studies, 34 where *in vivo* studies in mice and rats and 2 were *in vitro* studies. The three epidemiological studies reported from the same longitudinal study and suggested that MDMA exposure during pregnancy impairs neuromotor function in infants. In rat, postnatal exposure towards MDMA also caused locomotor deficits as well as impaired spatial learning that might be associated with decreased serotonin levels in the hippocampus. I*n vitro* MDMA caused cytotoxicity at high concentrations and effects on the serotonergic and neuritogenic alterations at lower concentrations which are in line with some of the *in vivo* alterations observed. Considering the adverse outcomes of developmental MDMA described in humans and in rodents we summarized the first putative AOP on developmental compound exposure leading to impaired neuromotor function in children. For generation of this AOP, MDMA exposure was taken as a model compound. In addition, we hypothesized a second AOP involving developmental disturbance of the dopaminergic system. However, further *in vitro* mechanistic studies are needed to understand the molecular initiating event(s) (MIE) triggering the downstream cascades and obtain consistent evidences causally linking the adverse outcome to effects at the cellular, organ and organism level.

Keywords: neurodevelopment; amphetamine derivative; pregnancy; AOP; motor function; epidemiological ToxRTool.

Abbreviation list

5-HIAA: 5-Hydroxyindoleacetic acid
5HT: serotonin
AIMS: Alberta Infant Motor Scales
AO: adverse outcome
AOP: adverse outcome pathway
BDNF: brain-derived neurotrophic factor
BRS: Behavioural Rating Scale
CWRU: Case Western Reserve University
DA: dopamine
DAISY: Drugs and Infancy Study
DART: developmental and reproductive toxicology database
DBH: dopamine beta-hydroxylase
DG: dentate gyrus
DIV: days in vitro
DNT: developmental neurotoxicity
DOPAC: 3,4-dihydroxyphenylacetic acid
e.o.d.: every other day
EB: embryoid bodies
ECVAM: European Centre for the Validation of Alternative Methods
GABA: gamma-aminobutyric acid
GD: gestational day
h a.i.: hours after injection
hiPSC: human induced pluripotent stem cell
HVA: homovanillic acid
i.p.: intraperitoneal
ICC: immunocytochemistry
IHC: immunohistochemistry
KE: key event
KEGG: Kyoto Encyclopedia of Genes and Genomes
KER: KE relationship
LOAEC: Lowest observed adverse effect concentration
LOAEL: Lowest observed adverse effect level
LSD: lysergic acid diethylamide
MADAM: N,N-dimethyl-2-(2-amino-4-methylphenylthio) benzylamine
MAP2: microtubule-associated protein 2
MDI: Mental Development Index
MDMA: 3,4-methylenedioxymethamphetamine
mESC: mouse embryonic stem cells
MHPG: 3-methoxy-4-hydroxyphenylglycol
MIE: molecular initiating event
MoA: mode-of-action
MWM: Morris Water Maze
NE: norepinephrine
NET: norepinephrine transporter
NGF: nerve growth factor
NMDAR: N-methyl-D-aspartate receptor
NNNS: NICU Network Neurobehavioural Scale
NT-3: neurotrophin-3
o.g.: oral gavage
PCPA: p-chlorophenylalanine
PDI: Psychomotor Index
PN month: postnatal month
PND: postnatal day
PPI: prepulse inhibition
s.c.: subcutaneous
S.D.: Sprague-Dawley
TH^+: tyrosine hydroxylase
UEL: University of East London
W: weeks

1. INTRODUCTION

The period of highest risk for women to develop a substance use disorder overlaps with the childbearing age (18-44), especially between the ages 18 and 29 (Compton et al., 2007; Forray, 2016). Furthermore, the percentage of pregnant women using illicit drugs has been increasing over the last years. According to the National Surveys on Drug Use and Health of the United States, the percentage of pregnant women reporting consumption of illegal drugs in the month before the survey increased from 5.1% in 2008 to 8.5% in 2017. Indeed, when focusing on the period of highest risk aforementioned (in this case 18 - 25 years of age) illicit drug use during the month before the survey reported by pregnant women even increased to 11.0% in 2017 (Substance Abuse and Mental Health Services Administration, 2018, 2009). Similar tendencies are observed in other countries, like Canada (Petrangelo et al., 2018), yet there is a huge gap of information on drug abusing women during pregnancy (EMCDDA, 2012). This is due to difficulties in data obtention most likely related to the social stigma associated with drug use during pregnancy.

Illicit drug use during pregnancy causes a public health concern because it is associated with increased health risks for the mother and the developing child. Which health adverse outcome is created largely depends on the substance consumed. For example, adverse effects related to prenatal exposure to legal drugs like alcohol are well described by lower birth rates, preterm birth or perinatal death (Bailey and Sokol, 2011) and fetal alcohol syndrome which includes intellectual disability, birth defects and characteristic dysmorphic facial features (Chudley et al., 2005). However, besides the data gap in exposure assessment, there is also little known on the adverse effects on development of many of the illicit drugs. One important example with sparse data yet public concern due to high consumption in the relevant age and sex group (EMCDDA, 2017) are amphetamine derivatives like MDMA (3,4-methylenedioxymethamphetamine).

MDMA or ecstasy or 'Molly' is a synthetic drug mainly consumed by teenagers and young adults for its psychotropic actions. These include psychostimulant and 'entactogen' effects enhancing emotional empathy and prosocial behaviour. Related to the entactogen effects of MDMA, several authors described that ecstasy users are more likely to engage in high risk sexual behaviours including casual and unprotected sex (Castilla et al., 1999; Mattison et al., 2001; May and Parrott, 2015; Palamar et al., 2018). MDMA users who become pregnant normally stop taking the drug after the first trimester, although some cases of prolonged consumption have also been described (Ho et al., 2001; Moore et al., 2010; Scott et al., 2010; Van et al., 1998).

Studies in pregnant rats demonstrated that MDMA crosses the placental barrier and reaches the fetal brain (Campbell et al., 2006). In addition, it was inferred that with a high probability MDMA is found in breast milk considering that it is a low-molecular-weight and hydrophobic molecule with a pKa of 10.4 (Cho et al., 2008). Several independent research groups observed adverse effects of MDMA on the developing rodent brain and few epidemiological or case report studies also described developmental neurotoxicity (DNT) of MDMA in humans. This drug has therefore been included in the list of reference compounds for alternative test methods to study their DNT potential (Aschner et al., 2017). However, the extent of the potential adverse outcomes and the mode-of-action(s) (MoA) underlying these DNT effects are enigmatic. So far, a variety of studies intended to clarify if the effects of MDMA on the developing nervous system are mediated through a similar MoA than adult neurotoxicity, i.e. causing disfunction of the serotonergic system, with serotonin depletion and loss of serotonin transporter (Parrott, 2013). Some of

these studies point out that the DNT MoA might be completely different than the one for adult neurotoxicity, yet this is currently unclear.

For improved risk perception and thus prevention of harm, comprehensive information is necessary that clearly links MDMA exposure of women during pregnancy to adverse neurodevelopmental outcomes in children. As a first step, the aim of this review is to collect and summarize articles published until November 2018 on DNT effects of MDMA in humans and animals *in vivo* as well as *in vitro*. From these data, hypothetical/putative adverse outcome pathways (AOP) will be summarized that highlight the main common findings and importantly, points out the existing knowledge gaps. This approach will help accelerating the acquisition of new knowledge on MDMA-induced DNT, especially information on the MoA, which is urgently needed for a deeper understandning of dose-effect relationships and thus a hazard and risk assessment based on mechanistic understanding.

2. REVIEW METHODOLOGY

2.1. Search Method

2.1.1. Databases

Literature search was performed in three databases covering different levels of specialization: 1) Web of Science: a multidisciplinary database including more than 34.200 journals, plus books, proceedings, patents, and data sets on the fields of biomedical sciences, natural sciences, engineering, social sciences, and arts and humanities (WebofScience, n.d.); 2) PubMed: a biomedical database including literature from MEDLINE, life science journals, and online books (PubMed, n.d.); 3) DART (developmental and reproductive toxicology database): a specific database belonging to the TOXNET database (toxicology data network) from the U.S. National Library of Medicine and including "journal references covering teratology and other aspects of developmental and reproductive toxicology" (DART, n.d.). The information gathering procedure used in this study is summarized in Figure 1.

2.1.2. Keywords

Searches performed in Web of Science and PubMed included two groups of truncated keywords: keywords related with "developmental neurotoxicity/exposure" AND "names and synonyms of MDMA and MDMA HCl obtained from ChemIDPlus (ChemIDplus, n.d.)"; while the search performed in DART only included "3,4-Methylenedioxymethamphetamine" name, as this database recognized the chemical name and automatically added synonyms and CAS number retrieved from ChemidPlus. The search in DART did not include the "developmental neurotoxicity/exposure" group of keywords because this database is already selective for references related to developmental toxicology. DART search was restricted to not include PubMed results to avoid duplicates (exclusion of the default option "Include PubMed Results"). The exact syntax with all keywords used in the three searches is detailed in Supplementary Information file 1. Timespan was not limited in any of the searches.

2.2. Exclusion criteria

All references obtained from the three searches (Web of Sciences: 153, PubMed: 104, DART: 42) were exported to a common Mendeley library (299 references). After excluding duplicates, 211

remaining articles were further screened based on title and abstract according to the following exclusion criteria:

- Secondary literature.
- Combined exposure to MDMA and other drugs.
- No evaluation of neurotoxic effects.
- Exposure does not occur during the developmental period (defined as gestational period in human studies, as gestation and lactation up to postnatal day 21 in rodent studies, and as exposure during cell differentiation for *in vitro* studies).
- Species different than rodents, rabbit, zebrafish, frog, primate and human.
- Articles withdrawn/retracted/removed.

A total of 118 articles were excluded according to these criteria leaving 93 articles for full text screening.

2.3. Classification and retrieval of selected studies

Selected 93 studies were classified in three groups: studies on the DNT of MDMA (1) in humans *in vivo*, (2) in animals *in vivo*, and (3) in *in vitro* models.

Full-text copies of the references included in groups 1, 2 and 3 were obtained from the following university libraries: Heinrich-Heine-Universität Düsseldorf, Universitat de Barcelona and Sapienza University of Rome and Fondazione Santa Lucia.

2.4. Information acquisition from full texts and harmonized evaluation of methodological quality

Full texts of the studies (13 from group 1, 65 from group 2 and 15 from group 3) were read and respective detailed study data gathered in a working table. While reading the full-text, article quality was evaluated and categorized using the "ToxRTool" (Schneider et al., 2009) available on the ECVAM website (http://ecvam.jrc.it, section "Publications"). This publicly available tool developed in Microsoft Excel® is based on the approach of Klimisch et al., (1997) to assess the reliability of toxicological data. The tool was used to harmonize the reliability assessment within the working group. The ToxRTool consists of two different excel sheets, one for *in vivo* and one for *in vitro* data, which were used for all studies included in groups 2 and 3, respectively. The criteria for these studies are grouped in: I) Test substance identification; II) Test system/organism characterization; III) Study design description; IV) Study results documentation; V) Plausibility of study design and results.

To the best of our knowledge, there is no ToxRTool available for evaluating the quality of human epidemiological studies. Therefore, we developed our own ToxRTool for these studies based on two guidelines for DNT risk assessment (Environmental Protection Agency, 1991; Travis et al., 2008). Criteria extracted from these guidelines were grouped following the same five categories as of the ToxRTool for *in vivo* and *in vitro* studies. This newly developed ToxRTool is available as a Supplementary information file 2.

While reading the full-text of the articles, inclusion/exclusion of studies to the review was revised based on the same six criteria presented in section 2.2 plus exclusion criteria "ToxRTool Category 3: not reliable" and single case report studies. Figure 1 summarizes the number of full-texts reviewed and the number of articles finally included/excluded in this review.

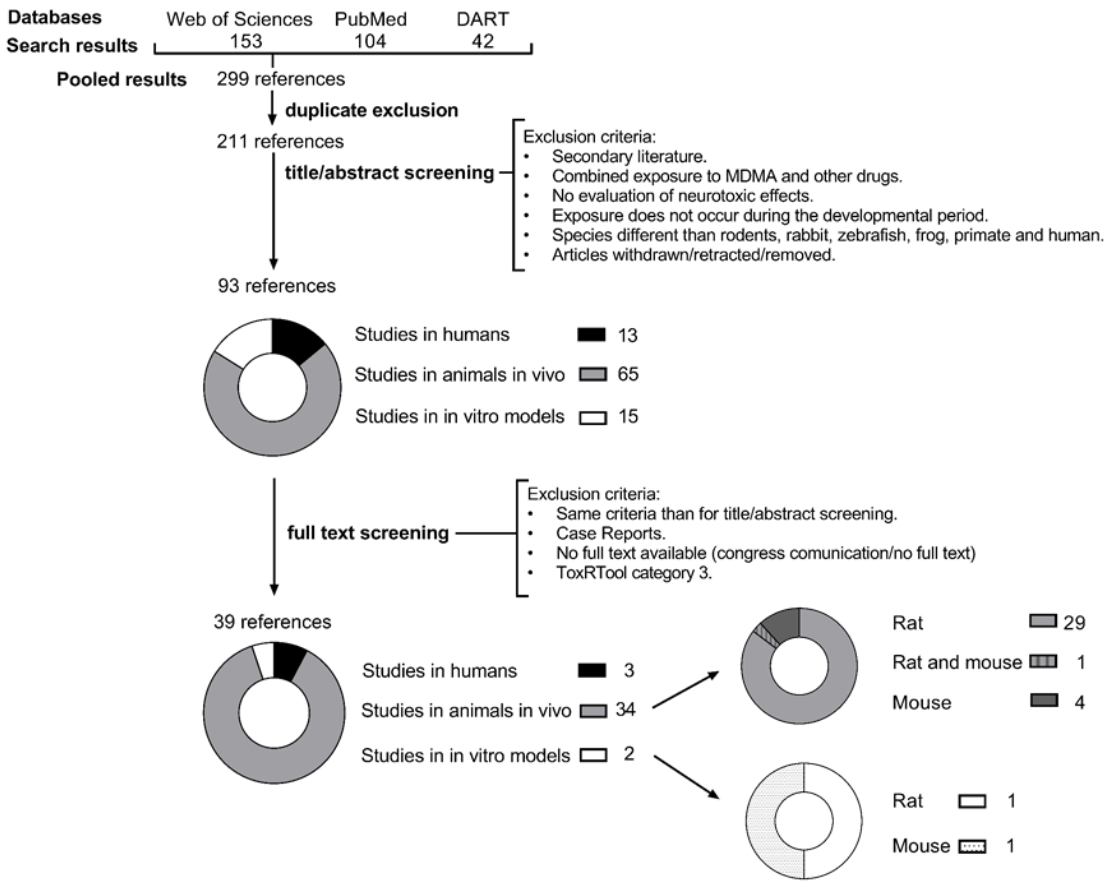

Figure 1. Summary of the search and screening strategy including the number of articles obtained at every screening step and detailing the species studied in vivo or in vitro in each article. No in vitro studies based on human cells were found.

3. RESULTS and DISCUSSION

3.1. Neurodevelopmental effects of prenatal exposure to MDMA in humans

A total of 13 human studies were identified by the systematic search. Ten of them were excluded for the following reasons: five because they were conference abstracts, one because it did not evaluate DNT, two because it was secondary literature, one because of low quality score in the ToxRTool (category 3) and one for being a single case report. The results of the three selected ones are summarized in Table 1.

Limited evidence is available on the neurodevelopmental effects of prenatal exposure to MDMA in humans. To the best of our knowledge, there is only one prospective epidemiological study evaluating neurobehavioural parameters in infants from birth up to 2 years of age after intrauterine exposure to MDMA. This longitudinal study resulted in 4 publications: the first describes the profile of drug intake of the mothers and will not be discussed here as it is not the focus of the present review (Moore et al., 2010); the second monitors the neurobehavioural outcomes of the infants at 1 and 4 months of age (Singer et al., 2012a); the third one describes the behavioural outcomes at one year of age (Singer et al., 2012b); and the fourth one compares the outcomes in a longitudinal study across the children at 1, 4, 12, 18, and 24 months of age (Singer et al., 2016). The results of this study suggest that MDMA exposure during pregnancy

impairs neuromotor function in infants. Below, we describe in detail the results of this epidemiological work and provide insights in its limitations and directions to be taken in human research of prenatal exposure to MDMA.

The prospective study of Singer and colleagues compared a cohort of 28 mothers who consumed MDMA during pregnancy with 68 mothers who did not. Regardless of their MDMA intake, all of them had a history of polydrug use and continued to use other substances during pregnancy. Mothers were recruited mainly through paid adverts as a part of the Case Western Reserve University (CWRU) and University of East London (UEL) Drugs and Infancy Study (DAISY) in the UK. The neurodevelopmental outcomes of the infants were monitored from birth to 2 years of age using a battery of standardized developmental scales that evaluate motor and cognitive functions (Singer et al., 2012a).

The main effect observed in MDMA-exposed infants was a motor delay detected as early as 1 month after birth, which persisted until 24 months of age. This effect manifested subtly at 1-month of age as a trend to more lethargic behaviours and hypotonia as measured by the NICU Network Neurobehavioural Scale (NNNS). The motor delay became more evident at 4 months of age, where MDMA-exposed children showed poorer motor quality in the Motor Quality Scale of the Behavioural Rating Scale (BRS) of the Bayley Development Scales and lower performance on the Alberta Infant Motor Scales (AIMS). Both of these scales measure gross motor maturation. Particularly, MDMA-exposed infants showed less coordination and more slow and delayed movements, and notably, this effect was dose-dependent (heavier vs lighter MDMA users; Singer et al., 2012a).

At 12 months of age, consistently with the early findings, MDMA-exposed children showed a lower Psychomotor Index (PDI) of the Bayley Development Scales, an index of gross and fine motor control and coordination. A third of the highly exposed children displayed a significant developmental delay (PDI <70) while the remaining were at risk (PDI <85). In comparison, only 10% of the lighter and non-exposed children showed a significant delay and a third were at-risk. The lower PDI was maintained in heavily exposed children at 18 and 24 months of age (Singer et al., 2012b). Importantly, MDMA exposure did not affect the cognitive domain. A lower Mental Development Index (MDI) was observed in children at 12 months of age; however, the authors hypothesized that this effect could be related to the deficit of fine motor skills (Singer et al., 2012b). This effect was no longer observed at later ages, and no other effects on language, emotional regulation or attention were observed (Singer et al., 2016).

Another effect of MDMA exposure reported by Singer and colleagues (2012a) is that MDMA-exposed infants were significantly more likely to be male (71% vs. 46%). Yet, the number of individuals is too low to draw a definite conclusion on MDMA effects on sex determination. They did not observe differences in other perinatal variables such as gestational length or probability of stillbirths, neither on other physical characteristics of the babies at birth (ie. birthweight, birth length; Singer et al., 2012a).

The results described in the UK cohort demonstrate a robust and reliable effect of prenatal MDMA exposure on motor development, evidenced by the fact that motor delay was detected by different developmental scales and sustained through age. Additionally, the effects were statistically significant despite the small sample size after controlling for multiple confounding factors, including home environment, maternal stress and exposure to other drugs. It is important to note that all women included in the study reported to use MDMA during the first two trimesters, decreasing its use as the pregnancy advanced, while only one woman kept

consuming during the 3rd trimester. Therefore, the motor effects observed can be attributable to exposures during the beginning and middle of pregnancy. Additional studies are necessary to evaluate the effects of MDMA exposure during the third trimester, which is not likely to be possible using epidemiological studies. Furthermore, little is known about potential interactions between drugs in polydrug users. In the UK cohort, women who took MDMA during pregnancy were more likely to also take marijuana, cocaine, LSD, and mushrooms while pregnant (Moore et al., 2010; Singer et al., 2016).

Taken all this together, more studies concerning prenatal exposure to MDMA are needed and a few considerations have to be taken into account. Despite the robustness of the UK cohort study, the sample of MDMA users, especially when divided between light (n= 15) and heavy users (n= 13; division based on a median split), was small. Recruiting larger samples is important to detect small effects with high variability, such as more subtle neurodevelopmental outcomes. Besides the inherent variability of neurodevelopmental parameters, another source of variation concerns the usage of MDMA between pregnant mothers with regards to duration, quantity or patterns of intake, which will in turn contribute to raise the variability in children outcomes. However, collecting large samples may be challenging because of social stigma associated to drug use, especially during pregnancy. As reported by the authors of the UK study this was a major drawback in recruiting subjects for their study. Another consideration refers to the method of exposure estimation. Although self-report may often be the only tool available to estimate drug intake, pregnancy follow-ups using hair or other biological samples would aid to validate self-reported usage and provide more accurate dosage estimates. Last, including a non-drug user control besides the polydrug control would be ideal to disregard effects of other drug exposure.

Table 1. Summary of studies on prenatal exposure to MDMA in humans

Reference/ type of study	Age evaluated	Infant Evaluation	Examiner	Statistical Analyses	Outcome Infants
(Singer et al., 2012a)	1 and 4 months (corrected for gestational age at birth)	Fetal growth (weight, length, head circumference, gestational age) Health information from hospital records NICU Network Neurobehavioural Scale (NNNS) Alberta Infant Motor Scales (AIMS) Bayley Scales of Infant Development III (Mental Development Index (MDI), and Psychomotor Index (PDI), Behavioural Rating Scale (BRS))	Same examiner masked to infant drug status and trained and certified in the procedure by gold standard reviewers All assessors were master's level psychology assistants or the equivalent and were masked to infant drug exposure.	MDMA vs non-MDMA: chi-square or Fisher's exact test and t- test or Wilcoxon Mann Whitney test Spearman correlation to assess relationships of amount and frequency of drug exposure to infant outcomes to determine covariates. Multiple linear regression to determine significant predictors of the outcome measures controlling for covariates For three group analyses (heavy, light, non-user) Analyses of Variance (ANOVA) was used	Child birth outcomes did not differ by group in gestation period, birthweight, prematurity Length and head circumference findings are inconclusive due to missing data MDMA-exposed infants were significantly more likely to be male (71% vs. 46%). NNNS 1mo: No differences between mean groups Non-significant trend to more lethargic behaviours and to manifest hypertonic responses Bayley 4mo: No differences in MDI or Attention/Arousal factor of the BRS BRS Motor Quality Scale: higher MDMA use over pregnancy predicting poorer motor quality: less coordinated and more likely to have slower and delayed movements Non-significant trend for heavily exposed MDMA infants to attain lower PDI scores than the other 2 groups (lighter/non-user). AIM 4mo: more heavily exposed MDMA infants performing less well than the non-MDMA or the lighter MDMA-exposed groups
(Singer et al., 2012b)	12 months old	Preschool Language Scale Bayley Scales of Infant Development III (MDI, PDI, BRS)	==	==	Infants with heavier exposure 5-point deficit in MDI and motor outcomes: most at-risk (PDI <85), and 1/3 significant developmental delay (PDI ,70) Non-significant trend for MDMA exposure to predict less orientation and engagement MDMA exposure was unrelated to language or emotional regulation outcomes
(Singer et al., 2016)	Longitudinal comparison 4, 12, 18 and 24 months	Bayley Scales of Infant Development III (MDI, PDI, BRS)	==	Effects of MDMA (heavy, light, none) evaluated using repeated measures mixed model approach with a random intercept. An unstructured covariance matrix used to account for correlated responses within a subject.	Effect of level of MDMA exposure on PDI over time: Heavier exposure 11-point deficit in PDI compared with lighter exposed children and a 6-point deficit compared to non-exposed children over the first two years of life No effect of MDMA on the MDI: prior effect seen at 12 mo no longer significant once the overall trajectory of development was considered Significant effects of exposure on BRS motor quality: heavier MDMA exposure twice as likely to be rated by examiners as demonstrating poorer motor quality The Attention/Arousal subdomain of the BRS was measured only at 4 months. Heavier MDMA-exposed infants were perceived as having poorer attentional skills than lighter exposed infants and ns trend to perform more poorly than non-exposed infants No test age by MDMA interaction was found, indicating that the effects of MDMA on the outcomes did not significantly vary over the first two years of life.

3.2. Neurodevelopmental effects of developmental exposure to MDMA in animals *in vivo*.

After the title and abstract screening, 65 articles on *in vivo* animal studies were included in the review. Full texts were collected and read for further evaluation according to the exclusion criteria and the ToxRTool – *in vivo*. From this analysis, 31 articles were excluded for the following reasons: one was a duplicate, two were secondary literature, three were not evaluating DNT, twenty-two were congress communications, one was excluded because of low quality (ToxRTool category 3), and two because the full-text could not be obtained.

All remaining 34 studies included in this group were performed in rodents, with 30 rat and 5 mouse studies (one study including both). Such a different proportion in the number of studies amongst these two species might be related to the knowledge acquired from adult neurotoxicity studies; adult neurotoxicity of MDMA is species-specific with induction of selective damage to serotonergic neurons in rats as well as most species examined including non-human primates (Green et al., 2003; Steele et al., 1994) and in contrast selective dopaminergic neurotoxicity in mice (O'Callaghan and Miller, 1994). According to Mueller and colleagues (2013), these different neurotoxicity profiles cannot be explained by differences in MDMA metabolism or pharmacokinetics of rats and mice. Probably, as the basis for these inter-species differences remain unknown, most study designs for developmental MDMA exposure choose rats because these belong to the rodent species which displays the most similarity in adult neurotoxicity to primate species.

Besides using different species, a huge variety of experimental designs was applied in the animal *in vivo* studies. For a comprehensive review on the large methodological diversity and little consistency among preclinical studies assessing the effects of amphetamine-type stimulants (including MDMA) during pregnancy and lactation the reader is referred to McDonnell-Dowling and Kelly (2015). In this article the high variability of doses administered in studies evaluating developmental effects of MDMA is also discussed. In general, MDMA doses administered in animal studies are much higher than the ones regularly taken by young recreational ecstasy users. However, it has been proven that the application of common interspecies scaling strategies to compare MDMA doses between rodents and humans are not valid because in humans MDMA has a saturable kinetics (de la Torre et al., 2000). Thus, according to toxicokinetic studies in humans and rats reviewed by Green et al. (2009), to produce a similar peak blood plasma to that seen in humans after a 2 mg/kg dose, four fold higher doses are required in rats. Besides, the rapid MDMA clearance in this rodent species is also used by some authors to explain that repeated injections in rats would be the best strategy to mimic single oral doses in humans. Despite the large methodological heterogeneity observed in doses, number of administrations per day and exposure period, we present a summary of the results focusing on common adverse outcomes observed in rodent studies.

Of the five studies performed in mice (Table 2 and Figure 2), three used a similar pattern of exposure: from fertilization or implantation to the end of lactation (gestational day (GD)1 or GD6 to postnatal day (PND)21) with one oral administration daily and a common maximum dose of 20 mg/kg b.w. (Cho et al., 2008; Kaizaki et al., 2014; Kwack et al., 2014). Cho and co-workers (2008) evaluated the effects of MDMA on neurogenesis using histological methods. They found a decreased number of BrdU labeled cells in the dentate gyrus 24h or 28 days after BrdU administration, indicating that developmental exposure to MDMA decreases the proliferation and survival of cells in the dentate gyrus. This observation is supported by similar findings in an acute dosage study with three i.p. doses of MDMA on PND6. In particular, they observed an increased cell degeneration determined by stereological cell counts in many of the 17 brain

regions analyzed, specially in the frontal, parietal and cingulate cortices, the septum, thalamus, hypothalamus and the cornu ammonis 1 region of the hippocampus. However, this effect was observed 24h after the exposure but not after 14 or 21 days (Dzietko et al., 2010). The other two studies with long gestational and postnatal exposure to MDMA evaluated different parameters of physical, functional and neuromotor maturation. While Kwack et al., (2014) did not find any adverse effect on physical development nor in motor reflexes, Kaizaki et al., (2014) linked MDMA exposure to delays in acquiring the righting reflex test, cliff-avoidance test and wire hanging maneuver, thus indicating alterations in reflexes and in motor development. However, the dose at which these behavioural adverse effects were observed (20 mg/kg b.w.), already produced a significant decrease in survival rate at PND4 and a significant decrease in body weight which makes it difficult to classify the adverse effects as specific DNT effects. The last study performed in mice (Eun et al., 2010) used a similar oral MDMA exposure paradigm, yet with a later onset of treatment (from GD15 to PND21, 20 mg/kg b.w.). Here, cell degeneration was not assessed because the study evaluated gene expression in the cerebral cortex of male and female offspring at PND77. Altered genes in mice developmentally treated with MDMA were related with 9 common KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways: the MAPK signaling pathway, focal adhesion pathway, insulin signaling pathway, calcium signaling pathway, regulation of actin cytoskeleton, Wnt pathway, neuroactive ligand–receptor interaction pathway, axon guidance and colorectal cancer signaling. Although the timepoint of gene expression analyses (PND77) was too late to predict a developmental MDMA MoA, similar studies examining earlier timpoints possibly in a species reacting more similar to humans could be the basis for designing *in vitro* experiments that unravel the MDMA MoA of neurodevelopmental toxicity.

Among the 30 studies performed in rats, the most common strain used was Sprague-Dawley. Only one study was performed in Long Evans and four in Wistar rats. A common limitation of these rat *in vivo* studies is the low number of dose-response studies that investigate more than two doses. Only five papers include three dose groups of MDMA plus the control to support the evaluation of dose-response relationships (Barenys et al., 2010; Broening et al., 2001, 1994; Dzietko et al., 2010; Vorhees et al., 2009, 2004). However, due to the overall low number of publications on the topic, single or two dose group studies were also considered in this review. Another limitation of most of the animal *in vivo* studies is the lack or reduced information on MDMA maternal or pup general toxicity. This deficiency makes the effect evaluation difficult to distinguish between DNT effects and general/maternal toxicity effects. Despite these limitations, there were common features seen in the rat *in vivo* studies that in the following paragraphs will be discussed according to five groups of studied endpoints, i.e. neurotransmission, behaviour, general development, effects on growth factors and other endpoints.

Neurotransmission

With twenty-one studies evaluating the effects of developmental exposure to MDMA on different neurotransmission related endpoints like concentrations of serotonin (5HT), dopamine (DA) or norepinephrine (NE) and their metabolites or binding to their transporters, this was the most studied group of endpoints among the articles reviewed (see Table 3 and Figure 3).

Three studies evaluated the effects of MDMA on NEergic neurotransmission, and all of them found a significant increase in NE concentration, either in hippocampus, prefrontal cortex or

nucleus accumbens (Broening et al., 2001; Skelton et al., 2012; Thompson et al., 2012). Two of these studies exposed animals postnatally and observed the alteration in the hippocampus but not in other brain areas (Broening et al., 2001; Skelton et al., 2012), while if exposure occurred prenatally the effect was observed in the prefrontal cortex and nucleus accumbens but not in the hippocampus (Thompson et al., 2012). The only study evaluating norepinephrine transporter (NET) binding reported a significant increase also only in the hippocampus, while the concentration of NE metabolite MHPG (3-Methoxy-4-hydroxyphenylglycol) was not affected according to Thompson and colleagues (2012). From these *in vivo* results in rats the question arises if a proposed MoA involving effects of MDMA on the NE system can be related to the adverse outcome (AO) observed in children. An accumulation of NEergic neurons is positioned in the locus coeruleus located in the pons *in vivo*. Extensive coeruleo-cortical innervation is found to a variety of brain regions including the motor cortex suggesting that NE modulates the activity of these projected brain areas (Counts and Mufson, 2012). Indeed, mice lacking the ability to produce NE by knocking out the dopamine beta-hydroxylase (DBH) gene demonstrate the importance of NE during development and suggest that motor output is likely to be regulated by NE at both the central and peripheral level (Thomas and Palmiter, 1997). However, reduced NE causes reduced motor functions. In the MDMA treated rats, mainly increasing NE was measured in rat brains raising the question if indeed actions on the NE system are related to the decreased motor functions seen in children prenatally exposed to MDMA as described above. However, the NE system also controls arousal including stress response, attention, and memory function (Counts and Mufson, 2012). The human *in vivo* study observed a lower MDI early that was not observed at older ages (Singer et al., 2012b). It might be that the human brain has the ability to compensate for a non-functional NE system during development. This was suggested earlier based on the observation that patients with genetic DβH-deficiency show remarkably normal cognitive functions (Jepma et al., 2011). In addition, there is an indication of NE dysregulation in human psychiatrial disease. NE was found hypersecreted in plasma in patients with unipolar depression and generalized anxiety (Sevy et al., 1989; Wyatt et al., 1971). If this dysregulation of the noradrenergic system is cause or consequence of these psychiatric diseases is not clear. However, long-term follow up of prenatally MDMA exposed children for their susceptibility to develop NE-related psychiatrial disorders seems desirable.

Among 13 studies evaluating DA concentration in different brain areas, 12 found no significant effects, including studies performed during pre- or postnatal periods (Aguirre et al., 1998; Broening et al., 2001, 1994; Cohen et al., 2005; Colado et al., 1997; Galineau et al., 2005; Koprich et al., 2003a, 2003b; Meyer and Ali, 2002; Schaefer et al., 2008, 2006, 2012; Skelton et al., 2012). Only one study evaluated DA transporter binding and also detected no significant effects (Galineau et al., 2005). Concerning the effects of MDMA on concentrations of DA metabolites in specific brain areas, the results of ten studies were inconsistent. MDMA affected 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations only at higher doses (administering 40 mg/kg b.w., 4x10 mg/kg b.w., 2x20 mg/kg b.w. or 2x15 mg/kg b.w.) (Broening et al., 1994; Koprich et al., 2003a, 2003b, Schaefer et al., 2012, 2008, 2006), while studies administering lower doses (10 mg/kg b.w., 2x10 mg/kg b.w. or 20 mg/kg b.w) caused no significant changes in DOPAC concentrations, independently of the administration period (pre- or postnatal) and length (1 to 10 days) (Aguirre et al., 1998; Cohen et al., 2005; Galineau et al., 2005; Meyer and Ali, 2002). For the positive studies, however, changes were not uniform. While some studies observed an increase in DOPAC concentration others measured decreases. Similarly, MDMA also affected another metabolite of DA, homovanillic acid (HVA). Three articles studying lower MDMA doses (10 mg/kg b.w., 2x10 mg/kg b.w. or 20 mg/kg b.w.) detected no significant changes

in HVA (Aguirre et al., 1998; Galineau et al., 2005; Meyer and Ali, 2002) while three articles including higher MDMA doses (40 mg/kg b.w., 2x20 mg/kg b.w. or 2x15 mg/kg b.w.) found significantly changed HVA concentrations in both directions, increased and decreased (Broening et al., 1994; Koprich et al., 2003a, 2003b). The reason for these inconsistent results on DA metabolite changes after MDMA exposure is not clear. As far as we can tell, there is not enough information to rule out that exposure scheme, timing or route of exposure or brain regions are related to the unequal effects of MDMA on DOPAC or HVA concentrations in rat brains. Nevertheless, we again ask the question if a proposed MoA involving effects of MDMA on the DAergic system can be related to the AO of decreased motor activity observed in children. Dopaminergic structures express connecting fibres to many different parts of the brain playing essential roles in aspects as simple as motivating basic movement and complex as cognition (Bissonette and Roesch, 2016). Moreover, DA is one of the earliest neurotransmitters occuring in the developing brain significantly shaping neuronal cytoarchitecture and circuitry by modulating cell proliferation, migration, and differentiation. Modifying DA receptor signaling during development alters amongst others tangential migration of GABAergic neurons possibly resulting in inappropriate neuronal excitation-inhibition balance in cortex. In addition, it modulates dendritic growth and the formation of dendritic spines, which, in the hyperdopaminergic DA transporter knockout mouse leads to the behavioural phenotype of hyperactivity (comprehensively reviewed in Money and Stanwood, 2013). Hence, developmental interference with the DA system is a possible MoA of MDMA causing behavioural abnormalities in children. Because dysregulation of the DAergic system also plays a fundamental role in neuropsychiatric disease later in life, follow-up studies of the children prenatally exposed towards MDMA in combination with human *in vitro* studies would shed more light on the mechanisms and consequences this exposure has in humans.

Finally, the most studied neurotransmitter in MDMA DNT studies was 5HT, with 16 articles evaluating its concentrations at different times after MDMA developmental exposure (Aguirre et al., 1998; Broening et al., 2001, 1995, 1994; Cohen et al., 2005; Colado et al., 1997; Darvesh and Gudelsky, 2004; Galineau et al., 2005; Koprich et al., 2003a, 2003b; Meyer and Ali, 2002; Schaefer et al., 2012, 2008, 2006; Skelton et al., 2012; St Omer et al., 1991). Among these studies only 8 found adverse effects of MDMA on 5HT concentrations in different brain areas. However, if the brain area studied is restricted to the hippocampus, 8 out of 10 articles detected a significant decrease in 5HT concentration, while in other brain regions results were less homogeneous. Concentrations of the 5HT metabolite 5-Hydroxyindoleacetic acid (5-HIAA) were evaluated in 12 studies, among which 7 detected an adverse effect, with a very similar pattern than 5HT concentration alterations. If the brain region again is restricted to hippocampus, 5 out of 7 studies detected a significant decrease, and in this case, 5 out of 6 studies also reported a significant decrease in the concentration of the metabolite in the striatum. Ten studies evaluated effects on serotonin transporter binding. Surprisingly, adverse effects were detected only in three out of the four experiments administering MDMA from PND1 to PND4 and earlier or later exposures did not find adverse effects in this endpoint. Similar to DA, 5HT plays two key roles during development: during early development it acts as a growth factor by regulating cell division, differentiation, migration, myelination, synaptogenesis, and dendritic pruning before taking on its second role as a neurotransmitter in the mature brain regulating sensory and motor function, cognition, attention, emotion, pain, sleep and arousal (reviewed in Brummelte et al., 2017; Shah et al., 2018). 5HT is also central to the development and function of two key stress response systems – the hypothalamic-pituitary–adrenal and the locus–coeruleus–NE systems that shape self-regulation and mental health across the lifespan and was already discussed

above (reviewed in Brummelte et al., 2017; Shah et al., 2018). From these functions two scenarios for how alteration of the 5HT system by MDMA can lead to the observed neurobehavioural effects in children can be envisioned. First, through a direct effect of MDMA on the serotoninergic system or secondly by indirectly interfering with the proper development of the locus coeruleus-NE system. Interestingly, the postnatal phase in rats is the most sensitive when it comes to MDMA interfering with serotonin transporter binding, as postnatal depletion of serotonin in rat pups via treatment with p-chlorophenylalanine (PCPA) leads to locomotor deficits (Vinay et al., 2005). MRI analyses in serotonin transporter *Slc6a4* knockout mice clearly shows that despite the small number of 5HT neurons and their localization to the brainstem, 5HT plays an important role in neuroanatomical organization (Ellegood et al., 2018). In terms of development of the serotonergic system the first postnatal weeks in rodents correspond to the 3rd trimester of pregnancy in humans (Suri et al., 2015).

Despite the multitude of studies describing effects of MDMA on neurotransmitter concentrations in developing brains, the precise MoA underlying the DNT of MDMA is still mysterious.

Behavioural endpoints

The second most studied group of endpoints following MDMA exposure was behaviour, evaluated in fourteen articles summarized inTable 4 and Figure 4 (Barenys et al., 2010; Broening et al., 2001; Canales and Ferrer-Donato, 2014; Cohen et al., 2005; Galineau et al., 2005; Heuland et al., 2010; Koprich et al., 2003b; Piper and Meyer, 2006; Schaefer et al., 2013; Skelton et al., 2012; St Omer et al., 1991; Thompson et al., 2009; Vorhees et al., 2009, 2004). Here, locomotor behaviour was studied after pre- or postnatal MDMA exposure. Interestingly, time of exposure is crucial for the adverse outcome: exposure during gestation either causes no adverse effects in locomotion or significant hyperactivity (Canales and Ferrer-Donato, 2014; Koprich et al., 2003b; St Omer et al., 1991; Thompson et al., 2009). In contrast, direct postnatal MDMA administration causes hypoactivity (Cohen et al., 2005; Skelton et al., 2012; Vorhees et al., 2009), independently of when during the postnatal period up to PND20 the exposure takes place. Therefore, among all exposure schemes reviewed, postnatal exposure towards MDMA produces the most similar effects in rat compared to human behaviour described in section 3.1. One major issue of pre- and postnatal exposure in rats compared to humans is the pre- and postnatal exposure routes. During gestation, in analogy to humans, the rat fetus is exposed to the test substance by transplacental transfer from the dam. However, postnatally rat pups' chemical exposure happens through breast milk or in most of the reviewed studies by direct subcutaneous administration. In contrast, during the corresponding rat postnatal phase humans are still developing in utero (Clancy et al., 2007, 2001) and thus exposed through the placental route. This can lead to different exposure doses of parent compounds and/or their metabolites between the species (Tsuji and Crofton, 2012). If these kinetic issues, translating neurodevelopmental time differences and/or species differences are part of the reason why pre- and postnatal MDMA exposure causes such opposite effects only in rat offspring, yet not in children is currently enigmatic.

Other behaviours commonly tested in a neurobehavioural testing battery are for example swimming/escape ability or spatial learning. No adverse effects were detected in any of the seven studies evaluating swimming/escape ability which included different pre- and postnatal exposure schemes (Broening et al., 2001; Cohen et al., 2005; Schaefer et al., 2013; Skelton et al.,

2012; St Omer et al., 1991; Vorhees et al., 2009, 2004). These results reflect an equal performance in swimming and motivation in MDMA treated pups than in controls and are helpful to interpret results of other behavioural tests requiring swimming skills and motivation to escape. Such a commonly used behavioural test that combines swimming and motivation to escape with spatial learning skills is the Morris Water Maze (MWM). Drugs increasing serotonergic stimulation, like selective serotonin reuptake inhibitors, can have a positive influence on motivation and swimming behaviour in adult rats and thus modulate the performance in the MWM (Bogdanova et al., 2013). However, six out of seven articles found that MDMA impaired spatial learning in the MWM independent of altered swimming and motivation behaviour, which in most cases depended on the exposure period, being the latest postnatal period (PND10 to PND20) the most sensitive one (Broening et al., 2001; Schaefer et al., 2013; Skelton et al., 2012; Thompson et al., 2009; Vorhees et al., 2009, 2004). MDMA developmental exposure decreases serotonin content in the hippocampus (see Neurotransmission endpoints), yet other studies evaluating the effects of drugs producing depletion of serotonin, like PCPA, did not detect alterations in these behaviours in adult animals (Page et al., 1999). As neurotransmitters have trophic functions early during development which are clearly distinct from synaptic signal transmission, adult neurotoxicity studies might not be representative for developmental exposure in their outcomes. Brain regions implied in MWM navigation include the striatum (Whishaw et al., 1987), prefrontal cortex (Mogensen et al., 1995), and especially the hippocampus (Morris et al., 1982), the latter being the brain region where most consistent effects of developmental MDMA exposure on neurotransmitter, especially 5HT concentrations were measured. Serotonergic lesions have already been related to severe spatial learning deficits in rats (Richter-Levin et al., 1994), which can be reduced by restoring the serotonergic innervation of the hippocampus by raphe grafts (Richter-Levin et al., 1994). If indeed MDMA acts via the serotonergic system on rat behavior, i.e. spatial memory, and if there is any relevance of this behavioural change for humans needs further investigations.

For all other behavioural tests studies were sparse and their results contradictory. Therefore, they could not be sufficiently evaluated within this review.

General developmental landmarks

Two of the studies analyzed included the evaluation of MDMA effects on general developmental landmarks (Table 5), as eye opening or incisor eruption (Heuland et al., 2010; St Omer et al., 1991). The inclusion of these parameters is valuable as it helps to discern between general developmental delay and specific neurodevelopmental effects. Yet within the two studies including general developmental landmarks, opposite results were produced. While one study reported delayed eye opening and incisor eruption (Heuland et al., 2010) the second one did not find any of these developmental delays (St Omer et al., 1991). Exposure period in the positive study was shorter than the negative study and contained the same time frame. Moreover, the studies shared the maximum dose of MDMA (10 mg/kg b.w.). One explanation of the differences in result could be the rat strain used; general development of the Sprague-Dawley rat was unaffected by MDMA, while Wistar rat pups showed the developmental delay. Although these results are not conclusive, inclusion of such general developmental endpoints in neurodevelopmental studies is advisable.

Effects on Growth Factors

Out of the 30 studies in rats only 4 evaluated the effects of postnatal MDMA exposure on growth factor signaling (Dzietko et al., 2010; Koprich et al., 2003a; Piper et al., 2009; Schaefer et al.,

2012). Growth factors studied were nerve growth factor (NGF), neurotrophin-3 (NT-3), and brain-derived neurotrophic factor (BDNF; see Table 6). None of the studies found alterations in NGF concentration or gene expression, while the only one evaluating effects in NT-3 detected a significant increase in its gene expression (Dzietko et al., 2010). All four studies included the evaluation of BDNF, but only two observed a significant increase in its gene or protein expression (Dzietko et al., 2010; Koprich et al., 2003a). In this case a general conclusion cannot be drawn from the data because exposure timing, strain and brain regions were all different in the studies.

Other endpoints

Among other endpoints tested (Table 7) it is worth mentioning two studies evaluating cell death and two more evaluating the effects of MDMA on neurite length and number of neurites per neuron. Cell death was detected in both studies after doses of 60 mg/kg, 3 x 20 mg/kg or 2x 10 mg/kg (Dzietko et al., 2010; Meyer et al., 2004), especially after early postnatal exposure (until PND6), but not after later exposure (PND13 or PND20). Adverse effects in neurite length and number of neurites per neuron were also detected in both studies (Thompson et al., 2012; Williams et al., 2014) but not in all areas analyzed. One of these studies included prenatal exposure to 2x15 mg/kg of MDMA while the other one included postnatal exposure to 2x20 mg/kg, so these results seem independent of the exposure period. During rodent late gestation, neurite outgrowth is modulated by DA (Money and Stanwood, 2013). Hence, a disturbance of the DAergic system by MDMA that is involved in neurite outgrowth may occur but has to be substantiated by experimental evidence.

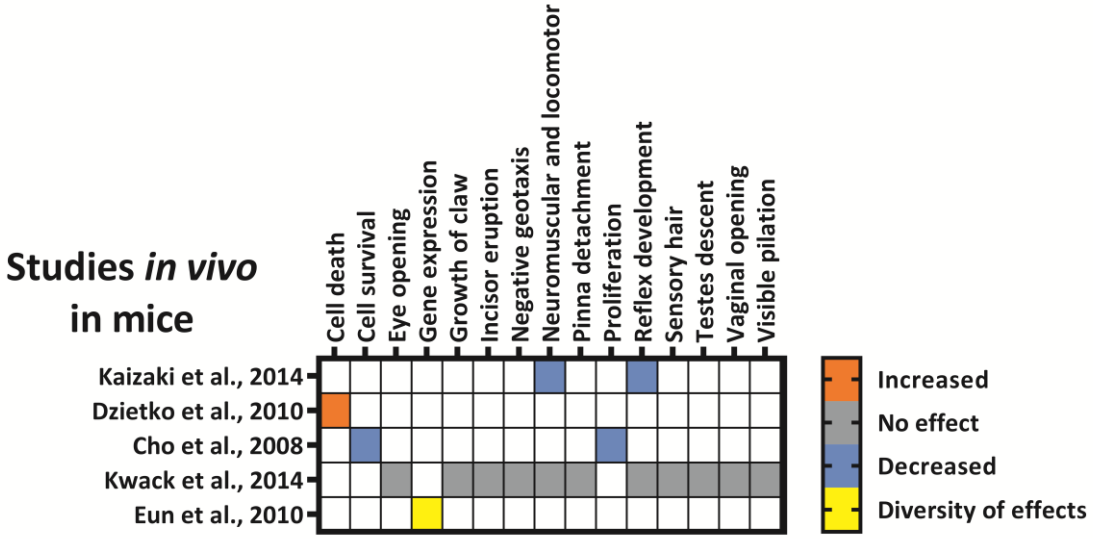

Figure 2. Heatmap summarizing the information about DNT studies of MDMA *in vivo* in mice collected in Table 2. Endpoints measured in a particular study are indicated with colored cells depending on the effect observed (increase – orange, decrease – blue, diverse effects – yellow) or the absence of effect (grey). For more information about the details of each specific study, readers are referred to Table 2.

Table 2. Summary of DNT studies of MDMA *in vivo* in mice.

Reference	Strain	Exposure period	Frequency of administration	Route	Endpoint Category	Endpoint	Analytical/test method	Measurement time-points	Time-point alteration is observed	Doses studied	LOAEL	Effect observed
(Kaizaki et al., 2014)	BALB/c	GD1-PND21	daily	oral	Behaviour	Reflex development	Righting reflex test	PND4 to PND14	PND10-PND14	20mg/10ml/kg	20mg/10ml/kg	Delayed development
	BALB/c	GD1-PND21	daily	oral	Behaviour	Reflex development	Cliff-avoidance test	PND9 to PND18	PND9-PND15	20mg/10ml/kg	20mg/10ml/kg	Delayed development
	BALB/c	GD1-PND21	daily	oral	Behaviour	Neuromuscular and locomotor	Wire hanging maneuver	PND10 to PND19	PND10-PND19	20mg/10ml/kg	20mg/10ml/kg	Lower ability
(Dzietko et al., 2010)	BL/6/BDNF+/-	PND6	single day	i.p.	Histology	Cell death	IHC	PND7	PND7	60 mg/kg	60 mg/kg	Increased
(Cho et al., 2008)	C57BL/6	GD6-PND21	daily	o.g.	Histology	Proliferation	BrdU incorporation	PND82	PND82	1.25 or 20 mg/kg	1.25 mg/kg	Decreased
	C57BL/6	GD6-PND21	daily	o.g.	Histology	Cell survival	Cell count	PND109	PND109	1.25 or 20 mg/kg	20 mg/kg	Decreased
(Kwack et al., 2014)	C57BL/6	GD6-PND21	daily	o.g.	General developmental landmarks	Sensory hair	External examination	PND4	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	General developmental landmarks	Pinna detachment	External examination	PND4	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	General developmental landmarks	Visible pilation	External examination	PND7	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	Behaviour	Reflex development	External examination	PND7	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	Behaviour	Reflex development	Surface righting reflex	PND9	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	Behaviour	Negative geotaxis	Negative geotaxis	PND9	-	1.25, 5 or 20 mg/kg	-	

	C57BL/6	GD6-PND21	daily	o.g.	General developmental landmarks	Incisor eruption	External examination	PND10	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	General developmental landmarks	Growth of claw	External examination	PND10	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	Behaviour	Reflex development	Midair righting reflex	PND14	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	Behaviour	Reflex development	Cliff avoidance	PND14	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	Behaviour	Neuromuscular and locomotor	Wire grasping	PND14	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	General developmental landmarks	Eye opening	External examination	PND14	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	Behaviour	Reflex development	Cornea reflex	PND20	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	General developmental landmarks	Testes descent	External examination	PND20	-	1.25, 5 or 20 mg/kg	-	
	C57BL/6	GD6-PND21	daily	o.g.	General developmental landmarks	Vaginal opening	External examination	PND30	-	1.25, 5 or 20 mg/kg	-	
(Eun et al., 2010)	C57BL/6N	GD15-PND21	daily	oral	Other	Gene expression	DNA microarray	PND77	PND77	20 mg/kg	20 mg/kg	Altered gene expression

GD: gestational day; IHC: immunohistochemistry; i.p.: intraperitoneal; o.g.: oral gavage; PND: postnatal day.

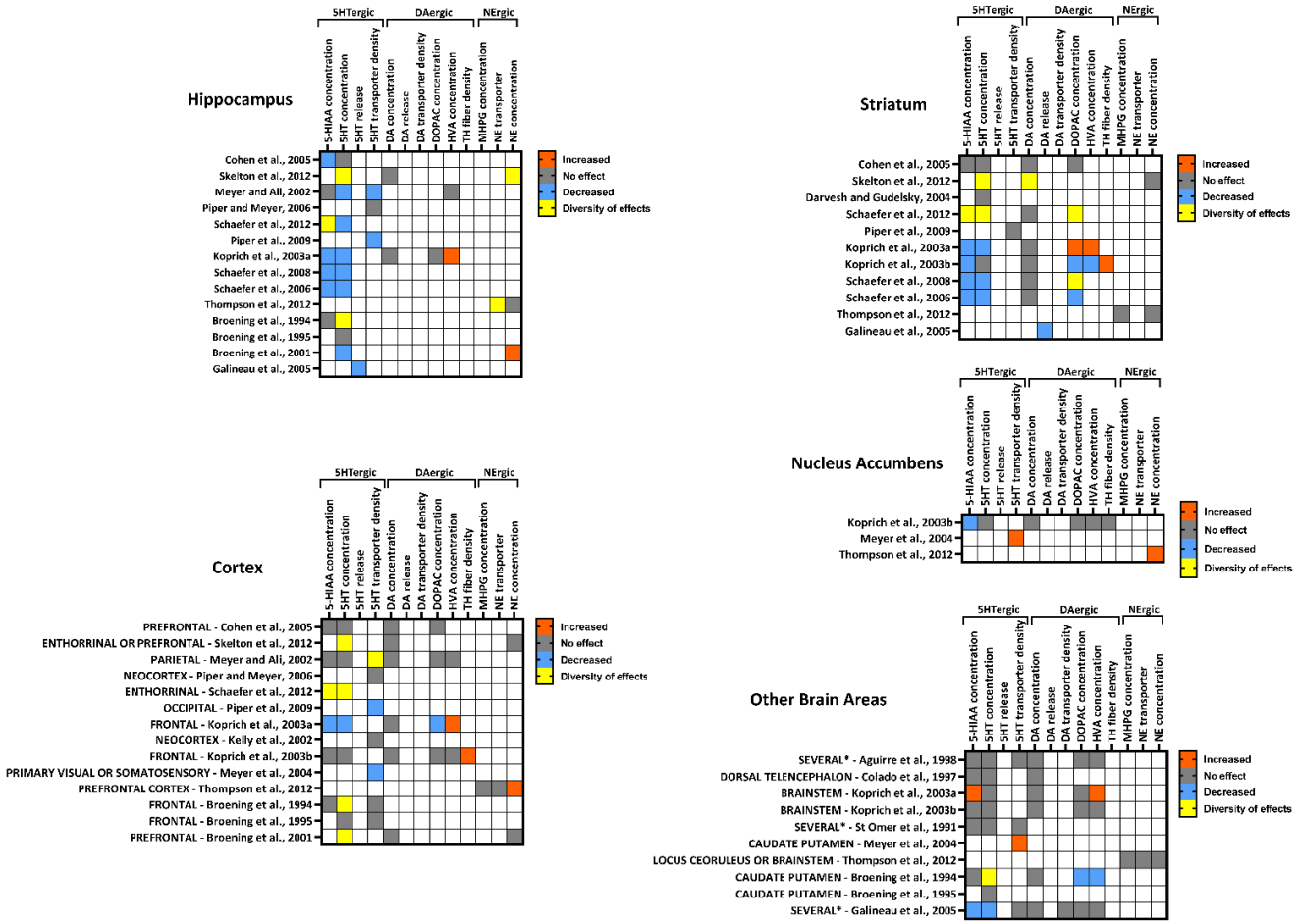

Figure 3. Heatmap panels summarizing the information about DNT studies of MDMA *in vivo* in rats evaluating neurotransmission endpoints collected in Table 3. Endpoints measured in a particular study are indicated with colored cells depending on the effect observed (increase – orange, decrease – blue, diverse effects – yellow) or the absence of effect (grey). Cortical areas evaluated in the 'Cortex' panel are indicated in capital letters before the reference of each study. For more information about the details of each specific study, and the different brain areas included in the three studies of the panel 'Other Brain Areas' indicated with SEVERAL*, readers are referred to Table 3.

Table 3. Summary of DNT studies of MDMA *in vivo* in rats evaluating neurotransmission related endpoints.

Reference	Strain	Exposure period	Frequency of administration	Route	Endpoint	Analytical/test method	Brain region	Measurement time-points	Time-point alteration is observed	Doses studied	LOAEL	Effect observed
(Cohen et al., 2005)	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND142	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Striatum	PND142	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Prefrontal cortex	PND142	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND142	PND142	2x 20mg/kg	2x 20mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Striatum	PND142	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Prefrontal cortex	PND142	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Striatum	PND142	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Prefrontal cortex	PND142	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DOPAC concentration	HPLC	Striatum	PND142	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DOPAC concentration	HPLC	Prefrontal cortex	PND142	-	2x 20mg/kg	-	-
(Skelton et al., 2012)	S.D.	PND 1–10	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND83	PND83	4x 10 and 4x 15 mg/kg	4x 10 mg/kg	Decreased
	S.D.	PND 6–15	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND83	-	4x 10 and 4x 15 mg/kg	-	-
	S.D.	PND 11–20	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND83	-	4x 10 and 4x 15 mg/kg	-	-
	S.D.	PND 1–10	daily	s.c.	5HT concentration	HPLC	Enthorrinal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
	S.D.	PND 6–15	daily	s.c.	5HT concentration	HPLC	Enthorrinal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
	S.D.	PND 11–20	daily	s.c.	5HT concentration	HPLC	Enthorrinal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
	S.D.	PND 1–10	daily	s.c.	5HT concentration	HPLC	Prefrontal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
	S.D.	PND 6–15	daily	s.c.	5HT concentration	HPLC	Prefrontal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
	S.D.	PND 11–20	daily	s.c.	5HT concentration	HPLC	Prefrontal cortex	PND83	PND83	4x 10 and 4x 15 mg/kg	4x 10 mg/kg	Decreased

S.D.	PND 1–10	daily	s.c.	5HT concentration	HPLC	Striatum	PND83	PND83	4x 10 and 4x 15 mg/kg	4x 10 mg/kg	Increased
S.D.	PND 6–15	daily	s.c.	5HT concentration	HPLC	Striatum	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 11–20	daily	s.c.	5HT concentration	HPLC	Striatum	PND83	PND83	4x 10 and 4x 15 mg/kg	4x 15 mg/kg	Increased
S.D.	PND 1–10	daily	s.c.	NE concentration	HPLC	Hippocampus	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 6–15	daily	s.c.	NE concentration	HPLC	Hippocampus	PND83	PND83	4x 10 and 4x 15 mg/kg	4x 15mg/kg	Increased
S.D.	PND 11–20	daily	s.c.	NE concentration	HPLC	Hippocampus	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 1–10	daily	s.c.	NE concentration	HPLC	Enthorrinal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 6–15	daily	s.c.	NE concentration	HPLC	Enthorrinal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 11–20	daily	s.c.	NE concentration	HPLC	Enthorrinal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 1–10	daily	s.c.	NE concentration	HPLC	Prefrontal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 6–15	daily	s.c.	NE concentration	HPLC	Prefrontal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 11–20	daily	s.c.	NE concentration	HPLC	Prefrontal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 1–10	daily	s.c.	NE concentration	HPLC	Striatum	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 6–15	daily	s.c.	NE concentration	HPLC	Striatum	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 11–20	daily	s.c.	NE concentration	HPLC	Striatum	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 1–10	daily	s.c.	DA concentration	HPLC	Hippocampus	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 6–15	daily	s.c.	DA concentration	HPLC	Hippocampus	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 11–20	daily	s.c.	DA concentration	HPLC	Hippocampus	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 1–10	daily	s.c.	DA concentration	HPLC	Enthorrinal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 6–15	daily	s.c.	DA concentration	HPLC	Enthorrinal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 11–20	daily	s.c.	DA concentration	HPLC	Enthorrinal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 1–10	daily	s.c.	DA concentration	HPLC	Prefrontal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 6–15	daily	s.c.	DA concentration	HPLC	Prefrontal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 11–20	daily	s.c.	DA concentration	HPLC	Prefrontal cortex	PND83	-	4x 10 and 4x 15 mg/kg	-	-
S.D.	PND 1–10	daily	s.c.	DA concentration	HPLC	Striatum	PND83	-	4x 10 and 4x 15 mg/kg	-	-

	S.D.	PND 6–15	daily	s.c.	DA concentration	HPLC	Striatum	PND83	-	4x 10 and 4x 15 mg/kg	-	-
	S.D.	PND 11–20	daily	s.c.	DA concentration	HPLC	Striatum	PND83	PND83	4x 10 and 4x 15 mg/kg	4x 15mg/kg	Increased
(Aguirre et al., 1998)	Wistar	GD6-GD20	e.o.d.	s.c.	DA concentration	HPLC	Striatum and hippothalamus	PND15	-	20 mg/kg	-	-
	Wistar	GD6-GD20	e.o.d.	s.c.	5HT concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND15	-	20 mg/kg	-	-
	Wistar	GD6-GD20	e.o.d.	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Frontal cortex and Hippoacampus	PND15	-	20 mg/kg	-	-
	Wistar	GD6-GD20	e.o.d.	s.c.	5-HIAA concentration	HPLC	Frontal cortex, striatum, hippocampus and -hippothalamus	PND15	-	20 mg/kg	-	-
	Wistar	GD6-GD20	e.o.d.	s.c.	HVA concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND15	-	20 mg/kg	-	-
	Wistar	GD6-GD20	e.o.d.	s.c.	DOPAC concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND15	-	20 mg/kg	-	-
	Wistar	PND14	e.o.d.	s.c.	5HT concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND21	-	20 mg/kg	-	-
	Wistar	PND21	e.o.d.	s.c.	5HT concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND28	-	20 mg/kg	-	-
	Wistar	PND14	e.o.d.	s.c.	5-HIAA concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND21	-	20 mg/kg	-	-
	Wistar	PND21	e.o.d.	s.c.	5-HIAA concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND28	-	20 mg/kg	-	-
	Wistar	PND14	e.o.d.	s.c.	DA concentration	HPLC	Striatum and hippothalamus	PND21	-	20 mg/kg	-	-
	Wistar	PND21	e.o.d.	s.c.	DA concentration	HPLC	Striatum and hippothalamus	PND28	-	20 mg/kg	-	-
	Wistar	PND14	e.o.d.	s.c.	HVA concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND21	-	20 mg/kg	-	-

	Wistar	PND21	e.o.d.	s.c.	HVA concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND28	-	20 mg/kg	-	-
	Wistar	PND14	e.o.d.	s.c.	DOPAC concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND21	-	20 mg/kg	-	-
	Wistar	PND21	e.o.d.	s.c.	DOPAC concentration	HPLC	Frontal cortex, striatum, hippocampus and hippothalamus	PND28	-	20 mg/kg	-	-
	Wistar	PND14	e.o.d.	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Frontal cortex and Hippoacampus	PND21	-	20 mg/kg	-	-
	Wistar	PND21	e.o.d.	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Frontal cortex and Hippoacampus	PND28	-	20 mg/kg	-	-
(Darvesh and Gudelsky, 2004)	S.D.	PND21	single day	s.c.	5HT concentration	HPLC	Striatum	PND28	-	20 mg/kg	-	-
(Meyer and Ali, 2002)	S.D.	PND1-PND4	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND25	PND25	2x 10 mg/kg	2x 10 mg/kg	Decreased
	S.D.	PND1-PND4	daily	s.c.	HVA concentration	HPLC	Hippocampus	PND25	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND25	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Hippocampus	PND25	PND25	2x 10 mg/kg	2x 10 mg/kg	Decreased
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Hippocampus	PND60	PND60	2x 10 mg/kg	2x 10 mg/kg	Decreased
	S.D.	PND1-PND4	daily	s.c.	5HT concentration	HPLC	Parietal cortex	PND25	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	DA concentration	HPLC	Parietal cortex	PND25	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	HVA concentration	HPLC	Parietal cortex	PND25	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	DOPAC concentration	HPLC	Parietal cortex	PND25	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	5-HIAA concentration	HPLC	Parietal cortex	PND25	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Parietal cortex	PND25	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Parietal cortex	PND60	PND60	2x 10 mg/kg	2x 10 mg/kg	Decreased
	Wistar	GD14-GD17	daily	s.c.	5HT concentration	HPLC	Dorsal telencephalon	PND7	-	2x 20 mg/kg	-	-

(Colado et al., 1997)	Wistar	GD14-GD17	daily	s.c.	DA concentration	HPLC	Dorsal telencephalon	PND7	-	2x 20 mg/kg	-	-
	Wistar	GD14-GD17	daily	s.c.	5-HIAA concentration	HPLC	Dorsal telencephalon	PND7	-	2x 20 mg/kg	-	-
(Piper and Meyer, 2006)	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	[^{3}H]citalopram binding assay	Cortex	PND70	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	[^{3}H]citalopram binding assay	Hippocampus	PND70	-	2x 10 mg/kg	-	-
(Schaefer et al., 2012)	S.D.	PND11	single day	s.c.	5HT concentration	HPLC	Hippocampus	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND21	PND21	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND15	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND16	PND16	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11	single day	s.c.	5HT concentration	HPLC	Enthorrinal cortex	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Enthorrinal cortex	PND21	PND21	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND15	daily	s.c.	5HT concentration	HPLC	Enthorrinal cortex	PND16	PND16	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11	single day	s.c.	5HT concentration	HPLC	Striatum	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Striatum	PND21	PND21	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND15	daily	s.c.	5HT concentration	HPLC	Striatum	PND16	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND60	PND60	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Enthorrinal cortex	PND60	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Striatum	PND60	-	4X 10 mg/kg	-	-
	S.D.	PND11	single day	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11	single day	s.c.	5-HIAA concentration	HPLC	Enthorrinal cortex	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11	single day	s.c.	5-HIAA concentration	HPLC	Striatum	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND21	PND21	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Enthorrinal cortex	PND21	PND21	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Striatum	PND21	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND15	daily	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND16	PND16	4X 10 mg/kg	4X 10 mg/kg	Decreased

	S.D.	PND11-PND15	daily	s.c.	5-HIAA concentration	HPLC	Enthorrinal cortex	PND16	PND16	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND15	daily	s.c.	5-HIAA concentration	HPLC	Striatum	PND16	PND16	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11	single day	s.c.	DA concentration	HPLC	Striatum	PND12	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Striatum	PND21	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND15	daily	s.c.	DA concentration	HPLC	Striatum	PND16	-	4X 10 mg/kg	-	-
	S.D.	PND11	single day	s.c.	DOPAC concentration	HPLC	Striatum	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	DOPAC concentration	HPLC	Striatum	PND21	PND21	4X 10 mg/kg	4X 10 mg/kg	Increased
	S.D.	PND11-PND15	daily	s.c.	DOPAC concentration	HPLC	Striatum	PND16	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND60	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Enthorrinal cortex	PND60	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Striatum	PND60	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Striatum	PND60	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DOPAC concentration	HPLC	Striatum	PND60	-	4X 10 mg/kg	-	-
(Piper et al., 2009)	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	[^{3}H]citalopram binding assay	Occipital cortex	PND11, PND30	PND30	2x 10 mg/kg	2x 10 mg/kg	Decreased
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	[^{3}H]citalopram binding assay	Hippocampus	PND11, PND30	PND11	2x 10 mg/kg	2x 10 mg/kg	Decreased
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	[^{3}H]citalopram binding assay	Striatum	PND11, PND30	-	2x 10 mg/kg	-	-
(Koprich et al., 2003a)	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Frontal cortex	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Striatum	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Brainstem	PND21	-	2x 20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Frontal cortex	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Striatum	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Brainstem	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increased

	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Frontal cortex	PND21	-	2x 20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Striatum	PND21	-	2x 20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Hippocampus	PND21	-	2x 20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Brainstem	PND21	-	2x 20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DOPAC concentration	HPLC	Frontal cortex	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	DOPAC concentration	HPLC	Striatum	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increased
	S.D.	PND11-PND20	daily	s.c.	DOPAC concentration	HPLC	Hippocampus	PND21	-	2x 20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DOPAC concentration	HPLC	Brainstem	PND21	-	2x 20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	HVA concentration	HPLC	Frontal cortex	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increased
	S.D.	PND11-PND20	daily	s.c.	HVA concentration	HPLC	Striatum	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increased
	S.D.	PND11-PND20	daily	s.c.	HVA concentration	HPLC	Hippocampus	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increased
	S.D.	PND11-PND20	daily	s.c.	HVA concentration	HPLC	Brainstem	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increased
(Kelly et al., 2002)	S.D.	GD15-GD18	daily	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Cortex	PND40	-	2x 20 mg/kg	-	-
	S.D.	PND10-PND13	daily	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Cortex	PND40	-	2x 20 mg/kg	-	-
	S.D.	PND15-PND18	daily	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Cortex	PND40	-	2x 20 mg/kg	-	-
	S.D.	GD15-GD18 and PND10-PND13	daily	s.c.	5HT transporter density	[^{3}H]paroxetine binding assay	Cortex	PND25	-	2x 20 mg/kg	-	-
(Koprich et al., 2003b)	S.D.	GD14-GD20	daily	s.c.	5HT concentration	HPLC	Frontal cortex	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	5HT concentration	HPLC	Striatum	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	5HT concentration	HPLC	Nucleus accumbens	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	5HT concentration	HPLC	Brainstem	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	5-HIAA concentration	HPLC	Frontal cortex	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	5-HIAA concentration	HPLC	Striatum	PND3 and PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Decreased

	S.D.	GD14-GD20	daily	s.c.	5-HIAA concentration	HPLC	Nucleus accumbens	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Decreased
	S.D.	GD14-GD20	daily	s.c.	5-HIAA concentration	HPLC	Brainstem	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	DA concentration	HPLC	Frontal cortex	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	DA concentration	HPLC	Striatum	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	DA concentration	HPLC	Nucleus accumbens	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	DA concentration	HPLC	Brainstem	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	DOPAC concentration	HPLC	Frontal cortex	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	DOPAC concentration	HPLC	Striatum	PND3 and PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Decreased
	S.D.	GD14-GD20	daily	s.c.	DOPAC concentration	HPLC	Nucleus accumbens	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	DOPAC concentration	HPLC	Brainstem	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	HVA concentration	HPLC	Frontal cortex	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	HVA concentration	HPLC	Striatum	PND3 and PND21	PND3 and PND21	2x 15 mg/kg	2x 15 mg/kg	Decreased
	S.D.	GD14-GD20	daily	s.c.	HVA concentration	HPLC	Nucleus accumbens	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	HVA concentration	HPLC	Brainstem	PND3 and PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Tyrosine hydroxylase fiber density	IHC	Frontal cortex	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
	S.D.	GD14-GD20	daily	s.c.	Tyrosine hydroxylase fiber density	IHC	Striatum	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
	S.D.	GD14-GD20	daily	s.c.	Tyrosine hydroxylase fiber density	IHC	Nucleus accumbens	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
(St Omer et al., 1991)	S.D.	GD6-GD18	daily	o.g.	5HT transporter density	[^{3}H]paroxetine binding assay	Cerebrum	PND29	-	2.5 and 10 mg/kg	-	-
	S.D.	GD6-GD18	daily	o.g.	5HT concentration	HPLC	Caudate nucleus, frontal cortex, or hippocampus	PND27	-	2.5 and 10 mg/kg	-	-

	S.D.	GD6-GD18	daily	o.g.	5-HIAA concentration	HPLC	Caudate nucleus, frontal cortex, or hippocampus	PND27	-	2.5 and 10 mg/kg	-	-
(Schaefer et al., 2008)	S.D.	PND11-PND15	daily	s.c.	DA concentration	HPLC	Striatum	PND16 and PND30	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND15	daily	s.c.	DOPAC concentration	HPLC	Striatum	PND16 and PND30	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND15	daily	s.c.	5HT concentration	HPLC	Striatum	PND16 and PND30	PND16	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND15	daily	s.c.	5-HIAA concentration	HPLC	Striatum	PND16 and PND30	PND16	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND15	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND16 and PND30	PND16	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND15	daily	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND16 and PND30	PND16 and PND30	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Striatum	PND21 and PND30	-	4X 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DOPAC concentration	HPLC	Striatum	PND21 and PND30	PND21	4X 10 mg/kg	4X 10 mg/kg	Increased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Striatum	PND21 and PND30	PND21	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Striatum	PND21 and PND30	PND21	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND21 and PND30	PND21	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND21 and PND30	PND21	4X 10 mg/kg	4X 10 mg/kg	Decreased
(Schaefer et al., 2006)	S.D.	PND11	single day	s.c.	DA concentration	HPLC	Striatum	PND12	-	4X 10 mg/kg	-	-
	S.D.	PND11	single day	s.c.	DOPAC concentration	HPLC	Striatum	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11	single day	s.c.	5HT concentration	HPLC	Striatum	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11	single day	s.c.	5-HIAA concentration	HPLC	Striatum	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
	S.D.	PND11	single day	s.c.	5HT concentration	HPLC	Hippocampus	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased

	S.D.	PND11	single day	s.c.	5-HIAA concentration	HPLC	Hippocampus	PND12	PND12	4X 10 mg/kg	4X 10 mg/kg	Decreased
(Meyer et al., 2004)	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	IHC	Primary visual cortex	PN month 9	PN month 9	2x 10 mg/kg	2x 10 mg/kg	Decreased
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	IHC	Primary somatosensory cortex	PN month 9	PN month 9	2x 10 mg/kg	2x 10 mg/kg	Decreased
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	IHC	Caudate-putamen	PN month 9	PN month 9	2x 10 mg/kg	2x 10 mg/kg	Increased
	S.D.	PND1-PND4	daily	s.c.	5HT transporter density	IHC	Nucleus accumbens	PN month 9	PN month 9	2x 10 mg/kg	2x 10 mg/kg	Increased
(Thompson et al., 2012)	S.D.	GD14-GD20	daily	s.c.	NE transporter	[^{3}H] nisoxetine	Hippocampus CA1	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	NE transporter	[^{3}H] nisoxetine	Hippocampus CA2	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
	S.D.	GD14-GD20	daily	s.c.	NE transporter	[^{3}H] nisoxetine	Hippocampus CA3	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
	S.D.	GD14-GD20	daily	s.c.	NE transporter	[^{3}H] nisoxetine	Hippocampus DG	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
	S.D.	GD14-GD20	daily	s.c.	NE transporter	[^{3}H] nisoxetine	Prefrontal cortex Cg3	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	NE transporter	[^{3}H] nisoxetine	Locus coeruleus	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	NE concentration	HPLC	Prefrontal cortex	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
	S.D.	GD14-GD20	daily	s.c.	NE concentration	HPLC	Striatum	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	NE concentration	HPLC	Nucleus accumbens	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
	S.D.	GD14-GD20	daily	s.c.	NE concentration	HPLC	Hippocampus	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	NE concentration	HPLC	Brainstem	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	MHPG concentration	HPLC	Prefrontal cortex	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	MHPG concentration	HPLC	Striatum	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	MHPG concentration	HPLC	Brainstem	PND21	-	2x 15 mg/kg	-	-
(Broening et al., 1994)	S.D.	PND10	single day	oral	5HT concentration	HPLC	Frontal cortex	PND17	-	10, 20, 40 mg/kg	-	-
	S.D.	PND10	single day	oral	5-HIAA concentration	HPLC	Frontal cortex	PND17	-	10, 20, 40 mg/kg	-	-
	S.D.	PND10	single day	oral	5HT concentration	HPLC	Hippocampus	PND17	-	10, 20, 40 mg/kg	-	-

	S.D.	PND10	single day	oral	5-HIAA concentration	HPLC	Hippocampus	PND17	-	10, 20, 40 mg/kg	-	-
	S.D.	PND10	single day	oral	5HT concentration	HPLC	Caudate Putamen	PND17	-	10, 20, 40 mg/kg	-	-
	S.D.	PND10	single day	oral	5-HIAA concentration	HPLC	Caudate Putamen	PND17	-	10, 20, 40 mg/kg	-	-
	S.D.	PND10	single day	oral	5HT concentration	HPLC	Frontal cortex	3, 6, 12, 24, 72, 120 and 168h after MDMA	3, 6, 12, 24h after MDMA	40 mg/kg	40 mg/kg	Decreased
	S.D.	PND10	single day	oral	5HT concentration	HPLC	Hippocampus	3, 6, 12, 24, 72, 120 and 168h after MDMA	3, 6, 12, 24h after MDMA	40 mg/kg	40 mg/kg	Decreased
	S.D.	PND10	single day	oral	5HT concentration	HPLC	Caudate Putamen	3, 6, 12, 24, 72, 120 and 168h after MDMA	3, 6, 12, 24h after MDMA	40 mg/kg	40 mg/kg	Decreased
	S.D.	PND10	single day	oral	5HT transporter density	[^{3}H]paroxetine binding assay	Frontal cortex	3, 6, 12, 24, 72, 120 and 168h after MDMA	-	40 mg/kg	-	-
	S.D.	PND10	single day	oral	DA concentration	HPLC	Caudate Putamen	3, 6, 12, 24, 72, 120 and 168h after MDMA	-	40 mg/kg	-	-
	S.D.	PND10	single day	oral	DOPAC concentration	HPLC	Caudate Putamen	3, 6, 12, 24, 72, 120 and 168h after MDMA	6h after MDMA	40 mg/kg	40 mg/kg	Decreased
	S.D.	PND10	single day	oral	HVA concentration	HPLC	Caudate Putamen	3, 6, 12, 24, 72, 120 and 168h after MDMA	3, 6, 12, 24h after MDMA	40 mg/kg	40 mg/kg	Decreased
(Broening et al., 1995)	S.D.	PND10	single day	oral	5HT concentration	HPLC	Frontal cortex	PND17	-	20, 40 mg/kg	-	-
	S.D.	PND10	single day	oral	5HT concentration	HPLC	Hippocampus	PND17	-	20, 40 mg/kg	-	-

	S.D.	PND10	single day	oral	5HT concentration	HPLC	Caudate Putamen	PND17	-	20, 40 mg/kg	-	-
	S.D.	PND10	single day	oral	5HT transporter density	[^{3}H]paroxetine binding assay	Frontal cortex	PND17	-	20, 40 mg/kg	-	-
(Broening et al., 2001)	S.D.	PND1-PND10	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND105	PND105	2x5, 2x10, 2x20 mg/kg	2x 5 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Hippocampus	PND105	PND105	2x5, 2x10, 2x20 mg/kg	2x 5 mg/kg	Decreased
	S.D.	PND1-PND10	daily	s.c.	5HT concentration	HPLC	Prefrontal cortex	PND105	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	5HT concentration	HPLC	Prefrontal cortex	PND105	PND105	2x5, 2x10, 2x20 mg/kg	2x 5 mg/kg	Decreased
	S.D.	PND1-PND10	daily	s.c.	NE concentration	HPLC	Hippocampus	PND105	PND105	2x5, 2x10, 2x20 mg/kg	2x 10 mg/kg	Increased
	S.D.	PND11-PND20	daily	s.c.	NE concentration	HPLC	Hippocampus	PND105	PND105	2x5, 2x10, 2x20 mg/kg	2x 5 mg/kg	Increased
	S.D.	PND1-PND10	daily	s.c.	NE concentration	HPLC	Prefrontal cortex	PND105	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	NE concentration	HPLC	Prefrontal cortex	PND105	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND1-PND10	daily	s.c.	DA concentration	HPLC	Prefrontal cortex	PND105	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	DA concentration	HPLC	Prefrontal cortex	PND105	-	2x5, 2x10, 2x20 mg/kg	-	-
(Galineau et al., 2005)	S.D.	GD13-GD20	daily	s.c.	DA concentration	HPLC	Whole brain	GD14, 16, 18, 20, PND0, 7, 14, 21	-	10 mg/kg	-	-
	S.D.	GD13-GD20	daily	s.c.	5HT concentration	HPLC	Whole brain	GD14, 16, 18, 20, PND0, 7, 14, 21	PND0	10 mg/kg	10 mg/kg	Decreased
	S.D.	GD13-GD20	daily	s.c.	DOPAC concentration	HPLC	Whole brain	GD14, 16, 18, 20, PND0, 7, 14, 21	-	10 mg/kg	-	-
	S.D.	GD13-GD20	daily	s.c.	HVA concentration	HPLC	Whole brain	GD14, 16, 18, 20, PND0, 7, 14, 21	-	10 mg/kg	-	-
	S.D.	GD13-GD20	daily	s.c.	5-HIAA concentration	HPLC	Whole brain	GD14, 16, 18, 20, PND0, 7, 14, 21	PND0	10 mg/kg	10 mg/kg	Decreased
	S.D.	GD13-GD20	daily	s.c.	5HT transporter density	[^{3}H]MADAM	Raphe nuclei, hypothalamus, somatosensory cortical areas, thalamus and hippocampus	GD18, 20, PND0, 7, 14, 21, 28, 70	-	10 mg/kg	-	-

S.D.	GD13-GD20	daily	s.c.	DA transporter density	[^{125}I]PE2I	Substantia nigra, ventral tegmental area, striatum, nucleus accumbens	GD18, 20, PND0, 7, 14, 21, 28, 70	-	10 mg/kg	-	-
S.D.	GD13-GD20	daily	s.c.	DA release	Microdialysis	Striatum	PND70	PND70	10 mg/kg	10 mg/kg	Decreased
S.D.	GD13-GD20	daily	s.c.	5HT release	Microdialysis	Hippocampus	PND70	PND70	10 mg/kg	10 mg/kg	Decreased

e.o.d.: every other day; GD: gestational day; IHC: immunohistochemistry; MHPG: 3-Methoxy-4-hydroxyphenylglycol; o.g.: oral gavage; PND: postnatal day; s.c.: subcutaneous; S.D.: Sprague-Dawley.

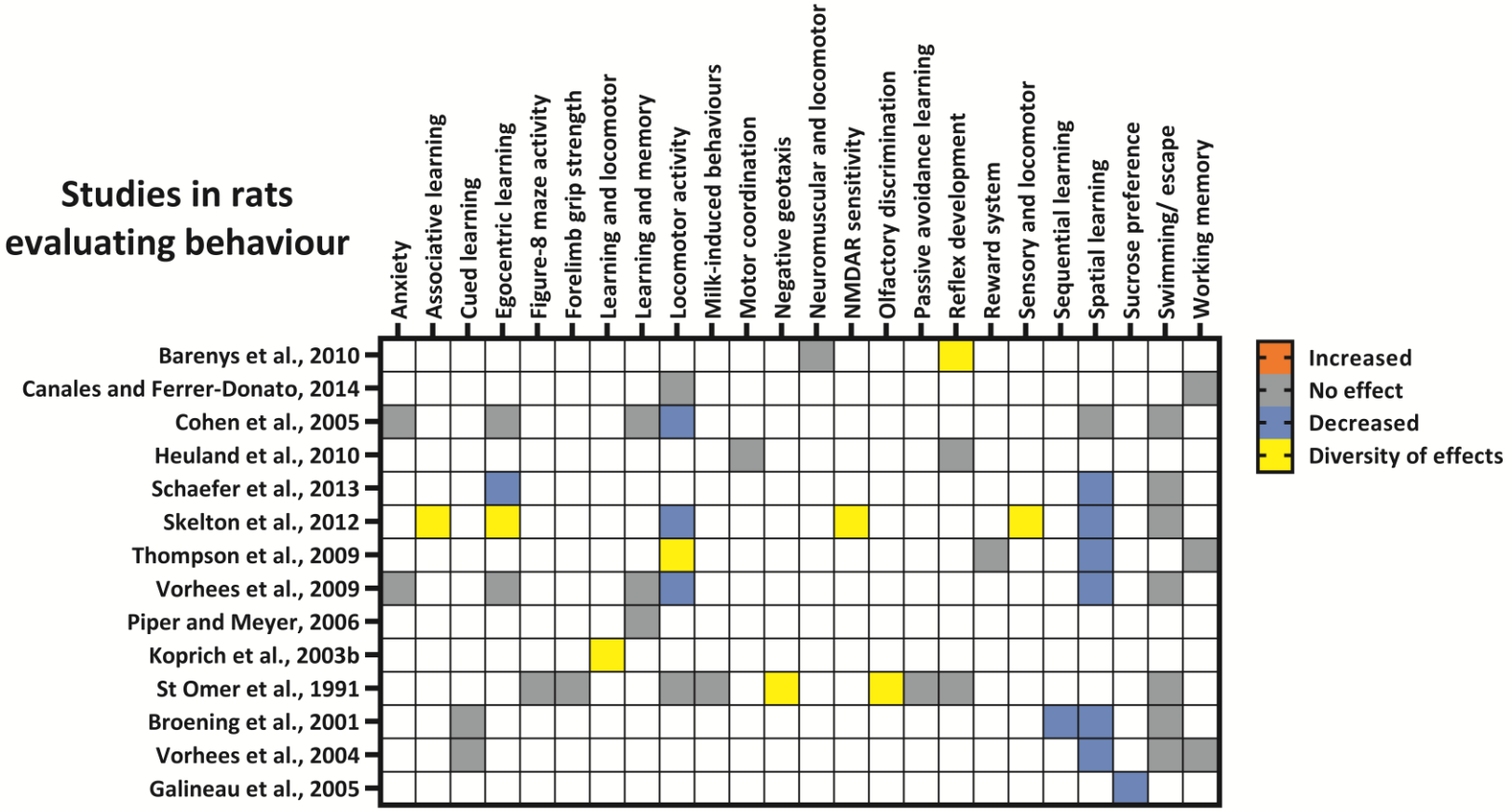

Figure 4. Heatmap summarizing the information about DNT studies of MDMA *in vivo* in rats evaluating behavior related endpoints collected in Table 4. Endpoints measured in a particular study are indicated with colored cells depending on the effect observed (increase – orange, decrease – blue, diverse effects – yellow) or the absence of effect (grey). For more information about the details of each specific study, readers are referred to Table 4.

Table 4. Summary of DNT studies of MDMA *in vivo* in rats evaluating behaviour related endpoints.

Reference	Strain	Exposure period	Frequency of administration	Route	Endpoint	Analytical/test method	Measurement time-points	Time-point alteration is observed	Doses studied	LOAEL	Effect observed
(Barenys et al., 2010)	S.D.	4 W pre-mating + 3 W gestation + 3 W lactation	three days per week	s.c.	Reflex development	Cliff-avoidance test	PND3	PND3	0.5, 5 and 10 mg/kg	10 mg/kg	Decreased
		4 W pre-mating + 3 W gestation + 3 W lactation	three days per week	s.c.	Reflex development	Tail-hang reflex	PND21	-	0.5, 5 and 10 mg/kg	-	-
		4 W pre-mating + 3 W gestation + 3 W lactation	three days per week	s.c.	Reflex development	Righting reflex test	PND21	-	0.5, 5 and 10 mg/kg	-	-
		4 W pre-mating + 3 W gestation + 3 W lactation	three days per week	s.c.	Neuromuscular and locomotor	Rotarod	PND22	-	0.5, 5 and 10 mg/kg	-	-
(Canales and Ferrer-Donato, 2014)	Long Evans	GD13–GD15	daily	o.g.	Working memory	Radial maze	PND97-PND100	-	2x 10 mg/kg	-	-
	Long Evans	GD13–GD15	daily	o.g.	Locomotor activity	Open-field test	PND110	-	2x 10 mg/kg	-	-
(Cohen et al., 2005)	S.D.	PND11-PND20	daily	s.c.	Spatial learning	MWM	PND115-PND125	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Egocentric learning	Cincinnati maze	PND126-PND132	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Learning and memory	Novel object recognition test	PND139	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Anxiety	Elevated Zero Maze	PND107	-	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Locomotor activity	Open-field test	PND140	PND140	2x 20mg/kg	2x 20mg/kg	Lower total distance Less time in center Increased thigmotaxis

	S.D.	PND11-PND20	daily	s.c.	Swimming/ escape	Straight channel	PND108	-	2x 20mg/kg	-	-
(Heuland et al., 2010)	Wistar	GD13-GD20	daily	s.c.	Reflex development	Fox Observational Battery	PND2-PND21	PND3,PND7, PND10, PND11	10 mg/kg	-	-
	Wistar	GD13-GD20	daily	s.c.	Motor coordination	Fox Observational Battery	PND2-PND21	PND10-PND28	10 mg/kg	-	-
(Schaefer et al., 2013)	S.D.	PND11-PND20	daily	s.c.	Swimming/ escape	Straight channel	PD61	-	4x 10 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Spatial learning	MWM	PD80-88	PND80-PND88	4x 10 mg/kg	4x 10 mg/kg	Worse acquisition, retention and reversal
	S.D.	PND11-PND20	daily	s.c.	Egocentric learning	Cincinnati maze	PD62-80	PND62-PND80	4x 10 mg/kg	4x 10 mg/kg	More errors Longer latencies
(Skelton et al., 2012)	S.D.	PND1-PND10	daily	s.c.	Sensory and locomotor	PPI	PND60	PND60	4x10 and 4x15 mg/kg	4x15 mg/kg	Increased startle response
	S.D.	PND6-PND15	daily	s.c.	Sensory and locomotor	PPI	PND60	PND60	4x10 and 4x15 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Sensory and locomotor	PPI	PND60	PND60	4x10 and 4x15 mg/kg	-	-
	S.D.	PND1-PND10	daily	s.c.	Swimming/ escape	Straight channel	PND61	-	4x10 and 4x15 mg/kg	-	-
	S.D.	PND6-PND15	daily	s.c.	Swimming/ escape	Straight channel	PND61	-	4x10 and 4x15 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Swimming/ escape	Straight channel	PND61	-	4x10 and 4x15 mg/kg	-	-
	S.D.	PND1-PND10	daily	s.c.	Egocentric learning	Cincinnati maze	PND62-PND80	PND62-PND80	4x10 and 4x15 mg/kg	-	-
	S.D.	PND6-PND15	daily	s.c.	Egocentric learning	Cincinnati maze	PND62-PND80	PND62-PND80	4x10 and 4x15 mg/kg	4x10 mg/kg	Worse performance
	S.D.	PND11-PND20	daily	s.c.	Egocentric learning	Cincinnati maze	PND62-PND80	PND62-PND80	4x10 and 4x15 mg/kg	4x10 mg/kg	Worse performance
	S.D.	PND1-PND10	daily	s.c.	Spatial learning	MWM	PND62-PND80	PND62-PND80	4x10 and 4x15 mg/kg	4x10 mg/kg	Worse acquisition
	S.D.	PND6-PND15	daily	s.c.	Spatial learning	MWM	PND62-PND80	PND62-PND80	4x10 and 4x15 mg/kg	4x10 mg/kg	Worse acquisition and reversal
	S.D.	PND11-PND20	daily	s.c.	Spatial learning	MWM	PND62-PND80	PND62-PND80	4x10 and 4x15 mg/kg	4x10 mg/kg	Worse acquisition, retention and reversal

	S.D.	PND1-PND10	daily	s.c.	Associative learning	Fear Conditioning	PND81-PND82	PND81-PND82	4x10 and 4x15 mg/kg	4x10 mg/kg	Worse cued fear conditioning
	S.D.	PND6-PND15	daily	s.c.	Associative learning	Fear Conditioning	PND81-PND82	PND81-PND82	4x10 and 4x15 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Associative learning	Fear Conditioning	PND81-PND82	PND81-PND82	4x10 and 4x15 mg/kg	-	-
	S.D.	PND1-PND10	daily	s.c.	Locomotor activity	Open-field test	PND81	PND81	4x10 and 2x15 mg/kg	4x10 mg/kg	Reduced locomotor activity
	S.D.	PND6-PND15	daily	s.c.	Locomotor activity	Open-field test	PND81	PND81	4x10 and 2x15 mg/kg	4x10 mg/kg	Reduced locomotor activity
	S.D.	PND11-PND20	daily	s.c.	Locomotor activity	Open-field test	PND81	PND81	4x10 and 2x15 mg/kg	4x10 mg/kg	Reduced locomotor activity
	S.D.	PND1-PND10	daily	s.c.	NMDAR sensitivity	MK-801 challenge in Open Field	PND82	PND82	4x10 and 4x15 mg/kg	4x10 mg/kg	Reduced MK-801-induced hyperactivity
	S.D.	PND6-PND15	daily	s.c.	NMDAR sensitivity	MK-801 challenge in Open Field	PND82	PND82	4x10 and 4x15 mg/kg	4x10 mg/kg	Reduced MK-801-induced hyperactivity
	S.D.	PND11-PND20	daily	s.c.	NMDAR sensitivity	MK-801 challenge in Open Field	PND82	PND82	4x10 and 4x15 mg/kg	4x10 mg/kg	-
(Thompson et al., 2009)	S.D.	GD14-GD20	daily	s.c.	Locomotor activity	Home cage activity	PND40-PND50	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Locomotor activity	Running wheel	PND36-PND39	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Locomotor activity	Open-field test	PND61-PND62	PND61-62	2x 15 mg/kg	2x 15 mg/kg	Hyperactivity
	S.D.	GD14-GD20	daily	s.c.	Reward system	High Fat Diet	PND132-PND145	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Reward system	Progressive ratio sucrose	PND58	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Reward system	Amphetamine sensitization	PND120	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Reward system	Cocaine Self-administration	PND54-PND105	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Working memory	Four-arm Spontaneous alternations	PND40	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Spatial learning	MWM	PND53-PND72	PND69-73	2x 15 mg/kg	2x 15 mg/kg	Preference for the local cue than for the

											previous platform location
(Vorhees et al., 2009)	S.D.	PND1-PND5	daily	s.c.	Anxiety	Elevated Zero Maze	PND60	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND6-PND10	daily	s.c.	Anxiety	Elevated Zero Maze	PND60	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND11-PND15	daily	s.c.	Anxiety	Elevated Zero Maze	PND60	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND16-PND20	daily	s.c.	Anxiety	Elevated Zero Maze	PND60	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND1-PND5 or PND6-PND10 or PND11-PND15 or PND16-PND20	daily	s.c.	Locomotor activity	Open-field test	PND61	PND61	4x10, 4x15, 4x20, or 4x25 mg/kg	4x15 mg/kg	Decreased distance
	S.D.	PND1-PND5	daily	s.c.	Learning and memory	Novel object recognition test	PND62-PND66	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND6-PND10	daily	s.c.	Learning and memory	Novel object recognition test	PND62-PND66	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND11-PND15	daily	s.c.	Learning and memory	Novel object recognition test	PND62-PND66	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND16-PND20	daily	s.c.	Learning and memory	Novel object recognition test	PND62-PND66	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND1-PND5	daily	s.c.	Swimming/ escape	Straight channel	PND67	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND6-PND10	daily	s.c.	Swimming/ escape	Straight channel	PND67	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND11-PND15	daily	s.c.	Swimming/ escape	Straight channel	PND67	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND16-PND20	daily	s.c.	Swimming/ escape	Straight channel	PND67	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND1-PND5	daily	s.c.	Egocentric learning	Cincinnati maze	PND68-PND83	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND6-PND10	daily	s.c.	Egocentric learning	Cincinnati maze	PND68-PND83	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND11-PND15	daily	s.c.	Egocentric learning	Cincinnati maze	PND68-PND83	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-
	S.D.	PND16-PND20	daily	s.c.	Egocentric learning	Cincinnati maze	PND68-PND83	-	4x10, 4x15, 4x20, or 4x25 mg/kg	-	-

	S.D.	PND1-PND5	daily	s.c.	Spatial learning	MWM	PND84-PND100	PND84-PND100	4x10, 4x15, 4x20, or 4x25 mg/kg	4x10 mg/kg	Impaired learning
	S.D.	PND6-PND10	daily	s.c.	Spatial learning	MWM	PND84-PND100	PND84-PND100	4x10, 4x15, 4x20, or 4x25 mg/kg	4x10 mg/kg	Impaired learning
	S.D.	PND11-PND15	daily	s.c.	Spatial learning	MWM	PND84-PND100	PND84-PND100	4x10, 4x15, 4x20, or 4x25 mg/kg	4x10 mg/kg	Impaired learning
	S.D.	PND16-PND20	daily	s.c.	Spatial learning	MWM	PND84-PND100	PND84-PND100	4x10, 4x15, 4x20, or 4x25 mg/kg	4x10 mg/kg	Impaired learning
(Piper and Meyer, 2006)	S.D.	PND1-PND4	daily	s.c.	Learning and memory	Novel object recognition test	PND69	-	2x 10 mg/kg	-	-
	S.D.	PND1-PND4	daily	s.c.	Learning and memory	Novel object recognition test	PND72	-	2x 10 mg/kg	-	-
(Koprich et al., 2003b)	S.D.	GD14-GD20	daily	s.c.	Learning and locomotor	Novel cage test	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Longer exploratory behaviour Higher locomotor activity
(St Omer et al., 1991)	S.D.	GD6-GD18	daily	o.g.	Reflex development	Righting reflex test	PND2 to PND5	-	2.5 and 10 mg/kg	-	-
	S.D.	GD6-GD18	daily	o.g.	Negative geotaxis	Negative geotaxis	PND7 to PND10	PND7 and PND10	2.5 and 10 mg/kg	2.5 mg/kg	Only in females
	S.D.	GD6-GD18	daily	o.g.	Locomotor activity and Swimming/ escape	Swimming performance	PND7 to PND20	-	2.5 and 10 mg/kg	-	-
	S.D.	GD6-GD18	daily	o.g.	Olfactory discrimination	Olfactory discrimination	PND9 - PND11	PND10 and PND11	2.5 and 10 mg/kg	2.5 and 10 mg/kg	Only males at 2.5 mg/kg in PND10 and only females at 10 mg/kg in PND11
	S.D.	GD6-GD18	daily	o.g.	Forelimb grip strength	Forelimb grip strength	PND14, PND17, PND22	-	2.5 and 10 mg/kg	-	-
	S.D.	GD6-GD18	daily	o.g.	Milk-induced behaviours	Milk-induced behaviours	PND6	-	2.5 and 10 mg/kg	-	-
	S.D.	GD6-GD18	daily	o.g.	Figure-8 maze activity	Figure-8 maze activity	PND21 - PND24	-	2.5 and 10 mg/kg	-	-
	S.D.	GD6-GD18	daily	o.g.	Passive avoidance learning	Passive avoidance learning	PND95 - PND98	-	2.5 and 10 mg/kg	-	-

(Broening et al., 2001)	S.D.	PND1-PND10	daily	s.c.	Swimming/ escape	Straight channel	PND60	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Swimming/ escape	Straight channel	PND60	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND1-PND10	daily	s.c.	Sequential learning	Multiple t-maze	PND63	PND63	2x5, 2x10, 2x20 mg/kg	2x10 mg/kg	Worst performance: errors increase
	S.D.	PND11-PND20	daily	s.c.	Sequential learning	Multiple t-maze	PND63	PND63	2x5, 2x10, 2x20 mg/kg	2x5 mg/kg	Worst performance: errors and latency increase
	S.D.	PND1-PND10	daily	s.c.	Cued learning	MWM	PND70	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Cued learning	MWM	PND70	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND1-PND10	daily	s.c.	Spatial learning	MWM	PND77	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Spatial learning	MWM	PND77	PND77	2x5, 2x10, 2x20 mg/kg	2x5 mg/kg	Worst performance: increase in latency, path length, cumulative distance (reduced trials for spatial learning) and average distance from target (reversed memory trials)
(Vorhees et al., 2004)	S.D.	PND11-PND20	daily	s.c.	Swimming/ escape	Straight channel	PND61	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Spatial learning	MWM	PND62 or PND77	PND62	2x5, 2x10, 2x20 mg/kg	2x5 mg/kg	Increased latency at 5 mg/kg, increased path lenght at 10 mg/kg, increased cumulative distance and increased average distance to target at 20 mg/kg (only when MWM performed before Barnes maze)
	S.D.	PND11-PND20	daily	s.c.	Cued learning	MWM	PND62 or PND77	-	2x5, 2x10, 2x20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Working memory	MWM	PND85	-	2x5, 2x10, 2x20 mg/kg	-	-

(Galineau et al., 2005)	S.D.	GD13-GD20	daily	s.c.	Sucrose preference		PND70	PND70	10 mg/kg	10 mg/kg	Decreased

GD: gestational day; MWM: Morris Water Maze; o.g.: oral gavage; PND: postnatal day; PPI: prepulse inhibition; s.c.: subcutaneous; S.D.: Sprague-Dawley; W: weeks.

Table 5. Summary of DNT studies of MDMA *in vivo* in rats evaluating general developmental landmarks related endpoints.

Reference	Strain	Exposure period	Frequency of administration	Route	Endpoint	Analytical/test method	Measurement time-points	Time-point alteration is observed	Doses studied	LOAEL	Effect observed
(Heuland et al., 2010)	Wistar	GD13-GD20	daily	s.c.	Eyes opening	External examination	PND2-PND21	PND15	10 mg/kg	10 mg/kg	Delay
	Wistar	GD13-GD20	daily	s.c.	Incisor eruption	External examination	PND2-PND21	PND9	10 mg/kg	10 mg/kg	Delay
(St Omer et al., 1991)	S.D.	GD6-GD18	daily	o.g.	Incisor eruption	External examination	PND7 to criterion	-	2.5 and 10 mg/kg	-	-
	S.D.	GD6-GD18	daily	o.g.	Eyes opening	External examination	PND12 to criterion	-	2.5 and 10 mg/kg	-	-

GD: gestational day; o.g.: oral gavage; s.c.: subcutaneous; PND: postnatal day; S.D.: Sprague-Dawley.

Table 6. Summay of DNT studies of MDMA *in vivo* in rats evaluating growth factor related endpoints.

Reference	Strain	Exposure period	Frequency of administration	Route	Endpoint	Analytical/test method	Brain region	Measurement time-points	Time-point alteration is observed	Doses studied	LOAEL	Effect observed
(Dzietko et al., 2010)	Wistar	PND6	single day	i.p.	BDNF	Gene expression	-	PND7	PND7 (6h a.i.)	20 mg/kg	20 mg/kg	Increase
		PND6	single day	i.p.	BDNF	Gene expression	-	PND7	PND7 (6h a.i.)	60 mg/kg	60 mg/kg	Increase
		PND6	single day	i.p.	NT-3	Gene expression	-	PND7	PND7 (6h a.i.)	60 mg/kg	60 mg/kg	Increase
		PND6	single day	i.p.	NGF	Gene expression	-	PND7	-	60 mg/kg	-	-
		PND6	single day	i.p.	BDNF	Protein expression	-	PND7	PND7 (12h a.i.)	60 mg/kg	60 mg/kg	Increase
(Schaefer et al., 2012)	S.D.	PND11	single day	s.c.	BDNF	HPLC	Striatum	PND12	-	4X 10 mg/kg	-	-
		PND11-PND20	daily	s.c.	BDNF	HPLC	Striatum	PND21	-	4X 10 mg/kg	-	-
		PND11-PND15	daily	s.c.	BDNF	HPLC	Striatum	PND16	-	4X 10 mg/kg	-	-
		PND11	single day	s.c.	NGF	HPLC	Striatum	PND12	-	4X 10 mg/kg	-	-
		PND11-PND20	daily	s.c.	NGF	HPLC	Striatum	PND21	-	4X 10 mg/kg	-	-
		PND11-PND15	daily	s.c.	NGF	HPLC	Striatum	PND16	-	4X 10 mg/kg	-	-
(Piper et al., 2009)	S.D.	PND1-PND4	daily	s.c.	BDNF	Immunoassay	Occipital cortex	PND11, PND30, PND65	-	2x 10 mg/kg	-	-
		PND1-PND4	daily	s.c.	BDNF	Immunoassay	Hippocampus	PND11, PND30, PND65	-	2x 10 mg/kg	-	-
		PND1-PND4	daily	s.c.	BDNF	Immunoassay	Striatum	PND11, PND30, PND65	-	2x 10 mg/kg	-	-
(Koprich et al., 2003a)	S.D.	PND11-PND20	daily	s.c.	BDNF	ELISA	Hippocampus	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increase
		PND11-PND20	daily	s.c.	BDNF	ELISA	Frontal cortex	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increase
		PND11-PND20	daily	s.c.	BDNF	ELISA	Striatum	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increase
		PND11-PND20	daily	s.c.	BDNF	ELISA	brainstem	PND21	PND21	2x 20 mg/kg	2x 20 mg/kg	Increase

GD: gestational day; i.p.: intraperitoneal; s.c.: subcutaneous.; PND: postnatal day; h a.i.; hours after injection; S.D.: Sprague-Dawley.

Table 7. Summary of DNT studies of MDMA *in vivo* in rats evaluating other endpoints.

Reference	Strain	Exposure period	Frequency of administratio	Route	Endpoint	Analytical/test method	Brain region	Measurement time-points	Time-point alteration is observed	Doses studied	LOAEL	Effect observed
(Canales and Ferrer-Donato, 2014)	Long Evans	GD3–GD15	daily	o.g.	Proliferation	BrdU incorporation	Dentate Gyrus	PND111	-	2x 10 mg/kg	-	-
(Cohen et al., 2005)	S.D.	PND11-PND20	daily	s.c.	Body Temperature	Temperature	-	P82–100	P82–100	2x 20mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Organ weight	Weight measure	Thymus	P142	-	2x 20 mg/kg	-	-
(Dzietko et al., 2010)	Wistar	PND6	single day	i.p.	Cell death	IHC	Globarl score including 17 brain regions	PND7	PND7	20, 40, 60 mg/kg	60 mg/kg	Increased
	Wistar	PND6	single day	i.p.	Cell death	IHC	Globarl score including 17 brain regions	PND7	PND7	3x 20 mg/kg	3x 20 mg/kg	Increased
	Wistar	PND13	single day	i.p.	Cell death	IHC	Globarl score including 17 brain regions	PND14	-	60 mg/kg	-	-
	Wistar	PND20	single day	i.p.	Cell death	IHC	Globarl score including 17 brain regions	PND21	-	60 mg/kg	-	-
(Darvesh and Gudelsky, 2004)	S.D.	PND21	single day	s.c.	Brain glycogen	Fluorimetric assay	Caudal quarter of the left cerebral hemisphere	PND28	PND28	20 mg/kg	20 mg/kg	Decreased (when evaluated at 24ºC, but not when evaluated at 17ºC)
(Colado et al., 1997)	Wistar	PND7	single day	s.c.	Lipid peroxidation	Malondialdehyde formation	Cortex	PND7	-	40 mg/kg	-	-
(Williams et al., 2014)	S.D.	PND11-PND20	daily	s.c.	Dendrite length	Golgi-Cox analysis	Enthorrinal cortex	PND60	PND60	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	Dendrite length	Golgi-Cox analysis	Nucleus Accumbens	PND60	PND60	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	Dendrite length	Golgi-Cox analysis	Dentate gyrus	PND60	PND60	2x 20 mg/kg	2x 20 mg/kg	Decreased

	S.D.	PND11-PND20	daily	s.c.	Dendrite length	Golgi-Cox analysis	Frontal cortex	PND60	-	2x 20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Dendritic branches per neuron	Golgi-Cox analysis	Enthorrinal cortex	PND60	-	2x 20 mg/kg	-	-
	S.D.	PND11-PND20	daily	s.c.	Dendritic branches per neuron	Golgi-Cox analysis	Nucleus Accumbens	PND60	PND60	2x 20 mg/kg	2x 20 mg/kg	Decreased
	S.D.	PND11-PND20	daily	s.c.	Dendritic branches per neuron	Golgi-Cox analysis	Dentate gyrus	PND60	PND60	2x 20 mg/kg	2x 20 mg/kg	Increased
	S.D.	PND11-PND20	daily	s.c.	Dendritic branches per neuron	Golgi-Cox analysis	Frontal cortex	PND60	-	2x 20 mg/kg	-	-
(Kelly et al., 2002)	S.D.	GD15-GD18	daily	s.c.	Cerebral glucose utilization	[^{14}C]-2-deoxyglucose	25 brain areas	PND90	PND90	2x 20 mg/kg	2x 20 mg/kg	Increased in locus coeruleus, inferior olive, nucleus ambiguus, trigeminal nucleus, hippocampus subiculum, thalamus anterior, hypothalamus medial, septal nucleus, globus pallidus
	S.D.	PND10-PND13	daily	s.c.	Cerebral glucose utilization	[^{14}C]-2-deoxyglucose	25 brain areas	PND90	-	2x 20 mg/kg	-	-
(Meyer et al., 2004)	S.D.	PND1-PND4	daily	s.c.	Cell death	IHC	Forebrain	PND5	PND5	2x 10 mg/kg	2x 10 mg/kg	Increased
	S.D.	PND1-PND4	daily	s.c.	Cell death	IHC	Hippocampus	PND5	PND5	2x 10 mg/kg	2x 10 mg/kg	Increased
(Thompson et al., 2012)	S.D.	GD14-GD20	daily	s.c.	Noradrenergic neurites	DBH	Prefrontal cortex Cg3	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
	S.D.	GD14-GD20	daily	s.c.	Noradrenergic neurites	DBH	Hippocampus CA1	PND21	PND21	2x 15 mg/kg	2x 15 mg/kg	Increased
	S.D.	GD14-GD20	daily	s.c.	Noradrenergic neurites	DBH	Hippocampus CA2	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Noradrenergic neurites	DBH	Hippocampus CA3	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Noradrenergic neurites	DBH	Hippocampus DG	PND21	-	2x 15 mg/kg	-	-
	S.D.	GD14-GD20	daily	s.c.	Noradrenergic neuron count	DBH	Locus coeruleus	PND21	-	2x 15 mg/kg	-	

DBH: dopamine beta-hydroxylase; GD: gestational day; IHC: immunohistochemistry; i.p.: intraperitoneal; o.g.: oral gavage; s.c.: subcutaneous; PND: postnatal day; S.D.: Sprague-Dawley.

3.3. *In vitro* studies on neurodevelopmental effects of MDMA.

Based on title and abstract screening according to the predefined criteria (see 2.2) 15 publications were selected from the *in vitro* studies for full text screening. During the full-text reading and acquisition of data 13 articles were excluded because of the following reasons: one did not include MDMA exposure, one was performed in chicken (see exclusion criteria), three obtained category 3 in ToxRTool (not reliable), and eight did not evaluate neurodevelopmental endpoints. Among the latter, two studies by Keating and colleagues were excluded, however, the authors demonstrated that MDMA affects folic acid uptake by human cytotrophoblasts and might thereby indirectly affect folic acid-dependent processes during brain development (Keating et al., 2009, 2007).

From the two publications that were selected based on the full text screen (Table 8), one publication used rat mesencephalic neuronal cultures from embryonic day four (E4), which were treated for 96 hours with MDMA starting from day in vitro (DIV) 0 (Lipton et al., 2008). The second one employed mouse embryonic stem cells (mESC, Royan B1) and treated them for 10 days during embryoid body formation (Meamar et al., 2010). Experimental cell models, species and endpoints were completely different between the two studies. This is the reason why they are discussed separately here. In rat mesencephalic neuronal cultures MDMA (LOAEC 0,75 µM) increased the number of TH^+ cells. This effect was stronger when MDMA was administered early, i.e. from 0 to 2 DIV with no exposure from 2 to 4 DIV, compared to late exposure, i.e. no MDMA between 0 and 2 DIV and exposure from 2 DIV to 4 DIV. In two *in vivo* studies, MDMA increased the number of TH^+ neurons on PND35 in the substantia nigra of prenatally (GD14-GD20) MDMA exposed rats (Lipton et al., 2008) as well as on PND21 (Koprich et al., 2003b). One of the earliest effects of DA in the developing brain is cell cycle regulation of specific neural progenitor cells. DA causes D1 receptor-dependent cell cycle inhibition with reduction of proliferation of a neural progenitor cells pool that is the source for most striatal neurons (Money and Stanwood, 2013).

The second *in vitro* study used embroid bodies, which were produced from mESC and subsequently differentiated towards the neural lineage due to retinoic acid treatment. MDMA exposure during the whole differentiation period (10 DIV) decreased neurite outgrowth (IC_{50} 50 µM) and MAP2 gene expression (LOAEC= 10 µM; Meamar et al., 2010). Shorter dendrites after postnatal MDMA exposure were also found at PND60 in rats in the nucleus accumbens, the dentate gyrus and the entorhinal cortex (Williams et al., 2014) indicating that neuronal cytoarchitectural changes induced by MDMA developmental exposure are long-lasting *in vivo*. Moreover, these cytoarchitectural changes appear in regions consistent with the spatial learning deficits described in the *in vivo* section (Williams et al., 2014). Neurite outgrowth can be affected by a variety of MoA, one being interference with the DAergic system (Money and Stanwood, 2013). As the *in vitro* study (Meamar et al., 2010) did not investigate the MoA of MDMA inhibiting neurite outgrowth, more data is clearly needed to unravel the molecular mechanism underlying MDMA DNT.

3.4 Generation of hypothetical AOPs from collected data

This review collected available data on the adverse effects of the recreational drug MDMA on the brain during development. The outcomes of human and rodent *in vivo* as well as rodent *in vitro* studies were collected, discussed, and subsequently assembled in hypothetical AOPs (Fig. 5). For general introduction to the AOP conceptual framework and specific vocabulary (MIE: molecular initiating event, KE: key event, KER: key event relationship and AO), the reader is referred to the following publications: (Ankley et al., 2010; Bal-Price et al., 2017). The anchors

of the hypothetical AOPs are the human AO, which is decreased motor function in children. In rodents *in vivo*, hypoactivity is observed in offspring when treated with MDMA postnatally. This AO in animals is closest to the human AO observed in the one epidemiological study. Based on the suggested early KE derived from the *in vitro* studies, we here suggest two hypothetical AOPs.

The first one (Figure 5) is based on the observation that the model compound for this AOP, MDMA, is transported to the cytoplasm by serotonin transporters competing with 5HT uptake and inducing back-transport of 5HT out of the cell (Hasenhuetl et al., 2018). This mechanism produces first an increase in extracellular 5HT but finally leads to a depletion of 5HT as a result of a combined effect in other targets like inhibition of tryptophan hydroxylase, the rate-limiting enzyme for 5HT synthesis (Capela et al., 2009). These effects are described in adult brains (Capela et al., 2009), but experimental results reviewed in this article suggest that this 5HT depletion also takes place during neurodevelopment specially after neonatal exposure to MDMA and in particular the hippocampus. Depletion of serotonin concentrations during development by the model compound PCPA leads to locomotor deficits (Vinay et al., 2005) linking this KE2 to an AO in animals. Supporting this link, neonatal intraventricular injections of the selective serotonin neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) leading to serotonin depletion in frontal cortex, hippocampus and striatum, also produced a reduction in locomotor activity in rats (Rok-Bujko et al., 2012). Serotonin deficiency during development causes alterations in neuroanatomical structures (KE4), one of them disturbing the development of brain stem projections into the lumbar enlargement of the spinal cord, resulting in poorer motor control (Vinay et al., 2005). If these disturbances are the reason for the AO seen after MDMA exposure in humans (KE4R to the human AO) is currently unknown. Over all, this is a hypothetical AOP with a high amount of uncertainty.

The second hypothetical AOP (Figure 5) involves the dopaminergic system and has even less supportive evidence than the first one. Here, altered DA receptor signaling during development alters amongst others tangential migration of GABAergic neurons possibly resulting in inappropriate neuronal excitation-inhibition balance in cortex, which could change motor activity. For example, the hyperdopaminergic DA transporter knockout mouse displays the behavioural phenotype of hyperactivity (Money and Stanwood, 2013). Much more data is needed to fill the gaps of this hypothetical AOP.

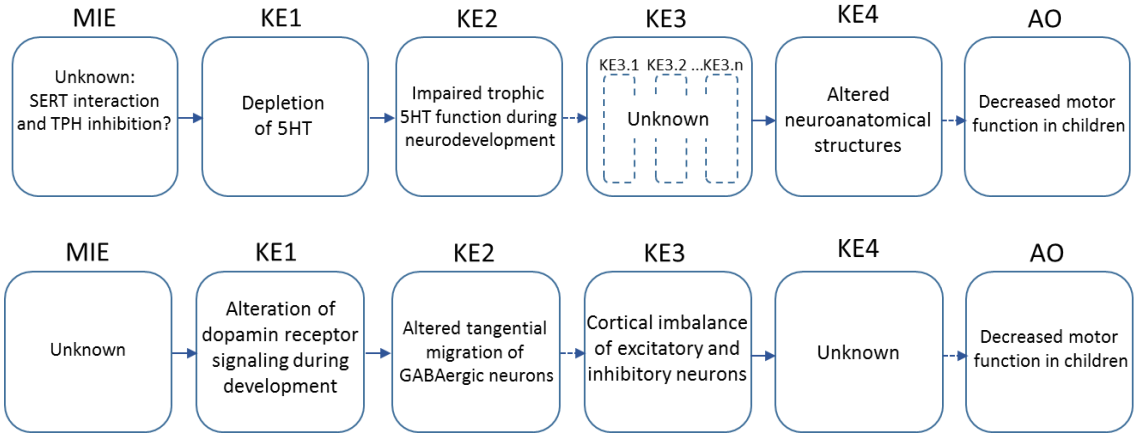

Figure 5. Hypothetical AOPs proposed from the data collection of this systematic review. (A) This AOP describes the interference of a compound with the serotonin transporter and enzyme synthesis during brain development leading to decreased motor functions in children. (B) The second AOP describes alteration of DA receptor signaling during development leading to a cortical imbalance of excitatory and inhibitory neurons in cortex causing decreased motor functinos in children.

This exercise revealed that there are huge data gaps in the understanding of basic biology as well as MDMA toxicology. There is no consistent evidence that links the adverse effects of MDMA across the different levels of organisations: cellular, organ and organism. From the AO point of view, postnatal exposure in rodents seems to reflect MDMA effects in exposed humans better than prenatal exposure. The reason for this and for the opposite effects seen after pre- and postnatal exposure in rodents are not known. Research needs for fully understanding how MDMA causes which adverse effects in humans should include further mechanistic *in vitro* studies, preferably in human developing brain cells, e.g. human induced pluripotent stem cell (hiPSC)-derived neural cells, or in alternative whole organisms, which allow the combination of mechanistic evaluations with behavioural assessments. Moreover, follow-up studies of prenatally exposed children is important because the transmitter systems altered by MDMA, i.e. the NE, DA and serotonergic systems, also determine psychiatric diseases later in life. Clearly more data are needed that shed light on the mechanisms and consequences of prenatal MDMA exposure in humans.

Table 8. Summary of DNT studies of MDMA *in vitro*.

Reference	Species	Cell type	Age at cell isolation	Exposure scheme	Exposure duration	Endpoint Category	Endpoint	Analytical/test method	Concentration range	Effective concentration (LOAEC)
(Lipton et al., 2008)	Rat	Mesencephalic neurons	E4	starting at 0 DIV	96 h	Dopaminergic differentiation	Tyrosine hydroxylase positves cells	ICC	0,75-750 µM	0,75 µM
						Apoptosis/cell death	Viability	MTT assay	0.75-750 µM	-
(Meamar et al., 2010)	Mouse	Embryonic stem cells (Royan B1)	-	During EB formation until 10 DIV	240 h	General neuronal endpoint	MAP2 expression	RT-qPCR	0.1-1000 µM	10 µM
						General neuronal endpoint	Neuronal morphology	ICC	0.1-1000 µM	IC_{50}= 50µM
				Starting at 4 DIV (after EB formation)	96 h	General neuronal endpoint	MAP2 expression	RT-qPCR	0.1-1000 µM	-
					96 h	General neuronal endpoint	Neuronal morphology	ICC	0.1-1000 µM	IC_{50}= 120µM

DIV: days *in vitro*; ICC: immunocytochemistry; MAP2: microtubule-associated protein 2.

REFERENCES

Aguirre, N., Barrionuevo, M., Lasheras, B., Del Rio, J., 1998. The role of dopaminergic systems in the perinatal sensitivity to 3, 4-methylenedioxymethamphetamine-induced neurotoxicity in rats. J Pharmacol Exp Ther 286, 1159–1165.

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J.E., Villeneuve, D.L., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29, 730–741. https://doi.org/10.1002/etc.34

Aschner, M., Ceccatelli, S., Daneshian, M., Fritsche, E., Hasiwa, N., Hartung, T., Hogberg, H.T., Leist, M., Li, A., Mundi, W.R., Padilla, S., Piersma, A.H., Bal-Price, A., Seiler, A., Westerink, R.H., Zimmer, B., Lein, P.J., 2017. Reference compounds for alternative test methods to indicate developmental neurotoxicity (DNT) potential of chemicals: example lists and criteria for their selection and use. ALTEX 34, 49–74. https://doi.org/10.14573/altex.1604201

Bailey, B.A., Sokol, R.J., 2011. Prenatal alcohol exposure and miscarriage, stillbirth, preterm delivery, and sudden infant death syndrome. Alcohol Res. Heal. J. Natl. Inst. Alcohol Abus. Alcohol. 34, 86–91.

Bal-Price, A., Lein, P.J., Keil, K.P., Sethi, S., Shafer, T., Barenys, M., Fritsche, E., Sachana, M., Meek, M.E., 2017. Developing and applying the adverse outcome pathway concept for understanding and predicting neurotoxicity. Neurotoxicology 59, 240–255. https://doi.org/10.1016/j.neuro.2016.05.010

Barenys, M., Gomez-Catalan, J., Camps, L., Teixido, E., de Lapuente, J., Gonzalez-Linares, J., Serret, J., Borras, M., Rodamilans, M., Llobet, J.M., 2010. MDMA (ecstasy) delays pubertal development and alters sperm quality after developmental exposure in the rat. Toxicol Lett 197, 135–142. https://doi.org/10.1016/j.toxlet.2010.05.009

Bissonette, G.B., Roesch, M.R., 2016. Development and function of the midbrain dopamine system: what we know and what we need to. Genes. Brain. Behav. 15, 62–73. https://doi.org/10.1111/gbb.12257

Bogdanova, O. V, Kanekar, S., D'Anci, K.E., Renshaw, P.F., 2013. Factors influencing behavior in the forced swim test. Physiol. Behav. 118, 227–239. https://doi.org/10.1016/j.physbeh.2013.05.012

Broening, H.W., Bacon, L., Slikker Jr., W., 1994. Age modulates the long-term but not the acute effects of the serotonergic neurotoxicant 3,4-methylenedioxymethamphetamine. J Pharmacol Exp Ther 271, 285–293.

Broening, H.W., Bowyer, J.F., Slikker Jr., W., 1995. Age-dependent sensitivity of rats to the long-term effects of the serotonergic neurotoxicant (+/-)-3,4-methylenedioxymethamphetamine (MDMA) correlates with the magnitude of the MDMA-induced thermal response. J Pharmacol Exp Ther 275, 325–333.

Broening, H.W., Morford, L.L., Inman-Wood, S.L., Fukumura, M., Vorhees, C. V, 2001. 3,4-methylenedioxymethamphetamine (ecstasy)-induced learning and memory impairments depend on the age of exposure during early development. J. Neurosci. 21, 3228–35.

Brummelte, S., Mc Glanaghy, E., Bonnin, A., Oberlander, T.F., 2017. Developmental changes in serotonin signaling: Implications for early brain function, behavior and adaptation. Neuroscience 342, 212–231. https://doi.org/10.1016/j.neuroscience.2016.02.037

Campbell, N.G., Koprich, J.B., Kanaan, N.M., Lipton, J.W., 2006. MDMA administration to pregnant Sprague–Dawley rats results in its passage to the fetal compartment.

Neurotoxicol. Teratol. 28, 459–465. https://doi.org/10.1016/j.ntt.2006.05.006

Canales, J.J., Ferrer-Donato, A., 2014. Prenatal exposure to alcohol and 3,4-methylenedioxymethamphetamine (ecstasy) alters adult hippocampal neurogenesis and causes enduring memory deficits. Dev Neurosci 36, 10–17. https://doi.org/10.1159/000356820

Capela, J.P., Carmo, H., Remiao, F., Bastos, M.L., Meisel, A., Carvalho, F., 2009. Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: an overview. Mol. Neurobiol. 39, 210–271. https://doi.org/10.1007/s12035-009-8064-1

Castilla, J., Barrio, G., Belza, M.J., de la Fuente, L., 1999. Drug and alcohol consumption and sexual risk behaviour among young adults: results from a national survey. Drug Alcohol Depend. 56, 47–53.

ChemIDplus, n.d. ChemIDplus [WWW Document]. URL https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp

Cho, K.O., Rhee, G.S., Kwack, S.J., Chung, S.Y., Kim, S.Y., 2008. Developmental exposure to 3,4-methylenedioxymethamphetamine results in downregulation of neurogenesis in the adult mouse hippocampus. Neuroscience 154, 1034–1041. https://doi.org/10.1016/j.neuroscience.2008.04.040

Chudley, A.E., Conry, J., Cook, J.L., Loock, C., Rosales, T., Leblanc, N., 2005. Fetal alcohol spectrum disorder: Canadian guidelines for diagnosis. Spectrum 172.

Clancy, B., Darlington, R.B., Finlay, B.L., 2001. Translating developmental time across mammalian species. Neuroscience 105, 7–17.

Clancy, B., Finlay, B.L., Darlington, R.B., Anand, K.J.S., 2007. Extrapolating brain development from experimental species to humans. Neurotoxicology 28, 931–937. https://doi.org/10.1016/j.neuro.2007.01.014

Cohen, M.A., Skelton, M.R., Schaefer, T.L., Gudelsky, G.A., Vorhees, C. V, Williams, M.T., 2005. Learning and memory after neonatal exposure to 3,4-methylenedioxymethamphetamine (ecstasy) in rats: interaction with exposure in adulthood. Synapse 57, 148–159. https://doi.org/10.1002/syn.20166

Colado, M.I., O'Shea, E., Granados, R., Misra, A., Murray, T.K., Green, A.R., 1997. A study of the neurotoxic effect of MDMA ('ecstasy') on 5-HT neurones in the brains of mothers and neonates following administration of the drug during pregnancy. Br J Pharmacol 121, 827–833. https://doi.org/10.1038/sj.bjp.0701201

Compton, W.M., Thomas, Y.F., Stinson, F.S., Grant, B.F., 2007. Prevalence, correlates, disability, and comorbidity of DSM-IV drug abuse and dependence in the United States: results from the national epidemiologic survey on alcohol and related conditions. Arch. Gen. Psychiatry 64, 566–576. https://doi.org/10.1001/archpsyc.64.5.566

Counts, S., Mufson, E., 2012. Locus Coeruleus, in: The Human Nervous System. pp. 425–438. https://doi.org/10.1016/B978-0-12-374236-0.10012-4

DART, n.d. DART - ToxNet.

Darvesh, A.S., Gudelsky, G.A., 2004. The relationship between hyperthermia and glycogenolysis in 3,4-methylenedioxymethamphetamine-induced serotonin depletion in rats. Neurotoxicol Teratol 26, 571–577. https://doi.org/10.1016/j.ntt.2004.03.008

de la Torre, R., Farre, M., Ortuno, J., Mas, M., Brenneisen, R., Roset, P.N., Segura, J., Cami, J., 2000. Non-linear pharmacokinetics of MDMA ('ecstasy') in humans. Br. J. Clin. Pharmacol. 49, 104–109.

Dzietko, M., Sifringer, M., Klaus, J., Endesfelder, S., Brait, D., Hansen, H.H., Bendix, I., Felderhoff-Mueser, U., 2010. Neurotoxic effects of MDMA (ecstasy) on the developing rodent brain. Dev Neurosci 32, 197–207. https://doi.org/10.1159/000313473

Ellegood, J., Yee, Y., Kerr, T.M., Muller, C.L., Blakely, R.D., Henkelman, R.M., Veenstra-

VanderWeele, J., Lerch, J.P., 2018. Analysis of neuroanatomical differences in mice with genetically modified serotonin transporters assessed by structural magnetic resonance imaging. Mol. Autism 9, 24. https://doi.org/10.1186/s13229-018-0210-z

EMCDDA, 2017. European Drug Report, European Union Publications Office. https://doi.org/10.1097/JSM.0b013e31802b4fda

EMCDDA, 2012. PREGNANCY, CHILDCARE AND THE FAMILY: KEY ISSUES FOR EUROPE'S RESPONSE TO DRUGS.

Environmental Protection Agency, 1991. Guidelines for Developmental Toxicity Risk Assessment, Risk Assessment Forum.

Eun, J.W., Kwack, S.J., Noh, J.H., Jung, K.H., Kim, J.K., Bae, H.J., Xie, H., Ryu, J.C., Ahn, Y.M., Park, W.S., Lee, J.Y., Rhee, G.S., Nam, S.W., 2010. Identification of post-generation effect of 3,4-methylenedioxymethamphetamine on the mouse brain by large-scale gene expression analysis. Toxicol Lett 195, 60–67. https://doi.org/10.1016/j.toxlet.2010.02.013

Forray, A., 2016. Substance use during pregnancy. F1000Research 5. https://doi.org/10.12688/f1000research.7645.1

Galineau, L., Belzung, C., Kodas, E., Bodard, S., Guilloteau, D., Chalon, S., 2005. Prenatal 3,4-methylenedioxymethamphetamine (ecstasy) exposure induces long-term alterations in the dopaminergic and serotonergic functions in the rat. Brain Res Dev Brain Res 154, 165–176. https://doi.org/10.1016/j.devbrainres.2004.10.012

Green, A.R., Gabrielsson, J., Marsden, C.A., Fone, K.C.F., 2009. MDMA: on the translation from rodent to human dosing. Psychopharmacology (Berl). 204, 375–378. https://doi.org/10.1007/s00213-008-1453-8

Green, A.R., Mechan, A.O., Elliott, J.M., O'Shea, E., Colado, M.I., 2003. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol Rev 55, 463–508. https://doi.org/10.1124/pr.55.3.3

Hasenhuetl, P.S., Bhat, S., Mayer, F.P., Sitte, H.H., Freissmuth, M., Sandtner, W., 2018. A kinetic account for amphetamine-induced monoamine release. J. Gen. Physiol. https://doi.org/10.1085/jgp.201711915

Heuland, E., Germaux, M.A., Galineau, L., Chalon, S., Belzung, C., 2010. Prenatal MDMA exposure delays postnatal development in the rat: a preliminary study. Neurotoxicol Teratol 32, 425–431. https://doi.org/10.1016/j.ntt.2010.03.006

Ho, E., Karimi-Tabesh, L., Koren, G., 2001. Characteristics of pregnant women who use Ecstasy (3,4-methylenedioxymethamphetamine). Teratology 63, 280.

Jepma, M., Deinum, J., L Asplund, C., Rombouts, S., T Tamsma, J., Tjeerdema, N., Spapé, M., Garland, E., Robertson, D., Wm Lenders, J., Nieuwenhuis, S., 2011. Neurocognitive Function in Dopamine-β-Hydroxylase Deficiency. Neuropsychopharmacology 36, 1608–1619. https://doi.org/10.1038/npp.2011.42

Kaizaki, A., Tanaka, S., Yoshida, T., Numazawa, S., 2014. Maternal MDMA administration in mice leads to neonatal growth delay. J Toxicol Sci 39, 33–39.

Keating, E., Gonçalves, P., Campos, I., Costa, F., Azevedo, I., Martel, F., 2007. Effect of pathological conditions, pharmacotherapy and drugs of abuse upon folic acid placental uptake. Faseb J 21.

Keating, E., Goncalves, P., Campos, I., Costa, F., Martel, F., 2009. Folic acid uptake by the human syncytiotrophoblast: Interference by pharmacotherapy, drugs of abuse and pathological conditions. Reprod. Toxicol. 28, 511–520. https://doi.org/10.1016/j.reprotox.2009.07.001

Kelly, P.A., Ritchie, I.M., Quate, L., McBean, D.E., Olverman, H.J., 2002. Functional consequences of perinatal exposure to 3,4-methylenedioxymethamphetamine in rat brain. Br J Pharmacol 137, 963–970. https://doi.org/10.1038/sj.bjp.0704961

Klimisch, H.J., Andreae, M., Tillmann, U., 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul. Toxicol. Pharmacol. 25, 1–5. https://doi.org/10.1006/rtph.1996.1076

Koprich, J.B., Campbell, N.G., Lipton, J.W., 2003a. Neonatal 3,4-methylenedioxymethamphetamine (ecstasy) alters dopamine and serotonin neurochemistry and increases brain-derived neurotrophic factor in the forebrain and brainstem of the rat. Brain Res Dev Brain Res 147, 177–182.

Koprich, J.B., Chen, E.Y., Kanaan, N.M., Campbell, N.G., Kordower, J.H., Lipton, J.W., 2003b. Prenatal 3,4-methylenedioxymethamphetamine (ecstasy) alters exploratory behavior, reduces monoamine metabolism, and increases forebrain tyrosine hydroxylase fiber density of juvenile rats. Neurotoxicol Teratol 25, 509–517.

Kwack, S.J., Yoon, K.S., Lim, S.K., Gwak, H.M., Kim, J.Y., Um, Y.M., Lee, J.D., Hyeon, J.H., Kim, Y.J., Kim, H.S., Lee, B.M., 2014. A one-generation reproductive toxicity study of 3,4-methylenedioxy-n-methamphetamine (MDMA, Ecstasy), an amphetamine derivative, in C57BL/6 mice. J Toxicol Env. Heal. A 77, 1431–1442. https://doi.org/10.1080/15287394.2014.951759

Lipton, J.W., Tolod, E.G., Thompson, V.B., Pei, L., Paumier, K.L., Terpstra, B.T., Lynch, K.A., Collier, T.J., Sortwell, C.E., 2008. 3,4-Methylenedioxy-N-methamphetamine (ecstasy) promotes the survival of fetal dopamine neurons in culture. Neuropharmacology 55, 851–859. https://doi.org/10.1016/j.neuropharm.2008.06.062

Mattison, A.M., Ross, M.W., Wolfson, T., Franklin, D., 2001. Circuit party attendance, club drug use, and unsafe sex in gay men. J. Subst. Abuse 13, 119–126.

May, A.L., Parrott, A.C., 2015. Greater sexual risk-taking in female and male recreational MDMA/ecstasy users compared with alcohol drinkers: a questionnaire study. Hum. Psychopharmacol. 30, 272–275. https://doi.org/10.1002/hup.2432

McDonnell-Dowling, K., Kelly, J.P., 2015. Sources of variation in the design of preclinical studies assessing the effects of amphetamine-type stimulants in pregnancy and lactation. Behav. Brain Res. 279, 87–99. https://doi.org/10.1016/j.bbr.2014.11.021

Meamar, R., Karamali, F., Sadeghi, H.M., Etebari, M., Nasr-Esfahani, M.H., Baharvand, H., 2010. Toxicity of ecstasy (MDMA) towards embryonic stem cell-derived cardiac and neural cells. Toxicol. Vitr. 24, 1133–1138. https://doi.org/10.1016/j.tiv.2010.03.005

Meyer, J.S., Ali, S.F., 2002. Serotonergic neurotoxicity of MDMA (ecstasy) in the developing rat brain. Ann N Y Acad Sci 965, 373–380.

Meyer, J.S., Grande, M., Johnson, K., Ali, S.F., 2004. Neurotoxic effects of MDMA ("ecstasy") administration to neonatal rats. Int J Dev Neurosci 22, 261–271. https://doi.org/10.1016/j.ijdevneu.2004.04.007

Mogensen, J., Pedersen, T.K., Holm, S., Bang, L.E., 1995. Prefrontal cortical mediation of rats' place learning in a modified water maze. Brain Res. Bull. 38, 425–434.

Money, K.M., Stanwood, G.D., 2013. Developmental origins of brain disorders: roles for dopamine. Front. Cell. Neurosci. 7, 260. https://doi.org/10.3389/fncel.2013.00260

Moore, D.G., Turner, J.D., Parrott, A.C., Goodwin, J.E., Fulton, S.E., Min, M.O., Fox, H.C., Braddick, F.M., Axelsson, E.L., Lynch, S., Ribeiro, H., Frostick, C.J., Singer, L.T., 2010. During pregnancy, recreational drug-using women stop taking ecstasy (3,4-methylenedioxy-N-methylamphetamine) and reduce alcohol consumption, but continue to smoke tobacco and cannabis: initial findings from the Development and Infancy Study. J Psychopharmacol 24, 1403–1410. https://doi.org/10.1177/0269881109348165

Morris, R.G., Garrud, P., Rawlins, J.N., O'Keefe, J., 1982. Place navigation impaired in rats with hippocampal lesions. Nature 297, 681–683.

Mueller, M., Maldonado-Adrian, C., Yuan, J., McCann, U.D., Ricaurte, G.A., 2013. Studies of (±)-3,4-methylenedioxymethamphetamine (MDMA) metabolism and disposition in rats and

mice: relationship to neuroprotection and neurotoxicity profile. J. Pharmacol. Exp. Ther. 344, 479–488. https://doi.org/10.1124/jpet.112.201699

O'Callaghan, J.P., Miller, D.B., 1994. Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse. J. Pharmacol. Exp. Ther. 270, 741–751.

Page, M.E., Detke, M.J., Dalvi, A., Kirby, L.G., Lucki, I., 1999. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology (Berl). 147, 162–167.

Palamar, J.J., Griffin-Tomas, M., Acosta, P., Ompad, D.C., Cleland, C.M., 2018. A comparison of self-reported sexual effects of alcohol, marijuana, and ecstasy in a sample of young adult nightlife attendees. Psychol. Sex. 9, 54–68. https://doi.org/10.1080/19419899.2018.1425220

Parrott, A.C., 2013. Human psychobiology of MDMA or "Ecstasy": an overview of 25 years of empirical research. Hum Psychopharmacol 28, 289–307. https://doi.org/10.1002/hup.2318

Petrangelo, A., Czuzoj-Shulman, N., Balayla, J., Abenhaim, H.A., 2018. Cannabis Abuse or Dependence During Pregnancy: A Population-Based Cohort Study on 12 Million Births. J. Obstet. Gynaecol. Can. https://doi.org/10.1016/j.jogc.2018.09.009

Piper, B.J., Farelli, J.D., Meyer, J.S., 2009. Dissociation between serotonin neurotoxicity and brain-derived neurotrophic factor induction following neonatal MDMA exposure in rats. Dev Neurosci 31, 90–94. https://doi.org/10.1159/000207497

Piper, B.J., Meyer, J.S., 2006. Increased responsiveness to MDMA in adult rats treated neonatally with MDMA. Neurotoxicol Teratol 28, 95–102. https://doi.org/10.1016/j.ntt.2005.09.002

PubMed, n.d. PubMed [WWW Document]. URL https://www.ncbi.nlm.nih.gov/pubmed

Richter-Levin, G., Acsady, L., Freund, T.F., Segal, M., 1994. Differential effects of serotonin and raphe grafts in the hippocampus and hypothalamus: a combined behavioural and anatomical study in the rat. Eur. J. Neurosci. 6, 1720–1728.

Rok-Bujko, P., Krzascik, P., Szyndler, J., Kostowski, W., Stefanski, R., 2012. The influence of neonatal serotonin depletion on emotional and exploratory behaviours in rats. Behav. Brain Res. 226, 87–95. https://doi.org/10.1016/j.bbr.2011.08.030

Schaefer, T.L., Ehrman, L.A., Gudelsky, G.A., Vorhees, C. V, Williams, M.T., 2006. Comparison of monoamine and corticosterone levels 24 h following (+)methamphetamine, (+/-)3,4-methylenedioxymethamphetamine, cocaine, (+)fenfluramine or (+/-)methylphenidate administration in the neonatal rat. J. Neurochem. 98, 1369–1378. https://doi.org/10.1111/j.1471-4159.2006.04034.x

Schaefer, T.L., Grace, C.E., Braun, A.A., Amos-Kroohs, R.M., Graham, D.L., Skelton, M.R., Williams, M.T., Vorhees, C. V, 2013. Cognitive impairments from developmental exposure to serotonergic drugs: citalopram and MDMA. Int J Neuropsychopharmacol 16, 1383–1394. https://doi.org/10.1017/s1461145712001447

Schaefer, T.L., Grace, C.E., Skelton, M.R., Graham, D.L., Gudelsky, G.A., Vorhees, C. V, Williams, M.T., 2012. Neonatal citalopram treatment inhibits the 5-HT depleting effects of MDMA exposure in rats. ACS Chem Neurosci 3, 12–21. https://doi.org/10.1021/cn2000553

Schaefer, T.L., Skelton, M.R., Herring, N.R., Gudelsky, G.A., Vorhees, C. V, Williams, M.T., 2008. Short- and long-term effects of (+)-methamphetamine and (+/-)-3,4-methylenedioxymethamphetamine on monoamine and corticosterone levels in the neonatal rat following multiple days of treatment. J. Neurochem. 104, 1674–1685. https://doi.org/10.1111/j.1471-4159.2007.05112.x

Schneider, K., Schwarz, M., Burkholder, I., Kopp-Schneider, A., Edler, L., Kinsner-Ovaskainen, A., Hartung, T., Hoffmann, S., 2009. "ToxRTool", a new tool to assess the reliability of toxicological data. Toxicol. Lett. 189, 138–144. https://doi.org/10.1016/j.toxlet.2009.05.013

Scott, K., Fagermo, N., Callaway, L., Lust, K., 2010. Illicit drug use in late pregnancy associated with stillbirth and eclampsia. Obs. Med 3, 113–114. https://doi.org/10.1258/om.2010.090061

Sevy, S., Papadimitriou, G.N., Surmont, D.W., Goldman, S., Mendlewicz, J., 1989. Noradrenergic function in generalized anxiety disorder, major depressive disorder, and healthy subjects. Biol. Psychiatry 25, 141–152.

Shah, R., Courtiol, E., Castellanos, F.X., Teixeira, C.M., 2018. Abnormal Serotonin Levels During Perinatal Development Lead to Behavioral Deficits in Adulthood. Front. Behav. Neurosci. 12, 114. https://doi.org/10.3389/fnbeh.2018.00114

Singer, L.T., Moore, D.G., Fulton, S., Goodwin, J., Turner, J.J.D., Min, M.O., Parrott, A.C., 2012a. Neurobehavioral outcomes of infants exposed to MDMA (Ecstasy) and other recreational drugs during pregnancy. Neurotoxicol. Teratol. 34, 303–310. https://doi.org/10.1016/j.ntt.2012.02.001

Singer, L.T., Moore, D.G., Min, M.O., Goodwin, J., Turner, J.J.D., Fulton, S., Parrott, A.C., 2016. Motor delays in MDMA (ecstasy) exposed infants persist to 2years. Neurotoxicol. Teratol. 54, 22–28. https://doi.org/10.1016/j.ntt.2016.01.003

Singer, L.T., Moore, D.G., Min, M.O., Goodwin, J., Turner, J.J.D., Fulton, S., Parrott, A.C., 2012b. One-Year Outcomes of Prenatal Exposure to MDMA and Other Recreational Drugs. Pediatrics 130, 407–413. https://doi.org/10.1542/peds.2012-0666

Skelton, M.R., Graham, D.L., Schaefer, T.L., Grace, C.E., Braun, A.A., Burns, L.N., Amos-Kroohs, R.M., Williams, M.T., Vorhees, C. V, 2012. Distinct periods of developmental sensitivity to the effects of 3,4-(+/-)-methylenedioxymethamphetamine (MDMA) on behaviour and monoamines in rats. Int. J. Neuropsychopharmacol. 15, 811–824. https://doi.org/10.1017/s1461145711000952

St Omer, V.E., Ali, S.F., Holson, R.R., Duhart, H.M., Scalzo, F.M., Slikker Jr., W., 1991. Behavioral and neurochemical effects of prenatal methylenedioxymethamphetamine (MDMA) exposure in rats. Neurotoxicol Teratol 13, 13–20.

Steele, T.D., McCann, U.D., Ricaurte, G.A., 1994. 3,4-Methylenedioxymethamphetamine (MDMA, “Ecstasy”): pharmacology and toxicology in animals and humans. Addiction 89, 539–551.

Substance Abuse and Mental Health Services Administration, 2018. Results from the 2017 National Survey on Drug Use and Health. Vol. I. Summ. Natl. Find.

Substance Abuse and Mental Health Services Administration, 2009. Results from the 2008 National Survey on Drug Use and Health: National Findings: Full Report and Detailed Tables.

Suri, D., Teixeira, C.M., Cagliostro, M.K.C., Mahadevia, D., Ansorge, M.S., 2015. Monoamine-sensitive developmental periods impacting adult emotional and cognitive behaviors. Neuropsychopharmacology 40, 88–112. https://doi.org/10.1038/npp.2014.231

Thomas, S.A., Palmiter, R.D., 1997. Disruption of the dopamine beta-hydroxylase gene in mice suggests roles for norepinephrine in motor function, learning, and memory. Behav. Neurosci. 111, 579–589.

Thompson, V.B., Heiman, J., Chambers, J.B., Benoit, S.C., Buesing, W.R., Norman, M.K., Norman, A.B., Lipton, J.W., 2009. Long-term behavioral consequences of prenatal MDMA exposure. Physiol Behav 96, 593–601. https://doi.org/10.1016/j.physbeh.2008.12.013

Thompson, V.B., Koprich, J.B., Chen, E.Y., Kordower, J.H., Terpstra, B.T., Lipton, J.W., 2012. Prenatal exposure to MDMA alters noradrenergic neurodevelopment in the rat. Neurotoxicol. Teratol. 34, 206–213. https://doi.org/10.1016/j.ntt.2011.09.005

Travis, K., Schnatter, R., Swaen, G., Money, C., Pallapies, D., Priem, P., Onyon, L., 2008. A proposed framework for the integration of human and animal data in chemical risk assessment. Toxicol. Lett. 180, S19–S20. https://doi.org/10.1016/j.toxlet.2008.06.729

Tsuji, R., Crofton, K.M., 2012. Developmental neurotoxicity guideline study: issues with methodology, evaluation and regulation. Congenit. Anom. (Kyoto). 52, 122–128. https://doi.org/10.1111/j.1741-4520.2012.00374.x

Van, T.M.R., Garbis, H., Reuvers, M., 1998. Ecstasy exposure during pregnancy. Teratology 58.

Vinay, L., Ben-Mabrouk, F., Brocard, F., Clarac, F., Jean-Xavier, C., Pearlstein, E., Pflieger, J.-F., 2005. Perinatal Development of the Motor Systems Involved in Postural Control. Neural Plast. 12, 131–139. https://doi.org/10.1155/np.2005.131

Vorhees, C. V, Reed, T.M., Skelton, M.R., Williams, M.T., 2004. Exposure to 3,4-methylenedioxymethamphetamine (MDMA) on postnatal days 11-20 induces reference but not working memory deficits in the Morris water maze in rats: implications of prior learning. Int. J. Dev. Neurosci. 22, 247–259. https://doi.org/10.1016/j.ijdevneu.2004.06.003

Vorhees, C. V, Schaefer, T.L., Skelton, M.R., Grace, C.E., Herring, N.R., Williams, M.T., 2009. (+/-)3,4-Methylenedioxymethamphetamine (MDMA) Dose-Dependently Impairs Spatial Learning in the Morris Water Maze after Exposure of Rats to Different Five-Day Intervals from Birth to Postnatal Day Twenty. Dev Neurosci 31, 107–120. https://doi.org/10.1159/000207499

WebofScience, n.d. Web of Science [WWW Document]. URL https://www.webofknowledge.com

Whishaw, I.Q., Mittleman, G., Bunch, S.T., Dunnett, S.B., 1987. Impairments in the acquisition, retention and selection of spatial navigation strategies after medial caudate-putamen lesions in rats. Behav. Brain Res. 24, 125–138.

Williams, M.T., Skelton, M.R., Longacre, I.D., Huggins, K.N., Maple, A.M., Vorhees, C. V, Brown, R.W., 2014. Neuronal reorganization in adult rats neonatally exposed to (+/-)-3,4-methylenedioxymethamphetamine. Toxicol Rep 1, 699–706. https://doi.org/10.1016/j.toxrep.2014.08.018

Wyatt, R.J., Portnoy, B., Kupfer, D.J., Snyder, F., Engelman, K., 1971. Resting plasma catecholamine concentrations in patients with depression and anxiety. Arch. Gen. Psychiatry 24, 65–70.