Abstract

Sex differences in behavioural patterns of drug abuse and dependence have been hypothesized to be a consequence of sexual dimorphisms in brain pathways, particularly within the dopaminergic reward circuitry. Yet, how potential sex differences are manifested at a neurochemical level remains unclear. Here, we use a meta-analysis approach to investigate whether animal studies robustly indicate a different regulation of striatal dopamine transmission in males and females. Data from 39 microdialysis experiments on female rats (n = 676) were extracted and statistically compared with data from 1523 male rats. All drugs of abuse, independent of their molecular mechanisms of action, notably increase extracellular dopamine concentrations in the nucleus accumbens (NAc) and caudate putamen (CPu). No significant sex differences in basal levels or in dopaminergic response to drugs of abuse were found. However, basal dopamine levels in CPu (but not NAc) were significantly altered by ovariectomy. In conclusion, there are no sex-dependent differences in basal dopamine levels within the NAc and CPu. Previously reported sex differences in the CPu seem to be a result of ovariectomy and may only to a lesser, non-significant degree be attributed to a sexual duality.

Keywords: drugs of abuse, dopamine, microdialysis, rat, scoping review.

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To date, pre-clinical findings suggest that ovarian hormones critical affect the reinforcing effects of drugs of abuse in females. In particular, acute oestradiol administration in female animals have been shown to enhance acquisition, motivation and escalation of drug intake, and increase the probability of relapse-like behaviours (Becker and Cha, 1989; Becker, 1990; Becker and Rudick, 1999; Hu, et al., 2004). These hormonal effects have been hypothesized to impact on sex differences in dopaminergic neurotransmission in striatum (Walker, et al., 2006; Riccardi, et al., 2011; Bobzean, et al., 2014). Previous studies have shown a lower level of cocaine-induced increase in dopamine (DA) levels in ovarioctomized female rats than castrated males (Hu, et al., 2004). Furthermore, oestradiol treatment in ovarioctomized rats was demonstrated to enhance stimulated dopamine release (Castner, et al., 1993; Cummings, et al., 2014; Becker, 2016). However, the effects of oestradiol were mostly on caudate putamen (CPu), whereas DA levels in nucleus accumbens (NAc) remained unaffected by hormonal treatment (Cummings, et al., 2014). This discrepancy in how dopamine and hormonal systems interacts, have been discussed in light of the different functions of each brain region. While NAc is associated with positive reinforcement and rewarding aspects of drug use (Spanagel and Weiss, 1999), CPu is merely engaged in habit formation and compulsive drug taking (Belin and Everitt, 2008; Willuhn, et al., 2012). However, these studies were conducted in male rats. Indeed, the questions remains of to what degree any data obtained from male animals can be used to interpret findings from female animals. Indeed, all reported sex differences are based on within-study statistics with relatively small number of animals and a global comparison of male-female findings of different studies is still missing.

A large-scale between-study analysis allows us to evaluate the robustness of conclusions made by individual studies and often leads to the generation of new mechanistic hypotheses (Flather, et al., 1997). In this study, we use an established (Noori, et al., 2018) hypothesis-free, global approach to statistically compare two large bodies of evidence, namely findings from all publications reporting on dopamine concentrations in NAc and CPu of male and/or female rats. To this end, we performed systematic data mining, extraction and analysis of basal levels of dopamine, as well as relative changes of DA in response to acute administration of drugs of abuse. Thereby, our search included studies that used in vivo microdialysis to determine extracellular DA concentrations in either male, female rats or both. This strategy allows an explorative investigation since it includes data that were not primarily obtained to analyse sex differences. The entirety of data was consistently integrated, meta-analysed and statistical tests including analysis of variance, cluster and sensitivity analyses were conducted to compare the findings with respect to sex but also to shine a light on effect modifiers and secondary factors.

**Methods**

A previously reported (Noori, et al., 2012a; Brand, et al., 2013; Fliegel, et al., 2013; Hirth, et al., 2016; Fritze, et al., 2017; Noori, et al., 2018), we have established a robust pipeline for standardized data mining, database creation and integrative analysis of pre-clinical neuropsychopharmacology experiments. This approach allows accurate extraction of maximum amount of data with a minimized chance for missing critical information and was therefore applied here to characterize basal dopamine concentrations as well as overlapping and distinct features of striatal dopaminergic response to systemically administered drugs of abuse in female rats.

**Study selection**

A systematic screening of the original research articles published until 31.04.2019 was performed on the online portal of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/pubmed/) based on the keywords rat (AND) microdialysis (OR) (female (OR) sex (OR) gender) (AND) (dopamine (OR) DA) (AND) (striatum (OR) nucleus accumbens (OR) caudate putamen (OR) reward) (AND) (alcohol (OR) ethanol (OR) (D.L)-amphetamine (OR) methamphetamine (OR) cocaine (OR) ketamine (OR) morphine (OR) nicotine (OR) phenylcyclidine (OR) PCP (OR) tetrahydradecanabinol (OR) THC). An identical set of keywords with the exception of (female (OR) sex (OR) gender) was used in our previous study (Noori, et al., 2018) to screen the same database for basal levels and drug-induced changes in dopamine concentrations in male animals. In order to consistently maximize the outreach of the search space within the constraints of the pubmed-database, we further screened the bibliographies of review and meta-analyses as well as identified articles for relevant references. The titles and abstracts of research articles were first screened by pairs of reviewers (LE and EM) independently. Subsequently, full text of any potentially eligible title and/or abstract were reviewed by both reviewers. Disagreements among reviewers were resolved through discussion.

**Inclusion and exclusion criteria**

The inclusion criteria were set strictly to achieve high level of consistency in article selection. In agreement with the guidelines for meta-analyses of pre-clinical studies (Vesterinen, et al., 2014), only original research articles were included. Meta-analyses, reviews, commentaries and other manuscript styles were excluded. However, the reference sections of such articles were screened for grey literature search, as described above. We focused our search on articles published in English language. Among these, studies were included only if they provided basal dopamine concentrations and/or relative changes in dopamine levels following acute administration of drugs of abuse with the striatal complex; that is NAc and CPu either numerically or in graphical manner. Measurements of other neurotransmitters or in other brain regions were excluded. Studies investigating chronic drug effects were only included if they either provided basal level prior to treatment or if they provided data for the acute drug effects as well. Only systematic drug administrations such as intraperitoneal or intravenous injections were included. Studies reporting local intracerebral injections of drugs into brain regions were excluded. Only microdialysis experiments in animals were included. In vitro microdialysis measurements were excluded. Furthermore, studies using animals other than outbred rats were included.
excluded. Furthermore, animals that received genetic, behavioural or surgical manipulations such as intracerebral lesions, prior baseline measurements and/or drug treatments were excluded. In particular, animals that underwent social and environmental manipulations such as stress by isolation, prolonged food restriction or altered temperature were excluded. Since this data-mining procedure was focused on female animals and data from male animals were excluded from our previous study (Noori, et al., 2013), we excluded studies from the current literature selection that were reporting data only on male rats. In addition to healthy drug-naïve animals, data were also collected from animals that were ovariectomized or pretreated with hormones and analysed post mortem to identify whether the manipulations would lead to any significant differences in dopaminergic overflow. We only included peer-reviewed publications. Data reported in conference abstracts and unpublished studies were excluded. If crucial information was missing, corresponding authors of eligible articles were contacted directly.

Data extraction
A previously reported (Noori, et al., 2012a; Brand, et al., 2013; Fliegel, et al., 2013; Hirth, et al., 2016; Fritze, et al., 2017; Noori, et al., 2018), three categories (i.e. biological, experimental procedure and outcome) of study variables and parameters were extracted using a structured template. The set of extracted biological parameters and variables is comprised of rat strain, state of consciousness, that is awake and freely moving or anaesthetized (anaesthetic agent and dosage), age or weight, oestrous cycle, ovariectomy (if yes, also time after it), hormonal pre-treatment (type of drug, dose and route of administration), and number of animals used in each experiment. The second category, namely the experimental procedure parameters and variables contains data on sampling rate (min), perfusion rate (µL/min), length (mm), outer diameter and molecular cut-off (kDa) of microdialysis membranes, recovery time following probe implantation (hrs), concentration in perfusate (nM) and dialysate matrix (e.g. Ringer solution), targeted brain region, neurochemical detection assay, route of drug administration, drug name and applied dose. The outcome variables were basal levels of dopamine, maximal drug dose effects (%) and the time Ti at which the maximum occurred; that is for a specific dose of the drug the absolute or relative changes of dopamine concentrations within a brain region were obtained. The drug effects were normalized to the basal levels if absolute values were provided, in order to obtain relative changes.

Anatomical nomenclature
A common issue of pre-clinical studies is the inconsistent use of anatomical nomenclature. While a few studies report accurate coordinates for probe placement, the designation of the targeted brain area often differs. In order to avoid false positives we unified in a cluster analysis the terminology using a coarse-grained nomenclature for brain regions (Noori, et al., 2012b; Noori, et al., 2017). Thereby, dorsal striatum, striatum, neostriatum and caudate were grouped as caudate putamen (CPu) and ventral striatum, nucleus accumbens shell/core were grouped as NAc.

Quality assessment
Several factors may influence the quality of the datasets and induce risks of bias. While a risk of bias assessment tool for animal intervention studies exists (Hooijmans, et al., 2014), it could not be utilized systematically in this study since numerous items related to performance and detection bias were not reported in the included studies. In addition to the items within the SYRCLE’s (www.SYRCLE.nl) risk of bias tool (Hooijmans, et al., 2014), we identified at least two more factors that could potentially affect our dataset. In different countries, clinically approved drugs have different trade names that if used, instead of the INN or International Union of Pure and Applied Chemistry designations, lead to inconsistencies in datasets. However, this issue did not occur in this study. In addition, the technical performance and completeness of reports vary among studies. In order to address this issue, we conducted a series of sensitivity analyses to assess the effect of effect modifiers and missing data on the final outcome of the meta-analysis. In order to investigate publication bias, study distribution was compared with the funnel-shape distribution. Since most studies were located near the average, it is safe to assume that most studies were of high precision and no indication towards the existence of publication bias was found.

Outcomes and effect modifiers
The primary outcomes were baseline levels and normalized alterations in extracellular dopamine concentrations (peak%baseline value) within the regions of interest. The time-point of extremal response (i.e. peak) was a secondary outcome. Numerous factors such as rat strain (e.g. Sprague-Dawley, Wistar, Lister-hooded), age, use of anaesthesia (Muller, et al., 2011), pre-treatment with hormones such as oestradiol, ovariectomy (OVX), number of animals, applied dose of drugs, route of drug administration (e.g. intravenous, intraperitoneal), and technical parameters such as length, outer diameter and molecular cut-off of the membranes, flow rate and calcium concentrations within artificial cerebrospinal fluid or Ringer’s solutions as well as the recovery time following implantation of the probes were considered as potential effect modifiers.

Meta-analysis
Dopamine concentrations in dialysate and percentage change in DA compared to baseline were the endpoints of our study. In general, each microdialysis experiment provides time-series of basal concentration and drug-induced changes (in minute scale) for a limited number of neurotransmitters and metabolites within one brain region. The baseline concentration depends on sampling time and perfusion rate and is therefore often reported in pg/µL or fmol/min units. Therefore, in the first step, all extracted basal levels were converted into nM (=fmol/µL) unit. To this end, if basal levels were provided in pg/µL, then the value was divided by the molecular weight of dopamine 153.18 g/mol (https://pubchem.ncbi.nlm.nih.gov/compound/Dopamine) and multiplied with 1000. If data were provided in fmol/min, then the data were divided by perfusion rate (in µL/min). For averaging the dopaminergic response to drugs, we extract the peak response (x) of a transmitter to the drug normalized to its baseline value and the time of the peak. As previously reported (Noori, et al., 2018), we conducted weighted (by inverse variance of each data point) meta-analyses of the extracted basal concentrations and drug effects (%) using fixed effect model. The choice of fixed versus random effect models depend strongly on the question at hand. If one can assume that the different studies are reporting an
estimate of the same effect then a fixed effect model is more appropriate (Vesterinen, et al., 2014). In this study, the meta-analysis was related consistently for each case to one effect. For instance, meta-analysis of basal values of dopamine in caudate putamen of female rats. Review Manager 5.3 (https://community.cochrane.org/help/tools-and-software/revman-5) was used for the calculation of heterogeneity scores ($I^2 = (Q- df)/Q \times 100\%$, where $Q$ is the chi-squared statistic and $df$ is its degrees of freedom) and tests for overall effects as well as generation of forest plots.

**Sensitivity analysis**

We used one-factor-at-a-time sensitivity analysis to evaluate the robustness of the weighted meta-analyses with respect to the above-mentioned effect modifiers. To this end, the dataset was divided into subgroups associated with a specific parameter, for instance adult versus adolescent rats and analysis of variance (ANOVA) was conducted ($p < 0.05$) to identify significant differences because of study design and specific choice of effect modifiers. While most of the parameters were reported consistently among the studies, two effect modifiers did not allow further analyses. The molecular cutoff of the membrane (if provided, within the range of 6–20 kDa) was in only 32% of studies reported, which does not allow reliable statistical comparisons. The second parameter was the recovery time following probe implantation. While this is a critical factor in determining basal transmitter levels, it was not consistently provided in a quantitative manner. Instead, the majority of the studies indicate an ‘overnight’ recovery, which lacks the necessary accuracy for statistical testing.

**Statistical analysis of sex-differences in baseline levels and drug-induced concentrations**

One-way analysis of variance ($\alpha < 0.05$) was used to statistically compare absolute or relative dopamine levels between NAc and CPu within each brain region with respect to sex. If multiple comparisons were conducted, then the level of significance was adjusted using Bonferroni-correction. For basal dopamine levels, data for male animals were obtained previously (Brand, et al., 2013). For drug-induced relative dopamine changes, data were extracted from syphad (www.syphad.com) database (Noori, et al., 2018). Since absolute values are used, a statistical outlier identification algorithm (isoutlier(A,’method’) in MATLAB R2018a) was applied prior to conducting the $F$-test. By default, an outlier is calculated by the method ‘median’ as a value that is more than three scaled median absolute deviations ($c \times \text{median}(|A_i - \text{median}(A)|) \text{ with } c = -1/(\sqrt{2}\text{erfcinv}(3/2)))$ away from median. Since the choice of the method is not unique, ‘quantiles’ and ‘grubbs’ methods were applied and the findings of ANOVA were compared a posteriori. Since the outcomes did not differ, all results are reported based on the least conservative algorithm with minimal a priori assumption, namely ‘quantiles’. All statistical analyses were conducted in non-blinded manner.

**Cluster analysis**

Drug effects on dopamine concentrations, dose of drugs, peak time and technical microdialysis parameters such as flow rate and calcium concentration of the perfusate, sampling time and length of the probe are considered as a function of continuous numerical variables. In turn, age, strain, state of consciousness, hormonal pre-treatment, ovariectomy, drug designation and route of administration were treated as discrete categorical variables. In order to discover patterns in mixed datasets derived from the literature, we applied a two-step clustering algorithm (https://www.ibm.com/support/knowledgecenter/bg/SSLVMB_24.0.0/spss/base/sdh_twoste p_main.html) using IBM SPSS statistics software. This clustering technique allows clustering data with both continuous and categorical attributes and uses a distance measure derived from a probabilistic model. The distance between two clusters is equivalent to the decrease in log-likelihood function. In a first step, a $k$-means procedure was applied to pre-cluster the data. Subsequently, we conducted a modified hierarchical agglomerative clustering procedure combining the objects sequentially to form homogeneous clusters. Furthermore, using Bayesian information criterion, the procedure indicates the importance of each variable (predictor) for the formation of a specific cluster. This information is reflected as the ‘goodness of cluster’ parameter associated to each variable, which takes values between 0 and 1, representing the least and most important variables in generating distinct clusters respectively.

**Results**

**Data distribution**

Systematic literature search identified in the first step 389 original publications. Out of these, 196 studies were relevant for data mining and data from 39 original research articles on in vivo microdialysis in female rat brain (covering studies involving 676 animals) were finally selected for the meta-analysis (Fig. 1; Table 1). No data could be found for ketamine, phencyclidine and tetrahydrocannabinol.

In general, the microdialysis experiments were conducted using comparable experimental parameters (Table 2). Moreover, 96% of studies were performed on adult animals and drugs were applied in 94% of cases intraperitoneal. Therefore, these variables were not considered as effect modifiers. While most studies used Sprague–Dawley rats (62%), sufficient data could be obtained from other strains such as Long–Evans (22%), and Holtzmann (11%) in order to statistically evaluate the impact of strain on dopaminergic response to drugs of abuse. Nonetheless, only 4% of all entries relate to experiments on Wistar rats, which are often used to assess the effect of drugs on neurochemical concentrations in male rats (Noori, et al., 2018). Therefore, special care needs to be applied in interpreting the overall findings of the present meta-analysis in light of previous investigations and our findings should be considered as relating mostly to Sprague–Dawley rats. Approximately one third of all experiments were conducted on ovariectomized rats, which provides sufficient data to analyse the effect of surgical pre-treatment on drug-induced changes in striatal dopamine concentrations.

**Basal dopamine levels**

As presented by the forest plots (Figs 2 and 3), 31 studies provide basal levels of dopamine in NAc (27 studies, mean $= 1.22 \pm 0.05$ nM, median $= 1.42$ nM, $n = 357$) and

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CPu (13 studies, mean = 2.42 ± 0.25 nM, median = 2.57 nM, \( n = 250 \)). While in average dopamine levels in CPu are over twofold larger than in NAc, there are no significant differences between the regions \( (p = 0.1, F_{1,44} = 2.79) \). Thereby, a critical effect modifier is the animal strain, as the basal levels of dopamine in Long–Evans (mean in NAc = 104.68 ± 112.92 nM; mean in CPu = 19.47 ± 9.24 nM) and Holtzmann animals (mean in NAc = 24.23 ± 17.59 nM; mean in CPu = 22.5 ± 7.74 nM) are significantly higher than Sprague–Dawley and Wistar in NAc \( (F_{3,24} = 6.47, p < 0.01) \) and CPu \( (F_{2,19} = 8.21, p < 0.01) \). Furthermore, the statistical comparison of basal dopamine concentrations between male and female (Sprague–Dawley) animals suggests that there are also no significant sex-specific differences in NAc \( (F_{1,65} = 2.09, p = 0.15) \) and CPu \( (F_{1,50} = 0.03, p = 0.84) \).

Impact of ovariectomy on striatal dopamine overflow

Ovarian hormones have been suggested to enhance dopaminergic transmission with reward circuitry and thereby modulate the neurochemical response to psychostimulants (Becker, 1990; Castner, et al., 1993). These investigations often apply hormones such as oestradiol to OVX rats prior to treatment with psychostimulant and subsequently measure the dopamine overflow in striatal regions. However, it remains unclear whether the ovariectomy procedure by itself alters basal dopamine level. The analysis of variance with respect to OVX-procedure suggests that basal concentrations of dopamine in caudate putamen but not nucleus accumbens \( (F_{1,11} = 0.002, p = 0.96) \) are affected significantly by ovariectomy. Indeed, basal dopamine in caudate putamen of OVX-rats is lowered significantly \( (F_{1,22} = 11.27, p < 0.01) \) to a level four times smaller than in non-operated female rats of comparable age.

Nicotine effects

Nicotine-induced changes in dopamine concentration were only reported within nucleus accumbens for systemic administration of the dose of 0.4 mg/kg in \( (n = 27) \) female rats (McCallum, et al., 2012; Eggan and McCallum, 2016; Eggan and McCallum, 2017). Thereby, nicotine increases dopamine levels by 169.44 ± 4.73 % in female animals. In average, no significant differences were found between DA
response to 0.4 mg/kg of nicotine in males and females ($F_{1,37} = 0.008, p = 0.93$).

**Morphine effects**

Alterations in striatal dopamine levels of female rats ($n = 81$) were measured for 5, 20 and 30 mg/kg of acute morphine injections (Maisonneuve, et al., 1991; Pearl, et al., 1996; Maisonneuve and Glick, 1999; Szumlinski, et al., 2000a; Steinmiller, et al., 2003; Velasquez, et al., 2019). Morphine at doses of 5 and 20 mg/kg increases dopamine levels in both regions nucleus accumbens (5 mg/kg: 194.29 ± 17.5%, 20 mg/kg: 212.5 ± 37.5%) and caudate putamen (5 mg/kg: 160.0 ± 15.27%) with no significant differences between the regions ($F_{1,7} = 1.47, p = 0.26$). Moreover, the morphine-induced changes in striatal dopamine concentrations were not different between male and female animals at both doses of 5 ($F_{1,13} = 0.31, p = 0.59$) and 20 mg/kg ($F_{1,6} = 0.03, p = 0.87$). For 30 mg/kg, only one study (Maisonneuve, et al., 1991) provided data suggesting a reduction of dopamine levels to 60% of baseline concentrations. For reliable statistical conclusions with respect to the effects of high morphine concentrations on female dopaminergic systems, more studies are necessary.

**Ethanol effects**

In total, five studies ($n = 165$) reported ethanol-induced changes in extracellular dopamine concentrations of female rat’s nucleus accumbens and caudate putamen (Blanchard, et al., 1993a; Blanchard, et al., 1993b; Blanchard and Glick, 1995; Campbell and McBride, 1995; Kohl, et al., 1998). Ethanol was applied in a range of 0.25–3 g/kg. However, while all doses increased dopamine levels in both regions, no dose–response relationship could be identified (0.25 g/kg: 215% in NAc and 147.5% in CPu; 0.5 g/kg: 172.33 ± 1.45% in NAc and 146.11 ± 3.05% in CPu; 1 g/kg: 120.06 ± 8.15% in NAc and 150.4 ± 30.87% in CPu; 2 g/kg: 158.65 ± 1.67% in NAc and 160% in CPu; and 3 g/kg: 200% in NAc). For 0.5 g/kg, the changes in nucleus accumbens were significantly higher than in caudate putamen ($F_{1,4} = 60.59, p < 0.01$), although neither lower nor higher concentrations showed any noticeable differences ($F_{1,5} = 2.01, p = 0.21$). Interestingly, there are no sex differences in dopaminergic response in nucleus accumbens to ethanol (0.5 g/kg: $F_{1,7} = 5.41, p = 0.053$; 1 g/kg: $F_{1,11} = 2.07, p = 0.18$; 2 g/kg: $F_{1,6} = 0.59, p = 0.81$).

**Cocaine effects**

Data were extracted ($n = 171$) for dopamine changes in NAc and CPu following 2–30 mg/kg and 10–20 mg/kg administration of cocaine respectively (Chapman, et al., 1992; Maisonneuve and Glick, 1992; Maisonneuve, et al., 1994; Johnson, et al., 2000; Kosten, et al., 2003; Shimamoto, et al., 2011; Holly, et al., 2012; Cummings, et al., 2014; Grote-wold, et al., 2014; Shimamoto, et al., 2015; Tobiansky, et al., 2016). In general, all doses of cocaine increase striatal dopamine levels. Although limited number of studies on cocaine-induced effects on caudate putamen exists, the results of data mining robustly indicate that cocaine increases dopamine levels in this region as well (10 mg/kg: 447.5 ± 37.7%; 15 mg/kg: 541 ± 125%; 20 mg/kg: 360%). In nucleus accumbens intraperitoneal cocaine injection is associated with a linear dose–response of positive slope (Fig. 4). Neither ovariectomy ($F_{1,7} = 2.32, p = 0.17$) nor the age ($F_{1,2} = 1.14, p = 0.39$) of the animals have any significant influence on the cocaine-induced dopamine alterations in NAc. In addition, there are no sex differences in accumbal dopaminergic response for different cocaine doses (10 mg/kg: $F_{1,75} = 0.08, p = 0.78$; 20 mg/kg: $F_{1,31} = 2.47, p = 0.13$).

**Amphetamine effects**

Data of 232 female rats were provided from 12 original studies (Robinson, et al., 1988; Becker and Cha, 1989; Robinson and Camp, 1990; Robinson and Camp, 1991; Maisonneuve and Glick, 1992; Castner, et al., 1993; Glick, et al., 1993; Becker and Rudick, 1999; Shoblock, et al., 2003; Geiger, et al., 2009; Shimamoto, et al., 2014; Cummings, et al., 2012; Shams, et al., 2016). Two-step cluster analysis suggests that psychostimulants, particularly amphetamines, induce the strongest impact on dopamine levels in striatum. However, no dose–response relationship could be identified (0.5 mg/kg: 920.83 ± 143.61% in CPu; 0.75 mg/kg: 400% in NAc and 350% in CPu; 1 mg/kg: 1625% in NAc; 1.25 mg/kg:

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**Table 1** Outcome of data mining process. In total, less than 10% of studies identified by keyword search (S) were included for data analysis. K and S denote the number of included and total number of identified studies respectively. N represents the number of animals included in the meta-analysis.

| Substance | k (S) | n | Dose range (mg/kg) |
|-----------|------|---|--------------------|
| Alcohol   | 5 (175)| 201| 0.25–3             |
| Amphetamine | 12 (103)| 240| 0.5–3             |
| Cocaine   | 13 (49)| 158| 2–30              |
| Morphine  | 6 (32)| 70 | 5–30              |
| Nicotine  | 3 (24)| 27 | 0.4               |

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**Table 2** Statistical distribution of experimental parameters related to microdialysis experiments.

| Parameter       | Median | Min | Max |
|-----------------|--------|-----|-----|
| Length (mm)     | 3      | 2   | 4   |
| Outer diameter (µm) | 250     | 216 | 500 |
| [Ca²⁺] (mm)     | 1.2    | 1.2 | 2.3 |
| Flow rate (µl/min) | 1       | 1   | 2   |
| Sample time (min) | 20      | 10  | 30  |

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880 ± 80% in NAc and 1030 ± 138.98% in CPU; 1.5 mg/kg: 555.56 ± 100% in NAc and 1000% in CPU; 2.0 mg/kg: 926.36 ± 405% in NAc and 840.48 ± 144.52% in CPU; 2.5 mg/kg: 1380.91 ± 452.35% in CPU; 3.0 mg/kg: 276.72 ± 1050% in CPU). In analogy to other drugs under investigation, dopaminergic response to amphetamine was compared with respect to sex. In contrast to previous studies (Becker, 1990; Castner, et al., 1993), no significant differences were found in how dopamine transmission was enhanced in male and female caudate putamen (0.5 mg/kg: $F_{1,7} = 1.42, p = 0.27$; 2 mg/kg: $F_{1,23} = 1.18, p = 0.29$; 2.5 mg/kg: $F_{1,7} = 2.47, p = 0.16$). Because of insufficient number of observations, a statistical comparison of male and female response to amphetamines within nucleus accumbens was not possible.

### Discussion

In recent years, increasing attention is rightfully paid to identify biological sex differences associated with neurological and psychiatric disorders (Smith and Dahodwala, 2014; Gogos, et al., 2019; Goldstein, et al., 2019; Manwani and McCullough, 2019). Thereby, the spatiotemporal scale of consideration and context are critical. While functional systems level studies question the presence of sexual dimorphisms within reward circuitry with respect to sexual arousal (Mitricheva, et al., 2019; Stark, et al., 2019), cellular and molecular sex differences associated with addiction may still be present within the same network (Sanchis-Segura and Becker, 2016; Huber, et al., 2018; Becker and Chartoff, 2019). In addition to context and scales of consideration, the...
proper sample size, choice of statistical methods and data convergence may further influence the outcome of investigations and thus, conclusions made on sex differences. Our scoping review and meta-analysis of pre-clinical studies suggest that sex differences were observed entirely in experiments with relatively small sample size between male and mostly ovariectomized female rats. However, the collective of all published studies provides the critical mass that is necessary for conducting second-order advanced statistical analyses. By comparing the overall findings on female rats with overall findings for male rats in a hypothesis-free and systematic manner, we show that there are no sex differences in basal dopamine concentrations in nucleus accumbens and caudate putamen. Moreover, there are no differences in the magnitude of response to drugs of abuse in both regions between males and females. Interestingly, the most critical effect modifier was ovariectomy (OVX), which is a common surgical procedure in studies that addressed sex differences in light of hormonal changes. OVX leads to a significant reduction of basal dopamine levels in caudate putamen but not in nucleus accumbens, which may account for previously reported dopaminergic sex differences in CPu but not NAc. The importance of basal neurotransmitter levels or generally initial brain state on the magnitude of drug response is a largely ignored factor in psychopharmacology. The theory of dynamical systems, which implicitly underlies any temporal measurement, states that the qualitative behaviour of a process strongly depends on its initial state (Perko, 2008). Consequently, a procedure such as OVX that leads to a significant change in basal levels in

![Fig. 3](image)

The outcome of meta-analysis and forest plot of basal dopamine levels (nM) in caudate putamen of 272 female rats. While in both brain regions the data show considerable heterogeneity $I^2 = 98\%$, the pattern of data distribution among included studies for caudate putamen (Robinson and Camp, 1990; Maisonneuve, et al., 1991; Robinson and Camp, 1991; Maisonneuve and Glick, 1992; Blanchard, et al., 1993a; Blanchard, et al., 1993b; Blanchard and Glick, 1995; Pearl, et al., 1996; Becker and Rudick, 1999; Maisonneuve and Glick, 1999; Cummings, et al., 2014; Shams, et al., 2016) indicates that the year of publication is a critical factor. In particular, studies published before 1995 report significantly ($p < 0.0001$) different basal dopamine levels that are in average approximately eightfold higher than publications in the following years.

![Fig. 4](image)

Bubble plot presentation of the dose–response relationship of cocaine-induced changes in dopamine levels of nucleus accumbens in female rats (radius of each bubble represents the number of animal in a study). Dopamine in nucleus accumbens responds linearly to the administration of increasing doses of cocaine. Linear regression of extracted data from 123 female rats suggests a positive relationship $y = 22.188x + 111.03$ between the applied cocaine dose ($x$) and relative changes of dopamine in NAc ($R^2 = 0.5635$).
comparison to non-ovariectomized females will affect all
following pharmacological manipulations. Therefore, statis-
tical tests of drug response in OVX and male rats are
objectively not optimal to address sex differences, even when
a hormonal pre-treatment was applied.
In spite of the absence of sex differences in striatal DA
system, extracellular dopamine levels were notably enhanced
by all drugs of abuse independent of the molecular modes of
action and the dose of the drugs. Indeed, as previously
reported (Noori, et al., 2018), dopaminergic system responds
to approximately 260 clinically approved and experimental
neuropsychiatric drugs, some of which having no direct
interactions with DA transmission. These findings raise the
question of dopamine possesses the necessary specificity to
be considered as a reliable marker for drug effects.
Big data and meta-analyses are powerful tools for
evidence-based medicine and have attracted increasing
attention in recent years beyond clinical applications, namely
in basic and pre-clinical research as well. Data derived from a
meta-analysis are useful for textbook knowledge, provide
better comparability of data given by the generalization of
already existing data, should be seen within the framework of
the 3R principle of animal experimentation, and assist
investigators for power analysis for designing experiments.
Generally, meta-analyses are highly sensitive to the outreach
of their underlying literature search and their input spaces.
Our study solely focused on studies that are indexed within
the National Library of Medicine database. The inclusion of
other databases such as EMBASE (https://www.embase.c
om/) could have potentially led to the inclusion of studies
that our search has missed and thus, the conclusions of our
study must be interpreted within the context of the data that it
included. In addition, the accuracy of the outcomes of a
meta-analysis strongly depends on the quality of the experimental procedures and reporting of the studies that it
integrates. Therefore, while big data and meta-analyses
approaches converge existing data into new knowledge and
hypotheses, an inherent limitation of our study is the quality
of its underlying data. There is large variability in perfusion
rates, exact positioning of microdialysis probe, age of the
animals and even in how the results are reported that may
affect the overall findings. One way to reduce the variability
in study design, which applies to both experimental studies
and systematic reviews, is to standardize the approaches and
to state the ‘research questions and analysis plan clearly prior
to observing the research outcomes-a process that is called
pre-registration’ (Nosek, et al., 2018). Nonetheless, the
unavoidably rough temporal resolution of microdialysis
experiments (i.e. sampling times in tens of minutes) limits
the interpretation of neurochemical changes with respect to
neuronal dynamics. An issue that is inherent to the technique
itself and indicates the urgent need for the development of
rapid neurochemical monitoring methods (Schwerdt, et al.,
2018).

In conclusion, our study suggests that there are no sex
differences in striatal dopaminergic overflow, although an
impact of ovariectomy on basal levels was found. However,
our study does not exclude the possibility of sex differences
in other spatiotemporal scales of consideration or other
components of reward circuitry and more studies are needed
to provide clarity on potential sexual dimorphism in the brain
related to addiction.

Author contributions
H.R.N. & R.S. designed the study. L.E. & E.M. collected the
data. H.R.N. analysed the data. R.S., E.M. & H.R.N. wrote
and reviewed the manuscript.

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All experiments were conducted in compliance with the ARRIVE
guidelines.

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