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Title:

Effects of early life stress on biochemical indicators of the dopaminergic system: a 3 level meta-analysis of rodent studies

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1 INTRODUCTION

During the perinatal period, the brain matures and is rapidly wired(Semple et al. 2013), rendering it particularly vulnerable to negative life experiences that might lastingly impact brain function and behavior(Levine 2005). This may contribute to the well-established observation that exposure to adverse conditions during childhood is a major risk factor for the later development of psychopathologies(Teicher et al. 2016), including schizophrenia and substance abuse(Agid et al. 1999; Enoch 2011; Dube et al. 2003; Scheller-Gilkey et al. 2002).

Prevailing evidence highlights that the dopamine (DA) system may be a prime candidate in mediating the influence of adverse events early in life on vulnerability to psychopathology(Gatzke-Kopp 2011). The DA system develops early during the embryonic period, matures throughout adolescence, and forms stable patterns during young adulthood(Money and Stanwood 2013). This prolonged development provides an extensive window of time in which adverse conditions early in life can tip the balance towards dysfunction(Money and Stanwood 2013). Indeed, alterations in this system have been consistently associated with mental disorders (for a review: (Money and Stanwood 2013)). For example, genetic variations of the DA degradation enzyme COMT are associated with schizophrenia and bipolar disorder as well as an increased risk for psychosis, autism and anxiety(Money and Stanwood 2013). In line, the DA receptor 2 is a major target for antipsychotics.

Overall, the associative studies in humans have led to the assumption that childhood adversities result in developmental alterations of the dopaminergic system. To investigate causality, preclinical studies using animal models have adopted behavioral early life stress (ELS) paradigms to mimic negative childhood conditions, aiming to understand the

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40 neurobiological substrate by which ELS adds to the development of DA system dysfunction.
41 Although extensive, the existing literature is quite heterogeneous: it uses disparate models
42 and outcome measures, and each study focuses on only a limited number of variables;
43 moreover, preclinical studies are frequently underpowered(Button et al. 2013). The resulting
44 findings are rather incoherent and difficult to interpret. This limitation hinders our
45 understanding of the entire biological system and its development, and delays translational
46 applicability.

47 To overcome these limitations, we performed a meta-analysis, a powerful method still
48 sparsely applied to preclinical research allowing to systematically synthesize the scientific
49 knowledge of a specific topic. Recent advances in the field of statistics such as the 3-level
50 approach (Cheung 2014b; Assink and Wibbelink 2016) along with their implementation in R
51 packages(Viechtbauer 2010; Cheung 2014a) now enable researchers to use more
52 sophisticated and robust methodology when analyzing meta-data. This method allows to
53 include multiple data-points from a single study (nesting), without necessarily knowing their
54 (often unreported) covariance. Ultimately, this substantially increases the flexibility of meta-
55 analysis applications and improves the validity of the conclusions drawn.

56 Here, we aimed to investigate whether preclinical studies support an effect of ELS on
57 dopaminergic signaling. We included diverse types and timings of ELS models(Fig 1), and we
58 operationalized the dopaminergic system by quantifying several biochemical markers in
59 mice and rats(Fig 2), across brain areas(Fig 3), considering possible confounders.

60 We determined whether the quality of the studies affected the estimation of the results. To
61 make our knowledge readily available to others, we organized all information in a freely
62 accessible open-source dataset and created a user-friendly web-app as a tool to guide future

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63 (pre)clinical research (e.g. power analysis calculation), thereby avoiding unnecessary
64 replication and limit animal experimentation.

65

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2 MATERIALS AND METHODS

The review adhered to SYRCLE (Systematic Review Center for Laboratory animal Experimentation) guidelines for protocol(De Vries et al. 2015), search strategy(Leenaars et al. 2012), and risk of bias assessment(Hooijmans et al. 2014).

2.1 THEORETICAL DEFINITIONS AND ASSUMPTIONS

We defined as *"individual comparison"* each test performed within a published study between a control group and an experimental group with a history of ELS. As often occurs in experimental studies(Aarts et al. 2014), multiple outcomes (*individual comparisons*) were collected from the same groups of animals (*nesting*).

We defined as *"experiment"* the ensemble of outcome measures from the same animals. According to this definition, each published study can report multiple experiments when conducted on different sets of animals. Similarly, experiments conducted on different sets of animals could potentially be reported in separate publications. For these reasons, we *nested* multiple individual comparisons belonging to the same animals within the same experiments, but considered *experiments* from the same publications as independent from each other.

2.2 SEARCH STRATEGY

A comprehensive literature search was conducted regarding *the effects of early life stress on biochemical indicators of dopaminergic signaling* on February 14th 2017. The search string was composed by the factors "dopamine", "early life stress" and "rodents" (Fig 1 and supplementary appendix S1). The search was conducted on the electronic databases PubMed (www.pubmed.com) and Web of Science (www.webofknowledge.com). For a flow chart of the entire methodology, see Fig 4.

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Studies' titles and abstracts were screened, and selected if the inclusion criteria were met (supplementary Table S2-1). In case of doubt, the full text was inspected. Eligible studies were evaluated by two independent reviewers (VB and RAS).

2.3 STUDY CHARACTERISTICS

2.3.1 Selection and data extraction

To limit subjectivity in the data gathering and entry process, data extracted from eligible studies were recorded in a standardized database (doi:10.17632/9x9fc3v26k.1). The following information was included: species, strain, sex, type and timing (relative to age) of the ELS model, outcome, time (relative to age) of outcome, technique used for outcome, brain area investigated, number of animals used, mean, standard deviation (SD) and standard error of the mean (SEM). If only SEM was reported, SD was calculated as $SEM * \sqrt{n}$, where n = amount of animals per group. If number of animals were reported as a range (e.g. 6-8 animals per group), we used the mean of this number (e.g. 7 animals per group). If a single control group was used to compare experimental groups in which ELS was induced with different models (e.g. handling and maternal deprivation), the sample size of the control group was equally divided as control for each experimental group (e.g. n=10 in not handled control becomes n=5 for control of maternal deprivation and n=5 for control of handling)(Vesterinen et al. 2014).

When the data was not reported numerically in the publication, we contacted two authors per manuscript. If no answer was received within three weeks and after a reminder, the

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authors were considered not reachable. Only 5 out of 56 contacted authors replied to our request. Given the low response rate, we estimated most of the data presented only in graphs with *Ruler for Windows* (https://a-ruler-for-windows.en.softonic.com/). We tested the accuracy of this method by comparing effect sizes calculated from either supplied data or evaluated with the ruler, and verified that they were highly correlated (R^2 = *.74*, supplementary Figure S2-1).

Concerning metabolites, some papers reported either concentrations, turnovers or both. In 97.5% of cases it was possible to calculate concentrations from turnovers with Pythagoras. Since concentrations and turnovers are related to the same information (though not identical), only concentrations were included in the analysis in order to avoid multi-collinearity. Turnover data-points are available in the MaDEapp *(Meta-Analysis on Dopamine and Early life stress)* for consultation.

2.4 Assessment of risk of bias in included studies

We used the SYRCLE tool to assess the risk of bias (supplementary Table S2-2)(Hooijmans et al. 2014). The criteria are based on the possible presence of selection bias (items 1, 2 and 3), performance bias (items 4 and 5), detection bias (items 7, 8, 9) and attrition bias (item 10). Furthermore, we added the item "*quality of control*" (item 6) to the category performance bias.

Since poor reporting of experimental details plays a role in heightening the quantified risk of bias, lack of reporting was scored as *unclear* risk of bias.

For quantitative inclusion in the analysis, amount of potential bias was operationalized by summing the risk of bias of each item according to the definition: "yes" = 0, "unclear" = 1,

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"no" = 2. This produced a continuous variable of integer increment between 0 (no risk bias) and 20 (maximum risk of bias), which was then scaled *(mean = 0)* to interpret the studies as of average risk of bias.

2.5 Data synthesis and statistical analysis

2.5.1 Effect size

We estimated the effect size for each individual comparison with *escalc* (R package *metafor*) as standardized mean difference with *Hedge's G* method, which includes a correction factor for small sample sizes(Vesterinen et al. 2014).

2.5.2 Study of heterogeneity

Heterogeneity was tested with the Cochran Q-test(Moreira et al. 2016) and I^2 statistics(Cheung 2014b). Study of the distribution of variance was conducted for models without moderators to determine how much variance could be attributed to differences between effect sizes within experiments (level 2) and to differences between experiments (level 3). Substantial distribution of heterogeneity at these levels further encouraged the use of moderators.

2.5.3 Model

We used a 3-level mixed effect model, which accounts for the anticipated heterogeneity of the studies as well as the dependency of effects within experiments. In our experimental design, the 3 levels correspond to variance of effect size between 1) animals, 2) outcomes and 3) experiments.

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153 Since pre- and post-natal models act on different times of development that are particularly
154 disparate regarding the array of environmental factors, we considered them as different
155 datasets and consequently analyzed them separately.

156 Effect sizes were considered outliers if their *z* score was above +3.29 or below -
157 3.29(Tabachnick and Fidell 2013), and removed from the analysis.

158 Since we hypothesized that the effect of ELS on DA signaling may not be evident from an
159 overall estimate, we defined *a priori* possible moderators of this effect. These belonged to
160 two different categories: biological and technical. The biological moderators were: outcome
161 measure used (e.g. DA and metabolites), brain area investigated, sex, species, age as a
162 continuous variable, and whether the outcome was at a RNA level/protein level/functional
163 (referred to as *method of assessment*). Specific regions within the brain areas were
164 investigated only in subgroup analysis due to the limited amount of observations. We
165 considered the type of ELS model and amount of potential bias as technical moderators.
166 These moderators may not underlie a biological difference, but can nevertheless explain
167 heterogeneity across studies. The postnatal ELS model 'handling' has been reported
168 repeatedly to cause effects in the opposite direction to those induced by other ELS
169 models(Levine 2005). We therefore multiplied each calculated effect size for handling by -1
170 (Vesterinen et al. 2014), so that the overall estimate would be in the same direction. We
171 verified that the same conclusions would have been drawn if handling was excluded as a
172 model (supplementary Figure S2-2).

173 To avoid multicollinearity among moderators, we firstly assessed each biological moderator
174 univariately. We set the significance level at $p<.10$ to test whether a moderator significantly
175 reduced the previously quantified heterogeneity. A less restrictive *p*-value was chosen to

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assure the inclusion of moderators that have a multivariate but not univariate effect(Hox 2010). Only interactions with at least 4 comparisons from 3 different publications from at least two different laboratories were quantitatively assessed. This explains why some of the keywords in our search string were not included in the final analysis (Fig 1).

2.5.4 Subgroup analysis

As the 3-level models revealed significant heterogeneity, we conducted subgroup analyses to further investigate its source. In particular, we tested the influence of the technical moderators (type of ELS model and amount of potential bias) as well as of the brain regions within the brain areas previously described (Fig 3), in subsets of the dataset with sufficient observations. For information on the type of ELS model used, please see supplementary appendix S2-1.

2.5.5 Sensitivity analysis and publication bias

According to the standards of meta-analyses, we should investigate the robustness of our effect sizes by performing analysis only on those studies that were blinded and randomized. Unfortunately, the amount of blinded and randomized studies was insufficient to proceed with this approach. As an alternative, we performed the analysis by including the amount of potential bias as a moderator. The results of this sensitivity analysis should be interpreted as the effects of ELS on biochemical markers of the dopaminergic system on studies of average risk of bias.

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To detect publication bias, funnel plots' asymmetry for each outcome variable was qualitatively evaluated. To the best of our knowledge, there are no available methods to quantify missing data (due for example to publication bias) in a multi-level setting(Assink and Wibbelink 2016). Nonetheless, we evaluated publication bias with Egger's regression(Egger et al. 1997). However, these results should be interpreted with caution as they are not based on a 3-level model. Lastly, we excluded those studies responsible for funnel plot asymmetry and conducted sensitivity analysis on the remaining dataset in the attempt to evaluate the influence of publication bias in the meta-analysis.

Data are presented as *Hedge's G* and 95% C.I. Data analysis was conducted with the computer program R (version 3.2.3)(Team 2015), with the aid of the following R packages: 1) *metafor*(Viechtbauer 2010) for conducting the analysis, 2) *ggplot2*(Wickham 2009) for graphics, and 3) *shiny*(Chang et al. 2017) to create the MaDEapp.

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3 RESULTS

3.1 STUDY SELECTION AND CHARACTERISTICS

3.1.1 Study selection and data extraction

The process of study selection is illustrated in the flow chart (Fig 4). The search string identified a total of 979 unique research papers. Statistical measurements (e.g. mean, SD and N) for quantitative analysis were extracted from 90 peer-reviewed publications (that met our pre-specified inclusion criteria as described in the methods section. Three publications(Arborelius and Eklund 2007; Kippin et al. 2008; Kosten, Zhang, and Kehoe 2005) were excluded from the analysis as it was not possible to extract nor infer any statistical measurement. Similarly, information was lacking from 23 comparisons of three other publications (Kikusui, Faccidomo, and Miczek 2005; Ognibene et al. 2008; Kirsten et al. 2012).

The included studies dated between 1996 and 2016, used ~2600 animals yielding a total of 1009 comparisons from 152 experiments. The publications were analyzed in two separate datasets, respectively using prenatal (41 publications) and postnatal (49 publications) ELS models. For a summary of experimental characteristics across studies see supplementary Table S3-1.

Four observations of the prenatal dataset (striatal Th (Baier et al. 2014), striatal DA(Basta-Kaim et al. 2011), striatal HVA(Z. D. Ling et al. 2004), striatal DR2(Son et al. 2007)) and 6 observations of the postnatal dataset (striatal DR1(Y. Zhang et al. 2013), striatal DR2(Y. Zhang et al. 2015), DAT in the VTA area(Tien et al. 2013), striatal DOPAC(Ognibene et al. 2008), cortical DA(Matthews et al. 2001) and limbic HVA(Kubesova et al. 2015)) were excluded from the analysis as outliers.

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3.1.2 List of included publications

The publications included in the analysis were:(Adrover et al. 2007; Alonso, Navarro, and Rodriguez 1994; Baharnoori, Bhardwaj, and Srivastava 2013; Baier et al. 2014; Bakos et al. 2004; Basta-Kaim et al. 2011; Berger et al. 2002; Bingham et al. 2013; Bitanihirwe et al. 2010; Brenhouse, Lukkes, and Andersen 2013; Cabib, Puglisi-Allegra, and D'Amato 1993; Cai et al. 2013; Camp, Robinson, and Becker 1984; Carboni et al. 2010; Chocyk et al. 2011; Kwok Ho Christopher Choy, De Visser, and Van Den Buuse 2009; K. H C Choy and van den Buuse 2008; Cory-Slechta et al. 2009; Cory-slechta et al. 2013; Dallé, Daniels, and Mabandla 2016; Delattre et al. 2016; L. W. Fan et al. 2011; L.-W. Fan et al. 2011; Gerardin et al. 2005; Gondré-Lewis et al. 2015; Gracia-Rubio et al. 2016; Granholm et al. 2011; Hall et al. 1999; Emily Hensleigh and Pritchard 2015; E. Hensleigh and Pritchard 2014; Hermel et al. 2001; Hill et al. 2014; J. W. Jahng et al. 2010; Jeong Won Jahng et al. 2012; Katunar et al. 2010; Kawakami et al. 2013; Kikusui, Faccidomo, and Miczek 2005; Kirsten et al. 2012; Kubesova et al. 2015; Lazzaretti et al. 2015; Lejeune et al. 2013; Li et al. 2014; Z. D. Ling et al. 2004; Z. Ling et al. 2004; Z. Ling et al. 2009; Liu et al. 2016; Lovic et al. 2013; Luchicchi et al. 2016; Madruga et al. 2006; Matthews et al. 2001; Meyer, Nyffeler, Schwendener, et al. 2008; Meyer, Nyffeler, Yee, et al. 2008; Novak et al. 2013; Ognibene et al. 2008; Oreland et al. 2011; Ozawa et al. 2006; Pallarés et al. 2013; Panagiotaropoulos et al. 2004; Pang et al. 2015; Papaioannou et al. 2002; Ploj, Roman, and Nylander 2003; Rentesi et al. 2013; Reynaert et al. 2016; Reznikov and Nosenko 1995; Romano-López et al. 2015; Eva Romero et al. 2007; E Romero et al. 2010; Rossi-George et al. 2011; Rots et al. 1996; Sasagawa et al. 2017; Silvagni et al. 2008; Silveira et al. 2010; Son et al. 2007; Tien et al. 2013; Vazquez et al. 2007; Vuillermot et al. 2012; Wang et al. 2009; Weston, Sobolewski, et al. 2014; Weston, Weston, et al. 2014; Winter et al. 2009; Womersley et al. 2011; Zager, Mennecier, and Palermo-Neto 2012; Zavitsanou et al. 2013; Y. Zhang et al. 2015;

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255 X. Y. Zhang, Kehoe, and Kosten 2006; Y. Zhang et al. 2013; X. Zhu et al. 2010; X. Zhu et al.

256 2011; Y. Zhu, Carvey, and Ling 2007; Zuckerman et al. 2003)

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3.2 META-ANALYSIS: PRENATAL ELS

3.2.1 Heterogeneity

Substantial heterogeneity was recorded in the prenatal dataset *(Q(378) = 954.969, p< .001)*, indicating that our search string identified a diverse range of experiments. In particular, 34.7% of variance could be attributed to within-sampling variance, 18.7% to within-experiment variance and 46.6% to between-experiment variance. These results suggested the use of moderators.

3.2.2 Moderators

Potential moderators (supplementary Table S3-2) - such as brain area, sex, and species - were selected prior the beginning of the study based on hypotheses of the ELS literature.

As identified with univariate analysis of potential moderators (supplementary Table S3-3), outcome measure *(F(7,371) = 3.956, p<.001)* and brain area investigated *(F(4,374) = 6.144, p<.001)* were significant moderators in the prenatal dataset, explaining 4.3% and 6% of variance respectively. There was no detectable moderating effect of sex, species, age used, and method of assessment (RNA, protein or functional level).

3.2.3 Model

The moderators that were univariately identified were included in the 3-level model to investigate the effects of ELS on markers of dopaminergic signaling. We hypothesized that the effects were dependent on the outcome analyzed and that they differed across brain areas.

Of the 20 interactions between outcome measure and brain area with enough data-points, only 2 reached statistical significance (Fig 5). For a summary of all interactions, see

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supplementary Table S3-4. In particular, in the striatal zone, Th was decreased *(Hedge's G(se) = -1.164(.295), p<.001*, supplementary Fig S3-1*)* while DOPAC was increased *(Hedge's G(se) = .323(.136), p = .018*, Fig 6A*)* following prenatal ELS.

Fig6B shows a cumulative forest plot of striatal DOPAC to exemplify that the chronological combining of the experiments shows consistency since 2010, and that subsequent experiments have not contributed to the direction nor the size of the effect. The cumulative forest plot does not correct for the multi-level structure of the model.

The interaction between outcome measure and brain area explained 22.2% of variance in the prenatal dataset. We identified 17 interactions with enough comparisons to further address heterogeneity *(Q(346) = 742.97, p<.001)*.

3.2.4 Subgroup analysis

Subgroup analysis was used to further investigate the unexplained heterogeneity deriving from the 3-level model. The type of ELS model and sub-brain areas were univariately evaluated as potential moderators for each interaction between outcome measure and brain area.

In the prenatal dataset, 15 interactions had enough observations to be considered for further subgroup analysis. Of these, 5 had a significant test of ELS model as moderator (supplementary Table S3-5), namely DOPAC and D1R in the striatal area, DA in the cortical area, and Th in the VTA area.

Subgroup analysis revealed that injection of LPS or PolyI:C has consistently different effects than maternal restraint. In particular, LPS and PolyI:C significantly increased DOPAC in the

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striatum *(LPS: Hedge's G(se) = .941(.229), p<.001; PolyI:C: Hedge's G(se) = .608 (.238), p=.016)*, while restraint did not *(Hedge's G(se)= .012(.207), p = .956*, Fig 6A*)*. Conversely, restraint decreased Th in the VTA area *(Hedge's G(se) = -.85(.348), p=.03)* whilst LPS injection did not *(LPS: Hedge's G(se) = .026(.188), p = .889,* supplementary Fig S3-2*)*. Concerning cortical DA, the effects of PolyI:C and restraint had opposite directions but did not reach statistical significance, whilst the LPS model could not be quantitatively evaluated. Concerning D1R, no significant effects of the subgroup analyses were recorded.

Sub-brain area was a significant moderator only for DA in the striatal area (including nucleus accumbens, dorsal and central striatum and nucleus caudatus; supplementary Fig S3-3). In particular, DA was increased in the nucleus accumbens after ELS *(Hedge's G(se) = .392 (.159), p = .01)* but it was unaffected in other parts of the striatum *(Hedge's G(se) = -.122 (.146), p = .40)*. For a summary of all subgroup analyses, see supplementary Table S3-6.

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3.3 META-ANALYSIS: POSTNATAL ELS

3.3.1 Heterogeneity

Our search identified a diverse range of experiments in the postnatal dataset, as shown by the substantial heterogeneity recorded *(Q(381) = 1061.278, p<.001)*. In particular, 33.1% to within-sampling, 46.9% to within-experiment, and 20.1% to between-experiment variance.

3.3.2 Moderators

Univariate analysis of potential moderators (supplementary Table S3-7, *p*-value significance set at <.10) identified outcome measure *(F(8, 373) = 9.139, p < .001)* and brain area investigated *(F(4,377) = 2.035, p = .089)* as significant moderators, explaining 10.3% and 0.8% of variance respectively. Other moderators, such as species and sex of the animal, had no detectable moderating effect.

3.3.3 Model

The moderators that were univariately identified were included in the 3-level model to investigate the effects of ELS on markers of dopaminergic signaling. We hypothesized that the effects were dependent on the outcome analyzed and that they differed across brain areas.

Of the 17 interactions with sufficient comparisons, 3 reached statistical significance (Fig 7, supplementary Table S3-8). In particular, in the striatal zone, DOPAC *(Hedge's G(se) = .541(.207), p = .009,* Fig 8*)*, HVA *(Hedge's G(se) = .555(.22), p=.012*, supplementary Fig S3-4*)* and DA *(Hedge's G(se) = .307(.147), p = .038,* supplementary Fig S3-5*)* were increased.

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335 The interaction between outcome measure and brain area explained 15.3% of variance in the
336 postnatal dataset. We identified 16 interactions with enough comparisons to further address
337 heterogeneity *(Q(345) = 898.4, p<.001)*.

338

339 ### 3.3.4 Subgroup analysis

340 The moderator effects of the ELS model used and sub-brain areas were evaluated with a
341 subgroup analysis.

342 In the postnatal dataset, 16 interactions had sufficient observations to be further analyzed. Of
343 these, 7 revealed a significant impact of the ELS model used (supplementary Table S3-9):
344 HVA, DOPAC and DA in the cortical area, HVA and DOPAC in the striatal area, DOPAC in the
345 limbic area, and DA in the VTA area.

346 Subgroup analysis showed that the effects of ELS model as moderator in striatal DOPAC (Fig
347 8) and HVA (supplementary Fig S3-4) were mainly due to handling. In particular, handling
348 decreased HVA *(Hedge's G(se) = -.778(.295), p = .03)* as well as DOPAC *(Hedge's G(se) = -*
349 *.77(.301), p = .029)* in the striatum, whilst separation of the mother from the pups had no
350 effect *(HVA: Hedge's G(se) = .08(.227), p = .735; DOPAC: Hedge's G(se) = -.205(.239), p = .411)*.

351 Sub-brain area was not a potential moderator in any of the interactions evaluated.

352 For a summary of all subgroup analyses, see supplementary Table S3-10.

353 ## 3.4 Sensitivity analysis

354 Sensitivity analysis was conducted to test the robustness of our findings. We examined
355 whether the quality of the studies included had an impact on the interpretation of our results.

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3.4.1 Quality of the studies: SYRCLE bias report

No publication reported information on all SYRCLE potential bias items. Overall, of the 90 publications, 37 (41%) reported randomization sequence of the animals in the experiments, 3 (3.3%) random housing allocation, 59 (65%) random group allocation, 49 (54.4%) random selection of the animals (Fig 9). In 11 (12%) publications the caretaker were reported blinded to the experimental condition, in 20 (22.2%) the experimenters blinded. Handling of incomplete data was reported in 42 publications (46.7%). 11 studies (12.2%) did not report sufficient information to evaluate the quality of the control group. Only 11 studies yielding a total of 117 comparisons reported being blinded and randomized.

3.4.2 Sensitivity analysis for potential bias

Since the amount of publications was insufficient to evaluate the robustness of our effects in a blinded and randomized dataset, we operationalized the amount of potential bias and performed the analysis again by including this factor as a moderator. Therefore, the results of this sensitivity analysis were interpreted as the effects of ELS on markers of DA signaling on studies of average bias.

The amount of potential bias was a significant moderator in the prenatal dataset *(F(1, 377) = 3.536, p = 0.061)*; yet, this did not affect the qualitative interpretation of the meta-analysis.

In the postnatal dataset, the test of moderators for amount of potential bias was not significant *(F(1, 380) = 0.500, p = 0.480)*. The interpretation of the results did not change, with the exception of DA in the striatal area, of which the effect size was decreased and the effect at a trend level *(Hedge's G(se)=-.289(.15), p = .057)*.

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3.4.3 Publication bias

Due to the lack of methods to quantitatively evaluate publication bias in a multi-level setting, we qualitatively estimated the risk for publication bias with funnel plots (Fig 10). Publication bias was more pronounced in the prenatal than the postnatal dataset. The same conclusion was reached when performing Egger's regression (no multi-level regression models): there was evidence for publication bias in the prenatal ($z = -5.014$, $p < .001$) but not in the postnatal ($z = -0.612$, $p = 0.54$) datasets. The presence of publication bias in the prenatal dataset may indicate an overestimation of the reported effect sizes.

Furthermore, we conducted an analysis of influential cases by removing studies with large standard error as well as residual values. Since the results did not change qualitatively, publication bias was considered low-to-moderate.

3.5 MaDEapp

Finally, we created a MaDEapp (https://vbonapersona.shinyapps.io/MaDEapp/), a web-based app with a user-friendly interface in which each researcher can perform his/her own meta-analysis on the topic of ELS and biochemical indicators of the dopaminergic signaling. The app offers the possibility to choose across a wide variety of options, such as outcome measures, brain areas, sex of the animals, type and timing of the ELS model. Based on the characteristics indicated, the app reports forest, funnel and cumulative plots. The forest plot includes a 3-level effect estimate (Hedge's G and CI), which can be used for future power calculation.

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For example, a researcher is interested in the effects of postnatal ELS on DR1 in the striatal area. In MaDEapp, the researcher selects the "postnatal" dataset, with "DR1" as outcome measure in the "striatal area" (Fig. 11). The resulting forest plot reports the estimated *Hedge's G (CI) = -.5 [-.91, -.1]*. The estimated effect size is smaller than 0. From this exploration, the researcher hypothesizes that postnatal ELS decreases DR1 expression in the striatal area. The effect size -.5 may be an overestimation of the real size of the effect due to potential (publication) bias. The researcher would then use an effect size of -.45 for power calculation for his/her future experiments.

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4 DISCUSSION

Schizophrenia and addiction are examples of psychiatric disorders reported to be linked to DA dysfunction. Childhood trauma is a well-documented risk factor(Teicher et al. 2016; Gatzke-Kopp 2011; Money and Stanwood 2013; Grace 2016). This clinical observation led to the hypothesis that the dopaminergic system mediates the risk of ELS. Although this link has been causally investigated in more than 90 rodent publications over 20 years, no consensus has yet been reached on the extent, directionality and specificity of this effect. Therefore, we performed a meta-analysis to question: Do rodent studies support long-lasting effects of ELS on biochemical indicators of the dopaminergic system? Overall, our results indicate that only a limited number of comparisons were significant suggesting that the effects of ELS on the dopaminergic system may not be apparent on a biochemical level.

4.1 METHODOLOGICAL CONSIDERATIONS

Dopaminergic signaling involves multiple interdependent elements (e.g. precursors, metabolites, receptors), which altogether contribute to the system as a whole. Data on these elements are sometimes gathered from the same animals, and are therefore dependent on each other(Aarts et al. 2014). In a meta-analysis setting, dependency implies overlap of information, which ultimately leads to an erroneous interpretation of the results(Rosenthal 1991; Van den Noortgate et al. 2013). To deal with this obstacle, several strategies have been adopted: from selecting only one effect size per study to ignoring the problem altogether(Cheung 2014b). Although sophisticated methods such as multivariate and multilevel analysis exist, these have the strong limitation that the needed covariance between the dependent effects is rarely reported in publications(Hox 2010). The 3-level approach that we used overcomes both limitations: it corrects for dependency of observations, without the

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use of covariances(Assink and Wibbelink 2016). To the best of our knowledge, this approach has never been used before in rodent literature. Although this powerful and practical method was initially created for human studies(Cheung 2014b), its applicability in preclinical research is warranted due to the multiple-outcome nature of such studies. The method is already available in the R packages *metafor* and *metaSEM*.

Together with the application of a 3-level mixed effect meta-analysis to preclinical literature, we here promote the use of tools to facilitate data exploration and advocate open science. We created MaDEapp, a freely available user-friendly app that allows to run a tailor-made meta-analysis on ELS and the dopaminergic system, depending on the specific question one has. Each individual can select a set of characteristics (e.g. prenatal/postnatal models, sex, age). The app returns a forest plot in which the total univariate 3-level estimate is presented, as well as a funnel plot to evaluate publication bias. This can be used to generate hypotheses, evaluate estimated sample sizes for power analysis, and explore which outcomes/brain areas have received most attention and which did not. We believe this app is a useful tool to guide future research on the topic.

MaDEapp and the analysis here presented are complementary. Meta-analysis is a statistical test, and it is limited by the frequency (*power*) and quality (*potential bias*) of the published data. In our analysis there was no significant effect of postnatal ELS on DR1 in the striatum *(p = .053)*; however, when we analyzed the same outcome univariately with MaDEapp, the confidence interval of the estimate did not include 0 and could be interpreted as "significant". This discrepancy may be due to a lack of power to confirm the effect in our analysis or due to an increased bias not corrected for in the more specific model used by the app. Therefore, non-significant results should be interpreted as lack of confirmation of an effect, not as

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454 evidence of no effect, since the meta-analysis could be underpowered to detect a specific
455 marker in a specific brain area. Alongside, the use of the app should be intended as
456 exploratory only and not as confirmatory.

457

458 ## 4.2 QUALITY OF THE STUDIES

459 Meta-analysis as a methodology is not simply the collection of statistical methods used to
460 achieve integration of available evidence. Its power lies in the application of systematic
461 scientific strategies to the literature review(Cornell and Mulrow 1999). In addition to
462 summarizing effects' estimates, it allows to evaluate the extent to which conclusions are at
463 risk of bias.

464 In our analysis, surprisingly few studies (12%) reported being randomized as well as blinded.
465 On the other hand, random allocation to group (41%) as well as blinded assessor (22.2%) was
466 comparable(Antonic et al. 2013) or better(Egan, Sena, and Vesterinen 2011) than previous
467 publications in neuroscience. Although it is likely that investigators did take measures to
468 reduce bias, lack of their reporting induced an unclear risk(Kilkenny et al. 2013; Landis et al.
469 2012) and hindered estimation of the value of the publications(Kilkenny et al. 2013). The
470 importance of quality of reporting has been an emerging issue in preclinical
471 research(Kilkenny et al. 2013; Landis et al. 2012). Despite the increased awareness, the quality
472 of reporting of the publications included in this meta-analysis has not improved since 2005
473 (supplementary Fig S4-1). Such evidence should encourage preclinical researchers as well as
474 reviewers to adhere to reporting guidelines such as ARRIVE(Kilkenny et al. 2013).

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Although imprecise reporting does not necessarily imply poor study quality, underpowered experiments seriously hamper research interpretation(Button et al. 2013). From the reported amount of animals included per experiment, we back-calculated the power at the beginning of the study, assuming at least one true positive effect per publication. We performed this analysis considering small (*Hedge's G* = .5), medium (*Hedge's G* = .8) or large (*Hedge's G* = 1) effect sizes (supplementary Fig S4-2). When considering a large effect size, 391 comparisons (38.7%) had power below chance level, and only 63 (6.2%) had power >.8, a cut-off value(Cohen 1992) generally aimed at in preclinical research. Although 43 papers (47%) had at least one comparison with power >.5, only 5 papers (5.5%) had at least one comparison with power >.8. This means that the vast majority of the experiments was not sufficiently powered to reliably detect an effect – an (already dramatic) best-case scenario given that the percentages were calculated assuming that the studies compared only two groups (t-tests) as well as a large and truly existing effect. Future preclinical studies should be grounded in power calculations based on realistically estimated effects. Although for each single study the amount of animals will be larger, overall higher power will lead to more reliable, reproducible and therefore higher quality research.

4.3 ELS CAUSES LIMITED ALTERATIONS ON BIOCHEMICAL MARKERS OF THE DA SYSTEM

In our analysis, we evaluated the dopaminergic system by including numerous biochemical markers across brain areas as well as potential moderators. These gave rise to a myriad of viable comparisons. Despite the extent, only a handful of significant effects were identified, thereby suggesting that biochemical indicators of the DA system well adapt to ELS interventions. Clearly, we cannot exclude the possibility that other indicators of the DA

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system (e.g. electrophysiological parameters or behavioral tests that critically depend on DA function) would have yielded clearer results. This awaits future investigation.

Prenatal and postnatal ELS were treated separately because the prenatal environment differs substantially from that postnatally. Nonetheless, both datasets shared consistent findings. Specifically, the striatal area was the most vulnerable: following prenatal ELS, Th was decreased and DOPAC increased; while postnatal ELS caused an increased in DOPAC as well as HVA. These changes were stable and reliable: the analysis used is adequately conservative and robust, and the effects survived sensitivity analysis and publication bias corrections. The stability of the effects is also qualitatively substantiated by the cumulative plots, which operationalize how subsequent experiments update our knowledge of the previously estimated effect size. Our results display that these were durable over time, and that replication after the initial 3-5 studies might not be very informative on these variables (except as a positive control in a study investigating another variable), as additional experiments did not alter the estimated effect. All in all, the sparse effects here reported are reliable and of medium size, suggesting that the system is damaged, which may in turn contribute to the vulnerability of ELS-dependent disorders.

The results can be interpreted as either hyper- or hypo-activation. For instance, the increase in metabolites can indicate an increase in the available amount of the substrate DA (hyperactivation) as well as an increased conversion rate causing less DA (hypoactivation). Similarly, the decrease in Th (precursor conversion enzyme) is not necessarily indicative of a decrease in DA function. Although our analysis suggests that postnatal ELS increases DA levels in the striatum, the effect is small in size and less robust that the other effects mentioned above. The mismatch between DA precursor and metabolism may suggest

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changes in the intermediate stage of DA conversion. For example, the DA converting enzyme COMT has been repeatedly shown to interact with ELS for the later development of psychiatric disorders(Lovallo et al. 2017; Klaus et al. 2017; Sheikh et al. 2017). On the other hand, L-DOPA – product of Th and precursor of DA – has been suggested to act as a novel transmitter itself or may have neurotropic functions, and thereby be transiently involved in perinatal developmental processes(Braun et al. 1999). Since the interaction between ELS and L-DOPA has not been further investigated, the link remains circumstantial.

We defined *a priori* several factors established in preclinical literature to be potential moderators of ELS effects. To our surprise, species, sex and method of assessment were not significant moderators. Although males, mice and protein as method of assessment were the most described conditions, plenty of observations were present for all groups. Nonetheless, the lack of evidence for a moderator effect should not be interpreted as evidence for absence: mice/rats should be chosen according to standard practice, both sexes should always be considered(Bale and Epperson 2015), and there is substantial evidence that a decrease in RNA level does not automatically result in a decrease in protein and therefore in function, as e.g. shown in a systems approach(Williams et al. 2016).

Lastly, the unexplained variance may not only indicate methodological differences, but also underlie additional biological moderators, such as sub-brain areas or differences across hemispheres (lateralization).

4.4 Translational potential?

The translational applicability of preclinical studies depends on the understanding of psychopathological clinical and intermediate phenotypes. For example, ELS is a main risk factor for schizophrenia as well as substance abuse disorder. However, these diseases have

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544 opposite intermediate phenotypes: while schizophrenia is supposed to be characterized by
545 hyperreactivity of the DA system although presumably to its afferent control(Grace 2016),
546 substance abuse may be linked to DA hypo(re)activation(Wise and Koob 2014). To what
547 extent do ELS studies in rodents accurately model these two conditions?

548 Three factors currently limit answering this question. Firstly, ELS in humans is a complex
549 concept, generally involving low socio-economic status, physical and/or psychological abuse,
550 poor living conditions and high caloric food (Teicher et al. 2016). Conversely, animal models
551 are extremely controlled and standardized pre- and postnatally. Although this facilitates the
552 definition of "traumatic early life" as well as the deriving caused effects, one can question its
553 ecological validity. Secondly, the dual hypo- / hyper- interpretation of the ELS-induced
554 phenotype in rodents prevents a whole-system level comparison, and restricts it to a micro
555 level, focusing on a particular compound in a particular brain area. Thirdly, the DA-dependent
556 changes in schizophrenia and addiction are most likely far more complex than the ones
557 observed following ELS in rodents. For example, our analysis failed to confirm any effect of
558 ELS on DA receptors. This was surprising, as changes in the availability of DA receptors is a
559 consistent characteristic across different types of addictions(Nutt et al. 2015) as well as in
560 schizophrenia(Sanyal and Van Tol 1997). Although the discrepancy could partly be due to a
561 power problem of the meta-analysis, these limitations challenge the reliability of ELS models
562 for translational purposes, at least with regard to these specific aspects of the
563 abovementioned human disorders. It cannot be excluded that more relevant models may
564 become apparent in light of different ELS theories(Daskalakis et al. 2013).

565 Lastly, although our study supports that ELS causes some changes in the DA system, these
566 associations remain at a correlational level in humans and should be interpreted as such.

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4.5 Limitations of the study

Despite our efforts to be as comprehensive as possible in the description of the effects of ELS on the DA system, we encountered several important limitations. Firstly, we investigated the DA system by evaluating the effects of ELS on biochemical markers. Although this provides a thorough conceptualization of the system, it does not supply a comprehensive functional evaluation. For example, the approach here reported is unable to operationalize DA innervations, projections and tone. ELS has been reported to alter DR3-signalling and neuronal activity in the lateral septum(Shin et al. 2018). Chronic stress in adulthood has been reported to change DA neurons' activity in a stressor-dependent manner(Valenti, Gill, and Grace 2012). These reports suggest that spontaneous activity, bursting and timing of dopaminergic firing may be susceptible to ELS action, yet they are not apparent from assessment of ligands, receptors and metabolites. Despite the high relevance of such measurements, these were excluded from the analysis as the publications on the topic are scarce and their integration not straightforward in a meta-analytic setting.

Secondly, the classification of "timing" of ELS to either prenatal or postnatal may be too reductionist, since neuronal circuits are shaped by experiences during critical periods of development of variable length (from days to years depending on the species) (Hensch 2005). The interested researcher can further explore this avenue by combining our dataset with RNA expression of Th or DA receptors found in the Allen Developing Mouse Brain Atlas(Thompson et al. 2014). Unfortunately, the literature so far published is insufficient to investigate how stress during specific postnatal days in which a certain RNA *X* is highly expressed uniquely alters its functioning later in life.

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590 Thirdly, due to insufficient data-points per outcome per brain area in several cases, a
591 meaningful quantitative estimation was not possible for all combinations of outcome*area.
592 All currently available measurements are reported as supplementary material and can be
593 further investigated via MaDEapp.

594 Fourthly, we included data only from published studies. Especially in the prenatal dataset,
595 there is evidence of low-to-medium publication bias as qualitatively estimated with a funnel
596 plot and sensitivity analysis, which may result in an overestimation of the effect sizes. Despite
597 the robustness of our methodology, this limitation should be considered in future power
598 calculations.

599 Lastly, we limited our analysis to baseline (i.e. unchallenged) conditions. Future studies should
600 focus on conditions where the DA system is challenged, as ELS manipulations can interact
601 with later life challenges to result in a pathological phenotype(Daskalakis et al. 2013).

602

603 To conclude, ELS induces a few yet robust effects on biochemical indicators of the DA system,
604 with – based on the currently available studies – the striatum being the brain area most
605 affected. Although the changes observed can be interpreted as both hypo- and hyper-
606 activation of the DA system, the effects were consistent across prenatal and postnatal ELS
607 models, sex, species and method of assessment.

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6 REFERENCES

Aarts, Emmeke, Matthijs Verhage, Jesse V Veenvliet, Conor V Dolan, and Sophie van der Sluis. 2014. "A Solution to Dependency: Using Multilevel Analysis to Accommodate Nested Data." *Nature Neuroscience* 17 (4): 491–96. doi:10.1038/nn.3648.

Adrover, Ezequiela, María Alejandra Berger, Adriana A. Pérez, Frank I. Tarazi, and Marta C. Antonelli. 2007. "Effects of Prenatal Stress on Dopamine D2 Receptor Asymmetry in Rat Brain." *Synapse* 61 (6): 459–62. doi:10.1002/syn.20389.

Agid, O, B Shapira, J Zislin, M Ritsner, B Hanin, H Murad, T Troudart, M Bloch, U Heresco-Levy, and B Lerer. 1999. "Environment and Vulnerability to Major Psychiatric Illness: A Case Control Study of Early Parental Loss in Major Depression, Bipolar Disorder and Schizophrenia [See Comments]." *Molecular Psychiatry* 4 (2): 163–72. doi:10.1038/sj.mp.4000473.

Alonso, S. J., E. Navarro, and M. Rodriguez. 1994. "Permanent Dopaminergic Alterations in the n. Accumbens after Prenatal Stress." *Pharmacology, Biochemistry and Behavior* 49 (2): 353–58. doi:10.1016/0091-3057(94)90433-2.

Antonic, Ana, Emily S. Sena, Jennifer S. Lees, Taryn E. Wills, Peta Skeers, Peter E. Batchelor, Malcolm R. Macleod, and David W. Howells. 2013. "Stem Cell Transplantation in Traumatic Spinal Cord Injury: A Systematic Review and Meta-Analysis of Animal Studies." *PLoS Biology* 11 (12). doi:10.1371/journal.pbio.1001738.

Arborelius, L., and M. B. Eklund. 2007. "Both Long and Brief Maternal Separation Produces Persistent Changes in Tissue Levels of Brain Monoamines in Middle-Aged Female Rats." *Neuroscience* 145 (2): 738–50. doi:10.1016/j.neuroscience.2006.12.007.

Assink, Mark, and Carlijn J. M. Wibbelink. 2016. "Fitting Three-Level Meta-Analytic Models in R: A Step-by-Step Tutorial." *The Quantitative Methods for Psychology* 12 (3): 154–74. doi:10.20982/tqmp.12.3.p154.

Baharnoori, Moogeh, Sanjeev K. Bhardwaj, and Lalit K. Srivastava. 2013. "Effect of Maternal Lipopolysaccharide Administration on the Development of Dopaminergic Receptors and Transporter in the Rat Offspring." *PLoS ONE* 8 (1): 18–23. doi:10.1371/journal.pone.0054439.

Baier, Carlos J., María Eugenia Pallarés, Ezequiela Adrover, María R. Katunar, Rita Raisman-Vozari, and Marta C. Antonelli. 2014. "Intrastriatal 6-OHDA Lesion Differentially Affects Dopaminergic Neurons in the Ventral Tegmental Area of Prenatally Stressed Rats." *Neurotoxicity Research* 26 (3): 274–84. doi:10.1007/s12640-014-9479-7.

Bakos, Jan, Roman Duncko, Aikaterini Makatsori, Zdeno Pirnik, Alexander Kiss, and Daniela Jezova. 2004. "Prenatal Immune Challenge Affects Growth, Behavior, and Brain Dopamine in Offspring." *Annals of the New York Academy of Sciences* 1018: 281–87. doi:10.1196/annals.1296.033.

Bale, Tracy L, and C Neill Epperson. 2015. "Sex Differences and Stress across the Lifespan." *Nature Neuroscience* 18 (10): 1413–20. doi:10.1038/nn.4112.

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Basta-Kaim, A., K. Fijał, B. Budziszewska, M. Regulska, M. Leśkiewicz, M. Kubera, K. Gołembiowska, W. Lasoń, and K. Wędzony. 2011. "Prenatal Lipopolysaccharide Treatment Enhances MK-801-Induced Psychotomimetic Effects in Rats." *Pharmacology Biochemistry and Behavior* 98 (2): 241–49. doi:10.1016/j.pbb.2010.12.026.

Berger, M A, V G Barros, M I Sarchi, F I Tarazi, and M C Antonelli. 2002. "Long-Term Effects of Prenatal Stress on Dopamine and Glutamate Receptors in Adult Rat Brain." *Neurochem Res* 27 (11): 1525–33. http://www.ncbi.nlm.nih.gov/pubmed/12512957.

Bingham, Brian C., C. S. Sheela Rani, Alan Frazer, Randy Strong, and David A. Morilak. 2013. "Exogenous Prenatal Corticosterone Exposure Mimics the Effects of Prenatal Stress on Adult Brain Stress Response Systems and Fear Extinction Behavior." *Psychoneuroendocrinology* 38 (11). Elsevier Ltd: 2746–57. doi:10.1016/j.psyneuen.2013.07.003.

Bitanihirwe, Byron K Y, Daria Peleg-Raibstein, Forouhar Mouttet, Joram Feldon, and Urs Meyer. 2010. "Late Prenatal Immune Activation in Mice Leads to Behavioral and Neurochemical Abnormalities Relevant to the Negative Symptoms of Schizophrenia." *Neuropsychopharmacology* 35 (12). Nature Publishing Group: 2462–78. doi:10.1038/npp.2010.129.

Braun, K., E. Lange, M. Metzger, and G. Poeggel. 1999. "Maternal Separation Followed by Early Social Deprivation Affects the Development of Monoaminergic Fiber Systems in the Medial Prefrontal Cortex of Octodon Degus." *Neuroscience* 95 (1): 309–18. doi:10.1016/S0306-4522(99)00420-0.

Brenhouse, Heather, Jodi Lukkes, and Susan Andersen. 2013. "Early Life Adversity Alters the Developmental Profiles of Addiction-Related Prefrontal Cortex Circuitry." *Brain Sciences* 3 (1): 143–58. doi:10.3390/brainsci3010143.

Button, Katherine S., John P. A. Ioannidis, Claire Mokrysz, Brian A. Nosek, Jonathan Flint, Emma S. J. Robinson, and Marcus R. Munafò. 2013. "Power Failure: Why Small Sample Size Undermines the Reliability of Neuroscience." *Nature Reviews Neuroscience* 14 (5). Nature Publishing Group: 365–76. doi:10.1038/nrn3475.

Cabib, Simona, Stefano Puglisi-Allegra, and Francesca R. D'Amato. 1993. "Effects of Postnatal Stress on Dopamine Mesolimbic System Responses to Aversive Experiences in Adult Life." *Brain Research* 604 (1–2): 232–39. doi:10.1016/0006-8993(93)90374-V.

Cai, Zhengwei, Lir-wan Fan, Asuka Kaizaki, Lu-tai Tien, Tangeng Ma, Yi Pang, Shuying Lin, Rick C.S. Lin, and Kimberly L Simpson. 2013. "Neonatal Systemic Exposure to Lipopolysaccharide Enhances Susceptibility of Nigrostriatal Dopaminergic Neurons to Rotenone Neurotoxicity in Later Life." *Developmental Neuroscience* 35 (2–3): 155–71. doi:10.1159/000346156.

Camp, D M, T E Robinson, and J B Becker. 1984. "Sex-Differences in the Effects of Early Experience on the Development of Behavioral and Brain Asymmetries in Rats." *Physiology & Behavior* 33 (3): 433–39. doi:10.1016/0031-9384(84)90166-5.

Carboni, E., V. G. Barros, M. Ibba, A. Silvagni, C. Mura, and M. C. Antonelli. 2010. "Prenatal Restraint Stress: An in Vivo Microdialysis Study on Catecholamine Release in the Rat

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698 Prefrontal Cortex." *Neuroscience* 168 (1). Elsevier Inc.: 156–66.
699 doi:10.1016/j.neuroscience.2010.03.046.

700 Chang, Wiston, Joe Cheng, JJ Allaire, Yihui Xie, and Jonathan McPherson. 2017. "Shiny: Web
701 Application Framework for R." https://cran.r-project.org/package-shiny.

702 Cheung, Mike W.L. 2014a. "MetaSEM: An R Package for Meta-Analysis Using Structural
703 Equation Modeling." *Frontiers in Psychology* 5 (OCT): 1–7. doi:10.3389/fpsyg.2014.01521.

704 ———. 2014b. "Modeling Dependent Effect Sizes with Three-Level Meta-Analyses: A
705 Structural Equation Modeling Approach." *Psychological Methods* 19 (2): 211–29.
706 doi:10.1037/a0032968.

707 Chocyk, A., A. Przyborowska, D. Dudys, I. Majcher, M. Ma??kowiak, and K. Wedzony. 2011.
708 "The Impact of Maternal Separation on the Number of Tyrosine Hydroxylase-Expressing
709 Midbrain Neurons during Different Stages of Ontogenesis." *Neuroscience* 182: 43–61.
710 doi:10.1016/j.neuroscience.2011.03.008.

711 Choy, K. H C, and Maarten van den Buuse. 2008. "Attenuated Disruption of Prepulse
712 Inhibition by Dopaminergic Stimulation after Maternal Deprivation and Adolescent
713 Corticosterone Treatment in Rats." *European Neuropsychopharmacology* 18 (1): 1–13.
714 doi:10.1016/j.euroneuro.2007.03.011.

715 Choy, Kwok Ho Christopher, Yvonne P. De Visser, and Maarten Van Den Buuse. 2009. "The
716 Effect of 'two Hit' Neonatal and Young-Adult Stress on Dopaminergic Modulation of
717 Prepulse Inhibition and Dopamine Receptor Density." *British Journal of Pharmacology*
718 156 (2): 388–96. doi:10.1111/j.1476-5381.2008.00008.x.

719 Cohen, Jacob. 1992. "A Power Primer." *Psychological Bulletin* 112 (1): 155–59.
720 doi:10.1037/0033-2909.112.1.155.

721 Cornell, J, and C Mulrow. 1999. *Meta-Analysis*. Edited by H J Ader and G J Mellenberg.
722 London: Sage.

723 Cory-Slechta, D. A., M. B. Virgolini, A. Rossi-George, D. Weston, and M. Thiruchelvam. 2009.
724 "Experimental Manipulations Blunt Time-Induced Changes in Brain Monoamine Levels
725 and Completely Reverse Stress, but Not Pb+/-Stress-Related Modifications to These
726 Trajectories." *Behavioural Brain Research* 205 (1): 76–87. doi:10.1016/j.bbr.2009.06.040.

727 Cory-slechta, Deborah A., Douglas Weston, Sue Liu, and Joshua L. Allen. 2013. "Brain
728 Hemispheric Differences in the Neurochemical Effects of Lead, Prenatal Stress, and the
729 Combination and Their Amelioration by Behavioral Experience." *Toxicological Sciences*
730 132 (2): 419–30. doi:10.1093/toxsci/kft015.

731 Dallé, Ernest, Willie M.U. Daniels, and Musa V. Mabandla. 2016. "Anti-Parkinsonian Effects of
732 Fluvoxamine Maleate in Maternally Separated Rats." *International Journal of*
733 *Developmental Neuroscience* 53. International Society for Developmental Neuroscience:
734 26–34. doi:10.1016/j.ijdevneu.2016.06.004.

735 Daskalakis, Nikolaos P., Rosemary C. Bagot, Karen J. Parker, Christiaan H. Vinkers, and E.R. de
736 Kloet. 2013. "The Three-Hit Concept of Vulnerability and Resilience: Toward

bioRxiv preprint doi: https://doi.org/10.1101/372441; this version posted July 19, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

737 Understanding Adaptation to Early-Life Adversity Outcome." *Psychoneuroendocrinology*
738 38 (9): 1858–73. doi:10.1016/j.psyneuen.2013.06.008.

739 De Vries, Rob B.M., Carlijn R. Hooijmans, Miranda W. Langendam, Marlies Leenaars, Merel
740 Ritskes-Hoitinga, and Kimberley E. Wever. 2015. "A Protocol Format for the Preparation,
741 Registration and Publication of Systematic Reviews of Animal Intervention Studies."
742 *Evidence-Based Preclinical Medicine* 1 (1): 1–9. doi:10.1002/ebm2.7.

743 Delattre, Ana Marcia, Bruno Carabelli, Marco Aur??lio Mori, Paula G. Kempe, Luiz E. Rizzo de
744 Souza, Silvio M. Zanata, Ricardo B. Machado, et al. 2016. "Maternal Omega-3
745 Supplement Improves Dopaminergic System in Pre- and Postnatal Inflammation-
746 Induced Neurotoxicity in Parkinson???S Disease Model." *Molecular Neurobiology*, 1–17.
747 doi:10.1007/s12035-016-9803-8.

748 Dube, Shanta R, Vincent J Felitti, Maxia Dong, Daniel P Chapman, Wayne H Giles, and Robert
749 F Anda. 2003. "Childhood Abuse , Neglect , and Household Dysfunction and the Risk of
750 Illicit Drug Use: The Adverse Childhood Experiences Study" 111 (3).
751 doi:10.1542/peds.111.3.564.

752 Egan, K. J., E. S. Sena, and H. M. Vesterinen. 2011. "Making the Most of Animal Data -
753 Improving the Prospect of Success in Pragmatic Trials in the Neurosciences." *Trials* 12
754 (Suppl 1). BioMed Central Ltd: no pagination. doi:10.1186/1745-6215-12-S1-A102.

755 Egger, M., G Davey Smith, M Schneider, and C Minder. 1997. "Bias in Meta-Analysis Detected
756 by a Simple, Graphical Test." *BMJ (Clinical Research Ed.)* 315 (7109): 629–34.
757 doi:10.1136/bmj.316.7129.469.

758 Enoch, Mary-Anne. 2011. "The Role of Early Life Stress as a Predictor for Alcohol and Drug
759 Dependence." *Psychopharmacology* 214 (1): 17–31. doi:10.1007/s00213-010-1916-6.

760 Fan, Lir-Wan, Lu-Tai Tien, Baoying Zheng, Yi Pang, Rick C S Lin, Kimberly L Simpson, Tangeng
761 Ma, Philip G Rhodes, and Zhengwei Cai. 2011. "Dopaminergic Neuronal Injury in the
762 Adult Rat Brain Following Neonatal Exposure to Lipopolysaccharide and the Silent
763 Neurotoxicity." *Brain, Behavior, and Immunity* 25 (2). Elsevier Inc.: 286–97.
764 doi:10.1016/j.bbi.2010.09.020.

765 Fan, Lir Wan, Lu Tai Tien, Rick C S Lin, Kimberly L. Simpson, Philip G. Rhodes, and Zhengwei
766 Cai. 2011. "Neonatal Exposure to Lipopolysaccharide Enhances Vulnerability of
767 Nigrostriatal Dopaminergic Neurons to Rotenone Neurotoxicity in Later Life."
768 *Neurobiology of Disease* 44 (3). Elsevier Inc.: 304–16. doi:10.1016/j.nbd.2011.07.011.

769 Gatzke-Kopp, Lisa M. 2011. "The Canary in the Coalmine: The Sensitivity of Mesolimbic
770 Dopamine to Environmental Adversity during Development." *Neuroscience and*
771 *Biobehavioral Reviews* 35 (3). Elsevier Ltd: 794–803. doi:10.1016/j.neubiorev.2010.09.013.

772 Gerardin, Daniela C C, Oduvaldo C M Pereira, Wilma G. Kempinas, Jorge C. Florio, Estef??nia
773 G. Moreira, and Maria M. Bernardi. 2005. "Sexual Behavior, Neuroendocrine, and
774 Neurochemical Aspects in Male Rats Exposed Prenatally to Stress." *Physiology and*
775 *Behavior* 84 (1): 97–104. doi:10.1016/j.physbeh.2004.10.014.

776 Gondré-Lewis, Marjorie C., Philippe J. Darius, Hong Wang, and Joanne S. Allard. 2015.

bioRxiv preprint doi: https://doi.org/10.1101/372441; this version posted July 19, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

"Stereological Analyses of Reward System Nuclei in Maternally Deprived/Separated Alcohol Drinking Rats." *Journal of Chemical Neuroanatomy*, no. 2015. Elsevier B.V. doi:10.1016/j.jchemneu.2016.02.004.

Grace, Anthony A. 2016. "Dysregulation of the Dopamine System in the Pathophysiology of Schizophrenia and Depression." *Nature Reviews Neuroscience* 17 (8). Nature Publishing Group: 524–32. doi:10.1038/nrn.2016.57.

Gracia-Rubio, Irene, Olga Valverde, Elena Martinez-Laorden, Maria Moscoso-Castro, M. Victoria Milanés, and M. Luisa Laorden. 2016. "Maternal Separation Impairs Cocaine-Induced Behavioural Sensitization in Adolescent Mice." *Plos One* 11 (12): e0167483. doi:10.1371/journal.pone.0167483.

Granholm, Ann Charlotte, Vandana Zaman, Jennifer Godbee, Michael Smith, Riad Ramadan, Claudia Umphlet, Patrick Randall, et al. 2011. "Prenatal LPS Increases Inflammation in the Substantia Nigra of Gdnf Heterozygous Mice." *Brain Pathology* 21 (3): 330–48. doi:10.1111/j.1750-3639.2010.00457.x.

Hall, F. S., L. S. Wilkinson, T. Humby, and T. W. Robbins. 1999. "Maternal Deprivation of Neonatal Rats Produces Enduring Changes in Dopamine Function." *Synapse* 32 (1): 37–43. doi:10.1002/(SICI)1098-2396(199904)32:1<37::AID-SYN5>3.0.CO;2-4.

Hensch, Takao K. 2005. "Critical Period Plasticity in Local Cortical Circuits." *Nature Reviews Neuroscience* 6 (11): 877–88. doi:10.1038/nrn1787.

Hensleigh, E., and L. M. Pritchard. 2014. "The Effect of Early Environmental Manipulation on Locomotor Sensitivity and Methamphetamine Conditioned Place Preference Reward." *Behavioural Brain Research* 268. Elsevier B.V.: 66–71. doi:10.1016/j.bbr.2014.03.045.

Hensleigh, Emily, and Laurel M. Pritchard. 2015. "Maternal Separation Increases Methamphetamine-Induced Damage in the Striatum in Male, but Not Female Rats." *Behavioural Brain Research* 295. Elsevier B.V.: 3–8. doi:10.1016/j.bbr.2014.12.008.

Hermel, E. E S, G. S. Severino, A. L. Cecconello, F. M. Pereira, G. L. Sanvitto, and A. B. Lucion. 2001. "Neonatal Handling and the Expression of Immunoreactivity to Tyrosine Hydroxylase in the Hypothalamus of Adult Male Rats." *Brazilian Journal of Medical and Biological Research* 34 (9): 1191–95.

Hill, Rachel A., Szerenke Kiss Von Soly, Udani Ratnayake, Maren Klug, Michele D. Binder, Anthony J. Hannan, and Maarten van den Buuse. 2014. "Long-Term Effects of Combined Neonatal and Adolescent Stress on Brain-Derived Neurotrophic Factor and Dopamine Receptor Expression in the Rat Forebrain." *Biochimica et Biophysica Acta - Molecular Basis of Disease* 1842 (11). Elsevier B.V.: 2126–35. doi:10.1016/j.bbadis.2014.08.009.

Hooijmans, Carlijn R, Maroeska M Rovers, Rob B M De Vries, Marlies Leenaars, Merel Ritskes-hoitinga, and Miranda W Langendam. 2014. "SYRCLE ' s Risk of Bias Tool for Animal Studies." *BMC Medical Research Methodology* 14 (1). BMC Medical Research Methodology: 1–9. doi:10.1186/1471-2288-14-43.

Hox, Joop J. 2010. *Multilevel Analysis: Techniques and Applications*. Second edi. New York, NY: Routledge.

bioRxiv preprint doi: https://doi.org/10.1101/372441; this version posted July 19, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

817 Jahng, J. W., V. Ryu, S. B. Yoo, S. J. Noh, J. Y. Kim, and J. H. Lee. 2010. "Mesolimbic
818 Dopaminergic Activity Responding to Acute Stress Is Blunted in Adolescent Rats That
819 Experienced Neonatal Maternal Separation." *Neuroscience* 171 (1). Elsevier Inc.: 144–52.
820 doi:10.1016/j.neuroscience.2010.08.063.

821 Jahng, Jeong Won, Sang Bae Yoo, Jin Young Kim, Bom Taeck Kim, and Jong Ho Lee. 2012.
822 "Increased Mesohippocampal Dopaminergic Activity and Improved Depression-like
823 Behaviors in Maternally Separated Rats Following Repeated Fasting/Refeeding Cycles."
824 *Journal of Obesity* 2012. doi:10.1155/2012/497101.

825 Katunar, Maria R., Trinidad Saez, Alicia Brusco, and Marta C. Antonelli. 2010. "Ontogenetic
826 Expression of Dopamine-Related Transcription Factors and Tyrosine Hydroxylase in
827 Prenatally Stressed Rats." *Neurotoxicity Research* 18 (1): 69–81. doi:10.1007/s12640-009-
828 9132-z.

829 Kawakami, S. E., I. M H Quadros, R. B. Machado, and D. Suchecki. 2013. "Sex-Dependent
830 Effects of Maternal Separation on Plasma Corticosterone and Brain Monoamines in
831 Response to Chronic Ethanol Administration." *Neuroscience* 253. IBRO: 55–66.
832 doi:10.1016/j.neuroscience.2013.08.031.

833 Keller-Wood, Maureen. 2015. "Hypothalamic-Pituitary-Adrenal Axis-Feedback Control."
834 *Comprehensive Physiology* 5 (July): 1161–82. doi:10.1002/cphy.c140065.

835 Kikusui, Takefumi, Sara Faccidomo, and Klaus A. Miczek. 2005. "Repeated Maternal
836 Separation: Differences in Cocaine-Induced Behavioral Sensitization in Adult Male and
837 Female Mice." *Psychopharmacology* 178 (2–3): 202–10. doi:10.1007/s00213-004-1989-1.

838 Kilkenny, Carol, William J. Browne, Innes C. Cuthill, Michael Emerson, and Douglas G. Altman.
839 2013. "Improving Bioscience Research Reporting: The Arrive Guidelines for Reporting
840 Animal Research." *Animals* 4 (1): 35–44. doi:10.3390/ani4010035.

841 Kippin, Tod E, Karen K Szumlinski, Zuzana Kapasova, Betsy Rezner, and Ronald E See. 2008.
842 "Prenatal Stress Enhances Responsiveness to Cocaine." *Neuropsychopharmacology :*
843 *Official Publication of the American College of Neuropsychopharmacology* 33 (4): 769–82.
844 doi:10.1038/sj.npp.1301447.

845 Kirsten, Thiago B., Gabriela P. Chaves-Kirsten, Lucas M. Chaible, Ana C. Silva, Daniel O.
846 Martins, Luiz R G Britto, Maria L Z Dagli, Andrea S. Torrão, João Palermo-Neto, and Maria
847 M. Bernardi. 2012. "Hypoactivity of the Central Dopaminergic System and Autistic-like
848 Behavior Induced by a Single Early Prenatal Exposure to Lipopolysaccharide." *Journal of*
849 *Neuroscience Research* 90 (10): 1903–12. doi:10.1002/jnr.23089.

850 Klaus, Kristel, Kevin Butler, Simon J. Durrant, Manir Ali, Chris F. Inglehearn, Timothy L.
851 Hodgson, Humberto Gutierrez, and Kyla Pennington. 2017. "The Effect of COMT
852 Val158Met and DRD2 C957T Polymorphisms on Executive Function and the Impact of
853 Early Life Stress." *Brain and Behavior* 7 (5): 1–12. doi:10.1002/brb3.695.

854 Kosten, Therese A, Xiang Yang Zhang, and Priscilla Kehoe. 2005. "Neurochemical and
855 Behavioral Responses to Cocaine in Adult Male Rats with Neonatal Isolation Experience."
856 *The Journal of Pharmacology and Experimental Therapeutics* 314 (2): 661–67.
857 doi:10.1124/jpet.104.081216.

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Kubesova, Anna, Hana Tejkalova, Kamila Syslova, Petr Kacer, Jana Vondrousova, Filip Tyls, Michaela Fujakova, Tomas Palenicek, and Jiri Horacek. 2015. "Biochemical, Histopathological and Morphological Profiling of a Rat Model of Early Immune Stimulation: Relation to Psychopathology." *PLoS ONE* 10 (1): 1–16. doi:10.1371/journal.pone.0115439.

Landis, Story C., Susan G. Amara, Khusru Asadullah, Chris P. Austin, Robi Blumenstein, Eileen W. Bradley, Ronald G. Crystal, et al. 2012. "A Call for Transparent Reporting to Optimize the Predictive Value of Preclinical Research." *Nature* 490 (7419). Nature Publishing Group: 187–91. doi:10.1038/nature11556.

Lazzaretti, Camilla, Grasielle Clotildes Kincheski, Pablo Pandolfo, and Natividade De S. 2015. "Neonatal Handling Causes Impulsive Behavior and Decreased Pharmacological Response to Methylphenidate in Male Adult Wistar Rats." *Journal of Integrative Neuroscience* 14 (4): 1–15. doi:10.1142/S0219635216500047.

Leenaars, M., C. R. Hooijmans, N. van Veggel, G. ter Riet, M. Leeflang, L. Hooft, G. J. van der Wilt, a. Tillema, and M. Ritskes-Hoitinga. 2012. "A Step-by-Step Guide to Systematically Identify All Relevant Animal Studies." *Laboratory Animals* 46 (1): 24–31. doi:10.1258/la.2011.011087.

Lejeune, Stéphanie, Nathalie Dourmap, Marie-Pascale Martres, Bruno Giros, Valérie Daugé, and Laurent Naudon. 2013. "The Dopamine D1 Receptor Agonist SKF 38393 Improves Temporal Order Memory Performance in Maternally Deprived Rats." *Neurobiology of Learning and Memory* 106 (November): 268–73. doi:10.1016/j.nlm.2013.10.005.

Levine, Seymour. 2005. "Developmental Determinants of Sensitivity and Resistance to Stress." *Psychoneuroendocrinology* 30 (10): 939–46. doi:10.1016/j.psyneuen.2005.03.013.

Li, Wai Yu, Yi Chun Chang, L. Jyuhn Hsiarn Lee, and Li Jen Lee. 2014. "Prenatal Infection Affects the Neuronal Architecture and Cognitive Function in Adult Mice." *Developmental Neuroscience* 36 (5): 359–70. doi:10.1159/000362383.

Ling, Z. D., Q. Chang, J. W. Lipton, C. W. Tong, T. M. Landers, and P. M. Carvey. 2004. "Combined Toxicity of Prenatal Bacterial Endotoxin Exposure and Postnatal 6-Hydroxydopamine in the Adult Rat Midbrain." *Neuroscience* 124 (3): 619–28. doi:10.1016/j.neuroscience.2003.12.017.

Ling, Zaodung, Qin A. Chang, Chong Wai Tong, Susan E. Leurgans, Jack W. Lipton, and Paul M. Carvey. 2004. "Rotenone Potentiates Dopamine Neuron Loss in Animals Exposed to Lipopolysaccharide Prenatally." *Experimental Neurology* 190 (2): 373–83. doi:10.1016/j.expneurol.2004.08.006.

Ling, Zaodung, Yuangui Zhu, Chong Wai Tong, Joshua A. Snyder, Jack W. Lipton, and Paul M. Carvey. 2009. "Prenatal Lipopolysaccharide Does Not Accelerate Progressive Dopamine Neuron Loss in the Rat as a Result of Normal Aging." *Experimental Neurology* 216 (2). Elsevier Inc.: 312–20. doi:10.1016/j.expneurol.2008.12.004.

Liu, Yen Wenn, Wei Hsien Liu, Chien Chen Wu, Yi Chen Juan, Yu Chen Wu, Huei Ping Tsai, Sabrina Wang, and Ying Chieh Tsai. 2016. "Psychotropic Effects of Lactobacillus Plantarum PS128 in Early Life-Stressed and Naïve Adult Mice." *Brain Research* 1631.

bioRxiv preprint doi: https://doi.org/10.1101/372441; this version posted July 19, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

899 Elsevier: 1–12. doi:10.1016/j.brainres.2015.11.018.

900 Lovallo, William R., Mary-Anne Enoch, Kristen H. Sorocco, Andrea S. Vincent, Ashley Acheson,
901 Andrew J. Cohoon, Colin A. Hodgkinson, and David Goldman. 2017. "Joint Impact of
902 Early Life Adversity and COMT Val158Met (Rs4680) Genotypes on the Adult Cortisol
903 Response to Psychological Stress." *Psychosomatic Medicine* 79 (6): 631–37.
904 doi:10.1097/PSY.0000000000000481.

905 Lovic, Vedran, Hiwote Belay, Claire-Dominique Walker, Christie L Burton, Michael J Meaney,
906 Marla Sokolowski, and Alison S Fleming. 2013. "Early Postnatal Experience and DRD2
907 Genotype Affect Dopamine Receptor Expression in the Rat Ventral Striatum."
908 *Behavioural Brain Research* 237. Elsevier B.V.: 278–82. doi:10.1016/j.bbr.2012.09.046.

909 Luchicchi, Antonio, Salvatore Lecca, Miriam Melis, Marta De Felice, Francesca Cadeddu,
910 Roberto Frau, Anna Lisa Muntoni, Paola Fadda, Paola Devoto, and Marco Pistis. 2016.
911 "Maternal Immune Activation Disrupts Dopamine System in the Offspring." *The*
912 *International Journal of Neuropsychopharmacology / Official Scientific Journal of the*
913 *Collegium Internationale Neuropsychopharmacologicum (CINP)* 19: pyw007.
914 doi:10.1093/ijnp/pyw007.

915 Madruga, Clarice, Léder L. Xavier, Matilde Achaval, Gilberto L. Sanvitto, and Aldo B. Lucion.
916 2006. "Early Handling, but Not Maternal Separation, Decreases Emotional Responses in
917 Two Paradigms of Fear without Changes in Mesolimbic Dopamine." *Behavioural Brain*
918 *Research* 166 (2): 241–46. doi:10.1016/j.bbr.2005.08.005.

919 Matthews, Keith, Jeffrey W. Dalley, Christine Matthews, Tung Hu Tsai, and Trevor W. Robbins.
920 2001. "Periodic Maternal Separation of Neonatal Rats Produces Region- and Gender-
921 Specific Effects on Biogenic Amine Content in Postmortem Adult Brain." *Synapse* 40 (1):
922 1–10. doi:10.1002/1098-2396(200104)40:1<1::AID-SYN1020>3.0.CO;2-E.

923 Meiser, Johannes, Daniel Weindl, and Karsten Hiller. 2013. "Complexity of Dopamine
924 Metabolism." *Cell Communication and Signaling* 11 (1): 34. doi:10.1186/1478-811X-11-
925 34.

926 Menegas, William, Joseph F. Bergan, Sachie K. Ogawa, Yoh Isogai, Kannan Umadevi
927 Venkataraju, Pavel Osten, Naoshige Uchida, and Mitsuko Watabe-Uchida. 2015.
928 "Dopamine Neurons Projecting to the Posterior Striatum Form an Anatomically Distinct
929 Subclass." *ELife* 4 (AUGUST2015): 1–30. doi:10.7554/eLife.10032.

930 Meyer, Urs, Myriel Nyffeler, Severin Schwendener, Irene Knuesel, Benjamin K. Yee, and Joram
931 Feldon. 2008. "Relative Prenatal and Postnatal Maternal Contributions to Schizophrenia-
932 Related Neurochemical Dysfunction after in Utero Immune Challenge."
933 *Neuropsychopharmacology* 33 (2): 441–56. doi:10.1038/sj.npp.1301413.

934 Meyer, Urs, Myriel Nyffeler, Benjamin K. Yee, Irene Knuesel, and Joram Feldon. 2008. "Adult
935 Brain and Behavioral Pathological Markers of Prenatal Immune Challenge during
936 Early/Middle and Late Fetal Development in Mice." *Brain, Behavior, and Immunity* 22 (4):
937 469–86. doi:10.1016/j.bbi.2007.09.012.

938 Money, Kelli M., and Gregg D. Stanwood. 2013. "Developmental Origins of Brain Disorders:
939 Roles for Dopamine." *Frontiers in Cellular Neuroscience* 7 (December): 1–14.

bioRxiv preprint doi: https://doi.org/10.1101/372441; this version posted July 19, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

940 doi:10.3389/fncel.2013.00260.

941 Moreira, Pedro Silva, Pedro R Almeida, Hugo Leite-Almeida, Nuno Sousa, and Patrício Costa.
942 2016. "Impact of Chronic Stress Protocols in Learning and Memory in Rodents:
943 Systematic Review and Meta-Analysis." *Plos One* 11 (9): e0163245.
944 doi:10.1371/journal.pone.0163245.

945 Novak, Gabriela, Theresa Fan, Brian F. O'Dowd, and Susan R. George. 2013. "Postnatal
946 Maternal Deprivation and Pubertal Stress Have Additive Effects on Dopamine D2
947 Receptor and CaMKII Beta Expression in the Striatum." *International Journal of*
948 *Developmental Neuroscience* 31 (3). International Society for Developmental
949 Neuroscience: 189–95. doi:10.1016/j.ijdevneu.2013.01.001.

950 Nutt, David J., Anne Lingford-Hughes, David Erritzoe, and Paul R.A. Stokes. 2015. "The
951 Dopamine Theory of Addiction: 40 Years of Highs and Lows." *Nature Reviews*
952 *Neuroscience* 16 (5). Nature Publishing Group: 305–12. doi:10.1038/nrn3939.

953 Ognibene, Elisa, Walter Adriani, Antonio Caprioli, Orlando Ghirardi, Syed F. Ali, Luigi Aloe, and
954 Giovanni Laviola. 2008. "The Effect of Early Maternal Separation on Brain Derived
955 Neurotrophic Factor and Monoamine Levels in Adult Heterozygous Reeler Mice."
956 *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 32 (5): 1269–76.
957 doi:10.1016/j.pnpbp.2008.03.023.

958 Oreland, Sadia, Karita Raudkivi, Lars Oreland, Jaanus Harro, Lotta Arborelius, and Ingrid
959 Nylander. 2011. "Ethanol-Induced Effects on the Dopamine and Serotonin Systems in
960 Adult Wistar Rats Are Dependent on Early-Life Experiences." *Brain Research* 1405.
961 Elsevier B.V.: 57–68. doi:10.1016/j.brainres.2011.06.031.

962 Ozawa, Kimiyoshi, Kenji Hashimoto, Takashi Kishimoto, Eiji Shimizu, Hiroshi Ishikura, and
963 Masaomi Iyo. 2006. "Immune Activation during Pregnancy in Mice Leads to
964 Dopaminergic Hyperfunction and Cognitive Impairment in the Offspring: A
965 Neurodevelopmental Animal Model of Schizophrenia." *Biological Psychiatry* 59 (6): 546–
966 54. doi:10.1016/j.biopsych.2005.07.031.

967 Pallarés, María Eugenia, Carlos Javier Baier, Ezequiela Adrover, Melisa Carolina Monteleone,
968 Marcela Adriana Brocco, and Marta Cristina Antonelli. 2013. "Age-Dependent Effects of
969 Prenatal Stress on the Corticolimbic Dopaminergic System Development in the Rat Male
970 Offspring." *Neurochemical Research* 38 (11): 2323–35. doi:10.1007/s11064-013-1143-8.

971 Panagiotaropoulos, Theofanis, Stavroula Pondiki, Agapi Papaioannou, Filaretos Alikaridis,
972 Antonis Stamatakis, Kyriaki Gerozissis, and Fotini Stylianopoulou. 2004. "Regulation of
973 Corticotropin and Stress Neonatal Handling and Gender Modulate Brain Monoamines
974 and Plasma Corticosterone Levels Following Repeated Stressors in Adulthood," 181–91.
975 doi:10.1159/000082516.

976 Pang, Yi, Lu Tai Tien, Hobart Zhu, Juying Shen, Camilla F. Wright, Tembra K. Jones, Samir A.
977 Mamoon, Abhay J. Bhatt, Zhengwei Cai, and Lir Wan Fan. 2015. "Interleukin-1 Receptor
978 Antagonist Reduces Neonatal Lipopolysaccharide-Induced Long-Lasting
979 Neurobehavioral Deficits and Dopaminergic Neuronal Injury in Adult Rats." *International*
980 *Journal of Molecular Sciences* 16 (4): 8635–54. doi:10.3390/ijms16048635.

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Papaioannou, A., U. Dafni, F. Alikaridis, S. Bolaris, and F. Stylianopoulou. 2002. "Effects of Neonatal Handling on Basal and Stress-Induced Monoamine Levels in the Male and Female Rat Brain." *Neuroscience* 114 (1): 195–206. doi:10.1016/S0306-4522(02)00129-X.

Ploj, K., E. Roman, and I. Nylander. 2003. "Long-Term Effects of Maternal Separation on Ethanol Intake and Brain Opioid and Dopamine Receptors in Male Wistar Rats." *Neuroscience* 121 (3): 787–99. doi:10.1016/S0306-4522(03)00499-8.

Rentesi, Georgia, Katerina Antoniou, Marios Marselos, Marika Syrrou, Zeta Papadopoulou-Daifoti, and Maria Konstandi. 2013. "Early Maternal Deprivation-Induced Modifications in the Neurobiological, Neurochemical and Behavioral Profile of Adult Rats." *Behavioural Brain Research* 244. Elsevier B.V.: 29–37. doi:10.1016/j.bbr.2013.01.040.

Reynaert, Marie Line, Jordan Marrocco, J??r??me Mairesse, Luana Lionetto, Maurizio Simmaco, Lucie Deruyter, Delphine Allorge, et al. 2016. "Hedonic Sensitivity to Natural Rewards Is Affected by Prenatal Stress in a Sex-Dependent Manner." *Addiction Biology* 21 (6): 1072–85. doi:10.1111/adb.12270.

Reznikov, A G, and N D Nosenko. 1995. "Catecholamines in Steroid-Dependent Brain Development." *The Journal of Steroid Biochemistry and Molecular Biology* 53 (1–6): 349–53. http://www.ncbi.nlm.nih.gov/pubmed/7626479.

Romano-López, Antonio, Mónica Méndez-Díaz, Fabio García García, Citlalli Regalado-Santiago, Alejandra E. Ruiz-Contreras, and Oscar Prospéro-García. 2015. "Maternal Separation and Early Stress Cause Long-Lasting Effects on Dopaminergic and Endocannabinergic Systems and Alters Dendritic Morphology in the Nucleus Accumbens and Frontal Cortex in Rats." *Developmental Neurobiology*. doi:10.1002/dneu.22361.

Romero, E, C Guaza, B Castellano, and J Borrell. 2010. "Ontogeny of Sensorimotor Gating and Immune Impairment Induced by Prenatal Immune Challenge in Rats: Implications for the Etiopathology of Schizophrenia." *Molecular Psychiatry* 15 (4). Nature Publishing Group: 372–83. doi:10.1038/mp.2008.44.

Romero, Eva, Carine Ali, Eduardo Molina-Holgado, Bernardo Castellano, Carmen Guaza, and José Borrell. 2007. "Neurobehavioral and Immunological Consequences of Prenatal Immune Activation in Rats. Influence of Antipsychotics." *Neuropsychopharmacology* 32 (8): 1791–1804. doi:10.1038/sj.npp.1301292.

Rosenthal, R. 1991. *Meta-Analytic Procedures for Social Research*. Sage.

Rossi-George, A., M. B. Virgolini, D. Weston, M. Thiruchelvam, and D. A. Cory-Slechta. 2011. "Interactions of Lifetime Lead Exposure and Stress: Behavioral, Neurochemical and HPA Axis Effects." *NeuroToxicology* 32 (1). Elsevier B.V.: 83–99. doi:10.1016/j.neuro.2010.09.004.

Rots, Nynke Y., Jeannette De Jong, Judith O. Workel, Seymour Levine, Alexander R. Cools, and E. Ronald De Kloet. 1996. "Neonatal Maternally Deprived Rats Have as Adults Elevated Basal Pituitary-Adrenal Activity and Enhanced Susceptibility to Apomorphine." *Journal of Neuroendocrinology* 8 (7): 501–6. doi:10.1046/j.1365-2826.1996.04843.x.

Sanyal, Suparna, and Hubert H.M. Van Tol. 1997. "Review the Role of Dopamine D4 Receptors

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in Schizophrenia and Antipsychotic Action." *Journal of Psychiatric Research* 31 (2): 219–32. doi:10.1016/S0022-3956(96)00039-8.

Sasagawa, Takayo, Noriko Horii-Hayashi, Akinori Okuda, Takashi Hashimoto, Cho Azuma, and Mayumi Nishi. 2017. "Long-Term Effects of Maternal Separation Coupled with Social Isolation on Reward Seeking and Changes in Dopamine D1 Receptor Expression in the Nucleus Accumbens via DNA Methylation in Mice." *Neuroscience Letters* 641 (February). Elsevier Ireland Ltd: 33–39. doi:10.1016/j.neulet.2017.01.025.

Scheller-Gilkey, Geraldine, Shannon M Thomas, Bobbi J Woolwine, and Andrew H Miller. 2002. "Increased Early Life Stress and Depressive Symptoms in Patients with Comorbid Substance Abuse and Schizophrenia." *Schizophrenia Bulletin* 28 (2): 223–31. doi:10.1093/oxfordjournals.schbul.a006933.

Semple, Bridgette D., Klas Blomgren, Kayleen Gimlin, Donna M. Ferriero, and Linda J. Noble-Haeusslein. 2013. "Brain Development in Rodents and Humans: Identifying Benchmarks of Maturation and Vulnerability to Injury across Species." *Progress in Neurobiology* 106–107 (12): 1–16. doi:10.1016/j.pneurobio.2013.04.001.

Sheikh, Haroon I., Katie R. Kryski, Yuliya Kotelnikova, Elizabeth P. Hayden, and Shiva M. Singh. 2017. "Catechol-O-Methyltransferase Gene (Val158met) Polymorphisms and Anxious Symptoms in Early Childhood: The Roles of Hypothalamus-Pituitary-Adrenal Axis Reactivity and Life Stress." *Neuroscience Letters* 659 (June). Elsevier: 86–91. doi:10.1016/j.neulet.2017.08.060.

Shin, Sora, Horia Pribiag, Varoth Lilascharoen, Daniel Knowland, Xiao Yun Wang, and Byung Kook Lim. 2018. "Drd3 Signaling in the Lateral Septum Mediates Early Life Stress-Induced Social Dysfunction." *Neuron* 97 (1). Elsevier Inc.: 195–208.e6. doi:10.1016/j.neuron.2017.11.040.

Silvagni, Alessandra, Virginia G. Barros, Cristina Mura, Marta C. Antonelli, and Ezio Carboni. 2008. "Prenatal Restraint Stress Differentially Modifies Basal and Stimulated Dopamine and Noradrenaline Release in the Nucleus Accumbens Shell: An 'in Vivo' Microdialysis Study in Adolescent and Young Adult Rats." *European Journal of Neuroscience* 28 (4): 744–58. doi:10.1111/j.1460-9568.2008.06364.x.

Silveira, P. P., A. K. Portella, S. A C N Assis, F. B. Nieto, L. A. Diehl, L. M. Crema, W. Peres, et al. 2010. "Early Life Experience Alters Behavioral Responses to Sweet Food and Accumbal Dopamine Metabolism." *International Journal of Developmental Neuroscience* 28 (1): 111–18. doi:10.1016/j.ijdevneu.2009.08.018.

Son, Gi Hoon, Sooyoung Chung, Dongho Geum, Sang Soo Kang, Wan Sung Choi, Kyungjin Kim, and Sukwoo Choi. 2007. "Hyperactivity and Alteration of the Midbrain Dopaminergic System in Maternally Stressed Male Mice Offspring." *Biochemical and Biophysical Research Communications* 352 (3): 823–29. doi:10.1016/j.bbrc.2006.11.104.

Swanson, L W. n.d. "The Projections of the Ventral Tegmental Area and Adjacent Regions: A Combined Fluorescent Retrograde Tracer and Immunofluorescence Study in the Rat." *Brain Research Bulletin* 9 (1–6): 321–53. http://www.ncbi.nlm.nih.gov/pubmed/6816390.

Tabachnick, Barbara G, and Linda S Fidell. 2013. *Using Multivariate Statistics*. 6, illustr ed.

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1062 Pearson Education,2013.

1063 Team, R Core. 2015. "R: A Language and Environment for Statistical Computing." *R*
1064 *Foundation for Statistical Computing*. Vienna, Austria. https://www.r-project.org/.

1065 Teicher, Martin H., Jacqueline A. Samson, Carl M. Anderson, and Kyoko Ohashi. 2016. "The
1066 Effects of Childhood Maltreatment on Brain Structure, Function and Connectivity."
1067 *Nature Reviews Neuroscience* 17 (10). Nature Publishing Group: 652–66.
1068 doi:10.1038/nrn.2016.111.

1069 Thompson, Carol L., Lydia Ng, Vilas Menon, Salvador Martinez, Chang Kyu Lee, Katie
1070 Glattfelder, Susan M. Sunkin, et al. 2014. "A High-Resolution Spatiotemporal Atlas of
1071 Gene Expression of the Developing Mouse Brain." *Neuron* 83 (2). Elsevier Inc.: 309–23.
1072 doi:10.1016/j.neuron.2014.05.033.

1073 Tien, Lu Tai, Asuka Kaizaki, Yi Pang, Zhengwei Cai, Abhay J. Bhatt, and Lir Wan Fan. 2013.
1074 "Neonatal Exposure to Lipopolysaccharide Enhances Accumulation of??-Synuclein
1075 Aggregation and Dopamine Transporter Protein Expression in the Substantia Nigra in
1076 Responses to Rotenone Challenge in Later Life." *Toxicology* 308. Elsevier Ireland Ltd: 96–
1077 103. doi:10.1016/j.tox.2013.03.014.

1078 Valenti, Ornella, Kathryn M. Gill, and Anthony A. Grace. 2012. "Different Stressors Produce
1079 Excitation or Inhibition of Mesolimbic Dopamine Neuron Activity: Response Alteration
1080 by Stress Pre-Exposure." *European Journal of Neuroscience* 35 (8): 1312–21.
1081 doi:10.1111/j.1460-9568.2012.08038.x.

1082 Van den Noortgate, Wim, José Antonio López-López, Fulgencio Marín-Martínez, and Julio
1083 Sánchez-Meca. 2013. "Three-Level Meta-Analysis of Dependent Effect Sizes." *Behavior*
1084 *Research Methods* 45 (2): 576–94. doi:10.3758/s13428-012-0261-6.

1085 Vazquez, Vincent, St??phanie Weiss, Bruno Giros, Marie Pascale Martres, and Val??rie Daug??
1086 2007. "Maternal Deprivation and Handling Modify the Effect of the Dopamine D3
1087 Receptor Agonist, BP 897 on Morphine-Conditioned Place Preference in Rats."
1088 *Psychopharmacology* 193 (4): 475–86. doi:10.1007/s00213-007-0789-9.

1089 Vesterinen, H. M., E. S. Sena, K. J. Egan, T. C. Hirst, L. Churolov, G. L. Currie, A. Antonic, D. W.
1090 Howells, and M. R. Macleod. 2014. "Meta-Analysis of Data from Animal Studies: A
1091 Practical Guide." *Journal of Neuroscience Methods* 221. Elsevier B.V.: 92–102.
1092 doi:10.1016/j.jneumeth.2013.09.010.

1093 Viechtbauer, M. 2010. "Conducting Meta-Analyses in R with the Metafor Package." *Journal of*
1094 *Statistical Software* 36 (3): 1–48. www.jstatsoft.org/v36/i03/.

1095 Vuillermot, S., E. Joodmardi, T. Perlmann, S. Ove Ogren, J. Feldon, and U. Meyer. 2012.
1096 "Prenatal Immune Activation Interacts with Genetic Nurr1 Deficiency in the Development
1097 of Attentional Impairments." *Journal of Neuroscience* 32 (2): 436–51.
1098 doi:10.1523/JNEUROSCI.4831-11.2012.

1099 Wang, Sabrina, Jia Ying Yan, Yu Kang Lo, Paul M. Carvey, and Zaodung Ling. 2009.
1100 "Dopaminergic and Serotoninergic Deficiencies in Young Adult Rats Prenatally Exposed
1101 to the Bacterial Lipopolysaccharide." *Brain Research* 1265. Elsevier B.V.: 196–204.

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1102 doi:10.1016/j.brainres.2009.02.022.

1103 Weston, Hiromi I., Marissa E. Sobolewski, Joshua L. Allen, Doug Weston, Katherine Conrad,
1104 Sean Pelkowski, Gene E. Watson, Grazyna Zareba, and Deborah A. Cory-Slechta. 2014.
1105 "Sex-Dependent and Non-Monotonic Enhancement and Unmasking of Methylmercury
1106 Neurotoxicity by Prenatal Stress." *NeuroToxicology* 41. Elsevier B.V.: 123–40.
1107 doi:10.1016/j.neuro.2014.01.009.

1108 Weston, Hiromi I., Douglas D. Weston, Joshua L. Allen, and Deborah A. Cory-Slechta. 2014.
1109 "Sex-Dependent Impacts of Low-Level Lead Exposure and Prenatal Stress on Impulsive
1110 Choice Behavior and Associated Biochemical and Neurochemical Manifestations."
1111 *NeuroToxicology* 44. Elsevier B.V.: 169–83. doi:10.1016/j.neuro.2014.06.013.

1112 Wickham, H. 2009. "Ggplot2: Elegant Graphics for Data Analysis." New York, NY: Springer-
1113 Verlag.

1114 Williams, E. G., Y. Wu, P. Jha, S. Dubuis, P. Blattmann, C. A. Argmann, S. M. Houten, et al. 2016.
1115 "Systems Proteomics of Liver Mitochondria Function." *Science* 352 (6291): aad0189-
1116 aad0189. doi:10.1126/science.aad0189.

1117 Winter, Christine, Anais Djodari-Irani, Reinhard Sohr, Rudolf Morgenstern, Joram Feldon,
1118 Georg Juckel, and Urs Meyer. 2009. "Prenatal Immune Activation Leads to Multiple
1119 Changes in Basal Neurotransmitter Levels in the Adult Brain: Implications for Brain
1120 Disorders of Neurodevelopmental Origin Such as Schizophrenia." *The International*
1121 *Journal of Neuropsychopharmacology* 12 (4): 513–24. doi:10.1017/S1461145708009206.

1122 Wise, Roy A, and George F Koob. 2014. "The Development and Maintenance of Drug
1123 Addiction." *Neuropsychopharmacology* 39 (2). Nature Publishing Group: 254–62.
1124 doi:10.1038/npp.2013.261.

1125 Womersley, Jacqueline S, Jennifer H Hsieh, Lauriston a Kellaway, Greg a Gerhardt, and
1126 Vivienne a Russell. 2011. "Maternal Separation Affects Dopamine Transporter Function in
1127 the Spontaneously Hypertensive Rat: An in Vivo Electrochemical Study." *Behavioral and*
1128 *Brain Functions : BBF* 7 (1). BioMed Central Ltd: 49. doi:10.1186/1744-9081-7-49.

1129 Zager, Adriano, Gregory Mennecier, and João Palermo-Neto. 2012. "Maternal Immune
1130 Activation in Late Gestation Enhances Locomotor Response to Acute but Not Chronic
1131 Amphetamine Treatment in Male Mice Offspring: Role of the D1 Receptor." *Behavioural*
1132 *Brain Research* 232 (1). Elsevier B.V.: 30–36. doi:10.1016/j.bbr.2012.03.036.

1133 Zavitsanou, Katerina, Victoria S. Dalton, Adam K. Walker, Cynthia S. Weickert, Luba Sominsky,
1134 and Deborah M. Hodgson. 2013. "Neonatal Lipopolysaccharide Treatment Has Long-
1135 Term Effects on Monoaminergic and Cannabinoid Receptors in the Rat." *Synapse* 67 (6):
1136 290–99. doi:10.1002/syn.21640.

1137 Zhang, Xiang Yang, Priscilla Kehoe, and Therese A. Kosten. 2006. "Neonatal Isolation Alters
1138 Estrous Cycle Effects on Ventral Striatal Extracellular Monoamine Levels." *Progress in*
1139 *Neuro-Psychopharmacology and Biological Psychiatry* 30 (3): 504–11.
1140 doi:10.1016/j.pnpbp.2005.11.023.

1141 Zhang, Yi, Yuting Wang, Lei Wang, Mei Bai, Xiuwu Zhang, and Xiongzhao Zhu. 2015.

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"Dopamine Receptor D2 and Associated MicroRNAs Are Involved in Stress Susceptibility and Resistance to Escitalopram Treatment." *International Journal of Neuropsychopharmacology* 18 (8): 1–10. doi:10.1093/ijnp/pyv025.

Zhang, Yi, Xiongzhao Zhu, Mei Bai, Li Zhang, Liang Xue, and Jinyao Yi. 2013. "Maternal Deprivation Enhances Behavioral Vulnerability to Stress Associated with MiR-504 Expression in Nucleus Accumbens of Rats." *PLoS ONE* 8 (7). doi:10.1371/journal.pone.0069934.

Zhu, Xiongzhao, Ting Li, Sufang Peng, Xiuling Ma, Xiaogang Chen, and Xiuwu Zhang. 2010. "Maternal Deprivation-Caused Behavioral Abnormalities in Adult Rats Relate to a Non-Methylation-Regulated D2 Receptor Levels in the Nucleus Accumbens." *Behavioural Brain Research* 209 (2). Elsevier B.V.: 281–88. doi:10.1016/j.bbr.2010.02.005.

Zhu, Xiongzhao, Sufang Peng, Sheng Zhang, and Xiuwu Zhang. 2011. "Stress-Induced Depressive Behaviors Are Correlated with Par-4 and DRD2 Expression in Rat Striatum." *Behavioural Brain Research* 223 (2). Elsevier B.V.: 329–35. doi:10.1016/j.bbr.2011.04.052.

Zhu, Yuangui, Paul M. Carvey, and Zaodung Ling. 2007. "Altered Glutathione Homeostasis in Animals Prenatally Exposed to Lipopolysaccharide." *Neurochemistry International* 50 (4): 671–80. doi:10.1016/j.neuint.2006.12.013.

Zuckerman, Lee, Moshe Rehavi, Rachel Nachman, and Ina Weiner. 2003. "Immune Activation during Pregnancy in Rats Leads to a Postpubertal Emergence of Disrupted Latent Inhibition, Dopaminergic Hyperfunction, and Altered Limbic Morphology in the Offspring: A Novel Neurodevelopmental Model of Schizophrenia." *Neuropsychopharmacology* 28 (10): 1778–89. doi:10.1038/sj.npp.1300248.

bioRxiv preprint doi: https://doi.org/10.1101/372441; this version posted July 19, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

7 FIGURE CAPTIONS

Fig 1. **Search string and inclusion criteria**. Graphical representation of the three main components of the search string. Items highlighted in bold were ultimately included in the analysis; other items were not included in the final analysis as explained in the methods' section.

Fig 2. **Graphical representation of signaling pathway**. Dopamine is synthesized by the enzyme tyrosine hydroxylase (Th). When released in the synaptic cleft, DA can 1) bind post-synaptic receptors (DR1-DR5), 2) bind auto-receptors, 3) bind dopamine transporters (DAT), 4) be converted to the metabolites DOPAC, 3MT and HVA by the action of the enzymes MAO and COMT(Meiser, Weindl, and Hiller 2013). Items in large (purple) font were included in the meta-analysis.

Fig 3. **Graphical representation DA system projections**. DA neurons are mainly situated in the midbrain, and can be subdivided with respect to their projection site(Grace 2016). In particular, DA neurons define separate populations of neurons that project to specific brain regions(Swanson, n.d.; Menegas et al. 2015). The major DA pathways are 1) mesocortical pathway, which defines projections from the VTA to the prefrontal cortex (PFC); 2) mesolimbic, from VTA to limbic system; 3) and nigrostriatal pathway, from substantia nigra (SN) to dorsal striatum, caudate nucleus and putamen. Other projections connect VTA to the hypothalamus, hippocampus and amygdala. Besides hosting dopaminergic neurons, these brain areas are involved in the feedback response to stress(Keller-Wood 2015).

Fig 4. **Flow-chart of study selection and analysis**.

Fig 5. **Summary of effects: prenatal dataset**. Boxplot representing the summary of effect estimates for every combination between outcome variable (biochemical markers) and brain area. White bars = enough comparisons for meaningful quantification (rule of thumb: at least 4 comparisons from 3 papers), black bars = number of comparisons insufficient for analysis.

Fig 6. **Striatal DOPAC in the prenatal dataset**. (A) Forest plot. Results of univariate and 3-level meta-analysis are reported (bottom diamonds), as well as subgroup analysis on the ELS model used. (B) Cumulative forest plot. This plot displays the accumulation of evidence over time as the individual comparisons are added in chronological order. M = males, F = females, Method = method of assessment, hip = hippocampus, N = amount of animals, SEM = standard error of the mean, CI = confidence interval. Following the name of the study, _n represents the number of the experiment, .n = represents which comparison within that experiment.

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Fig 7. **Summary of effects: postnatal dataset**. Boxplot representing the summary of effect estimates for every combination between outcome variable (biochemical markers) and brain area. White bars = enough comparisons for meaningful quantification (rule of thumb: at least 4 comparisons from 3 papers), black bars = number of comparisons insufficient for analysis.

Fig 8. **Striatal DOPAC in the postnatal dataset**. Forest plot. Results of univariate as well as 3-level meta-analysis are reported (bottom diamonds), as well as subgroup analysis on the ELS model used. * = effect sizes of handling were multiplied by -1 to maintain consistency directionality of the other models. A decrease in the graph identifies an increase in DOPAC in ELS animals. M = males, F = females, Method = method of assessment, hip = hippocampus, N = amount of animals, SEM = standard error of the mean, CI = confidence interval, MD = mother separated from the pups, LPS = injection of LPS. Following the name of the study, _n represents the number of the experiment, .n = represents which comparison within that experiment.

Fig 9. **Risk of bias assessment**. Each bar represents a different risk of bias item. Yes = measurements have been taken to avoid bias; no = no measurements were taken to avoid bias; unclear = not enough information provided in the paper to determine the risk of bias.

Fig 10. **Funnel plots**. Publication bias was evaluated by qualitatively assessing symmetry in funnel plot in the (A) prenatal and (B) postnatal datasets.

Fig 11. **MaDEapp**. Screen shot for visualization of MaDEapp, a web-based tool to perform *ad hoc* meta-analysis of the topic of ELS and biochemical indicators of the DA system. The app can be found at **website**.

ELS models

Prenatal

Injection LPS

Injection Poly I:C

Maternal immune activation

Restraint

Chronic unpredictable stress

Other injections (e.g. glucocorticoids)

Postnatal

Removal dam from litter (MD/MS)

Removal pup from litter (isolation)

Handling

Injection LPS (in pups)

Licking and grooming

Limited nesting and bedding

Communal housing

Other injections

Outcome measures

DA

DA precursors (tyrosine, **Th**, L-DOPA)

DA metabolites (DOPAC, HVA, 3-MT)

DA turnovers (DOPAC/DA, HVA/DA, 3-MT/DA)

DA receptors (DR1, DR2, DR3, DR4, DR5)

DA transporter (DAT)

Species

Mice

Rats

Degus

Mandarines voles

Other rodents

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Presynaptic neuron

L-Tyrosine

Th

L-DOPA

DA

Degradation

auto**DR**

DAT

Degradation

DOPAC

MAO

COMT

HVA

COMT

MAO

3-MT

DR1 **DR2** **DR3** DR4 DR5

Signal Transduction

Postsynaptic neuron

Legend

DA

DAT

DR

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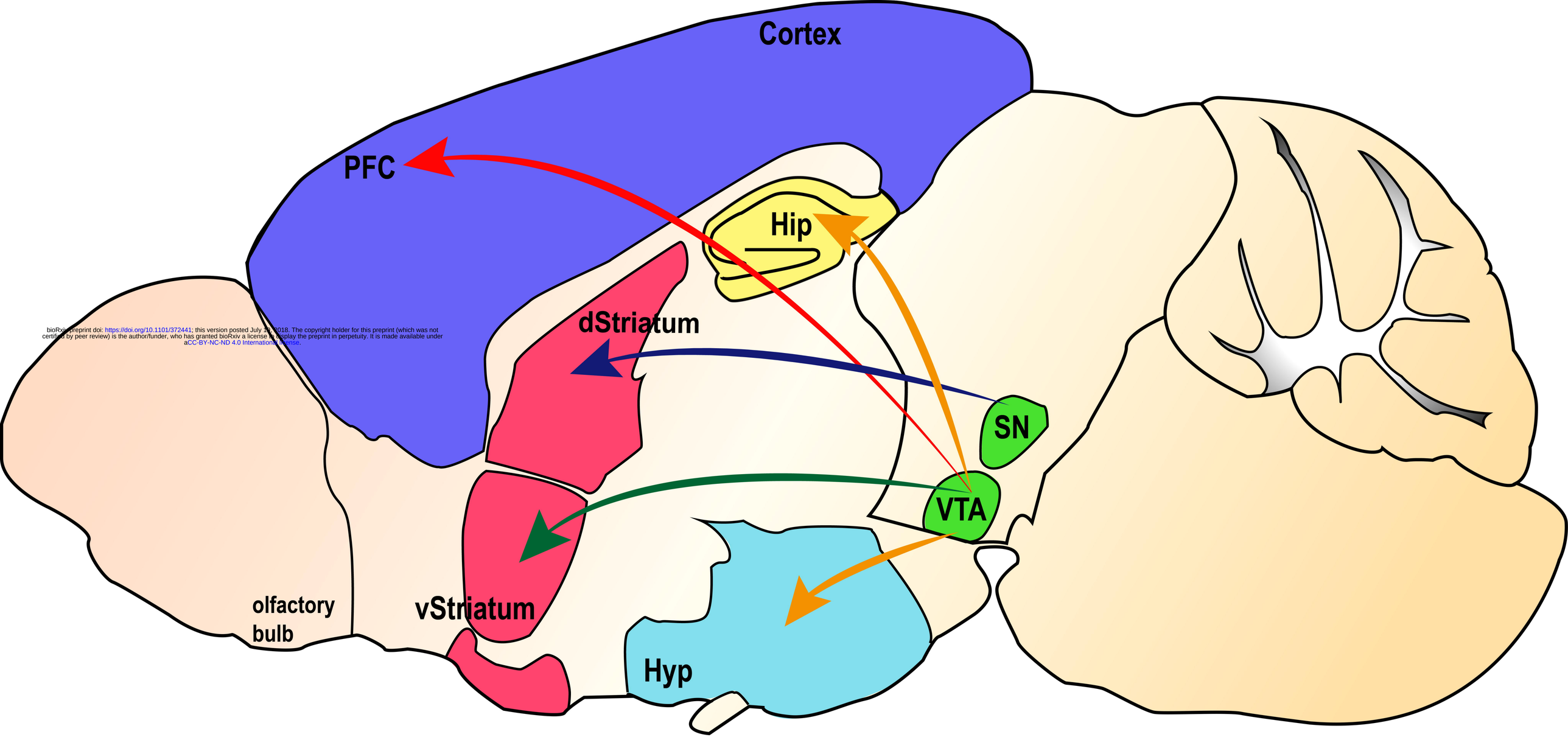

Identification

Screening

Eligibility

Data extraction

Data analysis

Sharing

979 unique publications

PubMed

Title selection

Abstract selection

Full text selection

93 publications

3 publications
no data available

90 publications
152 experiments
1009 comparisons

Prenatal
41 publications

Postnatal
49 publications

Outliers removal

Main analysis

Study of variance

Test of moderators

3-level model

Test of moderators

Subgroup models

Subgroup analysis

Risk of bias assessment

Publication bias assessment

Sensitivity analysis

Robustness results

MaDEapp

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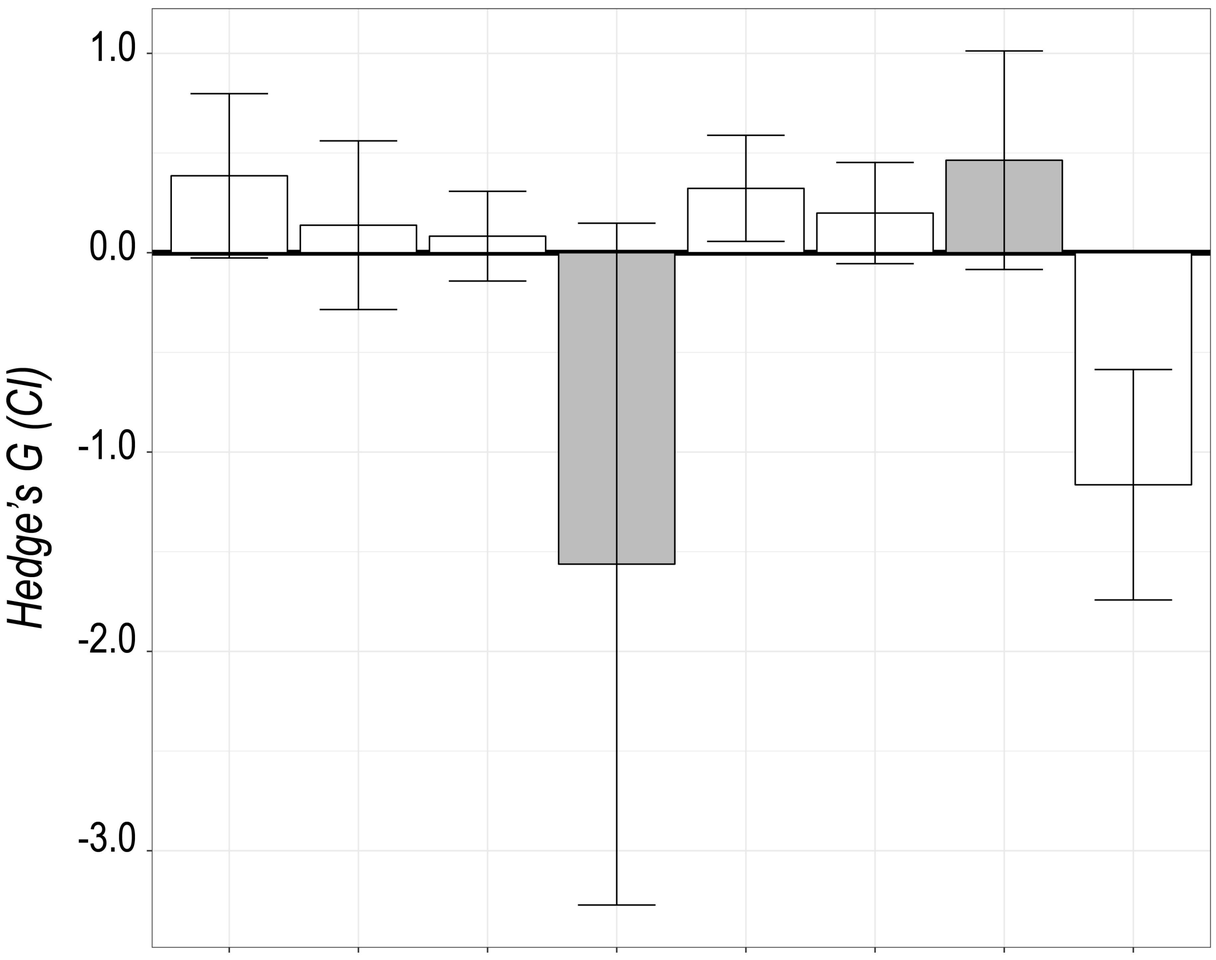
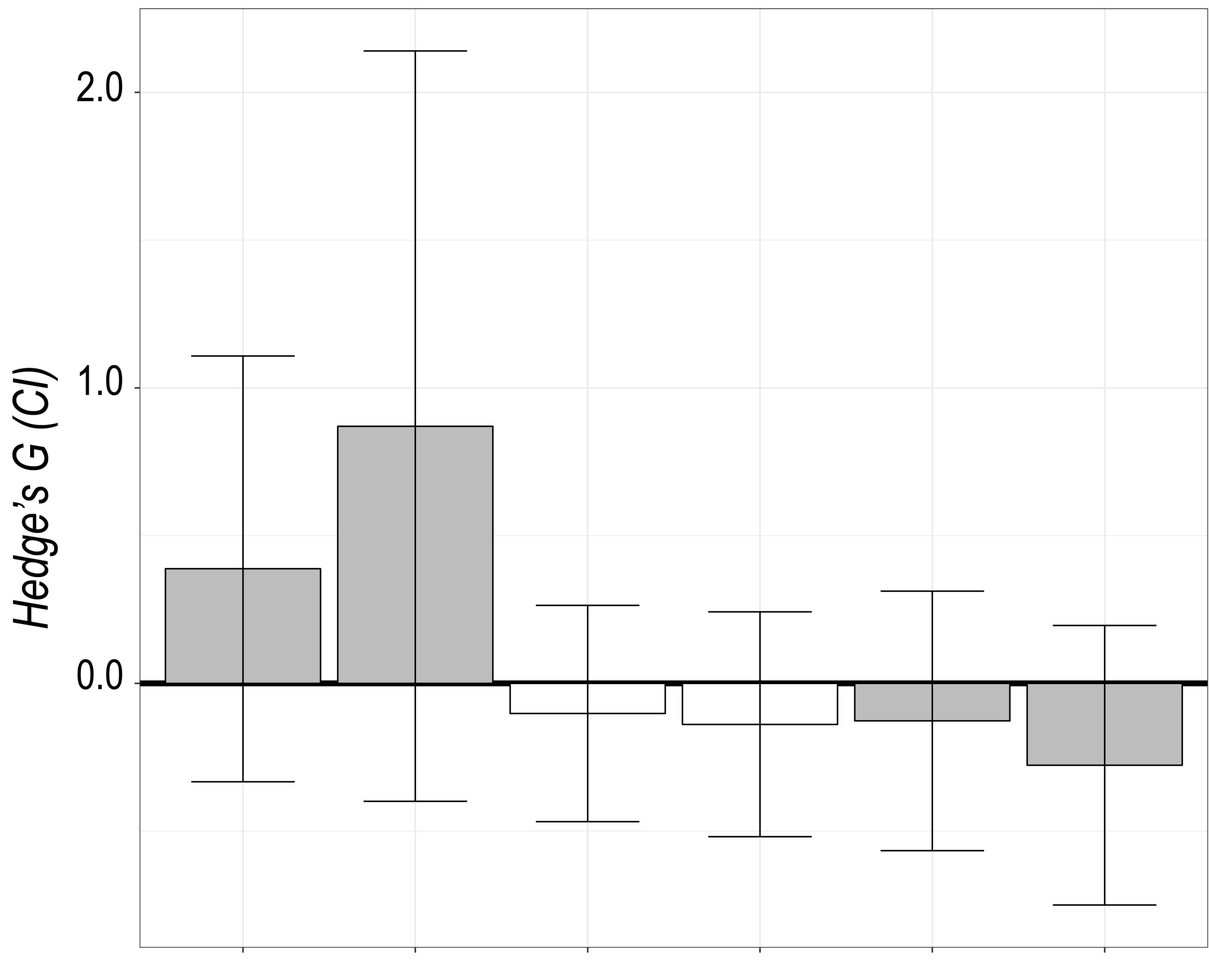
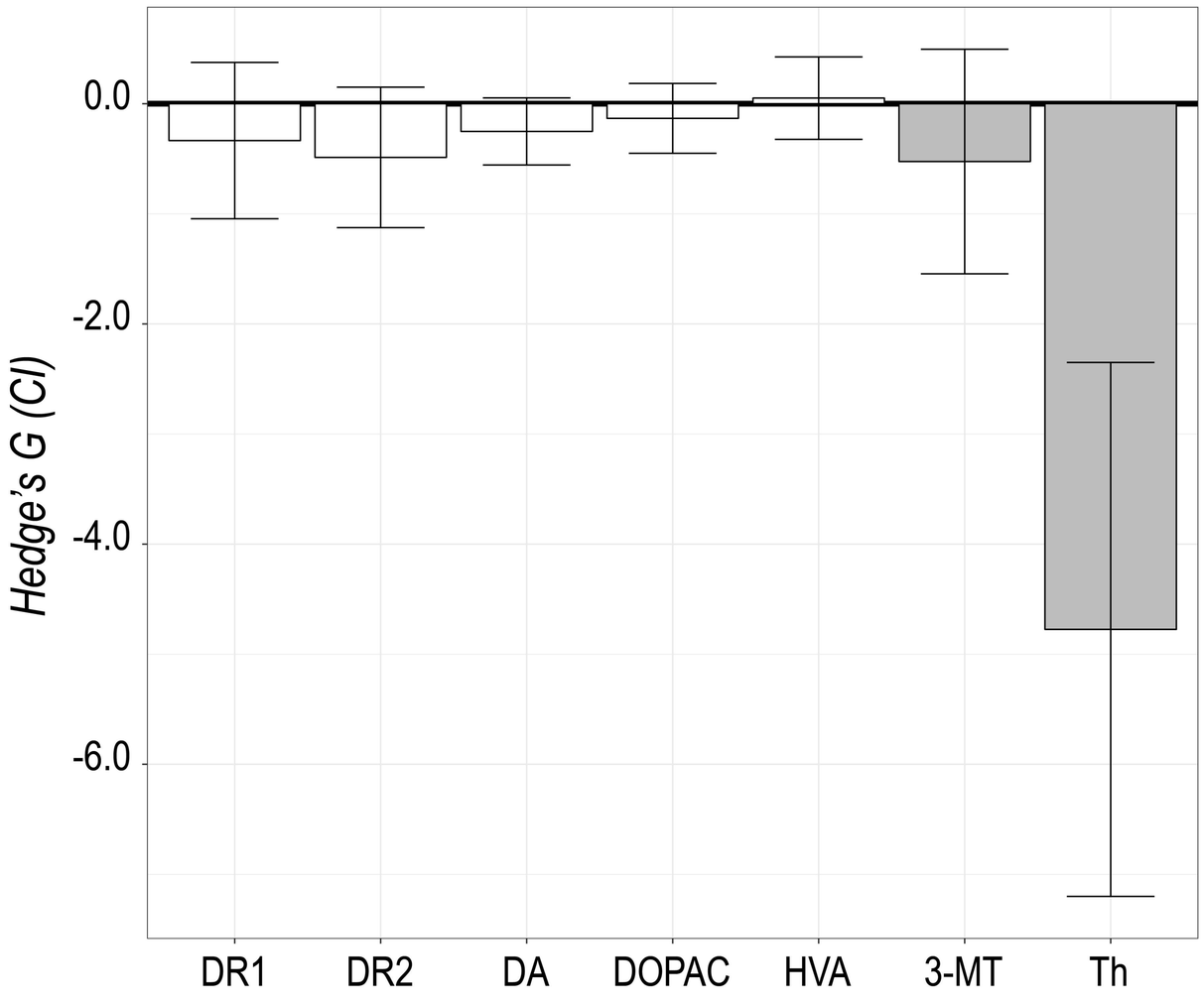
Striatal area



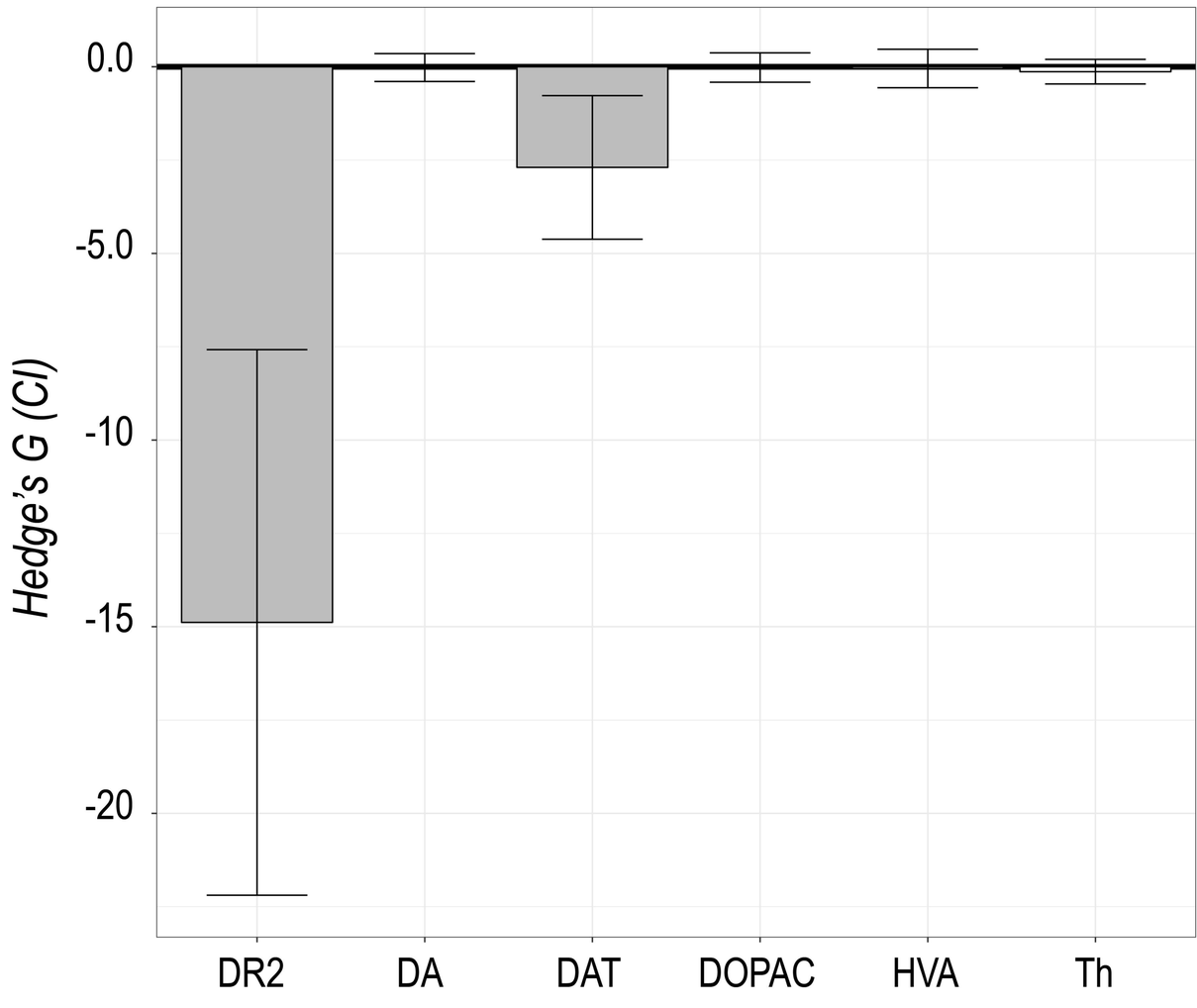
Limbic area



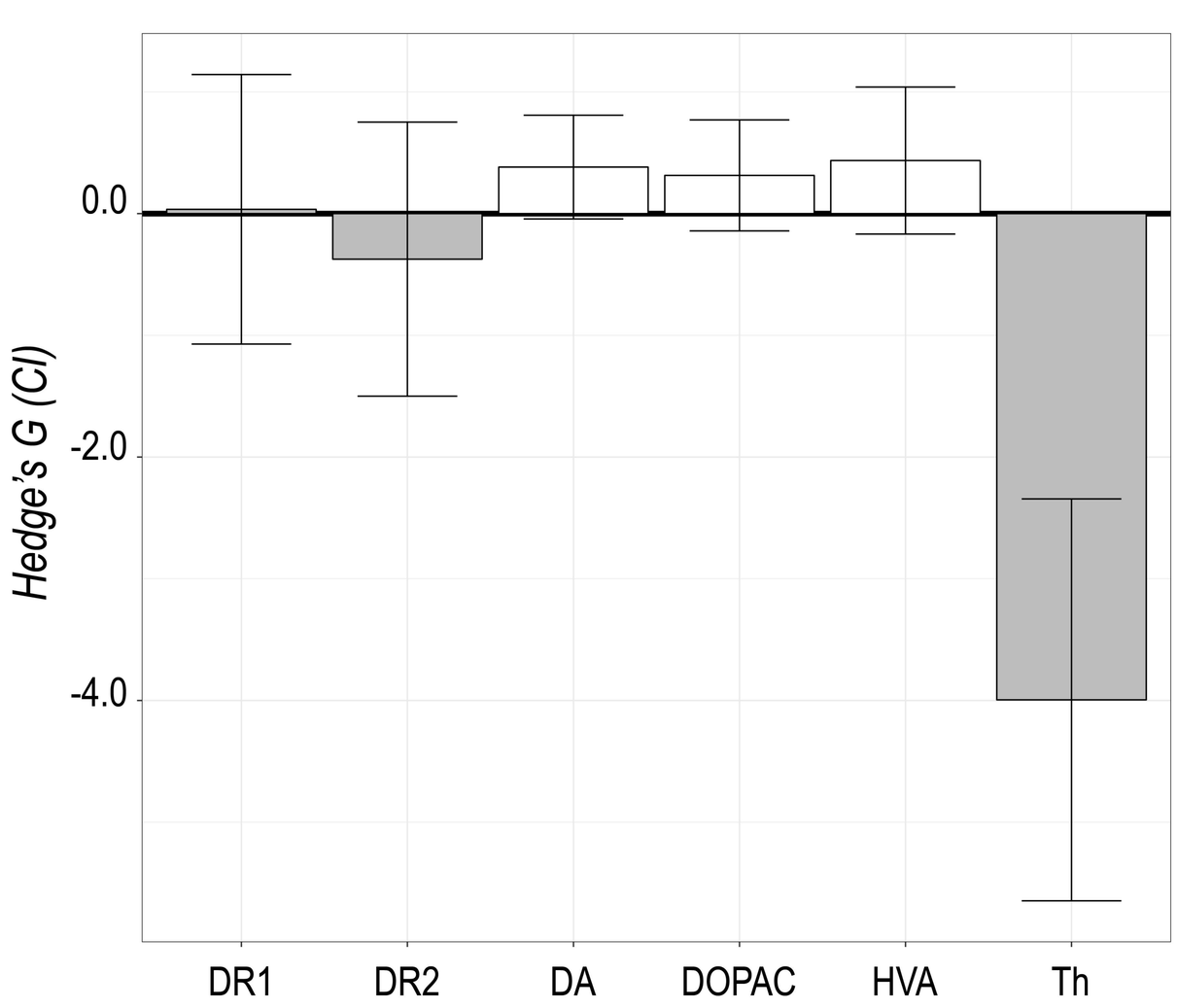
PFC area



VTA area



Hypothalamic area



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Legend

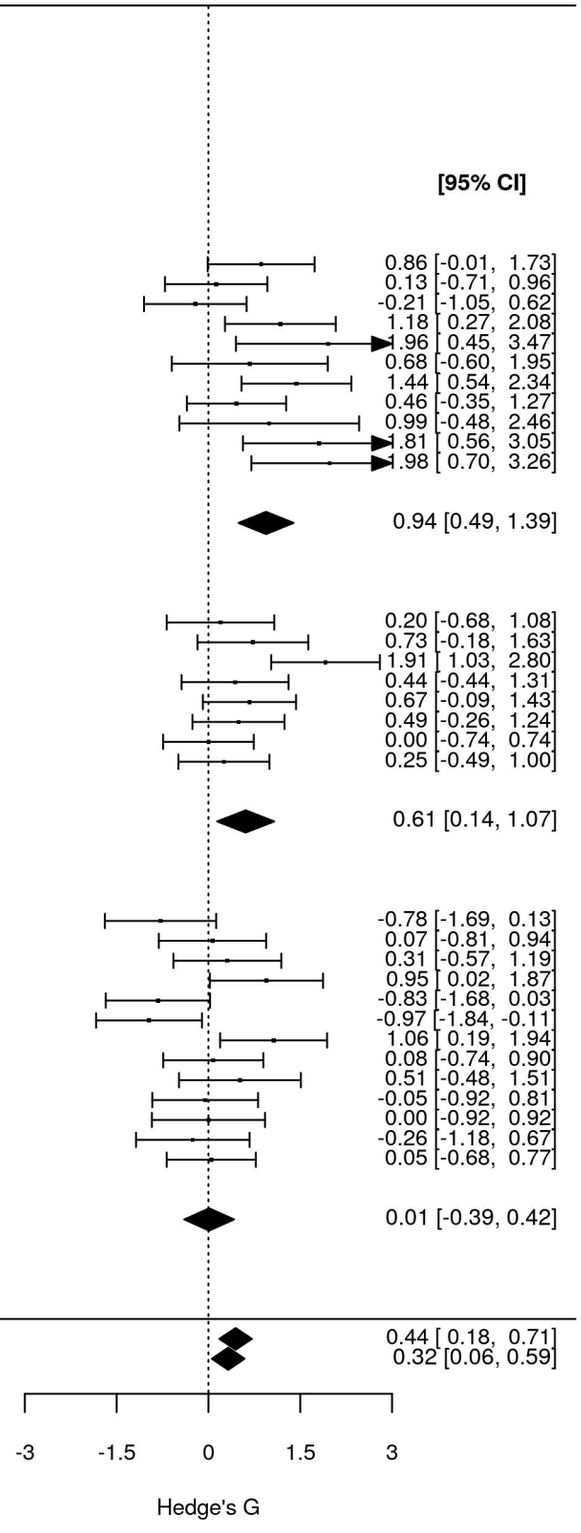
The combination outcome*area had sufficient comparisons for quantitative evaluation:

No

Yes

prenatal striatal DOPAC

Study	Sex	Method	Area	Control N	Control Mean	Control SEM	Experimental N	Experimental Mean	Experimental SEM	[95% CI]
1. LPS										
Romero 2010 _ 3.2	M	protein	striatum	11	40.7	4.3	11	60.7	8.5	0.86 [-0.01, 1.73]
Romero 2010 _ 3.1	M	protein	NAc	11	16.8	2.9	11	17.9	2.1	0.13 [-0.71, 0.96]
Romero 2010 _ 2.2	M	protein	striatum	11	43.6	5.8	11	40	3.7	-0.21 [-1.05, 0.62]
Romero 2010 _ 2.1	M	protein	NAc	11	21.9	3.9	11	47.4	8	1.18 [0.27, 2.08]
Romero 2010 _ 1.2	M	protein	striatum	5	81.6	6.1	5	113.7	7.1	1.96 [0.45, 3.47]
Romero 2010 _ 1.1	M	protein	NAc	5	57.6	9.4	5	71.2	6.6	0.68 [-0.60, 1.95]
Romero 2007 _ 1.2	M	protein	striatum	12	118	12	12	175	10	1.44 [0.54, 2.34]
Romero 2007 _ 1.1	M	protein	NAc	12	138	23	12	172	18	0.46 [-0.35, 1.27]
Delattre 2016 _ 2	M	protein	striatum	4	152	4	4	174	13	0.99 [-0.48, 2.46]
Basta 2011 _ 2	M	protein	striatum	7	1229.2	23.3	7	1409.3	44.1	1.81 [0.56, 3.05]
Basta 2011 _ 1	F	protein	striatum	7	1049	54.3	7	1313.7	39	1.98 [0.70, 3.26]
Subgroup: LPS										0.94 [0.49, 1.39]
2. Poly I:C										
Winter 2009 _ 1.2	M	protein	NAc	10	295	20	10	308	20	0.20 [-0.68, 1.08]
Winter 2009 _ 1.1	M	protein	caudate	10	304	27	10	362	21	0.73 [-0.18, 1.63]
Ozawa 2006 _ 2	P	protein	striatum	12	89	16	17	163	4	1.91 [1.03, 2.80]
Luchicchi 2016 _ 1	M	function	NAc	9	1627	236	12	2015	274	0.44 [-0.44, 1.31]
Bitanihirwe 2010 _ 2.2	F	protein	NAc	14	1.02	0.16	14	2.1	0.57	0.67 [-0.09, 1.43]
Bitanihirwe 2010 _ 1.2	M	protein	NAc	14	1.07	0.14	14	1.74	0.48	0.49 [-0.26, 1.24]
Bitanihirwe 2010 _ 2.1	F	protein	caudate	14	2.21	0.3	14	2.21	0.43	0.00 [-0.74, 0.74]
Bitanihirwe 2010 _ 1.1	M	protein	caudate	14	0.96	0.12	14	1.05	0.05	0.25 [-0.49, 1.00]
Subgroup: Poly										0.61 [0.14, 1.07]
3. restraint										
Weston 2014 _ 2.4	F	protein	striatum	10	114	10	10	84	13	-0.78 [-1.69, 0.13]
Weston 2014 _ 2.3	F	protein	NAc	10	136	15	10	139	12.5	0.07 [-0.81, 0.94]
Weston 2014 _ 1.4	M	protein	striatum	10	117	10	10	130	15	0.31 [-0.57, 1.19]
Weston 2014 _ 1.3	M	protein	NAc	10	83	9	10	118	13	0.95 [0.02, 1.87]
Weston 2014 _ 2.2	F	protein	striatum	12	40.6	4.2	11	30.6	2.2	-0.83 [-1.68, 0.03]
Weston 2014 _ 2.1	F	protein	NAc	12	40.1	3.1	11	25	5.6	-0.97 [-1.84, -0.11]
Weston 2014 _ 1.2	M	protein	striatum	12	30.16	3.3	11	50.53	7.2	1.06 [0.19, 1.94]
Weston 2014 _ 1.1	M	protein	NAc	12	25.5	2.6	11	26.45	4.2	0.08 [-0.74, 0.90]
Gerardin 2005 _ 1	M	protein	striatum	8	691.6	124.76	8	868.6	104.22	0.51 [-0.48, 1.51]
Cory 2013 _ 3	M	protein	striatum	12	18430	333.13	9	18352	562.21	-0.05 [-0.92, 0.81]
Cory 2009 _ 2	M	protein	NAc	9	54	11	9	54	9	0.00 [-0.92, 0.92]
Cory 2009 _ 1	F	protein	NAc	9	95	18	9	82	14	-0.26 [-1.18, 0.67]
ALONSO 1994 _ 1	F	protein	striatum	15	0.41	0.11	14	0.43	0.11	0.05 [-0.68, 0.77]
Subgroup: restraint										0.01 [-0.39, 0.42]
Univariate model										0.44 [0.18, 0.71]
Effect 3-level										0.32 [0.06, 0.59]



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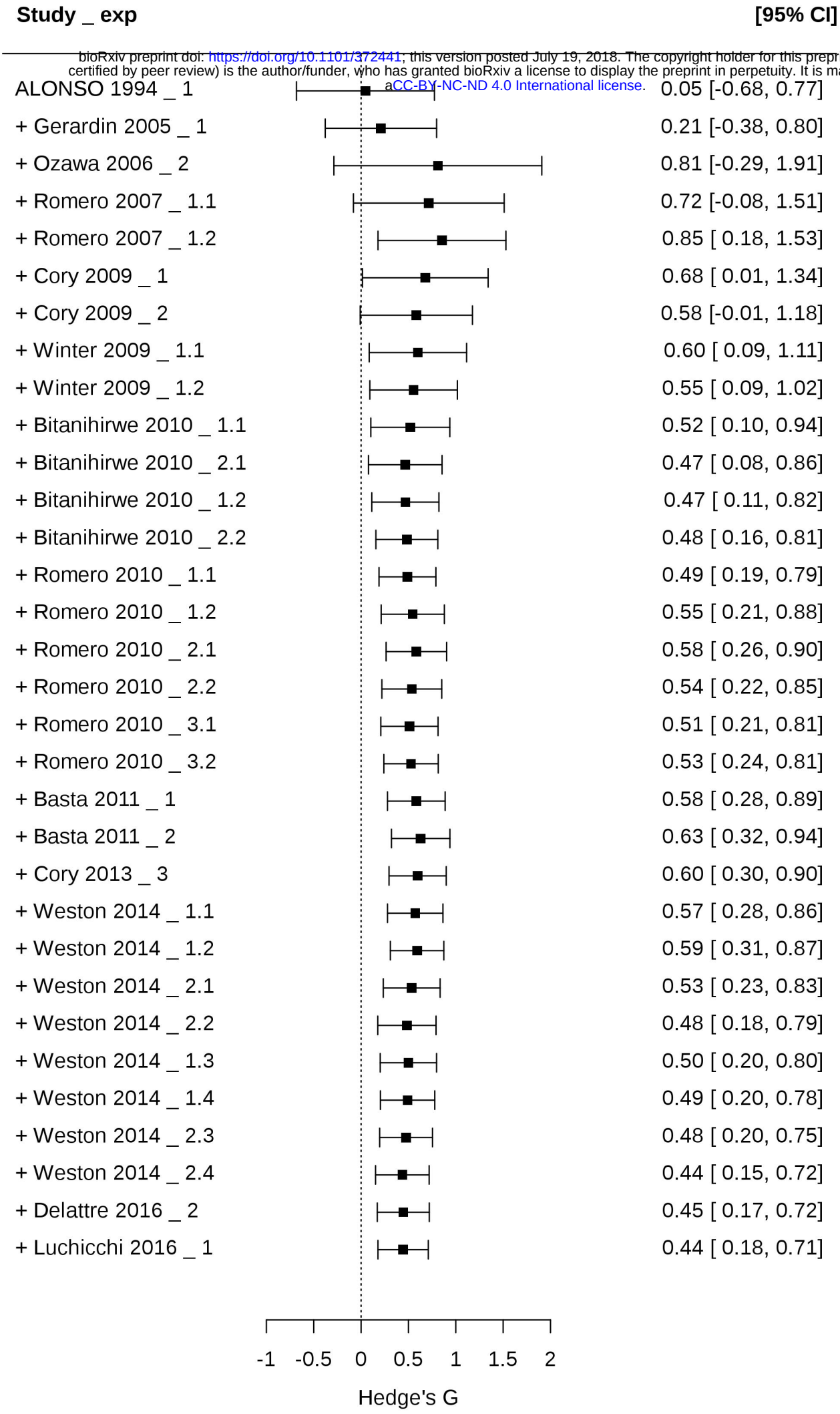

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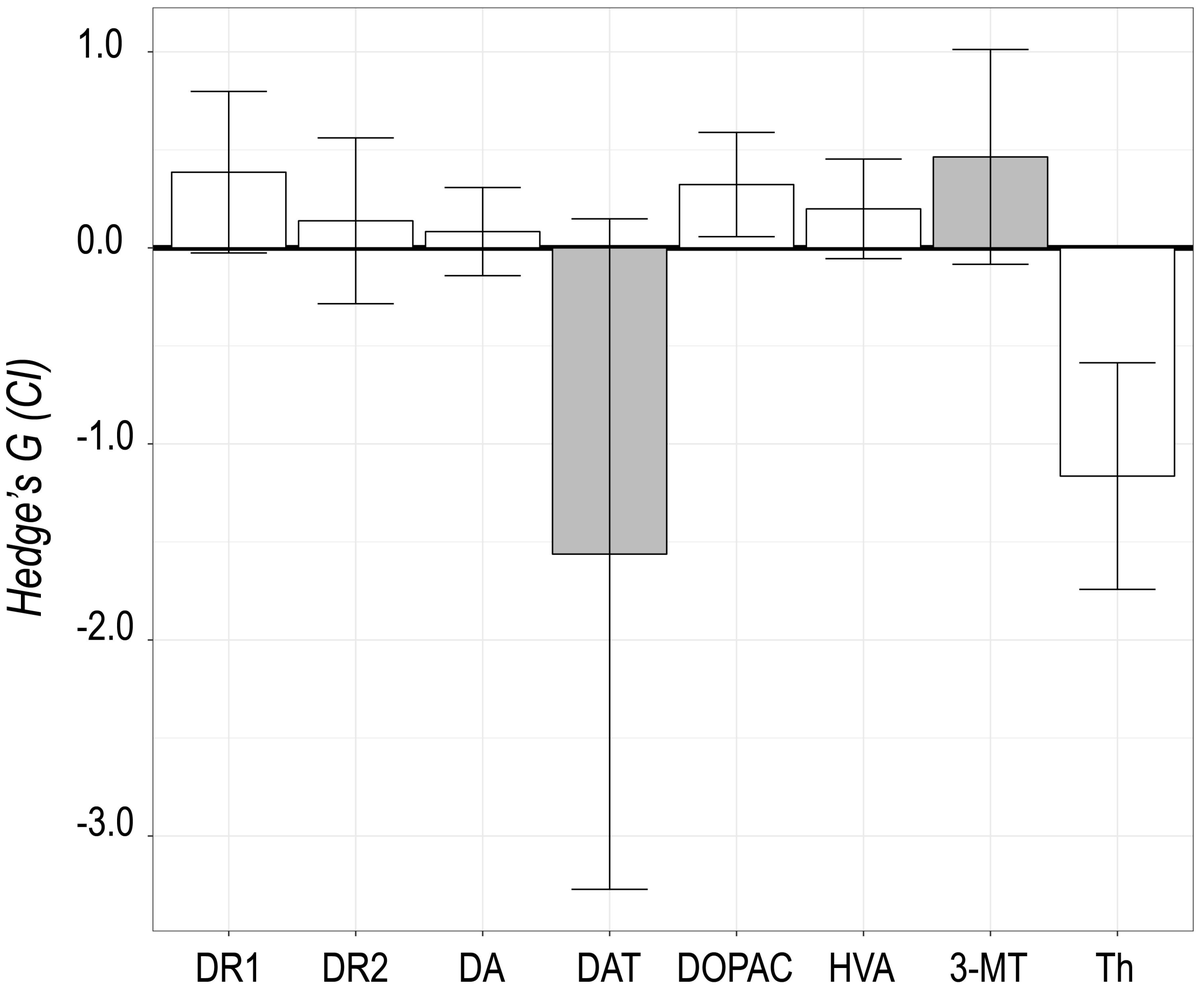
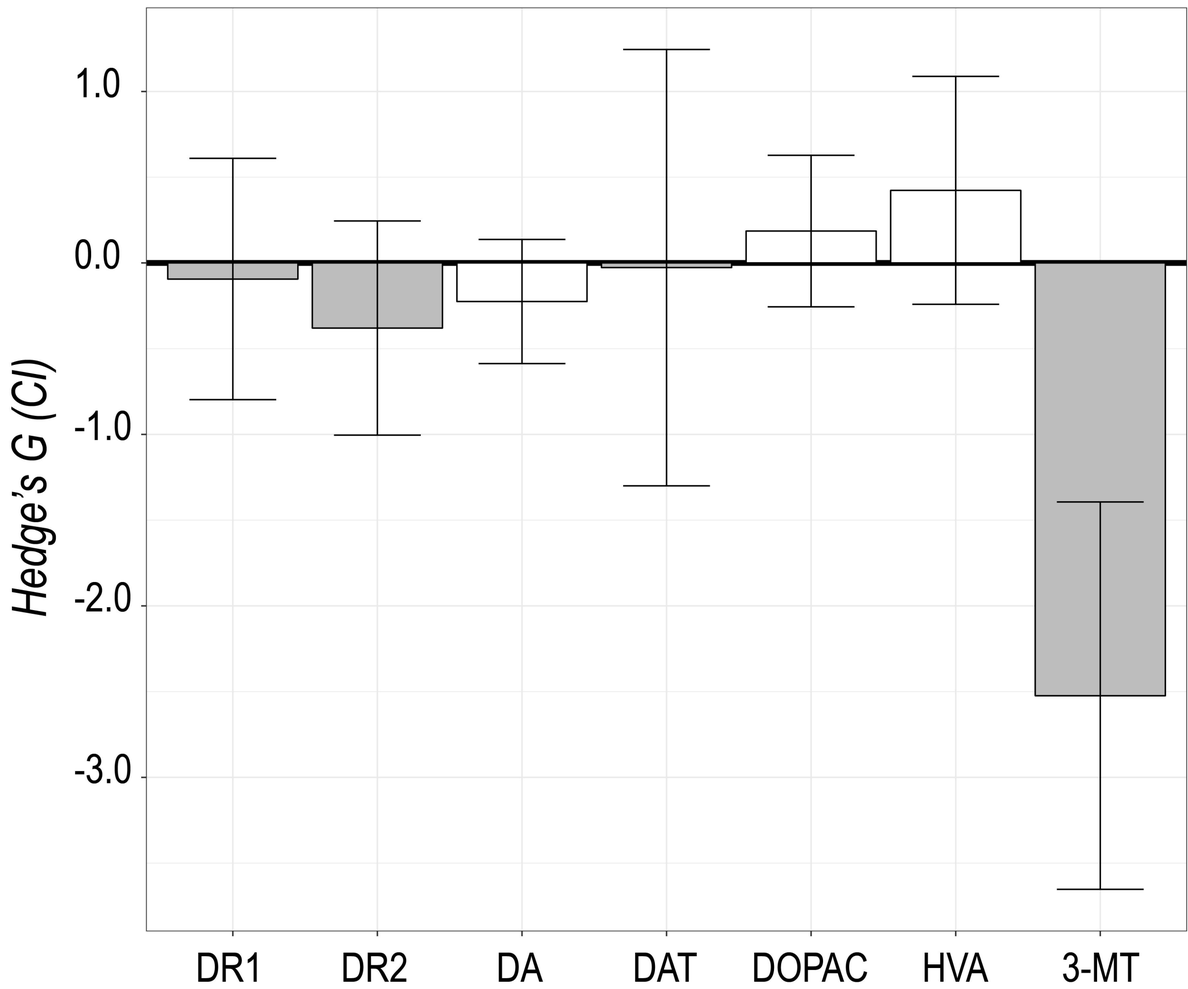
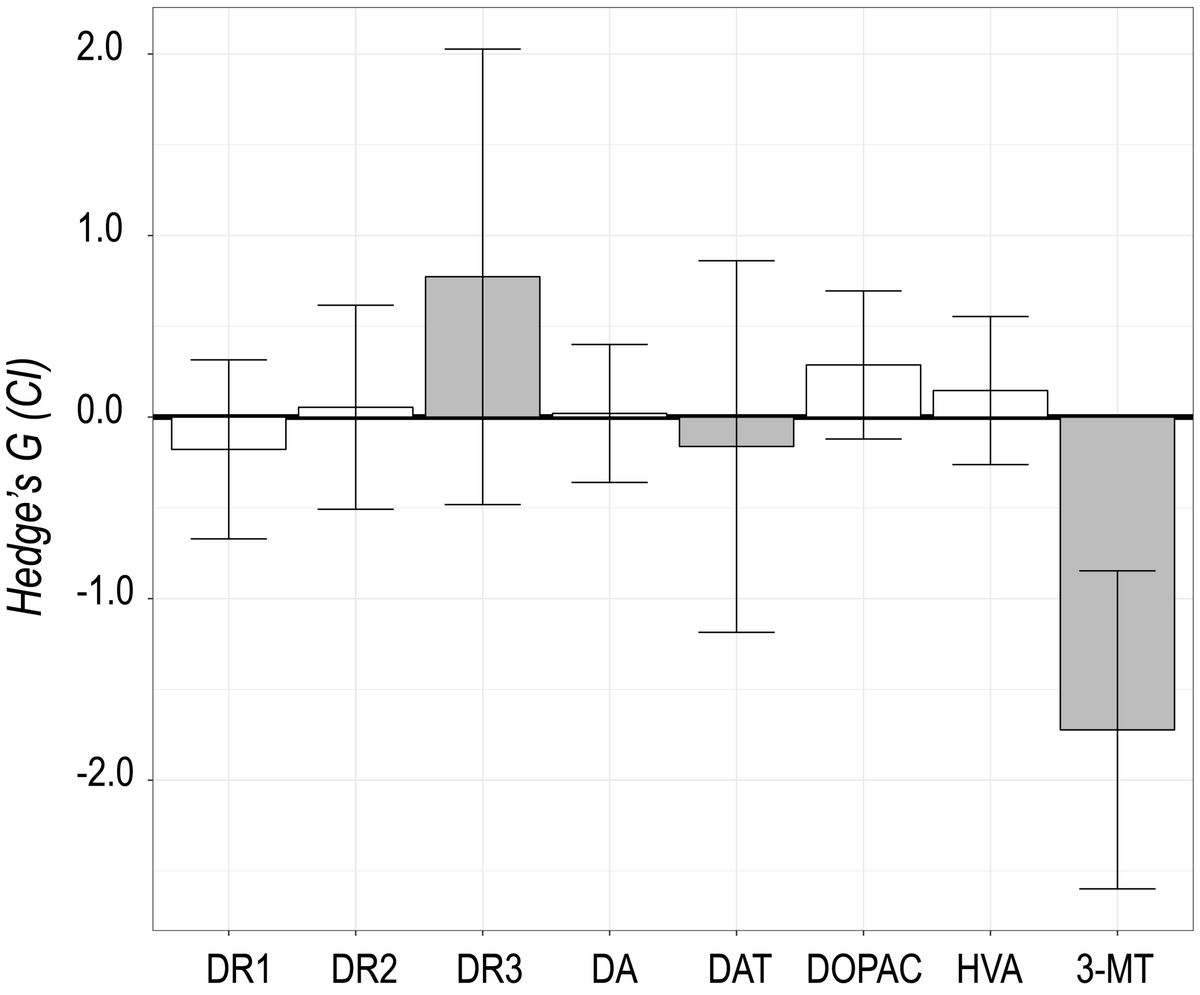
Striatal area



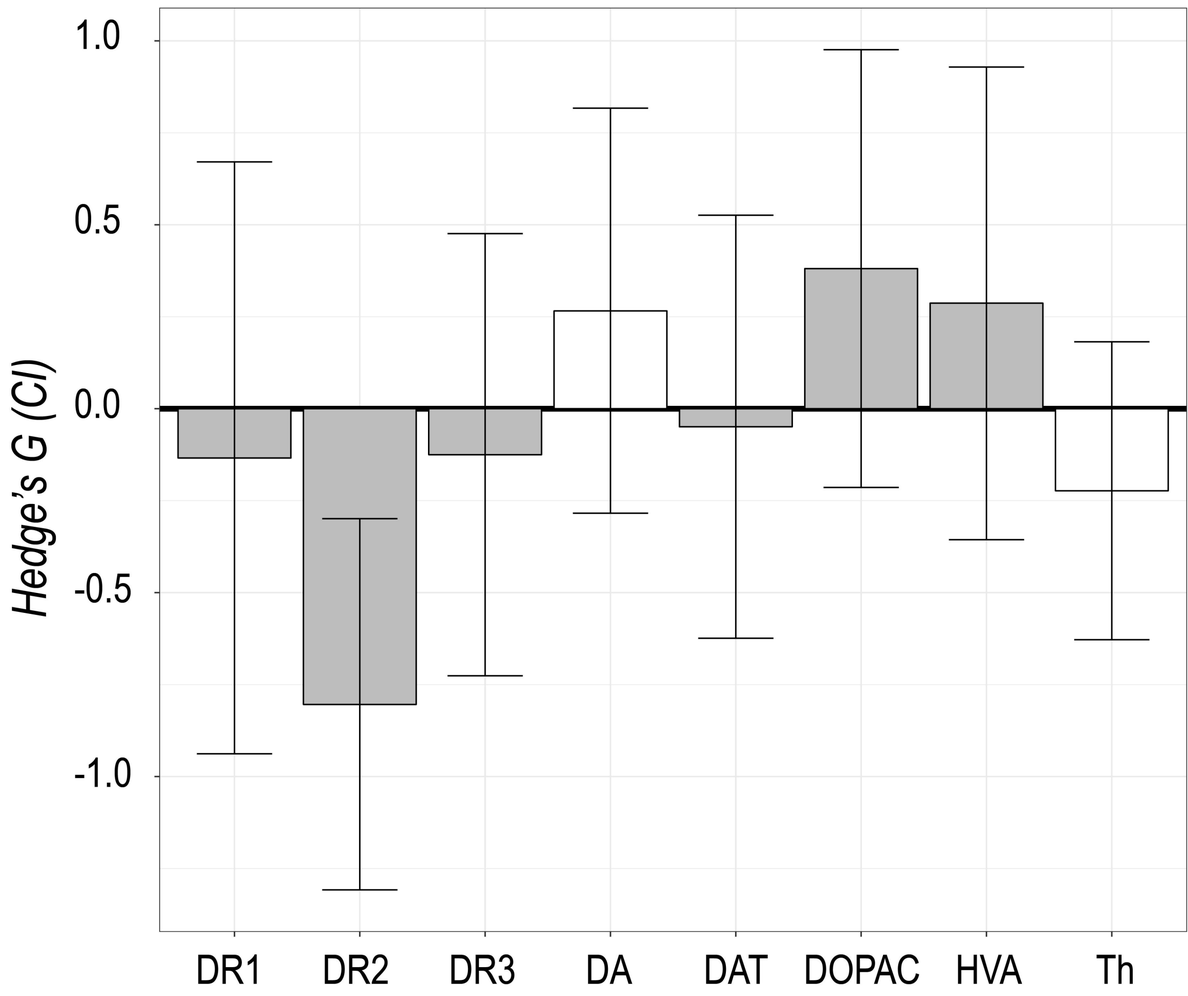
Limbic area



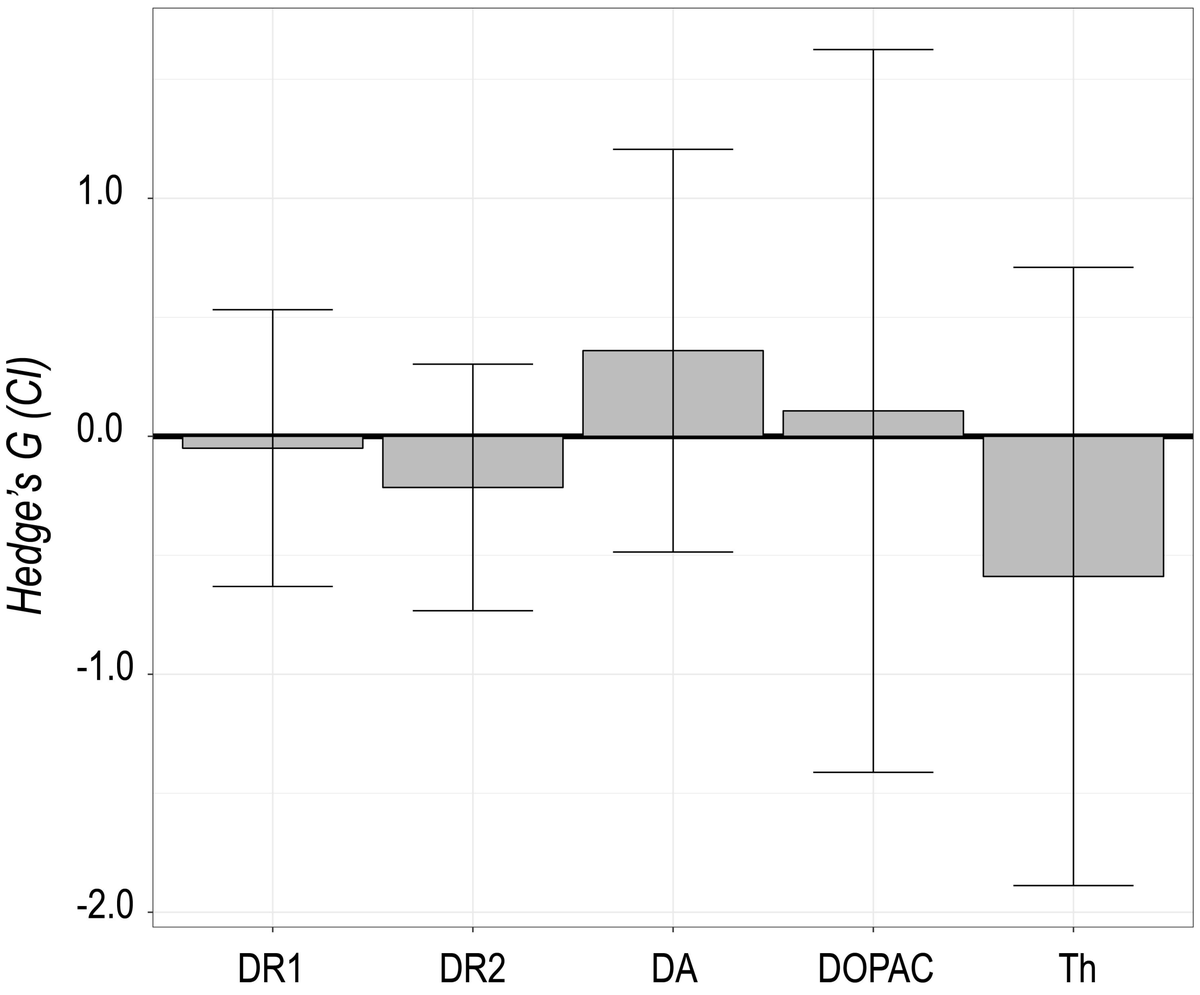
PFC area



VTA area

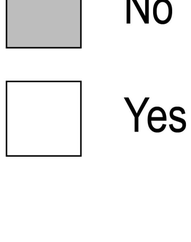


Hypothalamic area



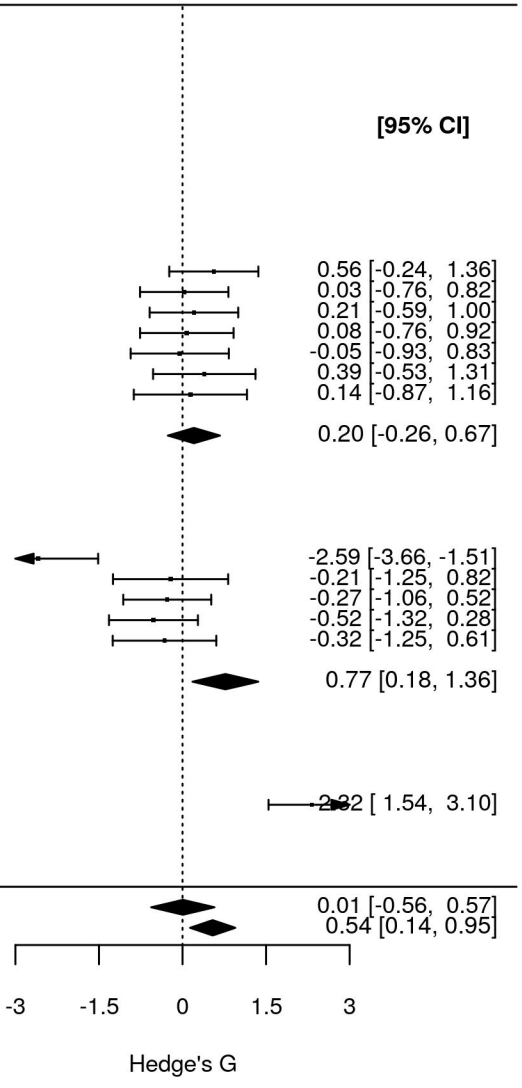
Legend

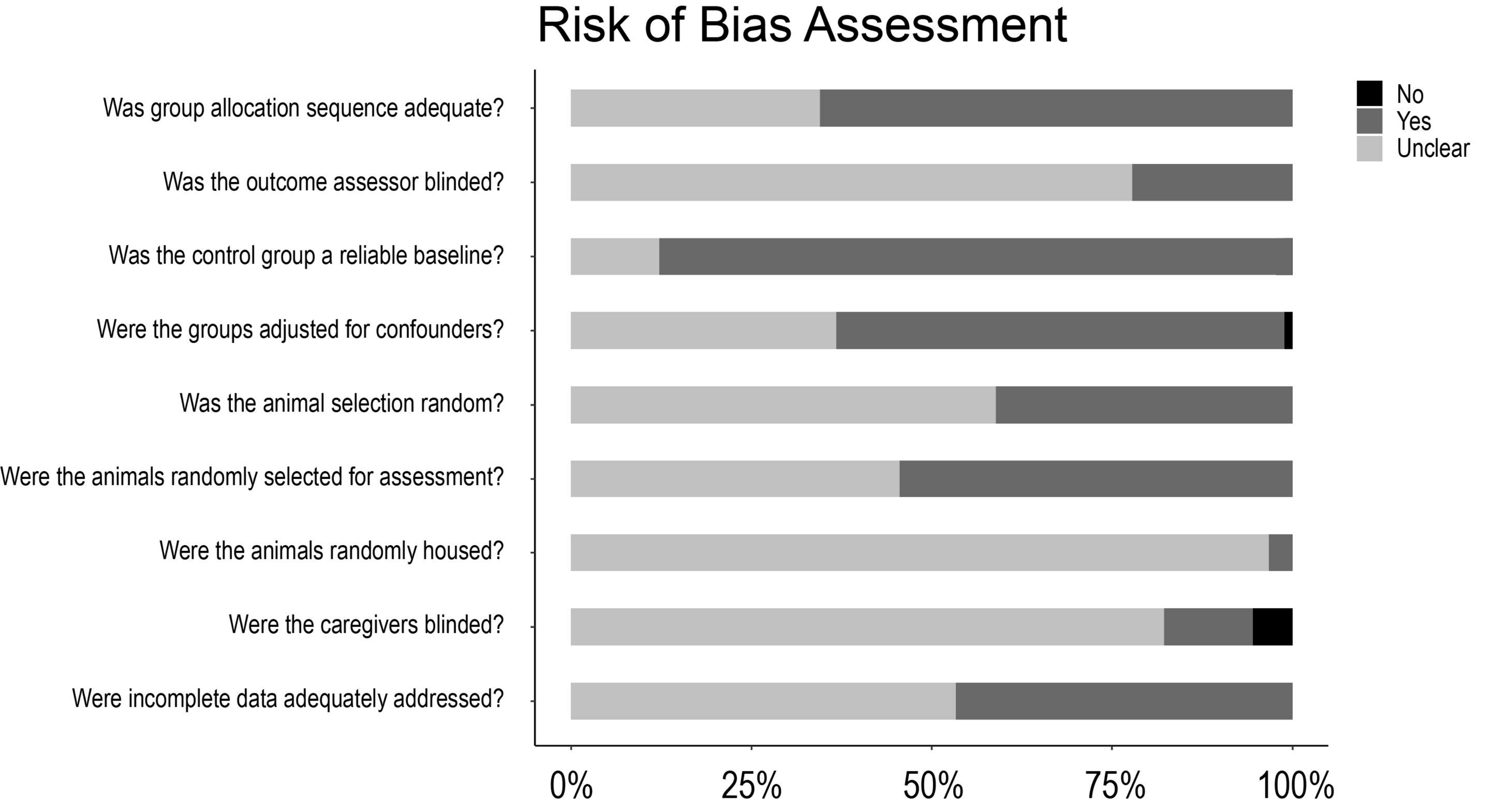
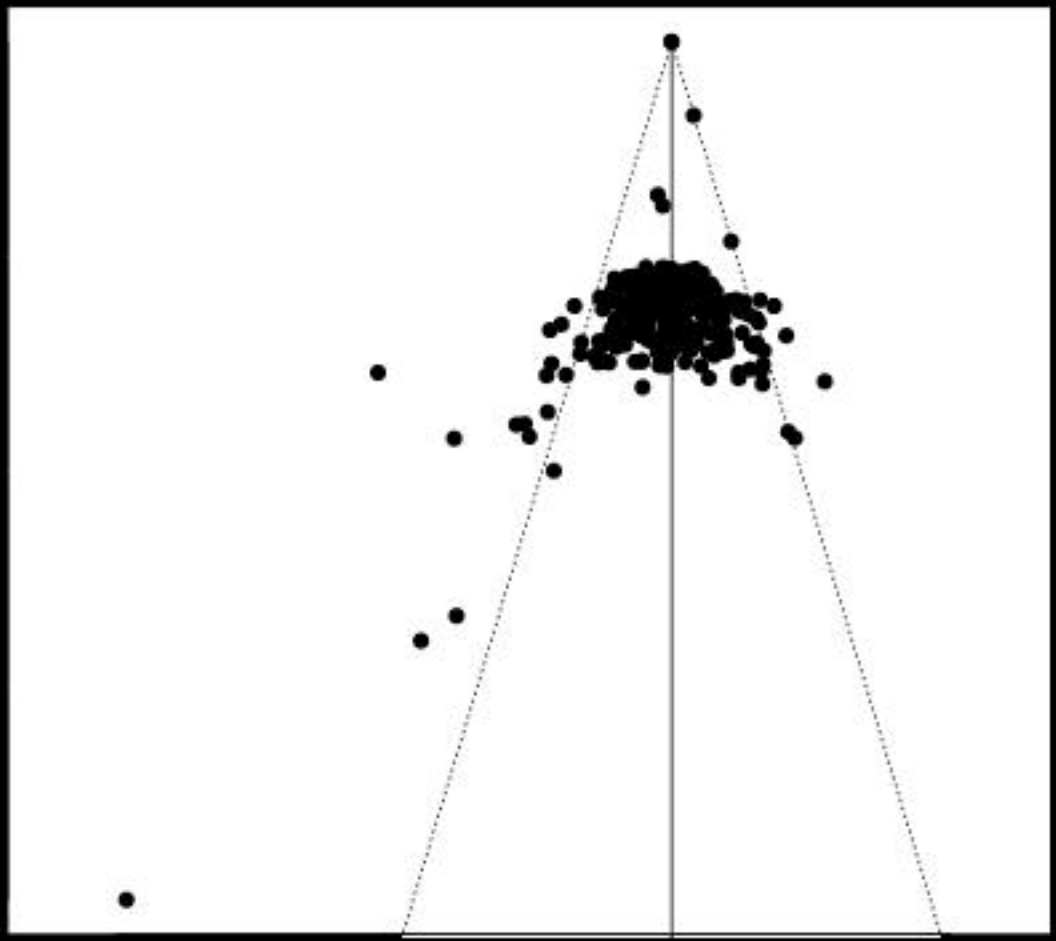
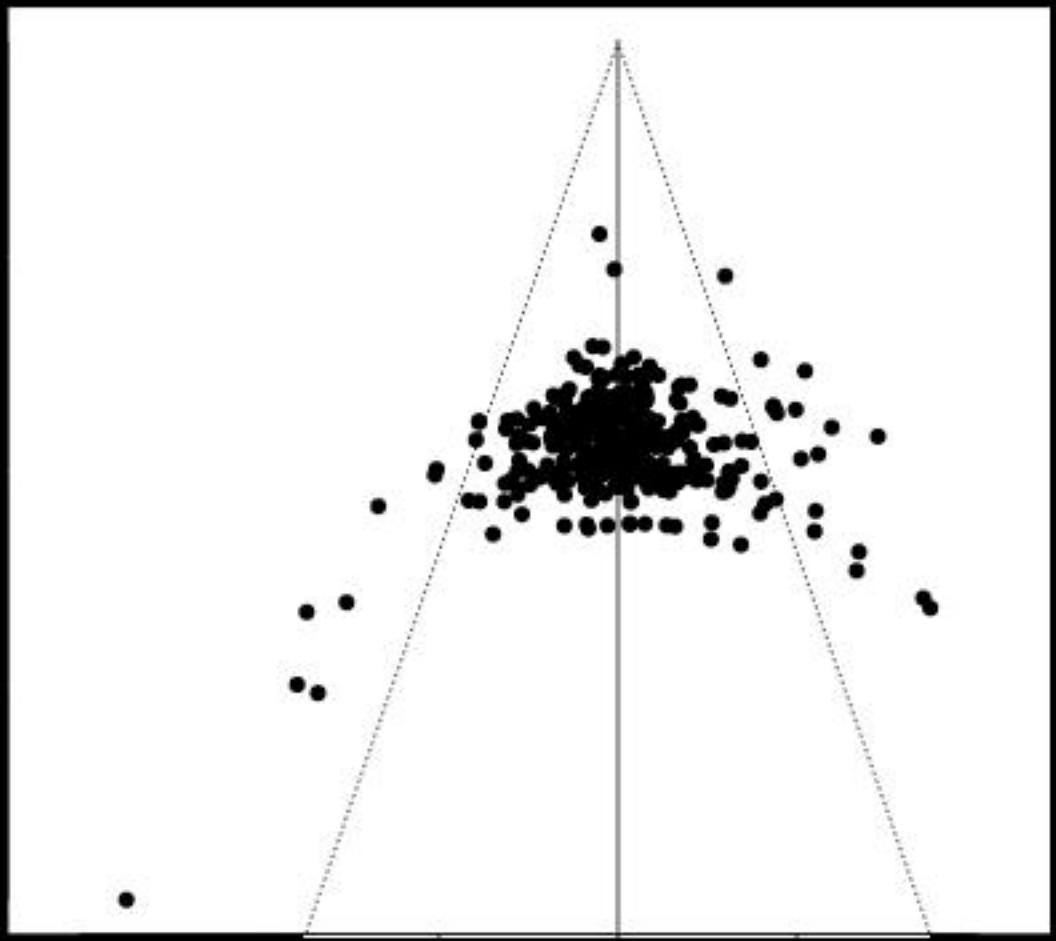
The combination outcome*area had sufficient comparisons for quantitative evaluation:



postnatal striatal DOPAC

Study	Sex	Method	Area	Control N	Control Mean	Control SEM	Experimental N	Experimental Mean	Experimental SEM	[95% CI]
1. MD										
Rentesi 2013 _ 2	M	protein	striatum	12	2.376	0.334	13	3.2	0.45	0.56 [-0.24, 1.36]
Oreland 2011 _ 2.2	M	protein	NAc	9	7.21	0.59	19	7.34	1.11	0.03 [-0.76, 0.82]
Oreland 2011 _ 2.1	M	protein	caudate	9	7.78	0.17	19	8.04	0.33	0.21 [-0.59, 1.00]
Liu 2016 _ 1	M	protein	striatum	10	417	95	12	466	224	0.08 [-0.76, 0.92]
Kawakami 2013 _ 2	M	protein	striatum	9	0.94	0.06	11	0.93	0.06	-0.05 [-0.93, 0.83]
Kawakami 2013 _ 1	F	protein	striatum	8	0.66	0.09	11	0.83	0.15	0.39 [-0.53, 1.31]
Jahng 2012 _ 1	M	protein	NAc	7	178	95	8	216	90	0.14 [-0.87, 1.16]
Subgroup: MD										0.20 [-0.26, 0.67]
2. handling *										
Silveira 2010 _ 1	M	protein	NAc	10	6746.3	436.8	15	3617.9	260.1	-2.59 [-3.66, -1.51]
Panag. 2004 _ 1	F	protein	striatum	5	1911	311.09	13	1392	729.14	-0.21 [-1.25, 0.82]
Oreland 2011 _ 1.2	M	protein	NAc	9	7.21	0.59	20	6.3	0.82	-0.27 [-1.06, 0.52]
Oreland 2011 _ 1.1	M	protein	caudate	9	7.78	0.17	20	7.04	0.36	-0.52 [-1.32, 0.28]
Cabib 1993 _ 1	M	protein	NAc	9	1089.6	42.8	9	1036	60.9	-0.32 [-1.25, 0.61]
Subgroup: handling										0.77 [0.18, 1.36]
3. LPS										
Kubesova 2015 _ 1	M	protein	striatum	19	15.43	0.5	24	25.4	1.08	2.32 [1.54, 3.10]
Univariate model										0.01 [-0.56, 0.57]
Effect 3-level										0.54 [0.14, 0.95]




Risk of Bias Assessment
Was group allocation sequence adequate?
Was the outcome assessor blinded?
Was the control group a reliable baseline?
Were the groups adjusted for confounders?
Was the animal selection random?
Were the animals randomly selected for assessment?
Were the animals randomly housed?
Were the caregivers blinded?
Were incomplete data adequately addressed?
No
Yes
Unclear
0%
25%
50%
75%
100%