PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Behavioral effects of methylphenidate in an animal model of attention-deficit/hyperactivity disorder, the spontaneously hypertensive rats: a systematic review and meta-analysis protocol
AUTHORS	**Douglas Teixeira Leffa (Corresponding Author)** **Alana Castro Panzenhagen** **Diego Luiz Rovaris** **Claiton Henrique Dotto Bau** **Luis Augusto Rohde** **Eugenio Horacio Grevet** **Gabriel Natan Pires**

VERSION 1 - REVIEW

REVIEWER 1	***Peter Paul Zwetsloot*** ***Universitair Medisch Centrum Utrecht***
REVIEW RETURNED	31-01-18

GENERAL COMMENTS	.In 'Behavioural effects of methylphenidate in an animal model of ADHD, the spontaneously hypertensive rats: a systematic review and meta-analysis protocol' Texeira Leffa et al. describe their protocol in detail for their systematic review and meta-analysis. This is important to guarantee that the methods used were declared upfront and should be applauded. The protocol is excellently written and includes most important aspects. There are some minor concerns and comments: 1. there is no final search mentioned with all search terms. Please add (in supplementary file) the complete final search that has been inserted in PubMed / Embase etc. Through this, you are absolutely sure that the search used in the primary paper is the same as the one in the protocol. 2. (2.2 p7) in '2.2 study selection' the authors state that they will do a full text screening if necessary. I would advice to always do a full text screening to be absolutely sure that your included paper meets al your inclusion criteria (sometimes abstracts can be misleading). 3. (2.2) Please state who the authors will be (with initials) who will conduct the search and who will act as third screener/referee. 4. (2.2) You exclude SHR substrains (stroke-prone SHR). Is there a reason to exclude these? Are they different from one another? In literature I can find authors saying that both are used as ADHD models. If there is a specific reason to exclude these, please elaborate on this and provide literature on this choice.

	5. (2.6.2) Please state how many studies you think you will approximately include, as this officially limits the amount of covariates you can examine. A general rule of thumb is 1 covariate per 10 studies included. If you choose to include more covariates, please state this in the primary paper, as this increases the chance of finding false-positive results. 6. (2.6.2, p10) you will perform a multivariate meta-regression model, in which studies with missing values will be excluded. please be aware that doing a 'complete case analysis' might bias your data and reduce the number of included studies. 7. (2.6.3) you mention sensitivity analysis, based on excluding one result at a time. If you want to do this, to get rid of any outliers I would suggest starting with a 'regular' outlier analysis on your outcome data-points (deviance residuals, Schoenfeldt residuals) and try to exclude those. (NB. This is not a request that certainly needs to be implemented, but just a suggestion.)

VERSION 1 – AUTHOR RESPONSE

Editor in Chief Comments to Author:
The reviewer has been some excellent suggestions. In addition, although you have clearly stated the aim of the systematic review in the introduction it would be beneficial to include your specific objectives to achieve this aim.

R: Thank you for your comment. Specific objectives were included in the introduction.

I suggest for section 2.5 you make it clear how you will ensure consistency in your relevance ranking of behavioural tests extracted (I assume this means that for each outcome only one behavioural test data point will be included?).

R: Yes, only one data point will be included for each behavioral test. We will rank the variables from each behavioral test subjectively according to their importance, and the relevance rank will be organized by one reviewer. Even though the ranking is organized in a subjectively way, the extraction of all papers will be based on the same rank, thus providing consistency to the method. A similar strategy has been previously used (1, 2).

I also suggest a short description of the pros and cons of this approach should be included in the discussion – i.e. why you have chosen this method rather than to include all data from a behavioural test to nest into a single data point for your outcome.

R: Thank you for your comment. The pros and cons of this approach have been incorporated in the discussion.

Associate Editor Comments to Author:
I agree with the suggestions for minor revisions as made by reviewer 1, and suggest that, if still possible, you try to incorporate these suggestions into your protocol.

Reviewer 1:
In 'Behavioural effects of methylphenidate in an animal model of ADHD, the spontaneously hypertensive rats: a systematic review and meta-analysis protocol' Texeira Leffa et al. describe their protocol in detail for their systematic review and meta-analysis. This is important to guarantee that the methods used were declared upfront and should be applauded. The protocol is excellently written and includes most important aspects. There are some minor concerns and comments:

1. there is no final search mentioned with all search terms. Please add (in supplementary file) the complete final search that has been inserted in PubMed / Embase etc. Through this, you are absolutely sure that the search used in the primary paper is the same as the one in the protocol.

R: Thank you for your comment. The complete final search has now been added as supplementary material.

2. (2.2 p7) in '2.2 study selection' the authors state that they will do a full text screening if necessary. I would advice to always do a full text screening to be absolutely sure that your included paper meets al your inclusion criteria (sometimes abstracts can be misleading).

R: Thank you for your suggestion. We do agree that abstracts can be misleading. In order to avoid the exclusion of a potentially eligible study and at the same time increase the efficiency of our search strategy, we decided to exclude studies without a full-text review only in those situation that an exclusion criteria is clearly defined (e.g. reviews or editorials, studies in human subjects). The information is now mentioned in “2.2 Study Selection”. We would like to highlight that this strategy is advised for both clinical and preclinical meta-analyses (3, 4).

3. (2.2) Please state who the authors will be (with initials) who will conduct the search and who will act as third screener/referee.

R: The authors responsible for the search are now described.

4. (2.2) You exclude SHR substrains (stroke-prone SHR). Is there a reason to exclude these? Are they different from one another? In literature I can find authors saying that both are used as ADHD models. If there is a specific reason to exclude these, please elaborate on this and provide literature on this choice.

R: The stroke-prone SHR are a substrain of SHR created by breeding animals presenting rapid increase in blood pressure at a younger age and severe hypertension, leading to a high incidence of stroke (5). Although few studies have been conducted using the stroke-prone SHR as a model of ADHD, biochemical (6) and genetic (7, 8) differences between the two strains have been reported. Those differences, together with the increase incidence of stroke in stroke-prone SHR, potentially leading to brain damage, prevented us from including the substrain in the analysis.

5. (2.6.2) Please state how many studies you think you will approximately include, as this officially limits the amount of covariates you can examine. A general rule of thumb is 1 covariate per 10 studies included. If you choose to include more covariates, please state this in the primary paper, as this increases the chance of finding false-positive results.

R: Thank you for your comment. Based on our previous experience with basic studies, we believe that at least 30 studies will be included in the final analysis. However, if this is not the case, a statement will be included in the primary paper.

6. (2.6.2, p10) you will perform a multivariate meta-regression model, in which studies with missing values will be excluded. please be aware that doing a 'complete case analysis' might bias your data and reduce the number of included studies.

R: Thank you for your comment. We do agree that this method may bias the final result, and have added a sentence in the discussion section.

7. (2.6.3) you mention sensitivity analysis, based on excluding one result at a time. If you want to do this, to get rid of any outliers I would suggest starting with a 'regular' outlier analysis on your outcome data-points (deviance residuals, Schoenfeldt residuals) and try to exclude those. (NB. This is not a request that certainly needs to be implemented, but just a suggestion.)

R: Thank you for your suggestion. Excluding one result at a time in order to observe how individual studies may affect the final effect size is also called the jackknife method (9), and it is commonly used

in meta-analysis. We have decided to maintain the method, and included more information about it in the text.

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VERSION 2 - Review

REVIEWER 1	Mira van der Naald Universitair Medisch Centrum Utrecht
REVIEW RETURNED	0506/2018

GENERAL COMMENTS	Recommendation Minor Revision Interesting research topic! 7. Note that using a standardized mean difference leads to distortion of the funnel plot. Please see the article by Zwetsloot et al for further details (doi: 10.7554/eLife.24260). 8. I am not in the position to answer this question. 11. Please elobarate how the results will be a measure to predictive validity. Especially describe how the results should be interpreted, taking into account that results of MPH in clinical setting are heterogeneous as well (as stated in the introduction). Furthermore, how will be dealt with heterogeneous internal validity in the analysis? 13. The protocol registered on SYRF mentions 9 items to be evaluated for quality assessment, but in the current manuscript one item is added, please explain why this item is added now. Furthermore, funding and conflict of interests are not addressed.

REVIEWER 2	Hendrik Gremmels Universitair Medisch Centrum Utrecht
REVIEW RETURNED	31/05/2018

GENERAL COMMENTS	Recommendation Major Revision The present article is a protocol for a for a systematic review and meta-analysis on the effects of methylphenidate on neurobehavioural characteritics in SHR rats. In the opinion of this reviewer this is an important and relevant subject, that is very suitable for a systematic review. It is commendable that the authors have decided to publish their protocol prior to conducting the review. From the cover letter I understand that the protocol has already undergone a round of review prior to my receiving it. Unfortunately I do not have access to this first review round and will consequently review the article 'de novo'. My apologies to the authors if some issues are addressed doubly.

	Regarding the geneneral structure of the protocol, the only remark that I have is whether the focus on SHR rats is not overly restrictive. SHR rats are perhaps the most common model for ADHD, but there are other (WKY substrains) that are also proposed models for ADHD. Is the question about predictive validity not better answered by showing that SHR rats show a more 'human-like' response to MPH than other rat strains? **Abstract**: The authors state that the main aim of the article is to to examine the predictive validity of SHR rats for ADHD, but they cannot answer this wholly with the present article. They can only show that one documentedly effective treatment in humans, MPH, may also work in the SHR model. This gives only a clue towards predictive validty, as there are other drugs (Amphetamines, Atomoxetine) that work in humans, and perhaps a few that work in SHR and not in humans. The abstract should focus on the actual article, i.e. the effect on MPH and not on speculative implications. For instance, the abstract does not even mention the recorded outcomes of the proposed meta-analysis. line 5 currently -> current **Introduction** In general the intro could be improved by information why face and construct validity are apparently not a problem in SHR rats. This could be nicely tied in with a bit describing the proposed mechanism of action of MPH. Par 2, line 4 mega-analysis-> meta-analysis line 6: spell out fMRI Par 2, last sentence: fix bad grammar Par 3, line 2: important->importantly Page 6 line 6 MPH on a molecular level **Methods** **2.2.** It would be better to use the 'conventional' steps in the PRISMA diagram instead of "Step 1-3". I.e. abstract screening , full-text etc. Step 3 should be reversed by specifying inclusion criteria criteria -> criterion 2.3. gender -> sex 2.5 The authors have decided to that they will include only one behavioural experiment from the same category and will choose the most relevant or the first. This seems very prone to bias and unclear. The first means the first reported in the article? This may

	be a negative bias as authors usually build up towards the more dramatic results in the article. On the other hand relevance decided by the authors of this article may also lead to bias. My suggestion would be to extract all of them and include the one with the largest effect size in the main analysis (effect of MPH). The results of different test variants (e.g. diff locomotion tests) can be presented in separate subgroup analyses (or if need be under sensitivity). Which test 'works' best (and thus has highest predictive validity) is an important question and can refine animal research. The last two sentences seem vague to me. "If the same animals were evaluated more than once in the same test, the last one will be selected for data extraction. If the manuscript divides the results by time, the first timepoint will be selected..." To my mind these say the exact opposite, please clarify. line 5: two attempt -> two attempts 2.6 Page 9, last line. Actually the four hypotheses are likely far from independent. Assuming a true underlying construct for ADHD and an effect of MPH thereupon, one expect that relative scores on the four outcome measures are highly correlated and therefore not independent. The Bonferroni correction is conservative and I don't oppose to it, but it may be overkill. Page 10. Any reason why the authors transform the values for locomotion? Common practice would be to show the negative values and put and additional 'favors MPH"/"favors control" on the axis. 2.6.2 The meta-regression test **are** independent hypotheses, and should be corrected for. The authors should specify a minimum number of studies (overall, or per arm) before conducting meta-analysis. Should the test-type (e.g. for locomotion) not be included in the investigation of heterogeneity?

Behavioral effects of methylphenidate in an animal model of attention-deficit/hyperactivity disorder, the spontaneously hypertensive rats: a systematic review and meta-analysis protocol

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Abstract

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent condition related to several negative outcomes. Although a strong neurobiological basis has been demonstrated, its pathophysiology is still poorly understood. In this sense, animal studies are essential in order to unravel the neurobiological factors associated with neuropsychiatric disorders, and the absence of proper animal models may be contributing to the currently challenges in the development of new treatments. The spontaneously hypertensive rats (SHR) are the most commonly used animal model of ADHD. However, its validity, and especially its predictive validity, has been questioned. In this study we aim at summarizing the evidence for the predictive validity of the SHR as an animal model of ADHD. The current protocol discloses the background, aims and methods of a systematic review and meta-analysis of studies reporting behavioral effects of methylphenidate (MPH), the most commonly prescribed treatment for ADHD, in the SHR. In addition, we plan to evaluate how pre-defined covariates influence the effects of MPH using meta-regression and sensitivity analyses.

Keywords: ADHD, SHR, meta-analysis, methylphenidate

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent neurodevelopmental disorder characterized by impairing levels of hyperactivity, impulsivity and inattention (1). Observational studies have shown that ADHD is related to several negative outcomes, including, among others, decreased quality of life (2), increased number of suicide attempts (3), less socioeconomic status (4), and increased mortality due to accidents (5).

A strong neurobiological basis has been repeatedly demonstrated in ADHD. Twin studies showed that ADHD has a heritability of about 70-80% (6), and candidate gene studies suggest mainly the influence of genes related to monoaminergic neurotransmission (7). A recent mega-analysis performed with more than 1500 ADHD patients found a reduced volume in the nucleus accumbens, amygdala, caudate, hippocampus and putamen in patients with the disorder (8). In addition, a meta-analysis of fMRI studies demonstrated reduced activation in distinct cortical region during attention and impulsivity tests (9). Although ADHD neurobiological basis has been confirmed through molecular genetics and neuroimaging studies, its pathophysiology is still poorly understood.

Animal models are considered a fundamental tool to unravel the neurobiological factors associated with neuropsychiatric disorders (10). More important, the absence of proper animal models has been proposed as a main factor driving the slow advancement of new treatments for neuropsychiatric disorders (10). An animal model should present predictive, face and construct validity in order to be considered a proper model of a disease (11). In ADHD, the spontaneously hypertensive rats (SHR) are widely considered the most appropriate model (12, 13). However, there is no consensus on the validity of the SHR as an animal model of ADHD.

Criticism has been demonstrated specially on the predictive validity of the SHR (14, 15), which might be measured by the ability of the animal model to respond to well documented treatment for the disorder (11). In ADHD, the most well documented treatment is the one

performed with stimulant medication (16). Among stimulants, methylphenidate (MPH), a dopamine transporter inhibitor, is the most commonly used (17). Although promoting overall clinical improvement, there is a substantial heterogeneity regarding the response to MPH treatment (18), and about 30% of patients do not present a clinical response (19). Therefore, a well-validated animal model is also essential in order to have a proper tool to understand the variability in the response to MPH in a molecular level. Therefore, in this study we intend to disclose the protocols for a systematic review and meta-analysis aiming at summarizing the MPH in the SHR. With this study we aim to answer the following question: does MPH treatment improve behavioral deficits presented by the SHR when compared to placebo?

2. Methods

Methods are described according to the format proposed by de Vries, Hooijmans (20). This protocol has been previously submitted for the CAMARADES Preclinical Systematic Review & Meta-analysis Facility (SyRF, http://syrf.org.uk/protocols/) with the title "Behavioral effects of methylphenidate in an animal model of attention-deficit/hyperactivity disorder, the spontaneously hypertensive rats: a systematic review and meta-analysis".

2.1. Search and study identification

We are going to include studies that administered MPH to SHR and evaluated one of the following behavioral outcomes: locomotion, attention, impulsivity or memory. Studies will be identified through a literature search using three different electronic databases: Medline, Scopus and Web of Science. The search strategy is going to be the following: (("SHR" OR "spontaneous* hypertensive rat*") AND ("MPH" OR "methylphenidate" OR "ritalin")). No search filters are going to be used. In addition, we are going to search the reference list of included studies.

2.2. Study selection

The study selection and inclusion is going to be performed with a pre-screening based on title and abstract, followed by a full text screening if necessary. Both will be conducted by two independent authors, and any disagreement will be discussed with a third author. There will be no date or language restrictions. The following steps will be conducted for the inclusion and exclusion of studies:

Step 1: All title and abstracts are going to be reviewed by two independent authors. Those reporting experimental studies evaluating the effects of MPH in the SHR are going to be selected for the next step. The following exclusion criteria will be applied: use of SHR substrains (e.g., stroke-prone SHR), MPH administered in brain slices, MPH administered only together with another drug, MPH self-administration. If the information is not sufficient to include or exclude the study, a full-text review will be conducted;

Step 2: Studies presenting behavioral outcomes are going to be selected for the next step. The behavioral outcomes are going to be categorized according to the author´s description. Studies with no behavioral outcomes will be excluded;

Step 3: Studies reporting locomotion, attention, impulsivity or memory outcomes are going to be included for final analysis. The following exclusion criteria will be applied: distinct behavioral outcomes from the ones previously presented, use of a crossover approach, not comparing the SHR-MPH to a SHR-placebo group. Studies using a crossover approach, meaning that the same rats will be used as active and placebo groups, will be excluded in order to avoid a carryover effect (21).

2.3. Study characteristics to be extracted

For the assessment of external validity, the following items will be extracted: study authors, year of publication, experimental groups, sample size, age and gender of animals, route of drug administration, MPH dosage in mg/kg, number of administrations per day, total days of treatment, behavioral test used, and outcome of interest.

2.4. Risk of bias assessment

Risk of bias assessment will be conducted by one review author using the SYRCLE´s risk of bias tool for animal studies (22). Any doubt in the assessment will be discussed with a second reviewer. Ten items will be evaluated in the quality assessment: three related to selection bias (sequence generation, baseline characteristic and allocation concealment); two related to performance bias (random housing and blinding); two related to detection bias (random outcome assessment and blinding); one related to attrition bias (incomplete outcome data); one related to reporting bias (selective outcome reporting); and one related to other sources of bias. Baseline characteristics will be comprised by information on sex and age of animals. The tenth item will address sources of bias beyond the ones covered by other domains. Each study will be evaluated considering the ten items, and for each item the study will be classified as presenting low, unclear or high risk of bias. Publication bias will be accessed using funnel plots and the Egger´s regression test.

2.5. Collection of outcome data

Continuous outcome data from behavioral tests will be extracted by two independent authors, an any disagreement will be discussed with a third author. Data will be extracted directly from the full-text article. When not reported in enough details, extraction will be done by graph estimation using a digital ruler, as previously described (23, 24). If both methods are not viable, the authors will be contacted. Two attempt will be performed within a two-week

interval. If no response is obtained, the article will be excluded. Each included article will be divided in experiments, defined as any case when a control group is compared to an experimental group. If the same animals were subjected to more than one behavioral experiment from the same category, only one will be included (either the most relevant or the first one to be performed). In addition, only one outcome will be extracted from each behavioral experiment. Whenever the article report multiple outcomes from the same behavioral test, the extraction will be performed according to a relevance rank organized by one reviewer considering the manuscript description. If the same animals were evaluated more than once in the same behavioral test, the last one will be selected for data extraction. If the manuscript divides the results by time, the first time point will be selected for extraction.

2.6. Data analysis

2.6.1. Meta-analysis

Studies will be grouped according to the behavioral outcomes reported (locomotion, attention, impulsivity or memory), and a meta-analysis will be performed for each group. Pooled effect sizes will be determined with standardized mean differences using Hedge´s G method with random-effects, allowing the comparison among distinct behavioral tests. The significance of pooled effect sizes will be determined using the Z-test. Since we will perform four independent meta-analyses, a Bonferroni correction will be applied, and a p-value $\leq$ 0.0125 will be considered as statistically significant. Individual study weights will be obtained using the inverse of the variance. Data will be transformed in order to obtain positive values for decreased locomotion or impulsive behavior and for increased attentional or memory performances.

Heterogeneity between studies will be assessed using both the Chi^2 and the I^2 tests, and a p-value ≤ 0.1 will be considered as statistically significant. A I^2 value on the order of 25%,

50% and 75% will be considered as low, moderate, and high heterogeneity, respectively (25). Standardized mean differences and heterogeneity values will be obtained using Review Manager (RevMan) version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

2.6.2. Meta-regression

A meta-regression will be conducted in order to evaluate potential sources of variability among studies. The following covariates will be selected based on biological plausibility and added to a random-effects meta-regression model: age of animals; route of drug administration; MPH total dosage. Age of animals will be extracted and transformed in "days" to be added as a continuous variable in the model. Route of drug administration will be added as a categorical variable using dummy variables. MPH total dosage will be calculated by multiplying the dosage received in mg/kg by the number of administrations per day and by the total days of treatment and added as a continuous variable. Covariates associated with the outcome with a $p \leq 0.1$ in a univariate analysis will be included in a final multivariate meta-regression model, in which an alpha of 5% will be established. Studies with missing values will be excluded from the meta-regression analysis. Meta-regression will be conducted using Stata 13.0 (College Station, TX: StataCorp LP), as previously described (26).

2.6.3. Sensitivity analysis

Sensitivity analyses will be performed in order to evaluate effect size differences related to main methodological decisions in our manuscript. The following sensitivity analyses will be performed for each behavioral category: (1) excluding one result at a time; (2) including only effect sizes extracted from the same behavioral test; (3) including studies with crossover designs; (4) including only one MPH dosage at a time, with a minimum of three studies using

the same dosage; (5) excluding studies presenting a concerning risk of bias, defined as either a high risk of bias in one category or an unclear risk of bias in 7 categories or more.

The objectives of our sensitivity analyses will be to (1) observe if any study skews the overall result; (2) evaluate if the effects of MPH are different for distinct behavioral tests; (3) evaluate possible changes in effect sizes due to a carryover effect from crossover studies; (4) observe if the effects of MPH are different for distinct dosages; (4) evaluate the impact of studies with a high risk of bias. Sensitivity analyses will not be corrected for multiple comparison, and the results will be interpreted as exploratory. A p-value ≤ 0.05 will be considered statistically significant for these analyses.

3. Discussion

The pathophysiology of ADHD is still mostly unknown, and a valid animal model is believed to be essential in order to advance this knowledge. Although the SRH are the most widely used animal model of ADHD, its validity has been questioned by different authors. The poor methodological description, high heterogeneity, and high probability of bias observed in preclinical research seems to be a relevant factor for the interpretation of the published literature (27). In this sense, an overall estimate of the available data obtained using systematic reviews and meta-analyses have become more important over the years. Although they have been already used in the last decades in order to guide clinical practice, systematic reviews and meta-analyses are relatively new in preclinical research (28).

The predictive validity represents the ability of the animal model to respond to well documented treatments for the disorder, and is essential for the characterization of a valid animal model (11). In the SHR, distinct behavioral responses have been reported after a treatment with MPH. For instance, van den Bergh, Bloemarts (14) reported no effects of MPH in the animal model of ADHD, while Tamburella, Micale (29) reported increased locomotion

after MPH administration. A deleterious effect of MPH in attentional performance of SHR has also been demonstrated (30). On the other hand, these results are contradicted by several studies showing decreased locomotion (31-34) and increased attention (31, 35-37) after treatment. Even though these divergences might be explained by methodological aspects including MPH dosage, route of drug administration and age of animals, no study has been conducted in order to evaluate this hypothesis.

By conducting this study, we expect to: (1) provide an overall estimate of the effect size of MPH on behavioral outcomes when administered to the SHR; (2) provide an estimate of the heterogeneity present in the published literature; and (3) identify covariates potentially related to this heterogeneity using meta-regression and sensitivity analyses. Our main hypothesis are: (1) MPH treatment will decrease locomotion and impulsivity, and increase attention and memory performance of the SHR; (2) a high heterogeneity will be found; (3) age of animals, route of drug administration and MPH total dosage are going to be statistically related to the effects.

To sum up, we believe that the results obtained from this study will be valuable in order to corroborate the predictive validity of SHR as an animal model of ADHD. Additionally, our results will provide data on the methodological strengths of published literature, which may aid the development of new experimental designs. In this way, our study will provide a background for the development of new research and for the advance of knowledge in the area.

4. References

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Behavioral effects of methylphenidate in an animal model of attention-deficit/hyperactivity disorder, the spontaneously hypertensive rats: a systematic review and meta-analysis protocol

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Abstract

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent condition related to several negative outcomes. Although a strong neurobiological basis has been demonstrated, its pathophysiology is still poorly understood. In this sense, animal studies are essential in order to unravel the neurobiological factors associated with neuropsychiatric disorders, and the absence of proper animal models may be contributing to the currently challenges in the development of new treatments. The spontaneously hypertensive rats (SHR) are the most commonly used animal model of ADHD. However, its validity, and especially its predictive validity, has been questioned. In this study we aim at summarizing the evidence for the predictive validity of the SHR as an animal model of ADHD. The current protocol discloses the background, aims and methods of a systematic review and meta-analysis of studies reporting behavioral effects of methylphenidate (MPH), the most commonly prescribed treatment for ADHD, in the SHR. In addition, we plan to evaluate how pre-defined covariates influence the effects of MPH using meta-regression and sensitivity analyses.

Keywords: ADHD, SHR, meta-analysis, methylphenidate

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent neurodevelopmental disorder characterized by impairing levels of hyperactivity, impulsivity and inattention (1). Observational studies have shown that ADHD is related to several negative outcomes, including, among others, decreased quality of life (2), increased number of suicide attempts (3), less socioeconomic status (4), and increased mortality due to accidents (5).

A strong neurobiological basis has been repeatedly demonstrated in ADHD. Twin studies showed that ADHD has a heritability of about 70-80% (6), and candidate gene studies suggest mainly the influence of genes related to monoaminergic neurotransmission (7). A recent mega-analysis performed with more than 1500 ADHD patients found a reduced volume in the nucleus accumbens, amygdala, caudate, hippocampus and putamen in patients with the disorder (8). In addition, a meta-analysis of fMRI studies demonstrated reduced activation in distinct cortical region during attention and impulsivity tests (9). Although ADHD neurobiological basis has been confirmed through molecular genetics and neuroimaging studies, its pathophysiology is still poorly understood.

Animal models are considered a fundamental tool to unravel the neurobiological factors associated with neuropsychiatric disorders (10). More important, the absence of proper animal models has been proposed as a main factor driving the slow advancement of new treatments for neuropsychiatric disorders (10). An animal model should present predictive, face and construct validity in order to be considered a proper model of a disease (11). In ADHD, the spontaneously hypertensive rats (SHR) are widely considered the most appropriate model (12, 13). However, there is no consensus on the validity of the SHR as an animal model of ADHD.

Criticism has been demonstrated specially on the predictive validity of the SHR (14, 15), which might be measured by the ability of the animal model to respond to well documented treatment for the disorder (11). In ADHD, the most well documented treatment is the one

performed with stimulant medication (16). Among stimulants, methylphenidate (MPH), a dopamine transporter inhibitor, is the most commonly used (17). Although promoting overall clinical improvement, there is a substantial heterogeneity regarding the response to MPH treatment (18), and about 30% of patients do not present a clinical response (19). Therefore, a well-validated animal model is also essential in order to have a proper tool to understand the variability in the response to MPH in a molecular level. Thus, in this study we intend to disclose the protocols for a systematic review and meta-analysis aiming at summarizing behavioral effects of MPH in the SHR. With this study we aim to answer the following question: does MPH treatment improve behavioral deficits presented by the SHR when compared to placebo? In order to achieve this aim, the following objectives were defined: (1) conduct an extensive literature research in order to select all studies evaluating behavioral effects of MPH in the SHR; (2) extract data; (3) summarize the data using meta-analysis; and (4) explore the influence of pre-defined variables using meta-regression and sensitivity analysis.

2. Methods

Methods are described according to the format proposed by de Vries, Hooijmans (20). This protocol has been previously submitted for the CAMARADES Preclinical Systematic Review & Meta-analysis Facility (SyRF, http://syrf.org.uk/protocols/) with the title "Behavioral effects of methylphenidate in an animal model of attention-deficit/hyperactivity disorder, the spontaneously hypertensive rats: a systematic review and meta-analysis".

2.1. Search and study identification

We are going to include studies that administered MPH to SHR and evaluated one of the following behavioral outcomes: locomotion, attention, impulsivity or memory. Studies will be identified through a literature search using three different electronic databases: Medline,

Scopus and Web of Science. The complete search strategy can be found in Supplementary material 1. No search filters are going to be used. In addition, we are going to search the reference list of included studies.

2.2. Study selection

The study selection and inclusion is going to be performed with a pre-screening based on title and abstract. If an exclusion criteria is not clearly observed (e.g., reviews or editorials, studies in human subjects), a full-text review will be performed. Both will be conducted by two independent authors (D.L. and A.P.), and any disagreement will be discussed with a third author (E.G.). There will be no date or language restrictions. The following steps will be conducted for the inclusion and exclusion of studies:

Step 1: All title and abstracts are going to be reviewed by two independent authors (D.L. and A.P.). Those reporting experimental studies evaluating the effects of MPH in the SHR are going to be selected for the next step. The following exclusion criteria will be applied: use of SHR substrains (e.g., stroke-prone SHR), MPH administered in brain slices, MPH administered only together with another drug, MPH self-administration. If the information is not sufficient to exclude the study, a full-text review will be conducted;

Step 2: Studies presenting behavioral outcomes are going to be selected for the next step. The behavioral outcomes are going to be categorized according to the author´s description. Studies with no behavioral outcomes will be excluded;

Step 3: Studies reporting locomotion, attention, impulsivity or memory outcomes are going to be included for final analysis. The following exclusion criteria will be applied: distinct behavioral outcomes from the ones previously presented, use of a crossover approach, not comparing the SHR-MPH to a SHR-placebo group. Studies using a crossover approach,

meaning that the same rats will be used as active and placebo groups, will be excluded in order to avoid a carryover effect (21).

2.3. Study characteristics to be extracted

For the assessment of external validity, the following items will be extracted: study authors, year of publication, experimental groups, sample size, age and gender of animals, route of drug administration, MPH dosage in mg/kg, number of administrations per day, total days of treatment, behavioral test used, and outcome of interest.

2.4. Risk of bias assessment

Risk of bias assessment will be conducted by one review (A.P.) author using the SYRCLE´s risk of bias tool for animal studies (22). Any doubt in the assessment will be discussed with a second reviewer (D.L.). Ten items will be evaluated in the quality assessment: three related to selection bias (sequence generation, baseline characteristic and allocation concealment); two related to performance bias (random housing and blinding); two related to detection bias (random outcome assessment and blinding); one related to attrition bias (incomplete outcome data); one related to reporting bias (selective outcome reporting); and one related to other sources of bias. Baseline characteristics will be comprised by information on sex and age of animals. The tenth item will address sources of bias beyond the ones covered by other domains. Each study will be evaluated considering the ten items, and for each item the study will be classified as presenting low, unclear or high risk of bias. Publication bias will be accessed using funnel plots and the Egger´s regression test.

2.5. Collection of outcome data

Continuous outcome data from behavioral tests will be extracted by two independent authors (D.L. and A.P.), an any disagreement will be discussed with a third author (E.G.). Data will be extracted directly from the full-text article. When not reported in enough details, extraction will be done by graph estimation using a digital ruler, as previously described (23, 24). If both methods are not viable, the authors will be contacted. Two attempt will be performed within a two-week interval. If no response is obtained, the article will be excluded. Each included article will be divided in experiments, defined as any case when a control group is compared to an experimental group. If the same animals were subjected to more than one behavioral experiment from the same category, only one will be included (either the most relevant or the first one to be performed). In addition, only one outcome will be extracted from each behavioral experiment. Whenever the article report multiple outcomes from the same behavioral test, the extraction will be performed according to a relevance rank organized by one reviewer. Variables from each behavioral test will be ranked subjectively according to their importance, and the one ranked highest will be extracted. If the same animals were evaluated more than once in the same behavioral test, the last one will be selected for data extraction. If the manuscript divides the results by time, the first time point will be selected for extraction.

2.6. Data analysis

2.6.1. Meta-analysis

Studies will be grouped according to the behavioral outcomes reported (locomotion, attention, impulsivity or memory), and a meta-analysis will be performed for each group. Pooled effect sizes will be determined with standardized mean differences using Hedge´s G method with random-effects, allowing the comparison among distinct behavioral tests. The significance of pooled effect sizes will be determined using the Z-test. Since we will perform four independent meta-analyses, a Bonferroni correction will be applied, and a p-value ≤

0.0125 will be considered as statistically significant. Individual study weights will be obtained using the inverse of the variance. Data will be transformed in order to obtain positive values for decreased locomotion or impulsive behavior and for increased attentional or memory performances.

Heterogeneity between studies will be assessed using both the Chi^2 and the I^2 tests, and a p-value ≤ 0.1 will be considered as statistically significant. A I^2 value on the order of 25%, 50% and 75% will be considered as low, moderate, and high heterogeneity, respectively (25). Standardized mean differences and heterogeneity values will be obtained using Review Manager (RevMan) version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

2.6.2. Meta-regression

A meta-regression will be conducted in order to evaluate potential sources of variability among studies. The following covariates will be selected based on biological plausibility and added to a random-effects meta-regression model: age of animals; route of drug administration; MPH total dosage. Age of animals will be extracted and transformed in "days" to be added as a continuous variable in the model. Route of drug administration will be added as a categorical variable using dummy variables. MPH total dosage will be calculated by multiplying the dosage received in mg/kg by the number of administrations per day and by the total days of treatment and added as a continuous variable. Covariates associated with the outcome with a $p \leq 0.1$ in a univariate analysis will be included in a final multivariate meta-regression model, in which an alpha of 5% will be established. Studies with missing values will be excluded from the meta-regression analysis. Meta-regression will be conducted using Stata 13.0 (College Station, TX: StataCorp LP), as previously described (26).

2.6.3. Sensitivity analysis

Sensitivity analyses will be performed in order to evaluate effect size differences related to main methodological decisions in our manuscript. The following sensitivity analyses will be performed for each behavioral category: (1) the jackknife method, a common procedure used to test the stability of the outcome after excluding one result at a time (27); (2) including only effect sizes extracted from the same behavioral test; (3) including studies with crossover designs; (4) including only one MPH dosage at a time, with a minimum of three studies using the same dosage; (5) excluding studies presenting a concerning risk of bias, defined as either a high risk of bias in one category or an unclear risk of bias in 7 categories or more.

The objectives of our sensitivity analyses will be to (1) observe if any study skews the overall result; (2) evaluate if the effects of MPH are different for distinct behavioral tests; (3) evaluate possible changes in effect sizes due to a carryover effect from crossover studies; (4) observe if the effects of MPH are different for distinct dosages; (4) evaluate the impact of studies with a high risk of bias. Sensitivity analyses will not be corrected for multiple comparison, and the results will be interpreted as exploratory. A p-value ≤ 0.05 will be considered statistically significant for these analyses.

3. Discussion

The pathophysiology of ADHD is still mostly unknown, and a valid animal model is believed to be essential in order to advance this knowledge. Although the SRH are the most widely used animal model of ADHD, its validity has been questioned by different authors. The poor methodological description, high heterogeneity, and high probability of bias observed in preclinical research seems to be a relevant factor for the interpretation of the published literature (28). In this sense, an overall estimate of the available data obtained using systematic reviews and meta-analyses have become more important over the years. Although they have

been already used in the last decades in order to guide clinical practice, systematic reviews and meta-analyses are relatively new in preclinical research (29).

The predictive validity represents the ability of the animal model to respond to well documented treatments for the disorder, and is essential for the characterization of a valid animal model (11). In the SHR, distinct behavioral responses have been reported after a treatment with MPH. For instance, van den Bergh, Bloemarts (14) reported no effects of MPH in the animal model of ADHD, while Tamburella, Micale (30) reported increased locomotion after MPH administration. A deleterious effect of MPH in attentional performance of SHR has also been demonstrated (31). On the other hand, these results are contradicted by several studies showing decreased locomotion (32-35) and increased attention (32, 36-38) after treatment. Even though these divergences might be explained by methodological aspects including MPH dosage, route of drug administration and age of animals, no study has been conducted in order to evaluate this hypothesis.

By conducting this study, we expect to: (1) provide an overall estimate of the effect size of MPH on behavioral outcomes when administered to the SHR; (2) provide an estimate of the heterogeneity present in the published literature; and (3) identify covariates potentially related to this heterogeneity using meta-regression and sensitivity analyses. Our main hypothesis are: (1) MPH treatment will decrease locomotion and impulsivity, and increase attention and memory performance of the SHR; (2) a high heterogeneity will be found; (3) age of animals, route of drug administration and MPH total dosage are going to be statistically related to the effects.

A detailed description of the methodology to be applied in systematic reviews and meta-analyses has many advantages, including expert external opinion on the methods, prevention of data fishing, and the possibility of helping researchers in future applications. Additionally, protocol publication is an opportunity to discuss potential sources of bias that

may be present in the future. In this sense, the following factors should be highlighted from our methodology. First, we are going to include only one data point for each behavioral test, which will be selected based on a subjective relevance rank. Although distinct approaches were available for the extraction (e.g. the combination of multiple variables into a single data point), we believe that the use of only one variable may ensure increased consistency among results. It is known that the same behavioral test can be performed in order to investigate distinct behavioral parameters. As an example, the open field test can be used to measure locomotion (39), anxiety-related behavior (40), or spatial memory (41), based primarily on which variable is collected. Therefore, even though our approach may present a risk of bias for being based on a subjective evaluation of importance, we believe that the grouping of multiple variables into a single data point would be an even greater threat since it would combine distinct information, thus increasing heterogeneity. Another important aspect is related to our meta-regression model. As previously mentioned, studies with missing values will be excluded from the analysis, which can bias the final results.

To sum up, we believe that the results obtained from this study will be valuable in order to corroborate the predictive validity of SHR as an animal model of ADHD. Additionally, our results will provide data on the methodological strengths of published literature, which may aid the development of new experimental designs. In this way, our study will provide a background for the development of new research and for the advance of knowledge in the area.

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Behavioral effects of methylphenidate in an animal model of attention-deficit/hyperactivity disorder, the spontaneously hypertensive rats: a systematic review and meta-analysis protocol

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Abstract

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent condition related to several negative outcomes. Although a strong neurobiological basis has been demonstrated, its pathophysiology is still poorly understood. In this sense, proper animal models are needed to further unravel the neurobiological basis of ADHD. The spontaneously hypertensive rats (SHR) are the most commonly used animal model of ADHD. However, its validity, and especially its predictive validity, has been questioned. The current protocol discloses the background, aims and methods of a systematic review and meta-analysis of studies reporting behavioral effects of methylphenidate (MPH), the most commonly prescribed treatment for ADHD, in the SHR. The following behavioral outcomes will be evaluated: locomotion, attention, impulsivity and memory. In addition, we plan to evaluate how pre-defined covariates influence the effects of MPH using meta-regression and sensitivity analyses.

Keywords: ADHD, SHR, meta-analysis, methylphenidate

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent neurodevelopmental disorder characterized by impairing levels of hyperactivity, impulsivity and inattention (1). Observational studies have shown that ADHD is related to several negative outcomes, including, among others, decreased quality of life (2), increased number of suicide attempts (3), less socioeconomic status (4), and increased mortality due to accidents (5).

A strong neurobiological basis has been repeatedly demonstrated in ADHD. Twin studies showed that ADHD has a heritability of about 70-80% (6), and significant genome-wide hits have been reported, especially in neurodevelopmental processes that are relevant to ADHD (7). A recent meta-analysis performed with more than 1500 ADHD patients found a reduced volume in the nucleus accumbens, amygdala, caudate, hippocampus and putamen in patients with the disorder (8). In addition, a meta-analysis of functional magnetic resonance imaging studies demonstrated reduced activation in distinct cortical region during attention and impulsivity tests (9). Even though molecular genetics and neuroimaging studies have been improving our understanding of the disorder, the pathophysiology of ADHD is still poorly understood.

Animal models are considered a fundamental tool to unravel the neurobiological factors associated with neuropsychiatric disorders (10). More importantly, the absence of proper animal models has been proposed as a main factor driving the slow advancement of new treatments for neuropsychiatric disorders (10). An animal model should present predictive, face and construct validity in order to be considered a proper model of a disease (11). In ADHD, the spontaneously hypertensive rats (SHR) are widely considered the most appropriate model (12, 13). Evidence has shown that the SHR present face validity (12, 13), which corresponds to the extent of similarities between the animal model and the disorder. The evaluation of construct validity, which represents the resemblance between the

etiological processes in patients and animal model, is still a challenge because the pathophysiology of ADHD is mostly unknown. In addition, there is no consensus on the predictive and construct validity of the SHR as an animal model of ADHD.

Construct validity depends on the understanding of the pathophysiology of the disorder, while predictive validity depends on the similarity between the response to treatment in both humans and animals. Criticism has been demonstrated on the predictive validity of the SHR (14, 15), which might be measured by the ability of the animal model to respond to well documented treatment for the disorder (11). In ADHD, the most well documented treatment is the one performed with stimulant medication (16). Among stimulants, methylphenidate (MPH), a dopamine transporter inhibitor, is the most commonly used (17). Although promoting overall clinical improvement, there is a substantial heterogeneity regarding the response to MPH treatment (18), and about 30% of patients do not present a clinical response (19). Therefore, a well-validated animal model is also essential in order to have a proper tool to understand the variability in the response to MPH on a molecular level. Thus, in this study we intend to disclose the protocols for a systematic review and meta-analysis aiming at summarizing behavioral effects of MPH in the SHR. With this study we aim to answer the following question: does MPH treatment improve behavioral deficits presented by the SHR when compared to placebo? In order to achieve this aim, the following objectives were defined: (1) conduct an extensive literature research in order to select all studies evaluating behavioral effects of MPH in the SHR; (2) extract data; (3) summarize the data using meta-analysis; and (4) explore the influence of pre-defined variables using meta-regression and sensitivity analysis. Since MPH is the pharmacological treatment most commonly prescribed for ADHD patients, our results will be important in order to reinforce the predictive validity of the SHR.

2. Methods

Methods are described according to the format proposed by de Vries, Hooijmans (20). This protocol has been previously submitted for the CAMARADES Preclinical Systematic Review & Meta-analysis Facility (SyRF, http://syrf.org.uk/protocols/) with the title "Behavioral effects of methylphenidate in an animal model of attention-deficit/hyperactivity disorder, the spontaneously hypertensive rats: a systematic review and meta-analysis".

2.1. Search and study identification

We are going to include studies that administered MPH to SHR and evaluated one of the following behavioral outcomes: locomotion, attention, impulsivity or memory. Studies will be identified through a literature search using three different electronic databases: Medline, Embase and Web of Science. The complete search strategy can be found in Supplementary material 1. No search filters are going to be used. In addition, we are going to search the reference list of included studies.

2.2. Study selection

The study selection and inclusion is going to be performed with a pre-screening based on title and abstract. If an exclusion criterion is not clearly observed (e.g., reviews or editorials, studies in human subjects), a full-text review will be performed. Both will be conducted by two independent authors (D.L. and A.P.), and any disagreement will be discussed with a third author (E.G.). There will be no date or language restrictions. We are going to include experimental studies evaluating the effects of MPH in the SHR in the following behavioral outcomes: locomotion, attention, impulsivity or memory. Only studies that administered MPH to SHR and have a control group will be included in the analysis. The following exclusion criteria will be applied: use of SHR substrains (e.g., stroke-prone SHR),

MPH administered in brain slices, MPH administered only together with another drug, and MPH self-administration. Studies using a crossover approach, meaning that the same rats will be used as active and placebo groups, will be excluded in order to avoid a carryover effect (21).

2.3. Study characteristics to be extracted

For the assessment of external validity, the following items will be extracted: study authors, year of publication, experimental groups, sample size, age and sex of animals, route of drug administration, MPH dosage in mg/kg, number of administrations per day, total days of treatment, behavioral test used, and outcome of interest.

2.4. Risk of bias assessment

Risk of bias assessment will be conducted by one review (A.P.) author using the SYRCLE´s risk of bias tool for animal studies (22). Any doubt in the assessment will be discussed with a second reviewer (D.L.). Ten items will be evaluated in the quality assessment: three related to selection bias (sequence generation, baseline characteristic and allocation concealment); two related to performance bias (random housing and blinding); two related to detection bias (random outcome assessment and blinding); one related to attrition bias (incomplete outcome data); one related to reporting bias (selective outcome reporting); and one related to other sources of bias. Baseline characteristics will be comprised by information on sex and age of animals. The tenth item will address sources of bias beyond the ones covered by other domains. Each study will be evaluated considering the ten items, and for each item the study will be classified as presenting low, unclear or high risk of bias. Publication bias will be accessed using funnel plots and the Egger´s regression test.

2.5. Collection of outcome data

Continuous outcome data from behavioral tests will be extracted by two independent authors (D.L. and A.P.), an any disagreement will be discussed with a third author (E.G.). Data will be extracted directly from the full-text article. When not reported in enough details, extraction will be done by graph estimation using a digital ruler, as previously described (23, 24). If both methods are not viable, the authors will be contacted. Two attempts will be performed within a two-week interval. If no response is obtained, the article will be excluded. Each included article will be divided in experiments, defined as any case when a control group is compared to an experimental group. If the same animals were subjected to more than one behavioral experiment from the same category (e.g. two distinct locomotion tests), both data will be extracted and the one with the largest effect size will be included in the analysis. In addition, only one outcome will be extracted from each behavioral experiment. Whenever the article reports multiple outcomes from the same behavioral test, the extraction will be performed according to a relevance rank organized by one reviewer. Variables from each behavioral test will be ranked subjectively according to their importance, and the one ranked highest will be extracted. If the same animals were evaluated more than once in the same behavioral test, the last one will be selected for data extraction. If the manuscript divides the results by time, the first time point will be selected for extraction.

2.6. Data analysis

2.6.1. Meta-analysis

Studies will be grouped according to the behavioral outcomes reported (locomotion, attention, impulsivity or memory), and a meta-analysis will be performed for each group. A minimum of three experiments will be required in order to conduct the meta-analysis. Pooled effect sizes will be determined with standardized mean differences using Hedge´s G method

with random-effects, allowing the comparison among distinct behavioral tests. The significance of pooled effect sizes will be determined using the Z-test. Since four statistical hypothesis tests will be performed, a Bonferroni correction will be applied in order to control the family-wise type I error rate, and a p-value ≤ 0.0125 will be considered as statistically significant. Individual study weights will be obtained using the inverse of the variance. Data will be transformed in order to obtain positive values for decreased impulsive behavior and for increased attentional or memory performances.

Heterogeneity between studies will be assessed using both the Chi^2 and the I^2 tests, and a p-value ≤ 0.1 will be considered as statistically significant. A I^2 value on the order of 25%, 50% and 75% will be considered as low, moderate, and high heterogeneity, respectively (25). Standardized mean differences and heterogeneity values will be obtained using Review Manager (RevMan) version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

2.6.2. Meta-regression

A meta-regression will be conducted for each behavioral outcome in order to evaluate potential sources of variability among studies. The following covariates will be selected based on biological plausibility and added to a univariate random-effects meta-regression model: age of animals; route of drug administration; MPH total dosage. Age of animals will be categorized in "adolescent" or "adult" before being included in the model, since some studies may not report the age in days or weeks. Animals 60 days old or more will be defined as "adults", while animals 28 to 60 days old will be defined as "adolescents" (26). Route of drug administration will be added as a categorical variable using dummy variables. MPH total dosage will be calculated by multiplying the dosage received in mg/kg by the number of administrations per day and by the total days of treatment and added as a continuous variable.

Covariates associated with the outcome with a $p \leq 0.1$ in a univariate analysis will be included in a final multivariate meta-regression model. Since up to four statistical hypothesis tests will be performed in the multivariate model, a Bonferroni correction will be applied in order to control the family-wise type I error rate. Studies with missing values will be excluded from the meta-regression analysis. Meta-regression will be conducted using Stata 13.0 (College Station, TX: StataCorp LP), as previously described (27).

2.6.3. Sensitivity analysis

Sensitivity analyses will be performed in order to evaluate effect size differences related to main methodological decisions in our manuscript. The following sensitivity analyses will be performed for each behavioral category: (1) the jackknife method, a common procedure used to test the stability of the outcome after excluding one result at a time (28); (2) including only effect sizes extracted from the same behavioral test, with a minimum of three experiments; (3) including studies with crossover designs; (4) including only one MPH dosage at a time, with a minimum of three studies using the same dosage; (5) excluding studies presenting a concerning risk of bias, defined as either a high risk of bias in one category or an unclear risk of bias in 7 categories or more.

The objectives of our sensitivity analyses will be to (1) observe if any study skews the overall result; (2) evaluate if the effects of MPH are different for distinct behavioral tests; (3) evaluate possible changes in effect sizes due to a carryover effect from crossover studies; (4) observe if the effects of MPH are different for distinct dosages; (5) evaluate the impact of studies with a high risk of bias. Sensitivity analyses will not be corrected for multiple comparison, and the results will be interpreted as exploratory. A p-value ≤ 0.05 will be considered statistically significant for these analyses.

3. Discussion

The pathophysiology of ADHD is still mostly unknown, and a valid animal model is believed to be essential in order to advance this knowledge. Although the SRH are the most widely used animal model of ADHD, its validity has been questioned by different authors. The poor methodological description, high heterogeneity, and high probability of bias observed in preclinical research seems to be a relevant factor for the interpretation of the published literature (29). In this sense, an overall estimate of the available data obtained using systematic reviews and meta-analyses has become more important over the years. Although they have been already used in the last decades in order to guide clinical practice, systematic reviews and meta-analyses are relatively new in preclinical research (30).

The predictive validity represents the ability of the animal model to respond to well documented treatments for the disorder, and is essential for the characterization of a valid animal model (11). In this sense, understanding the effects of MPH in the SHR is of great importance since MPH is the pharmacological treatment most commonly used in ADHD. However, we also want to highlight that other pharmacological approaches available for ADHD will not be included in our analysis, thus limiting our conclusions. In the SHR, distinct behavioral responses have been reported after a treatment with MPH. For instance, van den Bergh, Bloemarts (14) reported no effects of MPH in the animal model of ADHD, while Tamburella, Micale (31) reported increased locomotion after MPH administration. A deleterious effect of MPH in attentional performance of SHR has also been demonstrated (32). On the other hand, these results are contradicted by several studies showing decreased locomotion (33-36) and increased attention (33, 37-39) after treatment. Even though these divergences might be explained by methodological aspects including MPH dosage, route of drug administration and age of animals, no study has been conducted in order to evaluate this hypothesis.

By conducting this study, we expect to: (1) provide an overall estimate of the effect size of MPH on behavioral outcomes when administered to the SHR; (2) provide an estimate of the heterogeneity present in the published literature; and (3) identify covariates potentially related to this heterogeneity using meta-regression and sensitivity analyses. Our main hypothesis are: (1) MPH treatment will decrease locomotion and impulsivity, and increase attention and memory performance of the SHR; (2) a high heterogeneity will be found; (3) age of animals, route of drug administration and MPH total dosage are going to be statistically related to the effects.

A detailed description of the methodology to be applied in systematic reviews and meta-analyses has many advantages, including expert external opinion on the methods, prevention of data fishing, and the possibility of helping researchers in future applications. Additionally, protocol publication is an opportunity to discuss potential sources of bias that may be present in the future. In this sense, the following factors should be highlighted from our methodology. First, we are going to include only one data point for each behavioral test, which will be selected based on a subjective relevance rank. Although distinct approaches were available for the extraction (e.g. the combination of multiple variables into a single data point), we believe that the use of only one variable may ensure increased consistency among results. It is known that the same behavioral test can be performed in order to investigate distinct behavioral parameters. As an example, the open field test can be used to measure locomotion (40), anxiety-related behavior (41), or spatial memory (42), based primarily on which variable is collected. Therefore, even though our approach may present a risk of bias for being based on a subjective evaluation of importance, we believe that the grouping of multiple variables into a single data point would be an even greater threat since it would combine distinct information, thus increasing heterogeneity. Another important aspect is

related to our meta-regression model. As previously mentioned, studies with missing values will be excluded from the analysis, which can bias the final results.

To sum up, we believe that the results obtained from this study will be valuable in order to corroborate the predictive validity of SHR as an animal model of ADHD. Additionally, our results will provide data on the methodological strengths of published literature, which may aid the development of new experimental designs. In this way, our study will provide a background for the development of new research and for the advance of knowledge in the area.

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