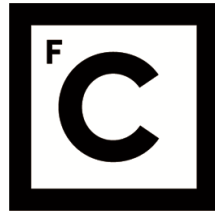


UNIVERSIDADE DE LISBOA

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**Ciências  
ULisboa**

**Animal use in Major Depressive Disorder: a necessary evil?  
Assessing the past to improve the future**

*“Documento Definitivo”*

**Doutoramento em Biologia**  
Especialidade Biotecnologia

Maria Constança Dias Pinheiro de Oliveira Carvalho

Tese orientada por:

Professor Doutor Andrew David Knight

Professor Doutor Luís António de Matos Vicente

Professor Doutor Tiago André Lamas Oliveira Marques

Documento especialmente elaborado para a obtenção do grau de doutor

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## **Nota Prévia**

A presente tese contém capítulos já publicados (Capítulos 2, 4, 5 e 7) e outros submetidos (Capítulos 3 e 6) de acordo com o Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, E no Despacho N.º 4624/2012 do Diário da República II série n.º 65 de 30 de março de 2012. A candidata realizou os trabalhos em colaboração, mas liderou e participou integralmente na concepção dos mesmos, desde a obtenção dos dados, análise estatística, discussão dos resultados e redação dos manuscritos.

Lisboa, 26 de janeiro de 2020

Maria Constança Dias Pinheiro de Oliveira Carvalho

Este trabalho é dedicado ao seguinte conjunto de pessoas:

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# Abstract

Animal models are widely used in research aimed at advancing human healthcare, although their utility for this purpose is more often presumed, than studied. In this thesis I evaluate the contribution of animal models to current knowledge of Major Depressive Disorder (MDD), a poorly understood mental disorder of multifactorial origin that affects thousands of people worldwide. My hypothesis is that if animal models are contributing meaningfully to medical advances, then animal studies will be well cited by human medical literature.

Accordingly, and after conducting a pilot study on ADHD (Chapter 2), I conducted a citation analysis on studies which used rats (Chapter 3) and non-human primates (NHP) (Chapter 4) as models for MDD research. The number of citations of these papers by human medical papers was low.

To determine if the low number of citations could be caused by the need for sufficient evidence to accumulate within a field, before a medical breakthrough can be reached, I determined if the citations were by papers on the same disorder, or on unrelated disorders (Chapter 5).

In an attempt to determine if low citation numbers are common to all indirect research approaches, I compared the number and relevance of citations of *in silico*, *in vitro* and NHP studies, by human medical papers. Other research approaches more effectively informed human research, than NHP models (Chapter 4). I also quantified the citations of other research methods by subsequent animal studies. Citations were low, contrary to common expectations that *in vitro* and *in silico* inform subsequent animal studies (Chapter 6).

Overall, these results indicate that animal models make poor contributions to human mental disorders research. This merits a change in the extant paradigm in biomedical research, at least in some human disorders, as proposed in Chapter 7.

**Key words:** animal use alternatives, Major Depressive Disorder, citation analysis, rats, non-human primates.



## Resumo

A experimentação animal é amplamente utilizada na investigação biomédica, nomeadamente na área da saúde mental. O objetivo desta tese é avaliar o contributo dos modelos animais para o conhecimento atual sobre as doenças mentais utilizando como estudo de caso a Perturbação Depressiva Major (PDM).

A doença mental pode ser descrita como um padrão comportamental derivado de causas biológicas e ambientais que provoca sofrimento e incapacidade funcional aos indivíduos afetados. Existem mais de 200 perturbações mentais, estas são particularmente complexas não só devido à sua origem multifatorial, mas também devido à multiplicidade dos seus sintomas- alguns impossíveis de mimetizar em modelos animais (e.g. sentimento de culpa excessiva no caso da PDM).

A PDM é o tipo mais grave e prevalente de depressão. Caracteriza-se pela existência de um episódio depressivo major. Durante este período com duração mínima de duas semanas, os indivíduos afetados podem experimentar alterações aos seus padrões habituais de sono e alimentação, humor deprimido, sentimentos de fadiga, perda de interesse e prazer nas atividades quotidianas, sentimentos de auto desvalorização, ideação suicida entre outros.

É fundamental compreender qual o contributo real da investigação com modelos animais para as doenças mentais. Esta compreensão é relevante não só pelas questões éticas levantadas pelo uso de animais em procedimentos- frequentemente invasivos e dolorosos- mas também pelos recursos alocados a este tipo de investigação, que poderiam estar afetos a outros modelos de investigação, mais promissores para a evolução da medicina.

Milhares de animais são utilizados anualmente para aumentar o nosso conhecimento sobre a PDM, porém o seu real contributo nunca foi sistematicamente avaliado, sendo esse o objetivo geral desta tese.

Se os estudos efetuados em modelos animais forem suficientemente relevantes é esperado que haja transferência de conhecimento de uma área para outra i.e. dos modelos animais para os humanos. Para testar esta hipótese foram formuladas um conjunto de previsões. Se os estudos efetuados em modelos animais forem suficientemente relevantes espera-se que:

- (Quase) todos sejam citados pelo menos uma vez nos artigos médicos sobre a patologia sobre a qual incidem;
- O número de citações em artigos com humanos seja elevado;

- As citações sejam importantes para a hipótese ou método dos artigos em que são citados;
- O número de citações recebidas pelos estudos efetuados em modelos animais seja superior ao número de citações recebidas pelos estudos efetuados noutros modelos indiretos;

O primeiro capítulo empírico desta tese (Capítulo 2) consiste num estudo piloto para testar e ajustar a metodologia escolhida. Assim, optei por estudar a Perturbação de Hiperatividade com Défice de Atenção (PHDA), uma patologia menos estudada que a PDM mas que partilha com esta características relevantes (e.g. o facto de ser uma doença de origem multifatorial com sintomas impossíveis de mimetizar em modelos animais). O facto de a PHDA ser menos estudada tornou possível analisar todos os artigos publicados sobre a mesma recorrendo a modelos animais. Assim, comecei por localizar no PubMed todas as publicações disponíveis sobre esta patologia que descrevem investigação original com modelos animais. Seguidamente, e recorrendo ao Web of Science, contabilizei todas as citações recebidas por estes artigos e determinei se as mesmas pertenciam a artigos de investigação com humanos, experimentação animal, artigos de revisão ou outro tipo de artigos (e.g. artigos de *in vitro*). Dos 211 artigos localizados, 43% nunca foram citados em publicações subsequentes de investigação humana. Adicionalmente, cerca de metade das 6,406 citações recebidas por estes artigos pertenciam a artigos de experimentação animal, enquanto apenas cerca de 8% provinham de artigos de investigação clínica. Os artigos humanos sobre PHDA que citaram os artigos analisados foram lidos cautelosamente por dois investigadores que determinaram qual a relevância do artigo citado para o artigo que o citava, concluindo que apenas cinco artigos foram relevantes para a hipótese em estudo. Este estudo conclui que o contributo da experimentação animal para a compreensão e tratamento desta patologia tem sido muito reduzido.

O Capítulo 3 descreve os resultados da análise de citações realizada em artigos originais sobre PDM que usaram ratas como modelos. Usando o PubMed e o Scopus localizei 178 publicações, citadas 8,712 vezes. Porém, tal como sucedeu no estudo descrito no Capítulo 2, apenas uma pequena percentagem (menos de 10%) das citações obtidas proveio de artigos originais de investigação com humanos, enquanto, mais de metade das citações recebidas pelos artigos localizados foram de artigos subsequentes de experimentação animal. Cerca de 30% dos artigos analisados não foram citados em nenhuma publicação médica com pacientes humanos e 49% não foram citados por nenhuma publicação médica sobre a PDM.

O quarto capítulo descreve um estudo comparativo entre várias metodologias. Mais concretamente localizei no PubMed todos os estudos recorrendo a modelos *in vitro*, *in silico* e

primatas não-humanos (PNH) focados na investigação da PDM. Com recurso ao Web of Science, analisei as citações dos artigos localizados, comparando as médias de citação de cada artigo. Foi possível concluir que os artigos que recorriam à metodologia *in vitro* eram, em média, os mais citados. No que se refere às citações feitas por artigos de medicina humana, tanto os artigos *in vitro* como os artigos *in silico* foram mais citados, em média, que os artigos de PNH. Para as citações de artigos de medicina humana sobre depressão, os artigos *in vitro* foram, em média, significativamente mais citados. Tal como no estudo descrito no Capítulo 2 desta tese, os artigos humanos sobre depressão que citaram os artigos em estudo foram lidos por dois investigadores, a fim de determinar a relevância dos mesmos para os artigos que os citam. Embora a amostra fosse insuficiente para obter uma potência de teste razoável, é visível uma diferença prática: A probabilidade de um estudo com PNH contribuir com relevância para um estudo subsequente em humanos é de apenas 16%, enquanto que tanto os artigos *in vitro* como os artigos *in silico* apresentam uma probabilidade de 25%.

O Capítulo 5 é um estudo piloto que inclui 50 publicações aleatórias (25 de PHDA e 25 de PDM). Após verificar que os estudos biomédicos com modelos animais são maioritariamente citados por estudos subsequentes que também recorrem a modelos animais tornou-se imperativo verificar se estes estudos eram na mesma doença ou se incidiam sobre outras doenças. Se a primeira opção se verificasse, isso poderia indiciar que há necessidade de acumular uma grande quantidade de dados até alcançar uma descoberta relevante para a investigação com pacientes humanos. No segundo caso, poderia indiciar a existência de um efeito lock-in, isto é, o paradigma alimentar-se a si mesmo por resistência à mudança. Os resultados deste estudo revelaram que a maioria dos estudos que citavam a amostra não eram na mesma doença indiciando a existência do efeito lock-in.

O sexto capítulo tem por objetivo testar se, pelo menos no caso da PDM, o paradigma vigente da investigação biomédica é, efetivamente, aplicado. As boas práticas e a legislação vigente em vários países requerem que sempre que possível os modelos animais sejam substituídos por métodos alternativos. Quando tal não é possível, o número de animais utilizados deve ser reduzido ao mínimo e o seu sofrimento deve ser, por todos os meios, minimizado. Assim, na investigação biomédica os modelos *in vitro* e *in silico* são habitualmente utilizados como um primeiro passo que irá determinar que medicamentos e/ou intervenções prosseguem para os ensaios com modelos animais. Os artigos analisados revelam que do total de citações recebidas pelos artigos *in vitro* apenas 18% são provenientes de artigos que recorreram a modelos animais. No que concerne aos modelos *in silico*, apenas 5% das citações recebidas por estes artigos são oriundas de artigos que utilizaram modelos animais. Os

resultados revelam que, pelo menos no que se refere à PDM, as recomendações legais e de boas práticas não estão a ser cumpridas. Porém a amostra é muito pequena (38 artigos *in vitro* e 29 *in silico*), pelo que as interpretações decorrentes dos mesmos devem ser feitas com cautela.

O Capítulo 7 é um artigo teórico que propõe uma mudança de paradigma na investigação biomédica. Atualmente a legislação que regula a investigação biomédica com animais baseia-se no utilitarismo, enquanto a investigação biomédica com seres humanos segue princípios deontológicos. Este capítulo explora as fragilidades do utilitarismo para esta finalidade, utilizando como exemplo os PNH, propondo que sejam adotados na investigação biomédica os mesmos princípios deontológicos que regem a investigação com seres humanos.

Os resultados desta tese indicam que a investigação com modelos animais contribuiu pouco para o conhecimento atual sobre a etiologia, patogénese e, sobretudo cura, da PDM. Os dados obtidos sugerem, também, que esta tendência pode ser comum a outras doenças mentais. Os resultados apontam, ainda, no sentido de haver outras abordagens de investigação mais promissoras para o desenvolvimento da medicina. Em face dos resultados apresentados parece necessária do ponto de vista ético, mas acima de tudo, do ponto de vista dos necessários impactos positivos para a própria medicina, uma mudança de paradigma na investigação biomédica. Considerando o exposto, espero, com este trabalho, contribuir para essa mudança de paradigma.

**Palavras-chave:** alternativas à experimentação animal, Perturbação Depressiva Major, Depressão Major, análise de citações, ratazanas, primatas não-humanos.

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# **CHAPTER 1**

## **Introduction**

# Chapter 1. Introduction

## Animal Experimentation

Animal experimentation can be defined as the use of non-human animals in scientific experiments that seek to control variables that affect the animals' behaviour or biological systems. Animal experimentation is at least 2500 years old (Mayir et al., 2016). It became more popular during the Renaissance, expanded substantially in the second half of the XIX century and has been increasing ever since (Knight, 2019).

At least 192.1 million animals are used annually worldwide in scientific procedures (Taylor & Alvarez, 2020). According to the latest available data (from 2015 to 2017) on the use of animals for scientific purposes within the European Union (EU), the majority of the 9.58 million animals were used for basic or applied research, as shown in Figure 1.

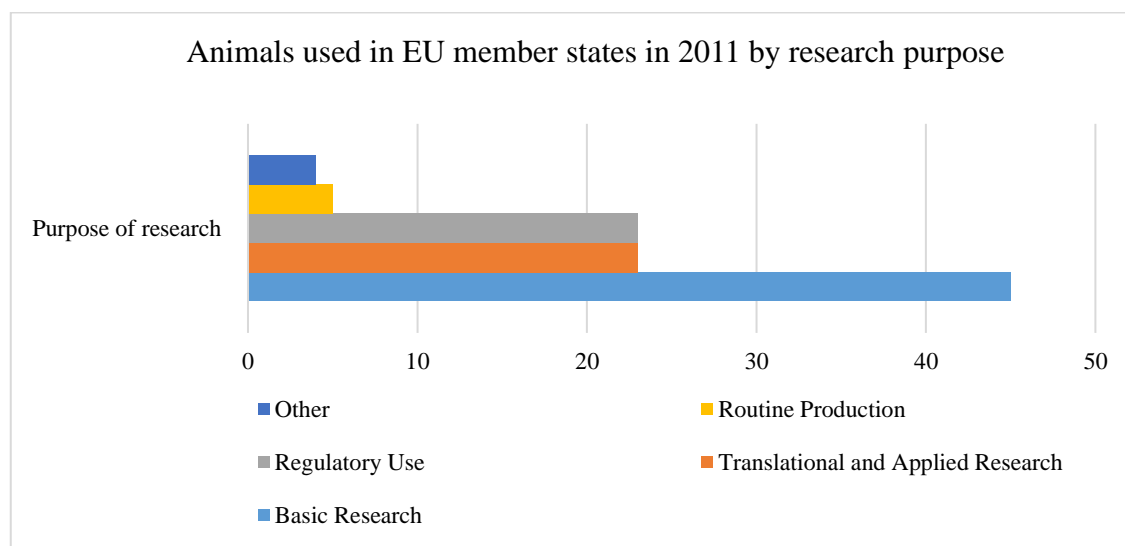


Figure 1. 1 Animals used in EU member states in 2011 by research purpose (adapted from EC, 2020)

Fundamental research corresponds to studies conducted with the aim of increasing scientific knowledge, without an obvious practical application (Organisation for Economic Co-operation and Development – OECD, 2015). It includes basic biomedical research which comprises all the research on mechanisms that underlie the formation and function of molecules, organs and functioning living organisms. This knowledge may later contribute to understand how disease, trauma, or genetic mutations modify normal physiological and behavioural processes (National Research Council – NRC, 2005).

Biomedical research (the research area dedicated to the study of the prevention, aetiology, pathogeneses and treatment of human disorders) is presumably the one that uses more animals either within basic biomedical research either in applied research.

Within biomedical research, animal experiments are used to a) understand physiology, mechanisms and function of biological tissues, organs or systems (fundamental research), b) provide insights into the biology of different disorders (research and development of human medicine), c) investigate the safety and efficacy of proposed treatments prior to exposure of humans in trials (toxicological and other safety evaluation and production and quality control of products for human medicine), d) educate and train physicians, e) diagnosis of diseases, f) oncological therapies, pharmaceutical research and development, combined drug testing and genetics (labelled as “Other” in Figure 1.1).

### ***Animal experimentation: a necessary evil?***

The controversy around the use of animals for research purposes is not new, it begun at least two centuries ago (for a review see Germain et al., 2017). For many years, the controversy around animal experimentation happened in the ethical domain and almost restricted to biomedical research. On the one hand, supporters of animal experimentation claimed that even though this practice may require animal suffering it is done for a greater good: to understand and ultimately cure human disorders. On the other hand, objectors to animal experimentations claimed that animals have intrinsic value and are not ours to be used, regardless of the potential benefits for human health (Germain et al., 2017).

In more recent years, a new line of argument within objectors to animal experimentation has arisen: the efficacy and transferability arguments. The drug development crises (i.e., decreasing number of drugs approved per million invested) gained public attention after the Food and Drug Administration (FDA) published a report stating that 92% of drugs that succeed in pre-clinical stages of drug development fail in human clinical trials (FDA, 2004). Since then a new era has begun: the discussion around the use of animals on biomedical research moved to a common ground: the defence of public health. Nonetheless the controversy has not decreased: while supporters of the use of animal models claim that they are crucial for understanding and ultimately cure human disorders, objectors to the use of animal models claim that the differences between humans and non-human animals are impossible to overcome (Germain et al., 2017). With this new focus of the discussion, it has moved from the ethical to scientific ground, and an effort to measure the contribution of animal experimentation to medical progress has begun and increased exponentially throughout the past few decades.

## **How can we measure the contributions of animal experimentation to medical progress?**

Society often relies on expert opinion to evaluate the contribution of animal models to biomedical progress, despite the fact that an opinion is not an evidence-based tool. In an interesting analysis on the claims about the contribution of animal models to biomedical progress, Matthews concluded that the popular statement “virtually every medical achievement of the last century has depended directly or indirectly on research with animals” (2008, p. 96) is, in fact, a declaration prepared by the Research Defence Society and signed by 500 researchers.

Surveys (e.g., Plous, 1996; Metzger, 2015) are a more systematic way of accessing opinions and may provide robust data on the perceptions about animal experimentations and its contribution to biomedical progress, but do not provide robust evidence on its actual contribution.

For many years, the assertions on the utility of animal models to biomedical research were based upon historical analysis, investigations into the development of treatments, and critical reviews of animal models. Historical accounts are disputed. A classical example is the discovery of the role of the pancreas in diabetes. Many claim that we owe this discovery to experiments conducted by Minkowski and von Mering with dogs in the second half of the XIX century (von Mering & Minkowski, 1889, as cited in Bliss, 1982). Others argue that this medical breakthrough was made by Thomas Cawley one-hundred years earlier while performing autopsies on patients who died from diabetes (Cawley, 1788, as cited in Fadali, 1996). In more recent years, retrospective analysis into the development of treatments have increased with contradictory results: some authors conclude that animal models were unhelpful or even harmful for human health (e.g., Bailey, 2008; Fadali, 1996; Greek & Menache, 2013), while others conclude the opposite (NRC, 2004).

Recently more objective tools to evaluate such contribution have arisen.

### ***Citation Analysis***

One of the best tools to assess the impact of animal research on the development of human medicine is citation analysis. In brief, a citation analysis is a way of estimating the impact of a paper by counting the number of times that paper has been cited. In a citation analysis one can also identify patterns of citation (e.g., what sort of paper cite the target paper) (Garfield & Merton, 1979). Assuming that the studies cited by authors guided and influenced their work

(Burright et al., 2005), then determining the frequency with which animal studies are cited within human medical papers allows us to measure the impact of animal research on medical progress. Several authors have conducted such citation analysis and have demonstrated low citation frequency (of papers using animal models) in later human medical papers, and, have subsequently concluded that animal experiments have made poor contribution towards advancing the biomedical progress (e.g., Dagg, 2000; Dagg & Seidle, 2004; Hackam & Redelmeier, 2006; Lindl et al., 2004; Shapiro, 1998).

### *Systematic reviews*

Systematic reviews are literature reviews focused on a research question that try to identify, appraise, select and synthesize all high quality research evidence relevant to that question. They are generally considered the best tool to produce evidence about the value of animal studies (Pound et al., 2004) not only because they are designed to include as much high quality evidence as possible, minimise sources of conscious or unconscious bias, but also because they evaluate experimental designs through rigorous and objective peer-reviewed protocols, applying the scientific method itself to the task of reviewing research evidence.

In the XXI century the number of systematic reviews shedding light on this issue has increased (e.g., Banwell et al., 2009; Corpet & Pierre, 2005; Macleod et al., 2005; O'Collins et al., 2011; Perel et al., 2007).

The above systematic reviews have revealed:

- Poor transferability of animal outcomes to human clinical trials;
- Significant methodological and design flaws in the clear majority of animal experiments;
- Insufficient reporting of experimental design and conduct details, which hinders reproducibility;
- Simultaneous occurrence of animal and clinical trials rather than sequentially as expected given that the animal experiments should be conducted first, to allow detection of possible toxicity. A recent study (Pound & Nicol, 2018) examined the same interventions as a previous systematic review from Perel et al. (2007) noting also that in some cases (e.g., Tirilazad, a drug to treat acute ischaemic stroke) animal trials continued to be conducted even after systematic reviews of the clinical trials on the same drugs were published.

These findings have worried the scientific community that has been making increasing efforts to correct the above mentioned flaws, either through refining animal experiments

(Fabian-Jessing, 2018; Garner, 2014) or implementing reporting checklists (e.g., Animals in Research: Reporting In Vivo Experiments – ARRIVE guidelines or Gold Standard Publication Checklist – GSPC scale, developed by Kilkenny et al., 2010 and Hooijmans et al., 2010, respectively). When Macleod, Sena and colleagues updated their systematic review performed in 2009 on the efficacy of interleukin-1 receptor antagonist in animal models of stroke (Banwell et al., 2009), they found out that more recent studies have corrected some methodological flaws, both in the experimental design and in reporting, making the animal trials more promising, in the authors' opinion (McCann et al., 2016).

Systematic reviews are increasingly encouraged within biomedical research resorting to animal models (Pound & Nicol, 2018). They help prevent unwarranted experimental duplication (Leenaars et al., 2012) and ensure the use of a suitable animal model (Hooijmans et al., 2010). However, even when systematic reviews are performed, if one of the search components is the animal species in which the experiment will be performed, as recommended by Leenaars et al. (2012), it is likely that experiments with the same goal but an alternative approach (e.g., *in vitro*, non-invasive experiments on human beings) will be excluded.

In other words, systematic reviews and reporting guidelines represent a huge step forwards for evidence-based research, as well as the achievement of 3Rs (Replacement, Reduction, Refinement) principles, as described by Russel and Burch (1959). However, they fail to guarantee that the first R (replacement) is properly achieved, i.e., they do not prevent the use of animals in experiments that could be performed without them. It is important to mention that replacement is the most important of the 3Rs, since the other two are seen as intermediate steps to reduce animal suffering whenever replacement cannot be achieved. As stated by Russel and Burch (1959, p. 34) “Replacement is always a satisfactory answer, but reduction and refinement should, whenever possible, be used in combination.”

To achieve the *replacement* goal, citation analysis are powerful tools, since they allow to identify research fields where the contribution of animal models are likely to fail, regardless of the methodological quality. For instances, Lindl and colleagues (Lindl et al., 2005; Lindl & Voelkel, 2011) have urged ethic committees for a restriction of harmful animal experiments after conducting a citation analysis on the publications derived from approved animal biomedical experiments performed at three German universities. The approved animal experiments stated in their application that the results would contribute to new human therapies or gain results with direct clinical impact. However, this was only the case in less than 1% of the citations. Similarly, Knight (2007) conducted a citation analysis on the contribution of chimpanzee studies to the development of medical treatment to human diseases concluding, not

only that the citation frequency of chimpanzee papers was low, but also that the cited papers contributed less to the human medical paper than papers resorting to other methods (e.g., *in vitro* papers). Interestingly, a recent citation analysis conducted by Adnan and Ullah (2018) also showed that *in vitro* studies were the most cited amongst the endodontic (dental specialty) research field.

## **Animal models in mental disorders research**

There are no certainties about the number of animals used on behalf of mental disorder research, since these disorders are investigated across different subjects (e.g., genetics, neurology, psychology). In 1998, Shapiro estimated that at least 8% of all animal research happened in psychology research. It is reasonable to assume that in genetics and neurology this number increases substantially. There is a growing number of transgenic models to study mental disorders which per se increases dramatically the number of animals used due to technicalities related to the generation of transgenic animal models (for a review see Bailey, 2019).

Mental disorders comprise a broad range of problems, with different symptoms. However, they are generally characterized by some combination of abnormal thoughts, emotions, behaviour and relationships with others (World Health Organization – WHO, 2018).

Mental disorders affect 970 million people worldwide (Ritchie & Roser, 2018) and it is estimated that they are responsible for 32.4% of years lived with disability (Vigo et al., 2016). Mental illness has relatively low direct costs in healthcare systems, but its indirect costs (translated in high productivity loss and impact on economic growth) are tremendous. It is estimated that the total economic costs of mental illness are higher than the costs of cancer or diabetes (Trautmann et al., 2016).

Mental disorders are complex, multifactorial origin disorders (Uher & Zwicker, 2017). They include behavioural symptoms easily observable in non-human animals (e.g., repetitive/restricted behaviours in Autism) as well as symptoms that are impossible to mimic in animal models (e.g., feeling of guilt in Major Depressive Disorder – MDD or auditory hallucinations giving commands in Schizophrenia). To overcome this limitation, researchers try to establish animal models that mimic clusters of observable symptoms (e.g., Yen et al., 2013), which are sometimes similar in very different human disorders. For example, weight loss can be a symptom of MDD but also of Nervous Anorexia (American Psychiatric Association – APA, 2013). Also the same animal model is used for totally different disorders. For instances DAT knock-out mice is used to model Attention Deficit Hyperactivity Disorder

(ADHD), but are also used to model Parkinson or Schizophrenia (Gainetdinov, 2008). Despite these limitations, researchers who resort to animal models within mental disorder research consider them invaluable tools within their research fields (e.g., Gainetdinov, 2008; Nilsson, 2019).

There are probably hundreds of opinion papers on the contribution of animal models to mental disorders (e.g., Papassotiropoulos & de Quervain, 2015; Richter-Levin et al., 2019; Söderlund & Lindskog, 2018). Some include retrospective analysis to the development of treatments (e.g., Menache, 2012) or extensive reviews (e.g., Shapiro, 1998). But, to my knowledge, apart from Shapiro's book (1998) which also includes empirical data, before this thesis there were no evidence-based papers aiming to evaluate the contribution of animal models to the understanding and treatment of mental disorders. Shapiro (1998) selected a sample of nine investigators from among those who published studies resorting to three animal models of eating disorders (sham feeding, tail pinch, and activity wheel) and resorted to Mc Ardle, an expert on citation analysis, to verify the number of citations and relevance of citations each original animal study to model human eating disorders received. He concluded that seven out of ten studies received no subsequent relevant citations (Shapiro, 1998).

### **MDD: a case study**

There are more than 200 classified mental disorders, grouped into 20 classifications such as neurodevelopmental disorders or anxiety disorders (APA, 2013). Here, I choose to focus on MDD as a case study.

MDD is the most severe of the eight forms of depression (APA, 2013). It is characterized by a constant depressed mood or diminished pleasure, along with four out of the following symptoms: substantial weight loss or gain; insomnia or hypersomnia; psychomotor agitation or retardation; tiredness or lack of energy; feelings of unimportance; disproportionate or inappropriate guilt; less ability to reason, concentrate or decide; persistent thoughts of death (for a more detailed review on MDD see Chapter 3). MDD is also the most prevalent and disabling depression type (Malhi & Mann, 2018).

It is particularly interesting and up-to-date to study the contribution of animal models to MDD research because common procedures to study MDD include the forced swim test or the learned helplessness protocols. These are procedures considered severe under the current European legislation (Directive 2010/63/EU), transposed to Portugal through Decree-Law 113/2013. The legislation clearly states that severe procedures should only be used when the



expected benefits are significant. Furthermore, it was recently pointed out that out of 47 different test drug compounds for antidepressant treatment, for which efficacy was tested via forced swim test, not even one is currently on the market to treat human depression (Trunnell, 2018). The lack of reliability (Trunnell, 2019) in addition to the severity of the procedures led to a recent call to ban this test (Reardon, 2019).

## Thesis aim and outline

The overall aim of this thesis is to evaluate the contribution of animal models to mental disorders research, using MDD as a case study. If animal model studies are sufficiently relevant we can expect knowledge transfer from one area to another, i.e., from animal to human studies.

More precisely if animal model studies are sufficiently relevant it is expected that:

- all animal papers are cited at least once in the medical articles on the same disorder;
- a substantial proportion of citations received by papers describing animal studies should be made by human medical papers;
- citations should be important to the hypothesis or method of the citing paper;
- the number of citations received from animal model studies should be greater than the number of citations received from studies performed in other indirect models.

The thesis aim and testing hypothesis are explored, tested and discussed in Chapters 2 to 5. The aim of each thesis chapter is described below.

- Chapter 2 is a published paper: Carvalho, C., Crespo, M. V., Bastos, L. F., Knight, A., & Vicente, L. (2016). Contribution of animal models to contemporary understanding of ADHD. *ALTEX – Alternatives to Animal Experimentation*, 33(3), 243-249. <https://doi.org/10.14573/altex.1507311>. It addresses the contribution of animal models towards current knowledge of ADHD. This is done through a citation analysis as previously described and a systematic qualitative analysis of citations, which consists in two independent raters evaluating the contribution of each animal research paper cited to the respective citing study. In this case, the contribution of each animal paper on ADHD cited in each human medical paper on ADHD that cited it. This study is considered a pilot study for this thesis. On the one hand, ADHD shares traits with MDD (e.g., barely understood complex disorder with multifactorial origins). On the other hand, the number of published papers on ADHD using animal models is small in comparison to MDD, which allowed me to assess all published papers (instead of a sample). No one has ever conducted such study on this disorder before. Being able to

analyse all the published papers on ADHD was valuable for several reasons: it presents new scientific data, which is very robust since it is not a sample but the whole population of published papers; it allows me to compare data from two mental disorders. If similar results are found amongst these disorders they hint the conclusions might be extrapolated for all complex and multifactorial origin mental disorders. Besides, this research allowed me to test and adjust the chosen methodology for the main research of my thesis. In this first study, I do not discriminate amongst the citations of subsequent animal research papers which are on ADHD and which are on other subjects. These data could have been useful for discussion, so I collected them in all MDD research. The lack of interrater reliability found in the initial qualitative analysis of citations led to a refinement of this methodology which was used later in this paper and in a paper describing MDD data (Chapter 4).

- Chapter 3 is the unpublished version of the subsequently published paper in *Frontiers in Psychology* on 14th July: Carvalho, C., Peste, F. Marques, T. A., Knight, A., & Vicente, L. (2020). The contribution of rat studies to the current knowledge of Major Depressive Disorder. *Frontiers in Psychology*, 11, 1486. <https://doi.org/10.3389/fpsyg.2020.01486>. Here we examine the contribution of rat studies to current knowledge of MDD through a citation analysis.
- Chapter 4 is a published paper: Carvalho, C., Varela, S. A., Bastos, L. F., Orfão, I., Beja, V., Sapage, M., Marques, T.A., Knight, A., & Vicente, L. (2019). The relevance of *in silico*, *in vitro* and non-human primate based approaches to clinical research on major depressive disorder. *Alternatives to Laboratory Animals*, 47(3-4), 128–139. <https://doi.org/10.1177/0261192919885578>. Here we conduct a citations analysis and qualitative analysis of citations on *in silico*, *in vitro* and non-human primate original papers aiming at understanding MDD.
- Chapter 5 is a published book chapter: Carvalho, C., Alves, D., Knight, A., & Vicente, L. (2019). Is animal-based biomedical research being used in its original context?. In K. Herrmann, & K. Jayne (Eds.), *Animal experimentation: Working towards a paradigm change* (pp. 376-390). Brill. [https://doi.org/10.1163/9789004391192\\_017](https://doi.org/10.1163/9789004391192_017). In this chapter we examine a small sample of ADHD and MDD papers to determine if the citations the papers received are by papers on the same disorder or for different topics.

- Chapter 6 is the unpublished version of the subsequently published paper in *PLoS ONE* on 24<sup>th</sup> June: Carvalho, C., Varela, S., Marques, T. A., Knight, A., & Vicente, L. (2020). Are in vitro and in silico approaches used appropriately for animal-based major depressive disorder research? *PLoS ONE*, 15 (6), e0233954. <https://doi.org/10.1371/journal.pone.0233954>. In this paper, I count the number of citations *in vitro* and *in silico* papers received from animal papers, comparing to the citations they received by *in silico*, *in vitro* and human medical papers.
- Chapter 7 is an opinion paper: Carvalho, C., Gaspar, A., Knight, A., & Vicente, L. (2019). Ethical and scientific pitfalls concerning laboratory research with non-human primates, and possible solutions. *Animals*, 9(1), 12. <https://doi.org/10.3390/ani9010012>. It discusses the ethical frameworks behind animal research and proposes a paradigm change. This is the only chapter/paper without empirical data.
- This thesis ends with an integrating discussion on the results obtained as well as their scientific and ethical implications – Chapter 8.

## Conflict of Interest Statement

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## **CHAPTER 2**

# **Contribution of Animal Models to Contemporary Understanding of Attention Deficit Hyperactivity Disorder**

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### Summary

Attention Deficit Hyperactivity Disorder (ADHD) is a poorly understood neurodevelopmental disorder of multifactorial origin. Animal-based research has been used to investigate ADHD etiology, pathogenesis and treatment, but the efficacy of this research for patients has not yet been systematically evaluated. Such evaluation is important given the resource consumption and ethical concerns incurred by animal use.

We used the citation tracking facility within Web of Science to locate citations of original research papers on animal models related to ADHD published prior to 2010 identified in PubMed by relevant search terms. Human medical papers citing those animal studies were carefully analyzed by two independent raters to evaluate the contribution of the animal data to the human studies.

211 publications describing relevant animal studies were located. Approximately half (3,342) of their 6,406 citations were by other animal studies. 446 human medical papers cited 121 of these 211 animal studies, a total of 500 times. 254 of these 446 papers were human studies of ADHD. However, only eight of the cited animal papers (cited 10 times) were relevant to the hypothesis of the human medical study in question. Three of these eight papers described results from both human and animal studies, but their citations solely referred to the human

data. Five animal research papers were relevant to the hypotheses of the applicable human medical papers.

Citation analysis indicates that animal research has contributed very little to contemporary understanding of ADHD. To ensure optimal allocation of Research & Development funds targeting this disorder the contribution of other research methods should be similarly evaluated.

Keywords: ADHD; animal models; citation analysis.

## **Introduction**

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder that affects around 2.2% of children worldwide, although considerable variation exists among different countries (Erskine et al., 2013).

Its main symptoms include failing to pay close attention to details, difficulties in listening and sustaining attention, difficulties in organization, as well as in following instructions, hyperactive behaviors that include running and climbing excessively, restlessness and excessive talking (APA, 2013). This can be a strongly disabling condition, since it significantly affects academic and professional outcomes as well as social and family bonds (APA, 2013).

There are no certainties about what causes this disorder, however, it is consensual that it has a multifactorial origin (APA, 2013). Several authors have suggested the involvement of different brain areas in the etiology of ADHD, namely fronto-striatal, fronto-parieto-temporal, fronto-cerebellar and fronto-limbic networks (Rubia et al., 2014). More recently, genetic studies have proposed the existence of some genetic propensity for this disorder (Martin et al., 2014). There is also evidence that family environment and exposure to harmful environmental substances play a role (Ni and Gau, 2014; Han et al., 2015; Neugebauer et al., 2015).

Even though the number of studies aiming to improve the comprehension of the etiology, pathogenesis, and evolution and ultimately cure of this disorder has increased in recent years, there is still a scarcity of relevant knowledge and an urgent need for more effective studies. This need is strengthened by recent studies that suggest that ADHD's prevalence might be increasing worldwide. For example, an American survey ascertained that from 1998-2000 through 2007-2009 the prevalence of ADHD in the US increased among children aged 5-17 years from 6.9% to 9.0% (Akinbami et al., 2011). Due to resource and financial constraints it is important to assess which research methods are the most promising in this field.

Since the mid-20th century animal research has been a very widely used biomedical research methodology. Furthermore, even though functional investigation methods of the brain are the leading technology in contemporary brain disorder research (Marcucci and Vandesen, 2006; Labate et al., 2013), the emergence and development of transgenic animal models has also led to an exponential growth of animal use in neuroscience research, including in ADHD (Porter et al., 2015). Within ADHD, animals are used to model ADHD-related behaviors and traits (Yen et al., 2013), to seek understanding of ADHD's biochemical pathways (Yen et al., 2013; Huang et al., 2015) as well as responses to putative drugs (Dudley et al., 2013) and other therapies (Ouchi et al., 2013).

However, the benefits of animal models have always been simply assumed. To date the contribution of animal models of ADHD have not been subjected to significant critical scrutiny within peer-reviewed literature. And yet their use is substantially consumptive of research resources and animals lives. To prevent the poor design and reporting of many animal experiments, tools for assessing methodological quality and experimental designs have emerged (Hooijmans et al., 2010; Kilkenny et al., 2012). These tools represent an important step forward towards evidence-based research as well as the achievement of Reduction and Refinement principles. However, they fail to guarantee that the first R (Replacement) is appropriately achieved, i.e., they do not prevent the use of animals in experiments that could be performed by non-animal means.

A systematic evaluation of the contribution of animal models to specific human disorders might prevent the use of animals in studies aiming for a better understanding of those disorders. To conduct such evaluation, we performed a citation analysis and a systematic qualitative analysis of citing publications. Assuming that the studies cited by authors guide and influence their work (Burright et al., 2005), citation analysis provides a partial measure of the impact of cited studies. Previous citation analyses in other fields have demonstrated poor contributions of animal studies to human medical papers (Hackam and Redelmeier, 2006; Knight, 2007). To our knowledge however, such a systematic qualitative analysis of citations has not yet been conducted in the ADHD field. The number of published animal studies on ADHD was small enough to allow us to perform a citation analysis on all published papers.

## Methods

### *Citation analysis*

The citation analysis was performed between January 2012 and December 2014. PubMed was searched for articles using animal models to investigate ADHD. We searched PubMed using Medical Subject Heading search terms (MeSH terms): “ADHD” AND title/abstract: “animal” OR “rat” OR “mice” OR “mouse” OR “Rattus” OR “Mus” OR “pig” OR “Cavia” OR “Sus” OR “rabbit” OR “Leporidae” OR “Drosophila” OR “primate” OR “monkey” OR “Macaca” OR “macaque” OR “Cebus” OR “dog” OR “Canis” OR “cat” OR “Felis”. MeSH terms are a comprehensive list of key terms related to each disorder designed to identify all relevant studies in an area (Uman, 2011). So, searching for ADHD retrieves other nomenclatures for the same disorder such as hyperkinetic disorder or minimal brain dysfunction.

We included journal papers, books, research reports and conference proceedings written in English or Portuguese. We restricted our search to publications prior to December 31, 2010, to allow adequate time for citation of articles. 543 articles were retrieved. Since our goal was to evaluate the impact of original animal research papers, we used PubMed filters to exclude review articles (“review”, “systematic review”, “meta-analysis”, “bibliography”) as well as opinion articles (“biography”, “autobiography”, “comment”, “editorial”, “interview”).

The remaining 211 papers (see supplementary file at <http://dx.doi.org/10.14573/altex.1507311s>) were subjected to a subsequent citation analysis using the cited reference search facility within Web of Science. For each animal study, we recorded the total number of times it was cited, and allocated each citation to one or more of seven categories (animal research papers, human papers, review articles, editorials, *in vitro* papers, *in silico* papers and non-invasive animal papers). Whenever it was not possible to define the category of the citing paper (due to language barriers or absence of the abstract), the paper was allocated as “not available”. If more than one category could be assigned to a paper (e.g., animal research and human paper), then that paper was allocated to multiple categories.

Using Pearson’s Chi-square goodness-of-fit test for distributions, we investigated whether there was a significant difference between the number of citations of the animal articles by human papers and by animal research papers. The Chi-square goodness-of-fit test is used to test whether a sample of observations has approximately the same frequency distribution as a specified probability distribution. This test is especially useful for assessing the distribution of discrete and categorical variables (Freund et al., 2010).

To evaluate the number of citations that the animal papers received we built density plots, i.e., relative frequency divided by bin width, using the statistical software R. A density plot is a graphical method for examining how well an empirically derived density function fits a theoretical density function for a specified probability distribution (Cox, 2005). In our data the papers cited more frequently received citation frequencies that were increasingly distant from each other, apparently following a geometric progression. Hence, it was more suitable to use logarithmic intervals. Owing to the occurrence of zero citations within human medical papers and the impossibility to use logarithm zero, we used 0.5 as the logarithm for the “No citations” cluster.

### *Systematic qualitative analysis of citations*

The total citations of animal studies by medical papers on humans (500) were encompassed in 446 articles on humans. Of the latter, 254 were papers on ADHD, and 192 were papers on other topics. 10 human ADHD papers were excluded from the subsequent qualitative analysis due to being either written in a language other than Portuguese or English, or because the papers were unavailable.

The remaining 244 papers on human ADHD were analyzed by two independent raters to evaluate the contribution of each animal research paper cited to the respective human study, as well as the goal of the latter.

To determine the foci of the human studies both raters allocated the human papers to one or more of the following categories defined prospectively:

1. *Clinical trials*: Papers aiming to test a new drug targeting ADHD.
2. *Treatment trials*: Papers aiming to study the effect of an existing drug in a new population. This category includes papers on drug-drug interaction and the use of a known drug for a new purpose.
3. *Genetics*: Papers aiming to explore specific genes, gene sequences or patterns that may be involved in the etiology of ADHD.
4. *Psychology*: Papers aiming to explore psychological variables that may be involved in the etiology of ADHD, including personality or cognitive traits and behavioral patterns.
5. *Epidemiology*: Papers aiming to understand natural or social environmental factors that might contribute to the etiology of ADHD.

6. *Neurology*: Papers that used fMRI, PET scans or other neurological examinations to study brain areas involved in ADHD.
7. *Comorbidities*: Papers aiming to identify and explore the interactions between ADHD and other disorders.
8. *Biochemistry*: Papers aiming to describe the biochemical changes that occur in ADHD.
9. *Physiology*: Papers aiming to describe physiological changes in ADHD.

Concerning the relevance of the animal papers cited, the two independent raters classified each animal study as being:

- *Redundant*: When the animal study was only mentioned amongst other studies as an example. When there were multiple studies used as an example of one or more points, the raters were instructed to only rate the study as redundant if there were older or human studies stating exactly the same points.
- *Minor Relevance*: When the animal study was cited in the discussion or introduction providing information not directly related to the hypothesis.
- *Relevant to the Hypothesis*: When the animal study was cited in the introduction, providing information relevant for the hypothesis explored in the human medical paper.
- *Relevant for Methods*: When the human paper used the same methodology as the animal paper, with the exception of species.

The above categories were defined prospectively and the same criteria were used by both raters.

Animal papers cited in clinical and treatment trials (human categories 1 and 2) were analyzed separately since we also wanted to determine if the animal data had translated to the human situation, i.e., when an animal study was used as a reference for the human trial, the raters independently investigated whether the animal results were in agreement with the human results.

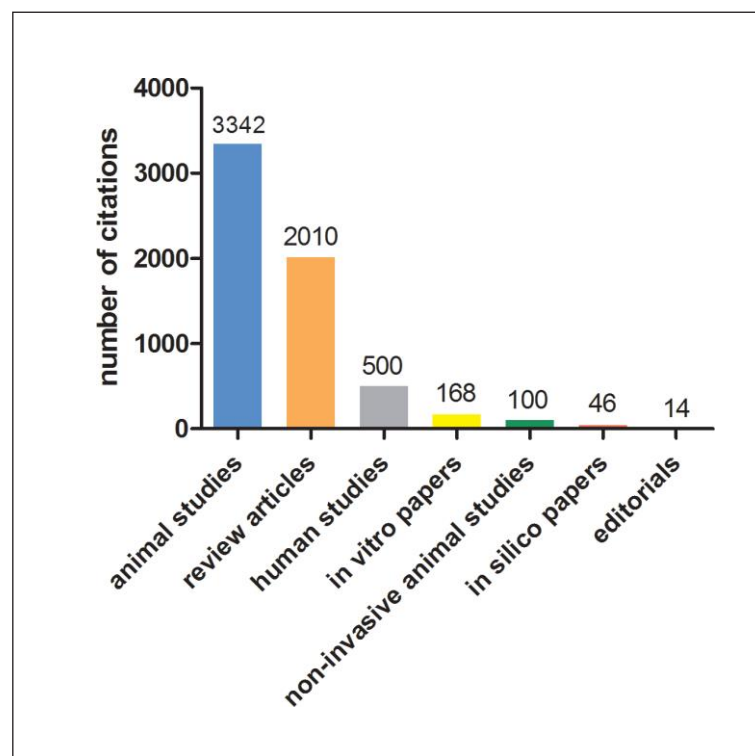
Whenever there was a disagreement between the raters either in determining the category of the human medical paper or in determining the relevance of the animal paper, a consensus was reached after detailed discussion.



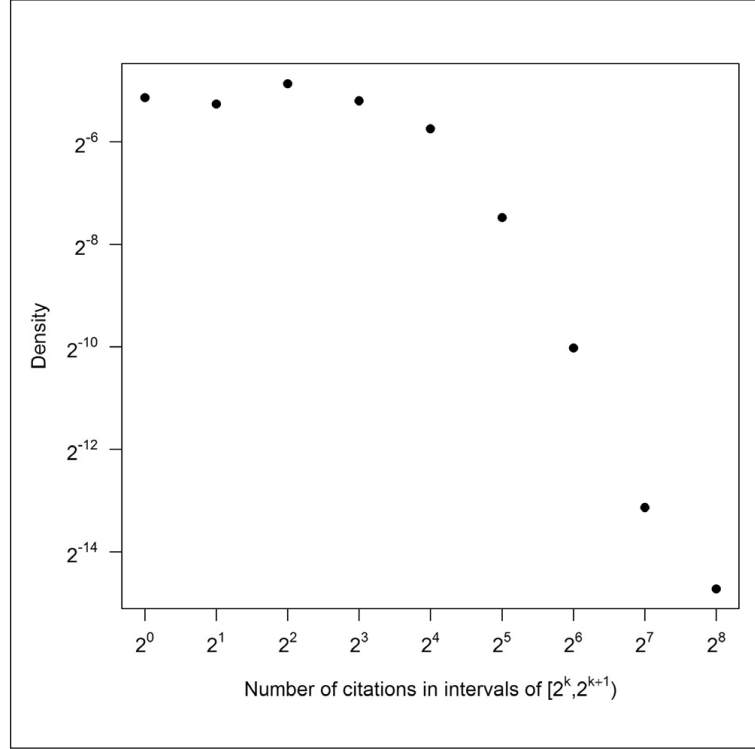
## Results

### *Citation analysis*

The 211 original animal studies focused on ADHD that were published before the end of 2010 and identified by PubMed search (see supplementary file at <http://dx.doi.org/10.14573/altex.1507311s>) were cited 6,406 times by December 2014. However, 43% of these animal studies were never cited in papers describing human studies. As shown in Figure 1, animal studies were mainly cited by other animal research papers (3,342), followed by review articles (2,010), human studies (500), *in vitro* papers (168), non-invasive animal papers (100), *in silico* papers (46) and editorials (14). Nine animal papers were cited in papers that included both animal research and human studies. 226 citing papers were unavailable for categorization due to being unavailable to us or written in a language other than English or Portuguese. Pearson's Chi-square test suggested that, by conventional criteria, the difference between the number of citations by animal research papers and by human studies was statistically significant (Chi-square = 2102.28;  $p < 0.0001$ ).

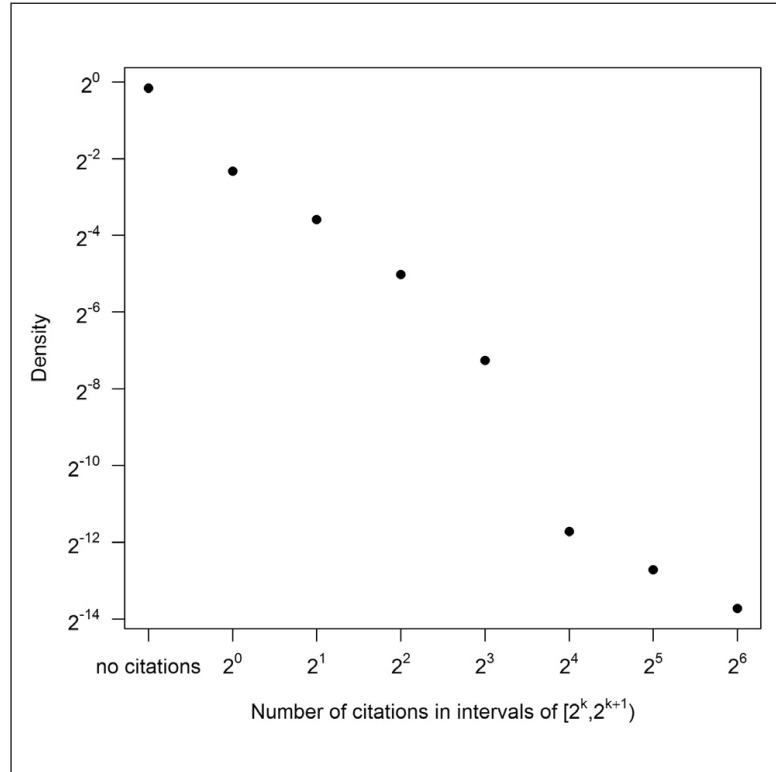


**Fig. 1:** Number of citations of animal papers on ADHD by category of citing papers



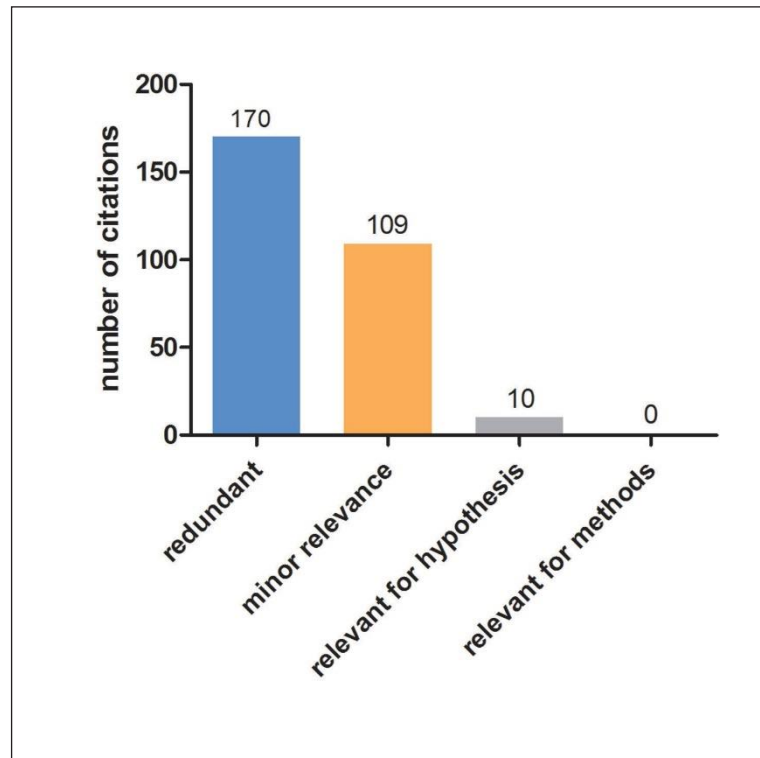
**Fig. 2: Density vs number of citations by all papers**

Each point represents the average number of citations within each interval. The intervals are defined by the power of two (e.g., the interval 23 includes articles that received from 8 citations to 15 citations). The use of  $[ )$  means that  $2k$  is included in the interval and  $2k+1$  is excluded from the interval.



**Fig. 3: Density vs number of citations by human papers**

Each point represents the average number of citations within each interval. The intervals are defined by the power of two (e.g., the interval 23 includes articles cited from 8 citations to 15). The use of  $[ )$  means that  $2k$  is included in the interval and  $2k+1$  is excluded from the interval.



**Fig. 4: Relevance of the animal papers cited by human papers on ADHD for the citing paper**

Figure 2 shows that below value  $2^5$  ( $< 32$  citations) the density plots were similar, meaning that a published animal paper focused on ADHD had a similar probability of being cited anywhere from one to 31 times. However, the likelihood of such a paper being cited 34 times or more descended abruptly. Figure 3 shows a more linear descending curve, evidencing that an animal paper on ADHD was likely to be cited very few times or not at all by human medical papers. The number of citations by human medical papers above value  $2^3$  (cited 16 times or more) was residual.

### *Systematic qualitative analysis of citations*

Of the 244 papers focused on human ADHD that cited animal studies, 81 were on genetics, 58 on treatment trials and on neurology each, 45 on psychology, 38 on comorbidity studies, 28 on biochemistry, 7 on epidemiology, 3 on clinical trials, and 2 on physiology. No pattern was identified between the categories of the human studies and the relevance of the animal papers cited.

Figure 4 presents a frequency histogram of the relevance categories of the animal papers cited in human papers in all categories except the clinical and treatment trials. The vast majority of citations of the animal papers was redundant or had minor relevance for the human paper.

No animal paper was relevant for the methods and only eight papers (cited 10 times) were relevant for the hypothesis explored in the human paper.

Of the eight animal papers considered relevant for the hypothesis, three were papers describing both animal research and human studies. Within these three papers, only the human studies were relevant for the citation in question. Therefore, five (2.3%) of the 211 animal studies focused on ADHD contributed to the hypothesis of a later human ADHD study.

The three clinical trials that cited animal papers did not use these animal studies for the hypothesis, methods or results. Therefore, investigation of translational research was not applicable.

Of the 58 treatment trials, four used animal papers for the hypothesis. The results in three out of four animal papers were in agreement with the results of the respective treatment trials.

## **Discussion**

To our knowledge, this paper provides the first systematic study of the contribution of animal-based research to contemporary understanding of ADHD.

We acknowledge that this study had several limitations:

Firstly, due to resource constraints we were unable to search a greater number of search engines (e.g., Web of Science, CAB Abstracts, Scopus) to increase the likelihood that we retrieved all animal papers investigating ADHD. We were similarly unable to examine the reference lists of retrieved papers in the hope of locating additional relevant papers. This means that some relevant publications may not have been located. Additional relevant studies may also exist in so-called “grey literature” such as unpublished reports of various kinds. However, it is reasonable to expect that most experiments that made a significant contribution to human healthcare advancements would have been published in a biomedical journal, and further, that most such journals would have been indexed in PubMed. Accordingly, we expect that our results are conservative, compared to the overall results that would have been achieved had it been possible to examine every single publication relevant to our research question.

Secondly, we used MeSH term search for ADHD, which means that all papers investigating this disorder should have been retrieved. However, we acknowledge that a minority of papers focused on this disorder may not have been labeled within PubMed standard MeSH terms for ADHD (e.g., due to labelling errors) and so may not have been located by our search.

Finally, we recognize that there is a level of difficulty in objectively determining the relevance of a cited paper to the paper citing it. Even though we have tried to avoid bias by using two raters, the initial assessment was sometimes divergent between the raters, requiring further discussion to reach a consensus. Hence we acknowledge that different raters using the same criteria might have rated some papers differently. However, we believe these would comprise only a small minority.

The citation analysis showed that 43% of the 211 animal studies were never cited by subsequent human studies and less than 8% of the total number of citations of the animal studies was by human medical papers. The systematic qualitative analysis narrowed that number further, since only eight animal papers (3.68%) seemed to be relevant to the hypothesis of a human medical study (Fig. 4). Only human data reported in three of these was actually relevant to the hypothesis. In sum, amongst the 57% of animal studies that were cited by human medical papers, the ones that may have significantly contributed to medical advances could be narrowed down to five articles, i.e., 2.3% of the overall total.

Those five articles were all published between the years 1999 and 2010 and all used genetically modified mice or rats as the animal model. However, this may simply have been a reflection of the animal species most used within the larger population of animal studies examined. These results suggest that more recent articles may be more effective than older ones. Only one gathered data from mice and a non-human primate model (rhesus monkeys), contradicting claims that the use of non-human primates is crucial for our understanding and treatment of the attention functions compromised in ADHD (e.g., Roelfsema and Treue, 2014).

Three of the five studies aimed to explore the mechanisms by which psychostimulants or other drugs act. One study aimed for a better understanding of dopaminergic pathways and the other study aimed to understand the effects of a knockout gene on visual-spatial abilities.

The animal studies appeared to influence mainly subsequent animal studies. This data emphasizes one of the major obstacles within contemporary scientific research: the segregation between research fields. If we exclude review papers and editorials, we can observe that the proportion of animal studies cited by original papers within other fields is considerably lower than the citations by other animal papers (Fig. 1). With respect to citation rates, there is a startling gap between animal and human studies.

In addition to animal research, the contribution of other research fields to the understanding, prevention and treatment of ADHD needs to be evaluated. Even though there are numerous reviews of candidate animal models for ADHD (Arime et al., 2011; Leo and

Gainetdinov, 2013), to our knowledge, there are no reviews of the contribution of other methods, e.g., *in silico* models.

The use of animal models in biomedical research consumes considerable research resources and raises serious ethical questions. These resources are then unavailable to other research methods or strategies for advancing healthcare. Hence, it is essential to ensure their efficiency and effectiveness.

Some studies have used citation analysis or systematic reviews to examine the contribution of animal models to other health disorders (Hackam and Redelmeier, 2006; Knight, 2007) and some of these studies (Pound et al., 2004; Knight, 2007), have implied that the citations of animal studies by human medical papers are often of little relevance for the human paper that was citing them. Even weaknesses of citation analysis identified by several researchers (Brooks, 1985; Garfield, 1998; Bornmann and Daniel, 2008) are fully addressed with a subsequent systematic qualitative analysis of citations.

Hence, our results suggest that animal studies rarely contributed significantly to contemporary understanding of ADHD.

In the future, ethics committees and funding agencies should consider this, prior to supporting the use of animal models in ADHD research. We hope that the methodology presented in this paper will be applied to similarly assess the contribution of animal research to other human disorders.

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### **Conflict of interest**

The authors declare that they have no conflicts of interest.

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### ***Supplementary Data***

#### List of 211 retrieved articles used for citation analysis

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## **CHAPTER 3**

# **The Contribution of Rat Studies to the Current Knowledge of Major Depressive Disorder**

## Chapter 3. The Contribution of Rat Studies to the Current Knowledge of Major Depressive Disorder

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### Abstract

**Objectives:** To examine the contribution of rat studies to the current understanding of Major Depressive Disorder (MDD), by counting the number of citations of original publications on this disorder resorting to rat models and their subsequent use in eight different research categories.

**Design:** Citation analysis.

**Study selection:** Publications prior to December 2013 that described original data using rat models within studies of MDD.

**Data sources:** To identify the publications, the bibliographic databases SCOPUS and PubMed were used. The citations analysis was made using the citing tracking facility within Scopus and Web of Science.



Data extraction: Resulting citations were thematically coded in eight categories, and descriptive statistics were calculated.

Results: 178 publications describing relevant rat studies were identified. They were cited 8,712 times. More than half (4,633) of their citations were by other animal studies. 794 (less than 10%) were by human medical papers.

Conclusions: Citation analysis indicates that rat model research has contributed very little to the contemporary understanding of MDD. This supports a paradigm change within this investigative research field.

Keywords: Major Depressive Disorder, animal models, animal use alternatives, citation analysis

## **Introduction**

Depression is the leading cause of disability worldwide (World Health Organization – WHO, 2019). Nowadays it is judged to affect more than 320 million people of all ages and genders (Global Burden of Disease 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016), even though it is more frequent in women than man (Ferrari et al., 2013).

Currently, and according to DSM-5 (American Psychiatric Association – APA, 2013), there are eight main forms of depression: MDD, Persistent Depressive Disorder, Dysphoric Disorder and Disruptive Mood Dysregulation Disorder, Substance/Medication-Induced Depressive Disorder, Depressive Disorder Due to Another Medical Condition, Other Specified Depressive Disorder. MDD is the most severe, prevalent and disabling depression type (Malhi & Mann, 2018). It is characterized by a persistent depressed mood or loss of pleasure, along with four out of the following symptoms: significant weight loss or gain; insomnia or hypersomnia; psychomotor agitation or retardation; fatigue or loss of energy; feelings of worthlessness or excessive or inappropriate guilt; diminished ability to think, concentrate or make decisions; recurrent thoughts of death (APA, 2013). For MDD to be diagnosed, the patient needs to fulfil five diagnostic criteria out of a pool of nine, which means that the same disorder may present differently in different subjects (Kaufman, 2018). The variety of both symptoms biomarkers has led to the recent suggestion that there might be several subtypes of MDD (Beijers et al., 2019).

MDD aetiology is not completely understood yet. Most authors agree that there is a combination of biological and environmental factors that determine the triggering of the disorder (Mandelli & Serretti, 2013). Biological factors to take into account include genes,

neurotransmitters and hormones, while environmental factors include childhood trauma, stressful life events, sexual abuse, low educational attainment and differences in personality traits.

Evidence suggests that there are genetic factors involved, but even though more than 100 candidate genes have been investigated, a clear connection between specific genes and MDD has not yet been established (Shadrina et al., 2018). Furthermore, studies suggest that variations in different genes, each with a minor effect, combine to increase the risk of developing this disorder (Wray et al., 2018). Most studies suggest that MDD patients have imbalanced brain chemistry at neurotransmitters level (Beijers et al., 2019). Amongst those, the majority of the studies indicate dopamine, norepinephrine and serotonin as the most implicated in MDD aetiology (Belujon & Grace, 2017). Others stress the involvement of glutamate (Réus et al., 2018). Nonetheless, some studies find no differences in neurotransmitters between MDD patients and healthy controls (for a review see Beijers et al., 2019). Similarly, it is widely accepted that hormones play a role in MDD aetiology (Druzhkova et al., 2019) but there is no clear-cut connection between a specific hormone secretion and MDD. For example, Asadikaram et al. (2019) found differences between MDD patients and healthy controls in hormone levels of adrenocorticotrophic hormone, testosterone, thyroid-stimulating hormone, free thyroxine index and cortisol/dehydroepiandrosterone sulfate (DHEA-S), while others consider vasopressin and oxytocin to play a pivotal role in MDD aetiology (for a review see Iovino et al., 2018).

Recent studies also suggest that there is a link between inflammation and MDD, suggesting that MDD has an inflammatory subtype (Beijers et al., 2019), but the claims that inflammation has a role in aetiology of MDD are still being disputed (Miller, 2018). The same happens with changes in gut microbiome in MDD patients (Winter et al., 2018). While the link between gut microbiome and depression is well documented, the question of the causality in the connection between the two remains to be robustly answered (Winter et al., 2018). It is important to mention that these patterns may be true for all biological changes found in MDD patients. It is almost impossible to determine if the biological changes caused MDD or if MDD caused the biological changes. Conversely, most environmental factors involved in MDD are definitely a primary cause. In this regard, the big unanswered question that remains is why does the same life event trigger MDD in one person and not in another.

Amongst the most documented environmental factors linked to MDD are childhood traumas, which also cause biological changes in the brain of MDD patients (Yu et al., 2019), stressful life events, sexual abuse, low educational attainment (Peyrot et al., 2013) and

personality traits (Bensaeed et al., 2014). Other disorders and traits are also strong predictors for MDD. For example, a big longitudinal study showed that people who present anxiety traits in their twenties are more prone to develop MDD in their thirties (Gustavson et al., 2018). Also Parkinsons', Migrains', Alzheimers' patients, amongst others, have high prevalence of MDD (Ketharanathan et al., 2014; Muneer et al., 2018; Tao et al., 2019).

It is not always possible to determine a proximal cause. MDD may have seasonal or peripartum onset, as well as being induced by other disorders (e.g., Parkinson) or substance ingestion, but it can also emerge without an obvious reason (APA, 2013).

MDD pathogenesis is as diverse as its aetiology. Even though there are several different treatment courses available (for a review see for example Pandarakalam, 2018), 50 to 60% of patients develop treatment resistant depression, i.e., do not enter remission even after trying different courses of treatment (Kraus et al., 2019), and only 52% of patients achieve a full recovery (Novick et al., 2017).

Due to its complexity, MDD is particularly hard to study, but its severity, prevalence and significant economic burden make it a moral and sociological imperative to keep investing this research field. Yet, its research funding has been scarce when compared to other disorders (e.g. cancer) (Ledford, 2014).

Randomised controlled trials (RCTs) are considered to be the gold standard for empirical research (Hariton & Locascio, 2018), namely in MDD's potential treatments and interventions (Monsour et al., 2019). But RCT are the final step, they are expensive and of limited use, since they are insufficient to predict the responses of more diverse populations of patients in real-life environments. Some authors consider observational longitudinal studies to be more useful in understanding the aetiology and pathogenesis of human disorders (Frieden, 2017) pointing out that they also overcome ethical and practical limitations of RCTs such as the insufficient study duration or the disregard of unpredictable variables that affect patients in their daily lives (Song & Chung, 2010). Others stress the importance of basic and applied research aiming to understand MDD's mechanisms in a controlled environment (e.g., Papassotiropoulos & de Quervain, 2015).

In this regard, advanced magnetic resonance imaging techniques used in patients and healthy controls can be a powerful tool regarding the physiological and metabolic characterization of brain tissue, in the same way that single photon emission computed tomography and positron emission tomography imaging modalities provide valuable data on brain function and activity (Tsougos et al., 2019).

Post-mortem studies as well as cell-based disease modelling are another valuable set of tools in understanding the biology of psychiatric disorders. As cellular biology techniques evolve new ways to generate and preserve human cells *in vitro* emerge (e.g. induced pluripotent stem cells, trans differentiation technologies for deriving neurons from adult humans, Vadodaria et al., 2018). However, they are insufficient to fully understand the pathways and progression of complex disorders. Some authors assert that systems biology might be the answer as it can integrate and model different levels of human experimental data – molecular, cellular, tissue, organ, clinical and population disorders (Langley, 2014). Others claim that the only way to overcome such limitations is resorting to animal models, which are seen as crucial for MDD research (e.g., Akil et al., 2018; Wang et al., 2017), despite their well-recognized limitations with respect to human predictively (Akil et al., 2018).

To overcome these limitations, combinations of different animal models are proposed (Akil et al., 2018), different transgenic lines of rats are generated (Bailey, 2019) and efforts are made to overcome the biological differences between species that keep emerging as extrapolation barriers (Hodge et al., 2019). All the above involve high economic costs and consume a tremendous amount of animal lives. The reason behind this is because it is assumed that animal use is unavoidable and its withdraw would jeopardize human health. However, very few studies have addressed the contribution of animal models to MDD research through significant critical scrutiny within peer-reviewed literature. Specifically, to our knowledge, the contribution of rats for this aim has never been evaluated in such terms, even though rodents are undoubtedly the most frequently used animals regarding this context. Even though mice are by far the most used rodents in biomedical research an initial search in PubMed, a search engine that comprises more than 30 million papers for biomedical literature, indicated that species within genus *Rattus* were highly used in MDD research, which made them an interesting case study. To evaluate the contribution of animal models to MDD research is important for ethical and economic reasons. As a society, we should make an informed decision on whether we should proceed refining animal models until we find a suitable one or if we should halt the current paradigm and invest more in other methods that might be more promising as well as cheaper and less ethically contentious (Carvalho et al., 2019).

To conduct such evaluation, we performed a citation analysis on original publications describing rat data within MDD research. A citation analysis as defined by Garfield & Merton (1979) consists in determining the number of citations target papers (in this case original papers resorting to rat models to study MDD) receive as well as determining citation patterns- in this case which sort of papers are citing the target papers (e.g. research papers, review papers).

Granting that the studies cited guide and influence authors work (Burright et al., 2005), such citation analysis can be used to evaluate the contribution of rat studies to current knowledge in MDD, as has been done for other disorders (e.g., Carvalho et al., 2016; Knight, 2007; Long et al., 2014) as well as for other species in regard to MDD research (Carvalho et al., 2019).

If rat studies are informing the human medical research community, then we expect that:

1. All of the papers would be cited at least once in subsequent human medical papers;
2. The proportion of citations by human medical papers would be substantially higher when compared to other research categories.

## Methods

The citation analysis was performed between January and August of 2019. PubMed and SCOPUS were searched for publications using rat models to investigate MDD. We searched PubMed using Medical Subject Heading search terms (MeSH terms): “Depressive Disorder, Major” AND “rat” OR “rodent”. MeSH terms are a comprehensive list of key terms made available by PubMed designed to identify all relevant studies in an area (Uman, 2011). So, searching for “Major Depressive Disorder” retrieves other nomenclatures for the same disorder such as melancholia. Similarly, the search term “rat” retrieves papers using all rat species. We used PubMed filters to exclude review articles (“review”, “systematic review”, “meta-analysis”, “bibliography”) as well as opinion articles (“biography”, “auto-biography”, “comment”, “editorial”, “interview”). Since Scopus does not have the MeSH term tool we used the search terms “Major depressive disorder” AND (“rat” OR “rattus”) in the search fields. We included journal papers, books, research reports and conference proceedings written in English or Portuguese, which are within our language proficiency. We restricted our search to publications prior to December 31, 2013, to allow adequate time for citation of articles to occur.

Since our goal was to evaluate the contribution of animal models - particularly rat models - to current knowledge of MDD, we excluded from our analysis all the papers that reported animal and human data, as well as papers reporting other species’ data (e.g. mice).

The retrieved papers were subjected to a subsequent citation analysis using the cited reference search facility within Scopus and Web of Science.

Web of Science is a major scientific citation indexing service that encompasses over 50,000 scholarly books, 12,000 journals and 160,000 conference proceedings. Scopus is the largest citation database; it covers nearly 36,377 titles from approximately 11,678 publishers.

For each rat study, we recorded the total number of times it was cited, and allocated each citation to one or more of eight categories, defined prospectively:

- Animals. This category included invasive procedures as defined by Knight (2011), i.e., interfering with bodily integrity (whether through puncture or incision) or production of genetically modified animals. This category also included severe procedures (as defined by current European Legislation Directive 2010/63/EU) commonly used in mental disorder research such as inescapable electroshock or isolating social animals for long periods.
- Humans. This category included papers that used human participants. They included clinical or treatment trials (either drug trials or non-pharmacological treatments), papers aiming to explore psychological, social, biochemical, physiological, genetic or neurological variables related to MDD; as well as papers aiming to understand the relationship between MDD and other disorders (co-morbidities) in human patients
- Reviews. This category included narrative reviews, systematic reviews, meta-analysis as well as extensive opinion papers that did not report original empirical data.
- Editorials. This category included editorials, comments and clinical guides.
- *In vitro*. This category included exclusively cell-line data. Whenever the source of the tissue or cell was a human participant (either alive or post-mortem) or a laboratory animal killed for such purpose the paper was allocated into “human paper” or “animal research paper”, respectively.
- *In silico*. This category included data obtained via computer simulations of human data.
- Social. This category included human surveys or other social perception papers.
- Non-invasive. This category included ethological research that relied solely on behavioral observation.

Whenever it was not possible to define the category of the citing paper (due to language barriers or absence of the abstract), the paper was denoted “not available” and removed from the sample. If more than one category could be assigned to a citing paper (e.g., animal research and human paper), then that paper was allocated to every appropriate category.

To evaluate if the proportions of citations made by human publications and animal publications on MDD were different we used a t-test. Results were considered statistically significant when  $P < 0.05$ . The analyses were performed in R 3.6.1 (R Core Team, 2019).

## Results

The 178 original rat studies focused on MDD that were published before the end of 2013 were cited 8,712 times by August, 2019. Of these 178, 87 (49%) studies were never cited in subsequent publications describing human studies on MDD, and 53 (30%) were never cited in any publications related to human research.

As shown in Figure 1, rat studies were mainly cited by other animal research papers (4,633), followed by review papers (2,909), human studies (794), *in vitro* papers (211), editorials (58), *in silico* papers (57), non-invasive animal papers (eight) and human social papers (one). 230 citations were unavailable to us due to assess or language barrier. These were removed from further analysis.

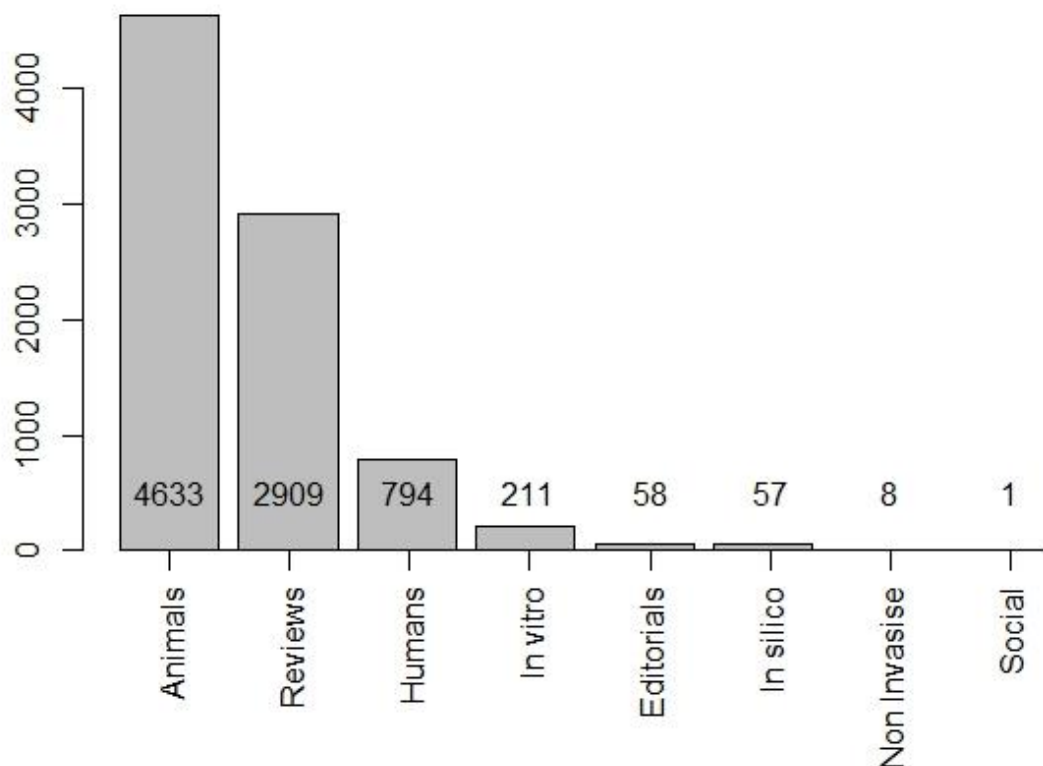


Figure 3.1 Frequency of citations by category established in this study

The proportion of citations by human medical papers is 9.1 % while the proportion of citations by animal experimentation papers is 53.2%. This corresponds to a mean difference between the proportions of citations by human and by animals of -44% ( $p < 0.001$ ). Beyond the statistical significance, this is certainly a considerable practical difference that reflects almost 83% ( $100 (9.1 - 53.2)/9.1$ ) less citations by human papers than by animal papers.

## Discussion

The majority of the rat papers located in this study were cited by subsequent animal research papers, but about half (49%) of the original papers retrieved were never cited in subsequent papers related to MDD in humans, and in fact about a third (30%) were never cited in any subsequent human studies.

Our citation analysis reveals that only around a tenth (9.1%) of the total number of citations were by human medical papers. This contradicts the assumption that citations made by human medical papers constitute a considerable proportion of the total number of citations, compared to the other categories, and raises doubts about the justification for these studies.

The results of our study are in agreement with previous studies that empirically evaluated the contribution of animal models to human healthcare, concluding that the contribution is poor (for a review see Knight, 2019). Clearly, biomedical research resorting to animal models is not normally considered significant, or particularly visible to, the human medical research community.

Supporters of animal models of human disorders claim that this happens: a) due to differences in the way basic animal work and human clinical trials are conducted, and propose a change to a translational biomarker-based approach within early steps of pre-clinical research (Garner, 2014); b) failings in study design, conduct, analysis and reporting (Pound & Ritskes-Hoitinga, 2018), which could be resolved with better reporting and better methodological quality (Fabian-Jessing et al., 2018).

Opponents of the use of animal models point out that animal models lack external validity i.e. findings derived in one setting, population or species cannot be reliably applied to other settings, populations and species, which is unavoidable since animal models: a) oversimplify complex human disorders and the conditions in which they occur; b) are unsuitable models due to species differences, proposing as a possible solution a shift towards human-based non-clinical research (Pound & Ritskes-Hoitinga, 2018).

This paradigm change towards human-based research is gaining more and more supporters (e.g., Langley, 2014; Pound & Ritskes-Hoitinga, 2018; Ram, 2019). But the resistance from animal researchers in face of this shift remains significant and is evident not only in the slowness to recognize the growing body of evidence against the use of animals as models, but also in the emphasis placed on refinement of animal use (Franco & Olsson, 2014) (the third R as defined by Russel and Burch (1959), instead of on the first and most important R – replacement with non-animal alternatives). Considering this, Frank (2005) proposed that



animal experimentation constitutes a good example of path dependency, which is a well-documented phenomenon that states that what has occurred in the past persists because of resistance to change.

Our results also show that more than half (53%) of the citations our target papers received were by subsequent animal papers, which strengthens the idea of the path dependency phenomenon described above. It can be argued that there is a need to have a substantial amount of animal research before achieving a critical mass that can lead to useful breakthroughs in human health, which might explain the high level of citations of animal papers by subsequent animal papers. Nonetheless, it does not explain the low level of citations by human papers, especially when this does not appear to be a trend in human-based approaches (*in vitro* and *in silico*) which received more citations by human medical papers in a small study we previously conducted (Carvalho et al., 2019). Furthermore, a recent citations analysis in another field of human health research also found *in vitro* papers to be the most cited papers, above reviews and animal experimentations papers (Adnan & Ullah, 2018).

We acknowledge that this study has certain limitations. Even though we used two big bibliographic databases to attempt to locate all publications that met our search criteria, we acknowledge that a small minority of relevant papers may not have been retrieved (e.g. due to labelling errors).

Similarly, we did not take note of certain citations such as self-citations, in-house citations and content-irrelevant citations. Such citation types were not considered significant in several studies and were similarly dismissed in recent studies (Huang et al., 2019).

Finally, we did not analyse the quality of citing papers as in previous studies (Carvalho et al., 2016; Carvalho et al., 2019). Doing so might have resulted in a lower number of rat papers cited by human studies into MDD, if only good quality citing papers were included. This might have resulted in an even lower, but a truer, indication of the contribution of rat models for current knowledge of MDD.

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## **CHAPTER 4**

# **The Relevance of *In Silico*, *In Vitro* and Non-Human Primate Based Approaches to Clinical Research on Major Depressive Disorder**



## Chapter 4. The Relevance of *In Silico*, *In Vitro* and Non-Human Primate Based Approaches to Clinical Research on Major Depressive Disorder

Carvalho, C., Varela, S. A. M., Bastos, L. F., Orfão, I., Beja, V. Sapage, M., Marques, T. A., Knight, A., & Vicente, L. (2019) The Relevance of *In Silico*, *In Vitro* and Non-Human Primate Based Approaches to Clinical Research on Major Depressive Disorder. *Alternatives to Laboratory Animals*, 47(3–4), 128–139. <https://doi.org/10.1177/0261192919885578>

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### Abstract

Major depressive disorder (MDD) is the most severe form of depression and the leading cause of disability worldwide. When considering research approaches aimed at understanding MDD, it is important that their effectiveness is evaluated. Here, we assessed the effectiveness of original studies on MDD by rating their contributions to subsequent medical papers on the

subject, and we compared the respective contribution of findings from non-human primate (NHP) studies and from human-based *in vitro* or *in silico* research approaches. For each publication, we conducted a quantitative citation analysis and a systematic qualitative analysis of the citations. In the majority of cases, human-based research approaches (both *in silico* and *in vitro*) received more citations in subsequent human research papers than did NHP studies. In addition, the human-based approaches were considered to be more relevant to the hypotheses and/or to the methods featured in the citing papers. The results of this study suggest that studies based on *in silico* and *in vitro* approaches are taken into account by medical researchers more often than are NHP-based approaches. In addition, these human-based approaches are usually cheaper and less ethically contentious than NHP studies. Therefore, we suggest that the traditional animal-based approach for testing medical hypotheses should be revised, and more opportunities created for further developing human-relevant innovative techniques.

Keywords: animal use alternatives, *in silico*, *in vitro*, major depressive disorder, non-human primate, three Rs

## Introduction

According to the World Health Organization, depression is the leading cause of morbidity worldwide. It affects more than 300 million people of all ages and is a major contributor to the overall global burden of disease.<sup>1</sup> People who suffer from depression are more prone to an early death either by suicide or through the development of other conditions such as cancer, heart disease or stroke.<sup>2,3</sup> In addition, these patients are also more prone to a number of other disorders (e.g. osteoporosis)<sup>4</sup> that, although not life-threatening, do significantly impact not only quality of life but also public health and national economies.

Accordingly, major investment has been dedicated to research aiming to improve the understanding of all eight forms of depression.<sup>5</sup> Major depressive disorder (MDD) is the most severe type and the third leading cause of long-term disability.<sup>6</sup> Besides, the few studies that have comprehensively investigated the impact of MDD in Europe (from 2004 to 2010) have shown that MDD was the costliest brain disorder in Europe, accounting for at least 1% of the total European economy.<sup>7,8</sup> In the United States, the economic burden of MDD alone was US\$210.5 billion in 2010.<sup>9</sup>

Clinical research is expensive, time-consuming and potentially ethically contentious. For instance, every patient who enrolls in a clinical trial is subject to an increased level of risk with respect to deviations from their regular clinical care, particularly with regard to the occurrence

of unexpected effects from exposure to a new treatment. Non-clinical (i.e. preclinical) research, often involving non-human animals and human-based *in vitro* and *in silico* approaches, is sometimes valuable in the early steps of biomedical research to simplify and accelerate drug and treatment discovery. However, to optimise the outcomes of this non-clinical research, it is crucial to evaluate the research approaches that might have the most potential for patient treatment results.

Animal-based research has been accepted as the ‘gold standard’ approach for preclinical biomedical research and testing since the second half of the 20th century.<sup>10</sup> Within this approach, non-human primate (NHP) research has been considered particularly relevant, due to the similarity between humans and NHPs. However, this similarity has led to NHPs being afforded various degrees of legal protection in different regions of the world. For example, Europe,<sup>11</sup> the United States<sup>12</sup> and New Zealand<sup>13</sup> have imposed considerable restrictions to the use of NHPs for scientific purposes. These restrictions are due to the understanding that subjecting NHPs to laboratory confinement alone, even before considering the use of any invasive or intrusive procedures, has resulted in psychosomatic injury, mutilation and physiological traits that have been compared to those exhibited by people with post-traumatic stress disorder.<sup>13–20</sup> Moreover, NHPs are expensive to acquire<sup>21</sup> and are the most expensive animals to maintain.<sup>22</sup> The legislation on animal use for experimental purposes of several countries (e.g. Directive 2010/63/EU) requires a cost–benefit assessment to be carried out prior to conducting a procedure on a non-human animal. For each project, the likely harm to the animal should be balanced against the potential benefits, and the project should only go ahead if the expected benefits outweigh the harms inflicted to the animals involved.

Considering all of the above, it is assumed that when research is conducted on NHPs, due to the ethical and economic concerns surrounding this practice, this research should provide highly relevant data that lead to concrete improvements in patient outcomes. While some authors assert that animal research approaches, and those involving NHPs in particular, are crucial for biomedical progress,<sup>23</sup> an increasing number of evidence-based papers show that the contribution of animal-based research to the advancement of human healthcare has been poor,<sup>24</sup> including in the case of MDD.<sup>25</sup> However, it is yet to be established whether this poor contribution is due to the intrinsic limitations of all non-clinical research, or whether human-based (*in vitro* and *in silico*) non-clinical research approaches are more effective in helping biomedical progress, at least when seeking to understand complex disorders of a multifactorial origin, such as MDD.

*In vitro* and *in silico* methods that directly rely on human-based knowledge and/or material are thought to potentially allow for faster development of medical treatments.<sup>26,27</sup> Usually, they are also more cost-effective than animal-based methods. However, despite yielding data of sufficient value to further disease understanding in humans, and providing the means to test new therapies, such non-animal methods are still judged against the standard biomedical research paradigm. Indeed, they are seen as incomplete on their own and considered to be preliminary steps prior to (often contradictory) animal testing.<sup>28,29</sup>

To shed light on this debate, the current study examines and compares the contribution of results from NHP studies, as well as from *in silico*-based and *in vitro*-based approaches, to clinical studies on MDD. This allows us to: (a) evaluate whether the low transferability of knowledge to clinical research is a common trait of all non-clinical research approaches; and (b) evaluate the specific relevance of NHP studies and human-based *in silico* and *in vitro* approaches to human clinical studies.

Considering the dominance of NHP studies within the current preclinical research paradigm, we expect the findings from these studies to have a higher contribution to subsequent clinical research than findings from *in silico*-based and *in vitro*-based studies. A similar or lower contribution from NHP studies would suggest that clinical research is becoming less reliant on this more costly and ethically questionable type of research, thus suggesting that the time for a paradigm shift has come.

## Methods

The design of this study was based on a previously developed method consisting of a quantitative citation analysis and a systematic qualitative analysis of citations.<sup>30</sup>

### *Quantitative citation analysis*

*Bibliographic search:* The citation analysis was performed between September 2016 and June 2017. The PubMed bibliographic database was searched for papers that described studies employing either NHPs, or *in vitro* or *in silico* research approaches, to investigate MDD. The following Medical Subject Heading (MeSH) search terms were used: ‘Depressive Disorder, Major’ AND MeSH terms: ‘primate’ OR ‘ape’ OR ‘macaque’ OR ‘macaca’ OR ‘rhesus’ OR ‘chimpanzee’ OR ‘bonobo’ OR ‘gorilla’ OR ‘gorila’ OR ‘Pan’ OR ‘orangutan’ OR ‘orangutan’ OR ‘Orang utan’ OR ‘orangutan’ OR ‘ourang-outang’ OR ‘Pongo’ OR ‘gibbon’ OR ‘Hylobates’ OR ‘Colobus’ OR ‘Baboon’ OR ‘Papio’ OR ‘Mandrillus’ OR ‘Mandrill’ OR

‘Cebus’ OR ‘Cebuella’ OR ‘Brachyteles’ OR ‘Loris’ OR ‘Nycticebus’ OR ‘lemur’ OR ‘Callithrix’ OR ‘in silico’ OR ‘computer model’ OR ‘mathematical model’ OR ‘computer simulation’ OR ‘in vitro’ OR ‘cell culture’ OR ‘culture technique’ OR ‘cell line’ OR ‘organ culture’ OR ‘tissue culture’.

MeSH terms are a comprehensive list of key terms related to each human disorder, designed to identify all relevant studies in a given area.<sup>31</sup> Thus, searching for ‘Depressive Disorder, Major’ retrieves other nomenclatures for the same disorder (e.g. Melancholia). There were no exclusive MeSH terms for NHPs, so the search retrieved additional papers with non-human animals that were excluded by manual sorting. All *in vitro*-based and *in silico*-based papers that used animal data (e.g. rat cell line data) were also excluded.

Papers from scientific journals, books, research reports and conference proceedings written in English, Portuguese or Italian were included (being within the authors’ linguistic fluencies). PubMed filters were used, in order to exclude review papers (‘review’, ‘systematic review’, ‘meta-analysis’, ‘bibliography’), as well as editorials and other types of non-research papers (‘biography’, ‘auto-biography’, ‘comment’, ‘opinion paper’, ‘interview’), since the aim of the study was to evaluate the impact of original data. The search was restricted to publications prior to 31 December 2011, to allow adequate time for subsequent citation of papers.<sup>32</sup> Nineteen NHP study-based papers, 29 *in silico*-based papers and 38 *in vitro*-based papers describing data from original MDD research were retrieved (see Appendix 1).

*Citation data:* A citation analysis on the retrieved papers was performed by using the cited reference search facility within the Web of Science bibliographic database. For each retrieved paper, the subsequent papers that cited it were identified, and three types of citation data were recorded:

- the total number of times that the retrieved paper was cited;
- the total number of times that the retrieved paper was cited per research category; and
- the total number of times that the retrieved paper was cited per research subject, that is, on MDD or other subjects, as detailed below.

Each citing paper was ascribed to one or more of the following eight research categories: ‘invasive animal research’; ‘human research’; ‘review’; ‘opinions’ (including editorials, comments or replies to comments); ‘*in vitro*’; ‘*in silico*’; ‘non-invasive animal research’ (e.g. observational studies with wild animals); and ‘other human studies’ (e.g. on social perceptions). The term ‘human research’ referred to any human-based research that might involve, among

other things, the analysis of biological samples, epidemiological and behavioural studies, medical case studies and clinical studies. A citing paper could be allocated to more than one category, if it described different research approaches. Whenever the category of the citing paper could not be defined (due to language barriers or absence of an abstract), the paper was labelled as ‘not available’ and removed from further analysis.

Among the categories ‘human research’, ‘*in silico*’, ‘*in vitro*’ and ‘invasive animal research’, it was also recorded whether the citing paper focused on MDD or on other subjects.

*Statistical analysis:* To test for differences between the numbers of citations across research approaches, three generalised linear models (GLMs), each with a Poisson response and a log link function, were implemented. Each model tested one of the following response variables: (a) the total number of citations; (b) the total number of citations by papers in the category ‘human research’; and (c) the total number of citations by papers in the category of ‘human research’ that focused specifically on MDD. In each model, the only explanatory variable was the type of research approach, of which there were three: NHP studies, *in silico*-based approaches and *in vitro*-based approaches. The GLM’s goodness of fit was evaluated by visual inspection of the diagnostic plots. Additionally, a Gaussian GLM was used to evaluate whether the proportions of citations by human research papers, and by human research papers specifically on MDD, were different across the three approaches. The analyses were performed in R 3.6.1,<sup>33</sup> by using the function *glm*. The results were considered significant when  $p < 0.05$ .

### *Systematic qualitative analysis of citations*

Citing papers featuring human research specifically on MDD were systematically analysed by two independent raters, to qualitatively evaluate the contribution of knowledge from NHP studies, or from *in vitro*-based or *in silico*-based research approaches, to the respective human clinical study. Each study was rated according to the following classes, which were defined prospectively, as in Carvalho et al.<sup>30</sup>:

- *Redundant*: when the cited study was only mentioned among other studies as an example. In the case where multiple studies were used as examples of one or more points, the raters were instructed to rate the study as redundant only if there were older or human studies stating exactly the same points.
- *Minor relevance*: when the cited study was cited in either the Discussion or the Introduction, to provide information not directly related to the hypothesis explored in the human study.

- *Relevant to the hypothesis*: when the cited study was cited in the Introduction, to provide information relevant to the hypothesis explored in the human study.
- *Relevant to the methods*: when the human study used the same methodology as that described in the cited paper, with the exception of species differences in the case of NHP study methods.

A paper considered to be ‘relevant’ could be both relevant for the hypothesis and the methods. The other options in the classes are mutually exclusive. In all cases, disagreement between the raters was resolved via detailed discussions until a consensus was reached.

Whenever it was not possible to assess the contribution of a cited paper to a human study due to unavailability of the full publication on the human study, the human research paper was labelled as ‘not available’ and removed from further analysis.

A statistical test was used for comparing proportions (Pearson’s  $\chi^2$  test implemented via R’s `prop.test` function), in order to assess differences between the three cited approaches (i.e. NHP studies, and *in vitro*-based and *in silico*-based approaches). Since, even for the pair with the largest difference, the null hypothesis of equal proportions could not be rejected under the usual significance levels, corrections for multiple comparisons were not attempted.

## Results

### *Citation analysis*

NHP study-based results: Nineteen publications featuring NHP studies in the field of MDD research were retrieved, which were subsequently cited 841 times in total. Of these 19 papers, five featured both human and NHP data.

The subsequent citing papers belonged to the following categories: invasive animal research (312); reviews (245); human research (152); *in vitro* research (81); *in silico* research (14); non-invasive animal research (6); and opinions, including editorials, comments or replies to comments (4). Eighty-five citing papers were not categorised due to being unavailable or written in a language other than English, Portuguese or Italian.

Of the 312 citations by animal research papers, 63 were specifically focused on MDD; of the 152 citations by human research papers, 71 were specifically focused on MDD.

*In silico-based approach results*: Twenty-nine publications describing the use of *in silico*-based approaches in the context of MDD research were retrieved, which were subsequently cited 806 times in total. Of these 29 papers, seven featured both patient data and computer

simulations. The subsequent citing papers belonged to the following categories: human research (317); *in silico* research (193); reviews (193); invasive animal research (44); *in vitro* research (17); and opinions (17). Fifty-eight citing papers were not categorised due to being unavailable or written in a language other than English, Portuguese or Italian.

Of the 317 citations by human research papers, 94 specifically focused on MDD; of the 193 citations by *in silico*- based research papers, 36 specifically focused on MDD.

*In vitro-based approach results:* Thirty-eight publications describing the use of *in vitro*-based approaches in the context of MDD research were retrieved, which were subsequently cited 2,574 times in total. All of the *in vitro*-based papers used samples of human biological material, mostly being obtained from MDD patients (in 34 out of the 38 studies).

The subsequent citing papers belonged to the following categories: *in vitro* research (1,239), resorting to the use of human biological material (789), laboratory animal biological material (373) or biological material from both sources (12); human research (978), of which 189 studies solely used human participants without concurrent use of *in vitro*-based research approaches; reviews (844); invasive animal research (464), of which 79 studies solely used live animals without concurrent use of *in vitro*-based research approaches; opinions (27); and *in silico* research (16). One hundred and fifty-four citing papers were not categorised due to being unavailable or written in a language other than English, Portuguese or Italian.

Of the 978 citations by human research papers, 482 specifically focused on MDD; of the 1,239 citations by *in vitro* research papers, 487 specifically focused on MDD.

### ***Comparison of citations of papers based on NHP studies, in vitro approaches and in silico approaches***

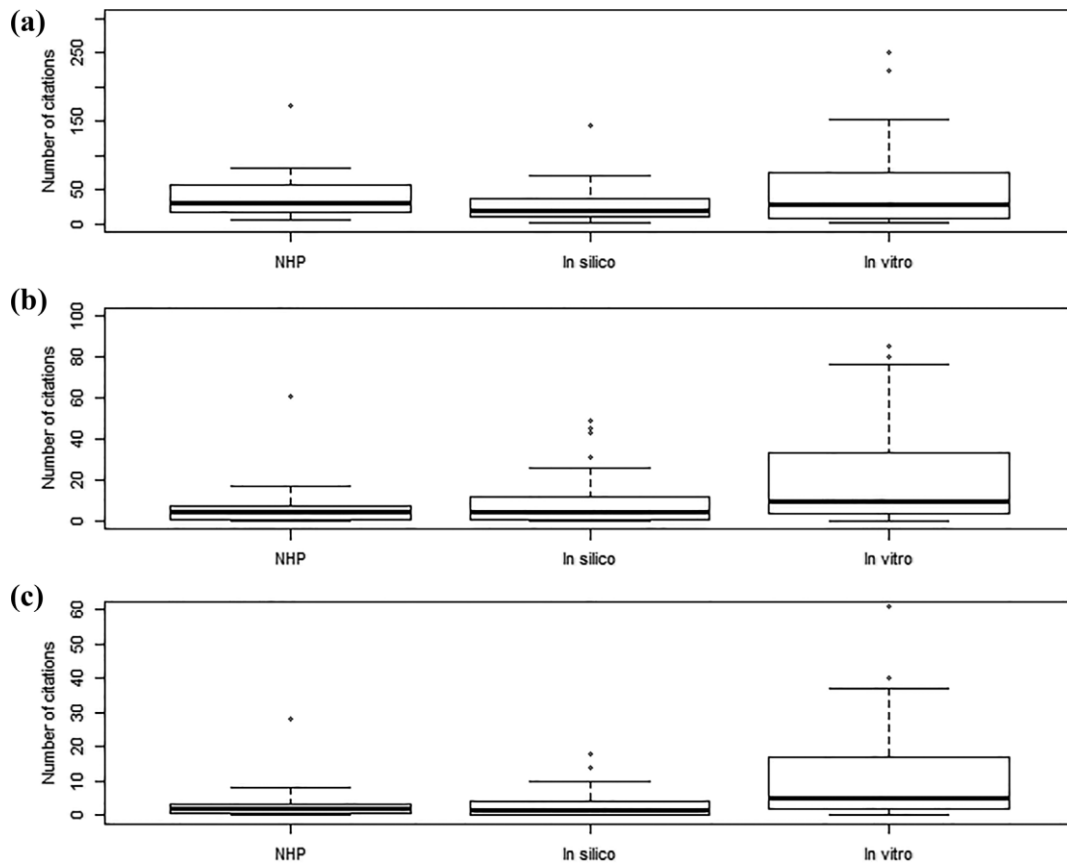
An inspection of the diagnostic plots showed no reason for concern with regard to the GLM fit. Among the papers using an *in vitro*-based approach, one was frequently cited (711 citations). We performed the analysis both with and without this potential outlier and found no significant differences between the two scenarios.

The GLM estimated the average number of citations per paper for each of the three approaches (Figure 1 (a)). Each NHP paper was cited 42.05 times (standard error (SE): 1.450). Papers based on *in silico* approaches were cited less frequently than this (26.87 times; i.e. - 15.18, SE: 0.946), and papers based on *in vitro* approaches were cited more frequently (69.57 times; i.e. +27.52, SE: 1.371). Both differences were statistically significant ( $p < 0.0001$ ).

With regard to the average number of subsequent citations by human research papers (Figure 1 (b)), each NHP paper was cited 2.03 times (SE: 0.08). In comparison, papers based



on *in vitro* and *in silico* approaches were more frequently cited (+ 1.09, SE: 0.09 and + 0.33, SE: 0.10, respectively). These differences were statistically significant ( $p < 0.001$ ).



**Figure 1.** The number of citations received by the retrieved papers, according to research approach. A bibliographic search was carried out to retrieve papers on MDD, which were categorised as based on NHP studies or *in silico* or *in vitro* approaches, according to the research method described. A citation analysis was then performed to identify papers that subsequently cited these retrieved papers. The graphs show: (a) the total number of times that the retrieved papers were cited, according to their research approach; (b) the number of times that the retrieved papers were cited by papers on human research, according to their research approach; and (c) the number of times that the retrieved papers were cited by papers on human research specifically focused on MDD, according to their research approach. For visualisation purposes, the largest observation in the ‘*In vitro*’ category was excluded from the data used to generate the graphs. MDD: major depressive disorder; NHP: non-human primate.

When looking at the average numbers of citations by human research papers specifically focused on MDD (Figure 1 (c)), each NHP paper was cited 1.27 times (SE: 0.12), which was not statistically different from the number of citations of papers based on *in silico* approaches (– 0.12, SE: 0.16). In these MDD-specific publications, papers based on *in vitro* approaches received, on average, more citations (+ 1.3, SE: 0.13) than papers based on NHP studies, and the difference was statistically significant ( $p < 0.001$ ).

The estimated proportion of citations of NHP papers by human research papers was 0.13 (SE: 0.05). This proportion was significantly higher for papers based on *in silico* approaches (+0.20, SE: 0.07,  $p$  0.004) and also for papers based on *in vitro* approaches (+ 0.30, SE: 0.07,  $p < 0.0001$ ).

The estimated proportion of citations of NHP papers by human research papers specifically focused on MDD was 0.06 (SE: 0.03), which was not significantly different from the proportion of citations of papers based on *in silico* approaches (+ 0.06, SE: 0.04,  $p = 0.1389$ ). The proportion of citations in these MDD-specific publications, of papers based on *in vitro* approaches (+ 0.14, SE: 0.04), was significantly different from that of the NHP papers ( $p = 0.001$ ).

### ***Systematic qualitative analysis of citations***

Of the 71 human research papers specifically focused on MDD that cited NHP papers, 50 (70%) were fully available for further analysis, along with 401 of the 482 (83%) human research papers on MDD that cited *in vitro*-based papers, and 58 of the 94 (62%) human research papers on MDD that cited *in silico*-based papers. It was judged that eight of 50 (16%), 15 of 58 (25%) and 100 of 401 (25%) of citations of papers based on NHP studies, *in silico* and *in vitro* approaches, respectively, were relevant to the hypothesis and/or the methods in the citing human research paper on MDD (see Table 1).

**Table 1.** The relevance of cited NHP study-based, *in silico*-based or *in vitro*-based papers to subsequent (i.e. citing) human research papers focused on MDD.<sup>a</sup>

Citations which are:	Papers based on NHP studies	Papers based on <i>in silico</i> approaches	Papers based on <i>in vitro</i> approaches	Total
Redundant or of minor relevance	42 (84%)	43 (75%)	301 (75%)	386
Relevant to the hypothesis or to the methods	8 (16%)	15 (25%)	100 (25%)	123
Total	50	58	401	509

MDD: major depressive disorder; NHP: non-human primate.

<sup>a</sup>The relevance or redundancy of the cited paper to the hypothesis or methods of the citing MDD paper was evaluated by two independent raters. Bold: total value.

The statistical test used to compare the proportions did not reveal any significant differences between the proportions of relevant citations between NHP–*in vitro*, NHP–*in silico* and *in vitro*–*in silico* ( $p = 0.31$ ,  $0.20$  and  $1$ , respectively).

## **Discussion**

We quantitatively and qualitatively analysed the contribution of NHP, *in vitro* and *in silico*-based research approaches to the contemporary understanding of MDD. Of the three approaches analysed, NHP studies seemed to be the approach that was least likely to contribute to furthering progress in this field of human medical research. Of the three, the human-based *in vitro* approach seemed to influence human research to the greatest extent, judging by the

number of citations. However, all three approaches seemed to be equally relevant in informing the hypothesis and/or methods of subsequent human research studies.

Overall, our results suggest that these less funded non-animal research approaches<sup>34</sup> are more or equally effective than heavily invested animal-based research in reaching their final goal — which is to inform clinical research to improve human healthcare. Our quantitative results showed that *in silico*-based and *in vitro*-based approaches contributed more than NHP study-based approaches to human medical research, as the proportion of cited papers featuring the former two approaches was higher than the proportion of cited papers featuring the latter. NHP study-based papers were mainly cited by other papers on animal experimentation, which suggests that they are mainly contributing to subsequent animal research rather than to advances in human healthcare. *In vitro* studies seemed to be the most effective approach, since this approach received significantly more citations in total, and by human research papers either specifically focused on MDD or on other general medical areas.

Of the five analysed NHP study-based papers that were relevant to the citing human research papers on MDD in terms of their hypothesis, method or both, one featured both NHP and human research data. This paper was cited twice, and both citing papers referred to the human research data rather than to the NHP data. Another one of these five NHP papers was considered relevant to the methods and was cited once. The citing paper described both human and rhesus monkey data, and the citation was relevant to the methods used with the rhesus monkeys. After excluding these cases, only three out of the 19 NHP studies were relevant to the hypothesis and/or methods of the subsequent human research studies on MDD.

The results of our citation analysis also suggest that the widely accepted approach to testing medical hypotheses — which relies on *in vitro*-based and *in silico*-based research as a preliminary step prior to animal testing — is not actually working as intended, since clinical papers tend to cite *in silico*-based and *in vitro*-based papers directly too. However, citations of *in silico*-based and *in vitro*-based papers in subsequent publications on human clinical studies of MDD constituted a low percentage (50% or less) of the total citations received in all three analysed categories. This may be explained by the complexity of MDD, which shares certain genetic factors, phenotypic traits and possible neurologic pathways with a number of other disorders. Hence, a human study on anorexia might cite a non-clinical study on MDD focused on weight loss, since weight change is one of the symptoms of MDD.

As to the qualitative results, the judged relevance of the initially retrieved papers to the publications subsequently citing them was low for all three analysed research approaches. Even though a higher percentage of cited *in silico*-based and *in vitro*-based papers were relevant to

the hypothesis and/or methods used by the citing clinical studies, the differences between the three approaches, in the extent of their judged relevance, were deemed insignificant. However, the size of the observed effect — where the proportion of citations of NHP-based papers was much lower than that of *in silico*-based or *in vitro*-based papers — suggests that, while not statistically significant (due to lack of statistical power), there might be a relevant practical difference.

Several important developments in *in vitro* technologies (e.g. organs-on-chips<sup>35</sup>) and in *in silico* technologies (e.g. advanced artificial intelligence based on sophisticated machine learning tools<sup>36</sup>) have been published since 2011. Such studies have been excluded from our analysis, in order to ensure that sufficient time is given to allow for subsequent citation of the resulting papers. However, it is reasonable to expect that these cutting-edge technologies are currently being widely used to generate and test new hypotheses in human medicine.<sup>27</sup> Similarly, induced pluripotent stem cells, even though they have been worked on and developed for more than a decade,<sup>37</sup> have only recently been recommended for MDD research.<sup>38</sup> In light of the above, it would be interesting to repeat the current study a decade from now to investigate whether this has led to an increase in the number of subsequent citations of *in vitro*-based and *in silico*-based papers on MDD, in both MDD-focused and general human research publications.

We recognise that our study has certain limitations. Due to resource constraints, we were unable to use a greater number of search engines (e.g. CAB Abstracts). This would have increased the likelihood of retrieving all *in silico*-based, *in vitro*-based and NHP study-based papers on MDD, which would have increased our sample size and thus made it more comprehensive. Similarly, we were unable to examine the reference lists of many of the retrieved papers, in order to locate additional relevant papers. This inevitably means that some relevant publications might not have been identified. Because the sample size was small, our results should be interpreted with this caveat.

Finally, we are aware of the difficulty in objectively determining the relevance of a cited paper to the publication citing it. We used two different raters, in order to attempt to decrease any error in subjective assessment. Occasionally, the raters differed in their initial assessment, indicating that, even when the same criteria are used for assessment, differences can sometimes arise. However, our experience suggests that these differences would relate to only a small proportion of the papers assessed. Despite the limitations in the citation search and in the systematic qualitative analysis of the citation value, we consider that the method we followed

is useful when evaluating the effectiveness of different research approaches. We hope that similar studies adopt this methodology, in order to investigate other medical disorders.

Our results suggest that the contribution of NHP studies to the current understanding of MDD is poor, and that other approaches with potentially superior relevance to humans should be used. Our results also shed light on the controversy around the efficacy of NHP-based research for investigating human disorders. This controversy is long-standing, with some authors claiming that their use is crucial for medical advancement,<sup>23</sup> while others assert the opposite.<sup>39,40</sup> However, ongoing scientific advances in non-animal methods for the acquisition of knowledge and the development of new treatments may provide future alternative solutions to help avoid the dilemmas and concerns surrounding NHP use.

## Conclusions

To our knowledge, this is the first study to compare the effectiveness of original studies involving the use of NHP, *in vitro* and *in silico* research approaches to inform the medical research community within the MDD field. Our results suggest that, in this field of medical research, human-based *in vitro* and *in silico* research approaches are more promising than NHP studies, in generating new hypotheses and methods for subsequent clinical research.

Given the scientific advances in human-based research methods, we suggest that our methodology could be used in the future to analyse the impact of more recent technologies in informing human medical research. Such analysis could examine if and how the standard paradigm for testing medical hypotheses is still being followed, from applied research, through animal use in preclinical testing, and on to clinical research and development. It could also provide further insight into how the ‘gold standard’ that considers *in vitro*-based and *in silico*-based research approaches as merely preliminary steps prior to animal testing could be challenged and revised. Given the scientific and ethical solutions that innovative human-based approaches are providing, with relatively little investment when compared to the investments in animal-based research, a reallocation of resources is clearly warranted in favour of researching and developing the use of such approaches as part of human medical research.

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Ethics approval was not required for this article.

## Informed consent

Informed consent was not required for this article.

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## Appendix 1: A list of retrieved articles

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## **CHAPTER 5**

# **Is Animal-Based Biomedical Research Being Used in Its Original Context?**



## **Chapter 5. Is Animal-Based Biomedical Research Being Used in Its Original Context?**

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### **Chapter 16**

## **Is Animal-based Biomedical Research Being Used in Its Original Context?**

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### **1. Introduction**

Since the second half of the twentieth century, non-human animals (hereinafter referred to as animals) have been widely used as models for researching human disorders. Historically, this occurred for two main reasons: a) animals are complex living systems; and b) it is considered less ethically-contentious as well as easier, quicker, and cheaper to use animals than humans. Their benefit for biomedical advancement is assumed even though systematic evaluations, though uncommon, suggest otherwise. It is crucial to evaluate whether animal-based biomedical research successfully benefits medical research—even through indirect

pathways—or if it is being used merely to justify further animal-based research. In this chapter we demonstrate that there is a lack of communication between animal-based research and clinical research. We discuss possible reasons for this and reflect on whether animal use in biomedical research is, indeed, fulfilling its primary purpose.

Humans share a long evolutionary story with the rest of the animal kingdom, which explains common physiological and behavioral traits and adaptations. For example, *basal ganglia*, a set of subcortical nuclei involved in several motor functions, are present throughout vertebrate taxa and are largely similar across species (Lee et al., 2015). Similarly, the rise of body temperature as a response to infection is shared by humans and other mammals (Nesse and Williams, 1996; Schaffner, 2006). Even poikilothermic (cold-blooded) animals, such as lizards, tend to move to warmer places when they are ill, until their body temperature is several degrees above normal (Nesse and Williams, 1996). The relatively recent decoding of genomes had shown an impressive number of genes shared between ourselves and taxonomically-distant species, such as the frog (Hellsten et al., 2010). These similarities provided the basis for the untested assumption that animals provide good research models for human disorders.

However, we know that minimal biological changes can create significant differences between species and individuals. For example, Darwin's finches comprise 14 closely related species that vary dramatically in their feeding habits, despite their biological proximity (Lack, 1947). Even amongst individuals of the same species, slight and almost undetectable differences can cause very different adaptive responses. For example, human beings with sickle cell trait may have increased protection from malaria but risk sudden death by hypoxia, when visiting high altitudes or performing intense physical exertion (Scheinin and Wetli, 2009; Webber et al., 2016), safe activities for most people.

Despite individual differences, it is obvious that human beings are the best biomedical model for human disorders. However, clinical research is time consuming and can have severe ethical constraints, which is one of the main reasons why animals are widely used as models for human disorders. Recent *in vitro* developments have allowed us to create cultures of human cells and tissues (e.g., Petropolis et al., 2016; Wilson, Ahearne and Hopkinson, 2015) that are considered superior to using animal samples for human-based research (Clemenson et al., 1998; Huhtala et al., 2008; Petropolis et al., 2016). Nonetheless, among the scientific community, the main obstacle to the total replacement of animal use in biomedical research is not a desire to study cells, tissues, or organs, but the desire to study entire, functioning bodily systems. This is considered necessary when objectives include understanding a drug effect in the whole

organism or trying to understand the etiology and pathogenesis of multifactorial disorders, such as mental disorders.

*In silico* techniques have been slowly addressing this issue, creating whole body simulations (e.g., Viceconti, Clapworthy and Jan, 2008; Viceconti, Henney and Morley-Fletcher, 2016). However, the availability of human data limits these models. For example, if a new disease arises, models may fail to predict accurately the response of the human body to the new pathogen due to lack of data. It should be noted that animal models also suffer from failure to predict human responses accurately. Despite the accepted potential of *in silico* techniques, unvalidated animal models are still commonly believed to be the best available, so far, for studying the entire, functioning human body.

Throughout the years, various authors have asserted that animal research has made only poor contributions to medical progress (e.g., Bailey, 2008; Fadali, 1996; Greek and Greek, 2003; Shapiro, 1998), while others have asserted the opposite (e.g., Illman, 2008; Shively and Clarkson, 2009; Perretta, 2009). Such assertions are based upon historical analyses, investigations into the development of various treatments, and critical reviews of animal model use. Historical accounts are disputed. A classic example is the discovery of the role of the pancreas in diabetes. Many claim that we owe this discovery to experiments conducted by Minkowski and von Mering with dogs, in the second half of the nineteenth century (cited in Bliss, 1982); whereas, others argue that this medical breakthrough was achieved by Thomas Cawley, 100 years earlier, while performing autopsies on patients who died from diabetes (cited in Fadali, 1996).

Investigations into the development of treatments are also controversial. A good example is the development of the poliomyelitis vaccine. Poliomyelitis is a viral disease that reached epidemic proportions in 1916. Some (e.g., Illman, 2008) state that it was the experiments performed on mice and monkeys that allowed scientists to understand its pathogenesis and develop a vaccine. Furthermore, both poliomyelitis vaccines (Salk vaccine and Sabin vaccine) were initially grown in monkey kidney tissue (Dowdle et al., 2003), reinforcing the perception of the central role of animal experiments in the development of poliomyelitis treatment (Illman, 2008). However, others (e.g., Fadali, 1996) claim that animal experiments delayed the vaccine's development. Rhesus monkeys, which provided a widely-used animal model for poliomyelitis, misled scientists to believe that the virus was transmitted via the respiratory, rather than the digestive route (Dowling, cited in Bailey, 2008), as earlier research on humans had suggested (see Fadali, 1996, for a review). This mistake led to an erroneous clinical trial in 1937, in which exposed children suffered olfactory damage (Parish, 1968). Also, the first poliomyelitis

vaccines, grown on monkey kidney cells, were responsible for the exposure of millions of American citizens to simian virus 40, found in rare human cancers (Pennisi, 1997). When it comes to non-human primates (NHPS), these disputes are even more contentious, because public opinion is less supportive of the use of NHPS in research (European Commission, 2010). Furthermore, as technology evolves, better methods become available, and the apparent historical necessity of animal experiments becomes of less relevance. For example, vaccines that used to be developed using animal tissues—at times suboptimally due to poor efficiency (e.g., rubella vaccine developed through duck embryo cells and dog kidney cells) or zoonosis (e.g., the simian virus that reached humans through the first polio vaccines)—are now being developed using human strains (Plotkin, 2017).

Recently more objective tools to assess the contribution of animal models to biomedical progress have emerged. Such is the case of systematic reviews, meta-analyses, and citation analyses. Systematic reviews are literature reviews focused on a research question that aim to identify, appraise, and synthesize all high-quality research evidence relevant to that question. They are generally considered the best tool to produce evidence about the value of animal studies (Pound et al., 2004), not only because they are designed to include all relevant information, drastically reducing the potential for bias; but also because systematic reviews evaluate experimental designs through rigorous and objective peer-reviewed protocols, such as the *Animal Research: Reporting In Vivo Experiments* (ARRIVE) guidelines that apply scientific method to the task of reviewing research evidence (Kilkenny et al., 2010). A meta-analysis can go even further by incorporating a statistical representation of all the reviewed studies as well.

In the past decades, the number of systematic reviews shedding light on the scientific value of animal studies has increased (e.g., Banwell, Sena and Macleod, 2009; Corpet and Pierre, 2005; Lucas et al., 2002; Macleod et al., 2005; Martić-Kehl et al., 2015; Perel et al., 2007). The systematic reviews have revealed: a) poor transferability of animal outcomes to human clinical trials (e.g., Perel et al., 2007); b) simultaneous occurrence of animal and clinical trials, rather than sequentially, as expected given that the animal experiments should be conducted first, to allow detection of possible toxicity (e.g., Lucas et al., 2002); and, c) significant methodological and design flaws in a clear majority of animal experiments (e.g., Martić-Kehl et al., 2015). Consequently, the use of ARRIVE or similar guidelines has become more common, which will hopefully lead to better protocols and reduce redundant studies. As for the poor transferability of animal outcomes to human trials, it can be argued that this is either a consequence of poor experimental design, and/or the fact that animal models are not suitable models for human beings (Bailey and Taylor, 2016).

Another way to determine the value of animal studies is citation analysis, which consists of determining the frequency with which a study is cited in subsequent papers. Several authors have conducted citation analyses on published papers, reporting data from animals used as models for human disorders (e.g., Carvalho et al., 2016; Knight, 2007; Long, Huang and Ho, 2014); results show that these papers have received very few citations in human medical papers. Again, it can be debated whether this occurs due to a false assumption that animal models are suitable models for human disorders or because of methodological errors, or both.

To try to address this issue, we performed a citation analysis on a small sample of papers reporting data from animals used to model two complex psychiatric disorders: attention deficit hyperactivity disorder (ADHD) and major depressive disorder (MDD).

ADHD is a chronic neurodevelopmental condition of multifactorial origin, marked by persistent inattention; hyperactivity; and, occasionally, impulsivity (American Psychiatric Association, APA, 2013). It affects 2.2% of children worldwide (Erskine et al., 2013); and it can be extremely disabling (APA, 2013). MDD is a complex psychiatric mood disorder characterized by a persistent feeling of sadness that seriously impairs normal day-to-day functioning and may even lead to suicide (APA, 2013). Mental disorders are the leading cause of years lived with disability worldwide, and 40.5% of this burden is caused by MDD alone (World Health Organization, 2008).

In this study we categorized the citations obtained into animal versus human studies and determined whether human-based and animal-based papers focused on the same disorder investigated by the animal study they were citing. This form of analysis is valuable for shedding light on whether animal-based research is being used to advance human healthcare, or whether it simply fuels further animal-based research. If animal studies are contributing to human healthcare advancements, then we would expect that:

1. The citations made in human-based papers should be a substantial proportion of total citations.
2. The citations should be made mainly by studies focused on the same disorder. Any substantial deviations would signal the possibility that animal-based research is not achieving its primary purpose.

## **2. Methods**

We conducted a citation analysis as defined by Garfield and Merton (1979). Briefly, in a citation analysis, one defines a number of target papers and conducts a search of all papers that

cite these target papers. The information obtained can include the total number of citations and patterns of citation. We used a total of 50 target papers: 25 non-human animal studies on ADHD, and 25 non-human animal studies on MDD.

The ADHD papers were selected from the citation analysis database created in the study by Carvalho et al. (2016). We included all papers reporting data collected with primate models (7 papers) and randomly selected 18 papers from the remaining papers, using the free online tool, *Research Randomizer* ([www.randomizer.org](http://www.randomizer.org)). The 25 studies were examined to determine the proportion of citations each paper received in human-based papers focused on ADHD, in human-based papers focused on other subjects, in animal-based papers focused on ADHD, and in animal-based papers focused on other subjects.

The MDD papers were obtained using PubMed to locate original articles using animal models to investigate major depressive disorder (similar to the protocol used in Carvalho et al., 2016). We searched PubMed using the following Medical Subject Heading (MeSH) search terms:

“Major Depressive Disorder” AND (title/abstract): “animal” OR “rat” OR “mice” OR “mouse” OR “Rattus” OR “Mus” OR “pig” OR “Cavia” OR “Sus” OR “rabbit” OR “Leporidae” OR “Drosophila” OR “primate” OR “monkey” OR “Macaca” OR “macaque” OR “ape” OR “rhesus” OR “chimpanzee” OR “bonobo” OR “gorilla” OR “Pan” OR “Orang Utan” OR “Pongo” OR “gibbon” OR “Hylobates” OR “Colobus” OR “Baboon” OR “Papio” OR “Mandrillus” OR “Mandrill” OR “Cebus” OR “Cebuella” OR “Brachyteles” OR “Loris” OR “Nycticebus” OR “Lemur” OR “dog” OR “Canis” OR “cat” OR “Felis.”

We found 33 published papers using NHPS as models and randomly selected seven, using the same randomizing tool. We found over 1,000 published papers using other animals as models and proceeded, as above, to randomly select 18 papers for the citation analysis. We recorded the number of citations each paper received from subsequent animal research papers and subsequent human research papers. We similarly analyzed the aim of the citing paper (whether it was focused on the same disorder or another), in both animal and human papers.

Using Fisher’s exact test (<http://www.kisnet.or.jp/nappa/software/star-e/freq/1x2.htm>), we investigated whether there was a significant difference between the number of citations of the target animal articles in human research papers and in animal research papers. We also verified whether there was a significant difference between the number of citations in

subsequent articles addressing the same disorder and subsequent articles addressing different topics. Differences were considered statistically significant if  $p < 0.05$ .

### 3. Results

Regarding our ADHD sample, the 25 original animal studies were cited 660 times. As shown in Figure 16.1, animal studies were mainly cited in other animal research papers (315), of which 82 focused on ADHD and 233 focused on different subjects. The sample resulted in 69 citations in human research papers, of which 30 focused on ADHA and 39 focused on different subjects. The remaining 345 citations were in review articles (198) or papers describing different methods, such as *in silico* or *in vitro* (147).

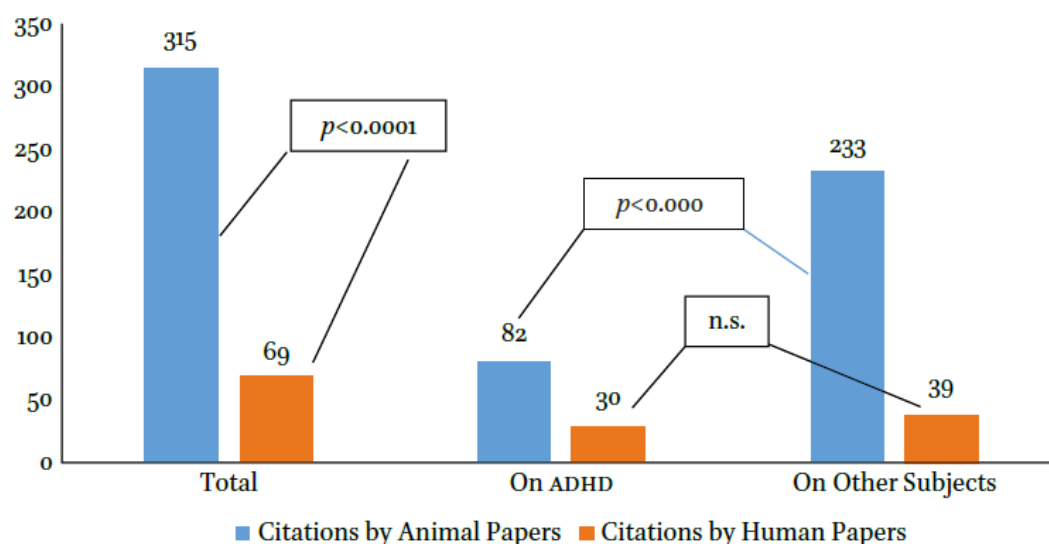


FIGURE 16.1 Citations of animal papers focused on ADHD.

The columns represent the number of citations of the 25 target papers in animal research papers (blue) and human research papers (orange). The total number (left), as well as the number of citations in papers studying ADHD (middle) and other subjects (right) are presented. Fisher's exact test p-values are also presented for each comparison made (n.s.= non-significant).

The number of citations in animal research papers was far greater than the number of citations in human research papers ( $p < 0.0001$ ). The number of citations in animal research papers focused on ADHD was lower than the number of citations in animal research papers focused on other subjects ( $p < 0.0001$ ). The difference between the number of citations in human research papers on ADHD and human research papers focused on other subjects was not statistically significant ( $p = 0.3355$ ).

Seven of the target papers reported NHP studies. These papers received 274 citations, 94 of which were in subsequent animal research papers and 48 were in human research papers.

The remaining 138 citations were in review papers (96) or papers describing different methods, such as *in silico* or *in vitro* (42). The difference between citations in animal research papers and human research papers was statistically significant ( $p=0.0001$ ). Of the 94 citations in subsequent animal papers, 21 were in papers focused on ADHD, and 73 were in papers focused on other issues. This difference was also statistically significant ( $p<0.0001$ ).

Of the 48 citations in human research papers, 15 were in papers focused on ADHD, and 33 were in papers describing other disorders. Fisher's exact test showed that in the case of NHP there was a statistically significant difference between the number of citations in papers on ADHD and papers focused on other subjects ( $p=0.0132$ ).

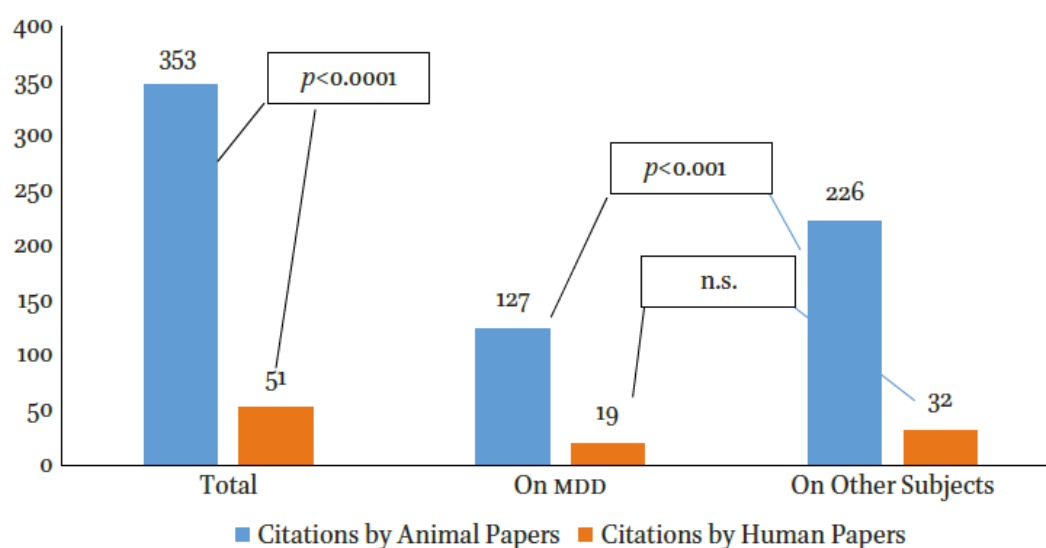


FIGURE 16.2 Citations of animal papers focused on MDD.

Regarding the MDD sample, the 25 target animal studies were cited 631 times. As shown in Figure 16.2, animal studies were mainly cited in other animal research papers (353), of which 127 focused on MDD, and 226 focused on different subjects. The sample received 51 citations in human research papers, of which 19 focused on MDD, and 32 focused on different subjects. The remaining 227 citations were in review articles (163) or papers describing different methods, such as *in silico* or *in vitro* (64).

The columns represent the number of citations in animal research papers (blue) and in human research papers (orange) of the 25 cited papers. The total number (left), as well as the number of citations in papers studying MDD (middle) and other subjects (right) are presented. Fisher's exact test p-values are also presented for each comparison made.

The number of citations in animal research papers was substantially greater than the number of citations in human research papers (Fisher's exact test,  $p<0.0001$ ). The number of citations in animal research papers focused on MDD was lower than the number of citations in



papers focused other subjects ( $p < 0.0001$ ). The difference between the number of citations in human research papers focused on MDD and papers focused on other subjects was not statistically significant ( $p = 0.0919$ ).

The seven papers reporting on NHP studies received 227 citations, 97 of which were in subsequent animal research papers, and 19 were in human research papers. This difference was statically significant ( $p = 0.001$ ). Of the 97 citations in subsequent animal papers, 13 were in papers on MDD, and 84 were in papers focused on other issues. This difference was statistically significant ( $p < 0.0001$ ). Of the 19 citations in human medical papers, six were in papers on MDD, and 13 were in papers focused on other subjects. This difference was not statistically significant ( $p = 0.1670$ ).

#### **4. Discussion**

Our results suggest that animal data is mainly used by subsequent animal papers. Another trend that emerged is that papers citing animal research (whether they focus on human medical research or not) focus on disorders that differ from the one targeted in the animal study cited. This trend is stronger in papers focused on animal research.

The tendency for animal research to be cited more in subsequent animal research has been previously described (e.g., Carvalho et al., 2016). This finding contradicts the previously stated assumption that citations in human-focused papers should constitute a substantial proportion of the total number of citations. Clearly, biomedical research focused on animal models does not seem to be considered important by, or particularly visible to, the human medical research community.

Our results also indicate that papers citing data collected from animal models do not necessarily target the disorder described in the animal paper. This difference appears to be more significant in animal research papers citing other animal research papers, than in human research papers that cite animal research. This contradicts the second assumption we tested: that citations should be made mainly by studies focused on the same disorder. This finding reinforces the concern that animal-based research is failing to shape meaningful healthcare advances for humans.

It can be argued that if the same animal model is used for different disorders, it may contribute more to medical research than predicted by the second assumption. For example, dat knock-out mice comprise a common model for ADHD but are also used to model Parkinson or schizophrenia (Gainetdinov, 2008). Nevertheless, the total citation frequency in human research

papers is still very low, regardless of the paper's area of focus (Carvalho et al., 2016; Knight, 2007; Long, Huang and Ho, 2014).

The fact that animal strains are used to model several disorders may help explain the intriguing tendency for animal research papers to be cited more often in papers addressing non-related subjects than in papers focused on the same disorder. This tendency was also apparent in human-based papers that cited animal-based papers focused on MDD. This may have occurred because there are 6–7 times more papers focused on MDD than on ADHD which may mean that the 25 papers on MDD were not a representative sample of MDD research. If this phenomenon was to recur with a larger sample, one could argue that this is due to the same animal strain being used for different purposes, as previously mentioned. If the strains used in MDD research are commonly used to model a greater number of disorders than strains used in ADHD research, it would be more probable that human studies focused on unrelated disorders cite studies in these strains. We did not verify this, and it should be explored in future studies.

Our data shows that even though the difference between the total frequency of citations by human papers focused on ADHD and paper focused on other subjects was not statistically significant, there was a bias regarding papers describing NHP models of ADHD. A close examination of the data allowed us to conclude that this bias was due to one paper, cited 18 times in human research papers, 17 of which focused on disorders other than ADHD. This particular paper described the behavioral changes caused by bicuculline microinjections in external *globus pallidus*, a brain structure involved in pathogenesis of ADHD but also in Tourette's syndrome. Most of the 18 citations this paper received in human papers were actually from papers related to Tourette's syndrome. If we discard this outlier, the data on NHP follows the same pattern as other ADHD papers.

Since our two assumptions have been challenged, we must discuss their causes and implications. One possible explanation for these results is that animal models only attempt to model specific symptoms or traits of complex human disorders. This oversimplification may lead to results that are non-applicable or of minimal use for human medicine. Another possible explanation is that funding is more easily attached to studies that claim to have the potential to advance human health. This may lead animal researchers to overestimate the applicability of their projects. A further possible explanation is that communication and sharing of ideas between clinical and preclinical research is insufficient. Moreover, previous studies have shown that clinical and preclinical trials can occur simultaneously (Pound et al., 2004), which emphasizes this lack of communication. Although it is difficult to define an optimum communication level, this issue must be raised in both communities in order to maximize

efficiency in scientific research as well as the promotion of animal welfare. An additional possibility is that a substantial amount of animal research is needed in order to achieve a critical mass that can lead to useful breakthroughs in human health. This is a theoretical possibility that is difficult to measure and properly test. However, even if proven correct, the financial and ethical implications of this assumption should be considered. Other methods may prove to be more efficient or ethically acceptable, and this comparison could lead to a re-evaluation of funding priorities. Finally, a conceivable possibility is that animal models are not suitable for biomedical research into complex human disorders. It may be possible that the uniqueness of some human disorders is just not feasibly simulated in non-human animals.

If our last suggested explanation is indeed correct, the implications must be considered. The funding currently allocated to these animal-based studies should still be available for science. While most of it would likely be redirected to other models of these disorders, some of it could be assigned either to other basic research fields or to the care of surplus animals.

Regardless of the possible explanations, our results indicate that animal-based research is failing to reach the human medical community, at least in the case of mental disorders, such as the ones we evaluated. This means that considerable financial investment and considerable suffering inflicted on the animals used for this purpose did not translate into direct medical advances. It would be interesting to survey the practitioners working with mental disorders to assess if this is due to lack of awareness of animal-based findings, or if they consider animal-based data to be inadequate or lacking in relevance.

In conclusion, our analysis suggests that most animal-based research, at least in the case of these mental disorders, is not currently being utilized by human-based researchers. Regardless of the reasons for this, the profound financial and ethical implications should lead to a re-evaluation of the current research paradigm, which is heavily reliant on invasive animal use.

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## **CHAPTER 6**

# ***Are *In Vitro* and *In Silico* Research Approaches Used Appropriately Prior to Conducting Animal Studies? A Major Depressive Disorder Case Study***



## Chapter 6. Are *in vitro* and *in silico* approaches used appropriately for animal-based major depressive disorder research?

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## Abstract

**Context:** The current biomedical research paradigm postulates that *in vitro* and *in silico* data inform animal studies that will subsequently inform human studies. The contribution of animal studies to human medical studies is disputed whereas the contribution of *in vitro* and *in silico* studies to animal studies is yet to be properly quantified.

**Objective:** To quantify the contribution of *in vitro* and *in silico* biomedical data on animal studies. If their contribution reflects the biomedical research paradigm, they should receive more citations from animal studies than from human medical studies.

**Method:** We examined a citation analysis database containing the number of citations received by 67 original *in vitro* and *in silico* papers on Major Depressive Disorder (MDD) organized in four research categories: other *in vitro* and *in silico* papers, animal papers and human medical papers. We determined the proportion of citations these papers received for each research category.

**Results:** The 38 *in vitro* papers received 2,574 citations. Of those, 18% were by animal papers and 40% were by human medical papers. The 29 *in silico* papers received 806 citations. Of those, 5% were by animal papers and 39% were by human medical papers.

**Conclusion:** The smaller proportion of citations by animal studies suggests that, at least within MDD, *in vitro* and *in silico* research is not substantially influencing animal studies, nor informing animal studies prior to human studies, contradicting the biomedical research paradigm. If a similar pattern occurs with other human disorders or drug testing, then the current animal-based biomedical research field is inconsistent with realities underpinning the development of human studies.

**Keywords:** Animal use alternatives; *in vitro*, *in silico*, biomedical research

## Introduction

Biomedical research heavily relies on animal studies, despite its ethical and clinical limitations (Herrmann, 2019).

The current standard paradigm for biomedical research and drug discovery and development requires scientists to test from the simplest to increasingly complex models before human studies and trials, as shown in Figure 6.1, kindly provided by Taylor (2019).

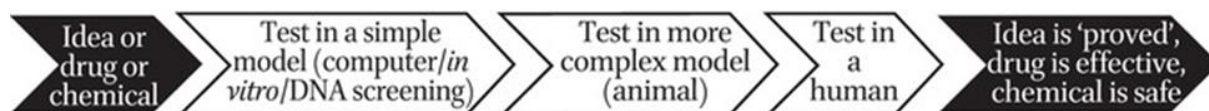


Figure 6.1 Current paradigm of biomedical research and drug discovery and development

Supporters of animal studies within biomedical research claim that 1) it is not possible to drop animal studies since that would jeopardize human health, and that 2) human-based methods (*in silico* and *in vitro*) are used in early steps of biomedical research to inform the animal research community, hence avoiding unnecessary or excessive use of animals. For example, purportedly, if a substance shows high levels of toxicity *in vitro* it will not go through animal testing (Choudhuri et al., 2017). In the same way, a drug that shows high toxicity levels in animal testing should not proceed to human trials. However, it has been demonstrated that human trials may sometimes run simultaneously with animal trials, rather than sequentially, as one would expect if animal trials were an essential step prior to human trials (Pound et al., 2004). It has also been demonstrated that *in vitro* and *in silico* data are more frequently directly cited in human medical papers than animal data, strengthening the idea that these research approaches are not necessarily being used as a step prior to animal studies (Carvalho et al., 2019a).

Still, the contribution of *in vitro* and *in silico* original research studies to animal studies targeting human disorders is yet to be determined. Hence, the aim of the current study is to assess if *in vitro* and *in silico* papers describing original data on a human disorder (MDD) are being appropriately cited in subsequent animal papers.

If *in vitro* and *in silico* studies are indeed seen as a prior step to animal studies in biomedical research, then we would expect that papers describing *in vitro* or *in silico* data on a human disorder should be cited more frequently by animal papers, than by human medical papers. If, on the contrary, this is not the case, other studies on other human disorders, drug testing or a broad review study should be conducted to confirm if the current theoretical paradigm for biomedical research is not being followed in practice, and if should be revised.

## Methods

We conducted a citation analysis as defined by Garfield and Merton (1979). Concisely, in a citation analysis, target papers are located first and then a search for all other papers citing the former is performed.

The information compiled comprises the total number of citations, and the patterns of citation. We used a total of 67 target papers of *in vitro* or *in silico* studies on MDD – utilising only human data, selected from the citation analysis database created in our previous study (Carvalho et al., 2019a). Using the citation tracking facility within Web of Science, we counted

the number of times each target paper was cited by subsequent papers in the following categories: `animal research papers`, `human medical papers`, `*in vitro* papers`, and `*in silico* papers`. Citing papers may have been assigned to more than one category if they described different research approaches (e.g. human and *in vitro*).

## Results

In total, 464 (18%) of the 2,574 citations received by the 38 *in vitro* papers were by invasive animal research papers and 978 (40%) by human medical papers. For the 29 *in silico* papers, 44 (5%) of the 806 citations were by invasive animal research papers and 317 (39%) by human medical papers.

As shown in Figure 6.2, the majority of citations received by both *in vitro* or *in silico* target papers were by papers employing the same research method, and by human medical papers. The proportion of citations by animal papers and the other research method were considerably lower. More importantly, the proportion of citations by animal papers was lower than by human medical papers.

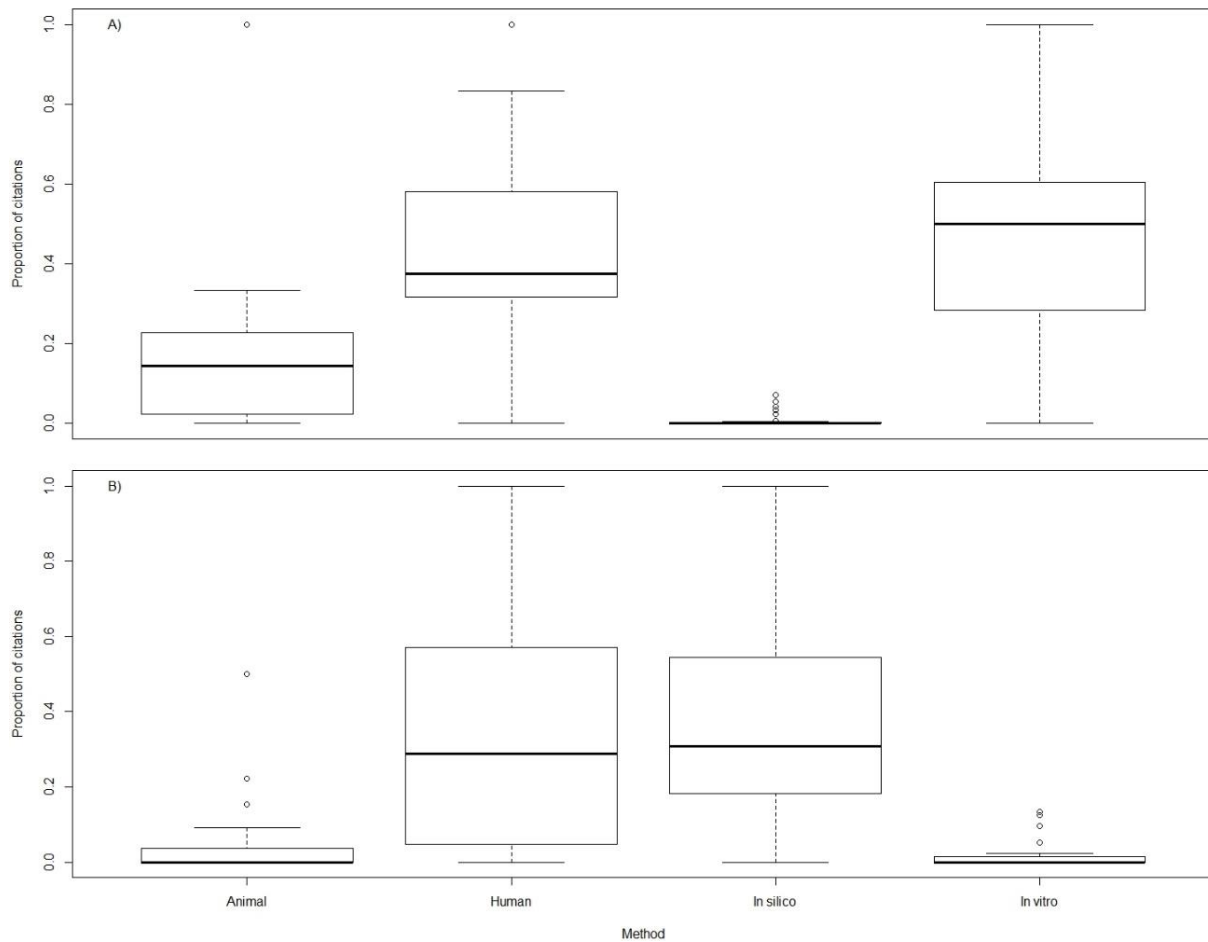


Figure 6.2. Citations of *in vitro* and *in silico* papers on MDD received by research category. A) shows the “*in vitro*” results and B) the “*in silico*” results.

## Discussion

The results of our citation analysis suggest that the standard approach to testing medical hypotheses – which postulates *in vitro* and *in silico* research are a step prior to animal testing – is not actually being followed, at least for MDD research. Clearly, MDD biomedical research utilising *in vitro* and *in silico* data does not seem to be considered important by, or at least more important to, the animal research community than to the human medical community.

One can argue that if the animal research community is not citing *in vitro* and *in silico* papers on MDD, these might be of limited use. However, that is inconsistent with their substantial use by the human medical community, which cites more this kind of research than the research based on animal studies (Carvalho et al., 2019a). Additionally, this lack of transferability of knowledge between the animal research and the human medical research communities is further enhanced by the fact that, in general, most citations received by animal

research papers are from studies within the same category, not from human medical papers (e.g. Carvalho et al., 2019b).

MDD is a complex human mental disorder with multifactorial aetiopathogenesis (Chiriță et al., 2015), so one cannot extrapolate that the citation patterns found here – that *in vitro* and *in silico* studies are not being used as a step prior to animal studies in MDD – will necessarily be replicated in other disorders that have just one cause (e.g. Down's syndrome). Hence, the next step should be the use of a similar approach targeting monofactorial disorders and drug trials. If, as whole, these studies produce similar results, then it would be compelling evidence that the standard protocols of biomedical research and drug discovery and development are not being followed, which supports the claims made by several authors (e.g. Herrmann, 2019) that the 3Rs (replacement, reduction, refinement) are not being addressed as well as required by law and by good research practices.

Sixty years ago Russell and Burch (1959) established the foundations of much current legislation regarding animal experimentation, with the formulation of the 3Rs principles. Even though the research community unanimously welcomes them, the focus of their application has predominantly been refinement, and not always in an effective way (Herrmann, 2019). In theory, the reduction principle depends upon the standard use of *in silico* and *in vitro* techniques prior to animal studies. If original data on human disorders from *in vitro* and *in silico* approaches are not being used by the animal research community, then the reduction principle is not being properly fulfilled. The reasons behind this must surely be multiple, but with our study there is one that became salient and deserves attention. *In vitro* and *in silico* approaches are, by definition, human-based methods, not animal-based methods, making it similarly difficult for animal studies to cite *in vitro* and *in silico* studies, as for human studies to cite animal studies. This highlights that the current paradigm of biomedical research and drug discovery and development includes two steps of knowledge transferability between the animal and the human models, neither of which appear to work well, which probably compounds the poor use of the 3Rs principles.

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## **CHAPTER 7**

# **Ethical and Scientific Pitfalls Concerning Laboratory Research with Non-Human Primates, and Possible Solutions**



# **Chapter 7. Ethical and Scientific Pitfalls Concerning Laboratory Research with Non-Human Primates, and Possible Solutions**

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## **Simple Summary**

Legislation and guidelines governing biomedical research with humans and non-human primates (NHPs) rely on different ethical frameworks. In this paper we argue that the main ethical framework used to assess and justify NHP experimentation is inadequate for its purpose. We propose a change of framework that we believe would benefit NHPs and improve research quality.

## Abstract

Basic and applied laboratory research, whenever intrusive or invasive, presents substantial ethical challenges for ethical committees, be it with human beings or with non-human animals. In this paper we discuss the use of non-human primates (NHPs), mostly as animal models, in laboratory based research. We examine the two ethical frameworks that support current legislation and guidelines: deontology and utilitarianism. While human based research is regulated under deontological principles, guidelines for laboratory animal research rely on utilitarianism. We argue that the utilitarian framework is inadequate for this purpose: on the one hand, it is almost impossible to accurately predict the benefits of a study for all potential stakeholders; and on the other hand, harm inflicted on NHPs (and other animals) used in laboratory research is extensive despite the increasing efforts of ethics committees and the research community to address this. Although deontology and utilitarianism are both valid ethical frameworks, we advocate that a deontological approach is more suitable, since we arguably have moral duties to NHPs. We provide suggestions on how to ensure that research currently conducted in laboratory settings shifts towards approaches that abide by deontological principles. We assert that this would not impede reasonable scientific research.

**Keywords:** non-human primate research; biomedical research; deontology; utilitarianism; animal use alternatives

## 1. Non-Human Primates in Laboratory Research

Since the mid twentieth century, non-human primates (NHPs) have been widely used in laboratory research, mostly in biomedical research [1], and mostly in the cognitive sciences [2].

In recent years, due to public pressure and legislation, the number of NHPs used in biomedical research has been significantly reduced in the European Union and the United States [3,4], but has increased dramatically in some other countries, particularly China [5].

Some researchers claim that the use of NHPs in biomedical research is crucial, due to their similarities with humans [6,7], and state that a total ban on such research would compromise medical advances in several fields, such as those focused on infectious diseases, cardiovascular diseases, endocrine diseases, reproductive diseases, neurological disorders, ophthalmic diseases [5,6] asthma, certain types of cancer [1], transplants [8] and psychiatric disorders [4]. However, for many years the presumed benefits of NHP research for medical advances were not subjected to rigorous critical evaluation. In recent years however, evidence-

based assessments have been conducted, frequently demonstrating that NHP models have provided disappointing contributions toward human medical advancements [9–12].

In the cognition and behaviour domains, studies with captive primates have made relevant contributions to psychology and neuroscience, as exemplified by Harlow’s experiments on “the nature of love” [13] or Selemon and Goldman Rakic’s [14] early brain topography studies. In both cases, as in many others, discoveries were made which incurred high costs to NHP subjects, including suffering and death. The ecological validity of behavioural and cognitive studies conducted on captive NHPs has also been questioned [15].

## **2. Similarities between Humans and Non-Human Primates**

Moral concerns raised by the use of NHPs in intrusive or invasive research result from their sentience, consciousness and affective states. In those aspects, NHPs are very similar to humans, which makes it reasonable to give them similar protection to that afforded to human subjects. However, they are very different in other aspects, so they are not necessarily good models for human biology.

From the mid-twentieth century onwards, it became clear that NHPs and humans shared so many traits that trying to categorise any trait as wholly human became something of a futile exercise. Below, we present a few examples of studies that brought a greater awareness of the high similarity (and evolutionary continuity) between humans and NHPs.

Some of the very first “humanlike” capabilities that attracted considerable scientific interest were the discoveries that NHPs build and use tools [16,17], solve new problems, and develop and pass on cultural behaviour [18,19]. NHPs of several species have also demonstrated the ability to recognise themselves in a mirror—an ability that has been largely interpreted as evidence of self-awareness [20,21]. This was once thought to be a uniquely human ability. All NHPs that have been studied to date, from rhesus monkeys to chimpanzees and gorillas, have also been shown to have distinct personalities with complex behavioural patterns, as occurs with humans [22]. Furthermore, all NHPs establish strong social bonds [23,24], and most live in complex societies [25]. Like humans, NHPs experience and display emotions [26], strong mother–infant and other familial bonds [27] and are capable of experiencing empathy and behaving sympathetically (e.g., [28,29]). In addition, an increasing amount of evidence has accumulated that they have notions of justice and unfairness [30]. NHPs communicate effectively through vocalisations, gestures, and facial expressions [31–35]. They possess a linguistic and lateralised brain which allows them to learn and use sign language,

among other skills [36,37]. NHP cognitive abilities have been astounding us for many years. NHPs can create lasting memories [38,39], possess mathematical skills [40], and can even outperform university students in numerical memory [41]. They can solve complex problems that require intelligence [42–44].

These skills are not exclusive to NHPs and can be found across a number of other non-human animal species. However, NHP behaviour and skills are among the most well documented. The fact that they are our closest living relatives has probably facilitated the recognition and social acceptance of their cognitive abilities and emotional lives and has probably made them a preferred target for cognitive research.

Collectively, these and many other studies addressing primate cognition, emotion, and social behaviour have become the scientific basis for arguing that NHPs should be afforded a significant moral status, for some authors [45,46]. It has also been pointed out that the similarities between humans and NHPs are the main ethical obstacle regarding the laboratory confinement and use of NHPs [47]. This is indeed a controversial issue within the scientific community, and for the wider public [48,49], but the recognition that there are significant ethical concerns to be addressed is nearly universal.

Because of their anatomical and physiological similarity to humans [50], as well as such cognitive, behavioural, and social similarities, NHPs have been portrayed as ideal animal models for some biomedical and cognitive research problems.

However, such similarities do not automatically make NHPs ideal models for humans within biomedical research [15]. For example, major evolutionary jumps have occurred since the last common ancestor humans shared with chimpanzees, with homologous brain areas being recruited in humans for new functions, and new structures emerging altogether [51].

### **3. The Ethical Frameworks of Deontology and Utilitarianism**

Biomedical research, with both humans and non-human animals, presents considerable ethical challenges, since it is not uncommonly invasive or intrusive, causing pain, stress or discomfort. For example, xenotransplantation experiments are classified under the current legislation as “severe” procedures, since they are likely to compromise the general health of the animal in the case of organ rejection. However, NHPs are still presently used in this sort of research [52,53].

In modern human societies, laws should express and enforce society’s moral codes [54]. Legislation and ethical guidelines have arisen to guide scientists through ethical dilemmas and

prevent forms of abuse that were more common in the past. Classical examples include the use of orphans to carry smallpox live vaccine through arm-to-arm transportation across the Atlantic Ocean during the 19th century—this involves vaccinating a child and then transferring the vaccine to another as soon as the infectious pustule forms [55]; medical research conducted with prisoners by German doctors; and the infamous Tuskegee research, in which African-Americans that had syphilis unknowingly were not given treatment so the doctors could study the natural progress of the disease in rural American areas between 1932 and 1972 [56]. After World War II and the subsequent Nuremberg trials, rules and principles to guide research with human beings emerged. The general rules that guide modern research with human subjects were written by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization (WHO) in 1982 and revised in 1993 and 2002 [57]. The International Ethical Guidelines for Biomedical Research Involving Human Subjects—which has been transposed into legislation or guidelines in most countries, established four basic ethical principles: respect for persons, beneficence, non-maleficence and justice.

Respect for persons includes the principle of autonomy (as described by Beauchamp and Childress [58]), and the protection of individuals with impaired autonomy [57]. The beneficence principle refers to the obligation to maximise benefit, whilst the non-maleficence principle refers to minimising harm [57,58], in keeping with the utilitarianism view (see section below), except that in this case the permissible harm must be mild, regardless of expected benefits [58,59]. The principle of justice requires the equitable distribution of resources, which in the case of biomedical research translates to an equal distribution of burdens and benefits amongst research participants [58,60].

The same principles are stated in The Belmont Report, a document created in the USA in 1979 by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. This is a critical document for those involved in basic and clinical research with human beings [61].

Regarding clinical research, the regulations and mechanisms mentioned above seem to be effective in solving most of the ethical challenges [62], but that is not the case with invasive animal experiments, particularly those using NHPs [63,64]. The main reason for these inconsistencies seems to be the use of different frameworks to evaluate and guide research with humans and NHPs [65]. Guidelines and legislation that regulate human research rely on mostly deontological principles, while those that regulate animal research rely on utilitarianism.

The use of different ethical frameworks for humans and NHPs may even result in opposing ethical recommendations: genetic experiments, which are often restricted from an ethical point of view when it comes to human beings [59,66], may be encouraged from an ethical point of view when it comes to NHPs [6,67].

In the next sections, we briefly describe both ethical frameworks and analyse how each applies to current NHP research.

### ***3.1. Deontology***

Deontological ethics is the normative ethical position often associated with the philosopher Immanuel Kant, which judges the morality of an action based on the action's adherence to a rule or rules [68,69]. The underlying assumption is that something is good because it is the right thing to do [70]. Deontology stands for principles that must be fulfilled regardless of their consequences [71] and, according to Kant, there are hypothetical imperatives, which apply to someone who wishes to achieve a certain goal, and categorical imperatives, which are universal, absolute, and unconditional requirements that must be obeyed in all circumstances. Subsequent deontological philosophers, such as Ross [72], included the concept of moral relativism in deontology, which states that the morally right act is relative to the circumstances.

Ross [72] also postulated seven *prima facie* duties: (1) duty of beneficence (help other people to increase their pleasure, improve their character, and so on); (2) duty of non-maleficence (avoid harming other people); (3) duty of justice (to ensure people get what they deserve); (4) duty of self-improvement (to improve oneself); (5) duty of reparation (to repay someone for acting wrongly towards them); (6) duty of gratitude (to benefit people who have benefited oneself); (7) duty of promise-keeping (to act according to explicit and implicit promises, including the implicit promise to tell the truth). It is noteworthy that current legislation and guidelines on research with human subjects encompass the first three.

Deontology was established as an anthropocentric and rationalist framework due to the firm belief of rationalist philosophers that humankind is separated from the rest of animal kingdom by an exclusive capacity of reasoning. Since we now know that reasoning is not an exclusively human capacity, there is no reason why deontology should not be applied to non-human animals, as proposed by the American philosopher Tom Regan [73]. His theory of animal rights [73] asserts that every individual who is the subject of a life has inherent value. Such an animal is worthy of moral consideration, regardless of his/her species. According to Regan [73], individuals that fulfil the following criteria are “subjects of a life”: those who have

beliefs and desires, perception, memory, and a sense of future, including their own future, an emotional life together with feelings of pleasure and pain, preference and welfare interests, the ability to initiate an action in pursuit of a goal, a psychophysical identity over time; and an individual welfare in the sense that their experiential life fares well or ill for them. According to Regan [73], “subjects of a life” ought to be respected and must not be treated as means to an end.

### 3.1.1 Deontology in Contemporary NHP Research

Within biomedical research, NHPs are usually seen as merely means to an end. For example, xenotransplantation research aims for engineered animals lacking certain antigens so that their organs can be used for transplantation into human patients, with a reduced chance of immune rejection [8]. In such cases, the animal is being used as a means to an end. Although the same institutions (e.g., CIOMS) that wrote regulations and legislations to conduct clinical research also did so for animal research [74], the deontological principles that guided the former are totally absent in the latter. Nevertheless, European Directive 2010/63/EU on the protection of animals used for scientific purposes states that “the performance of procedures that result in severe pain, suffering or distress, which is likely to be long-lasting and cannot be ameliorated, should be prohibited” [75]. This incorporates, to some extent, the non-maleficence principle.

Even though the beneficence and non-maleficence principles can be found—to a certain extent—in some NHP laboratory research, the principle of justice is totally absent. As for the principle of autonomy, this can be found occasionally in cognitive research projects, when the test apparatus is built or presented in a way that animals enrol in the experiment of their own volition [41].

Several authors have recommended that NHPs (amongst other animals) involved in biomedical research should receive ethical consideration similar to that granted to humans, as well as analogous protection [46,60,76,77]. Most deontological guidelines require that the participant give informed consent prior to participation in research [59], but there are humans who cannot give valid informed consent (e.g., children or mentally incompetent adult patients). In such cases, the same deontological principles apply to research conducted on them, resorting to legal guardians, and specific legislation with greater restrictions. In most countries, humans who cannot consent may only be engaged in research that benefits them directly [78,79].

According to the legislation in most countries, research protocols for human studies—especially for humans who cannot provide consent—must be approved by independent experts (e.g., paediatricians in the case of children). Similar criteria could be used for NHPs who cannot

consent, but who can effectively communicate their wishes through behavioural traits interpretable by an experienced primatologist. Additionally, if NHPs who are enrolled in a research experience had legal guardians, whose consent was mandatory prior to commencing research—as occurs with humans unable to understand or communicate informed consent [60,77], and as occurs in studies involving owners together with their companion animals [80]—then we believe that research involving NHPs would likely become more transparent and less exploitative than has sometimes reportedly been the case [81].

In sum, within research involving human subjects unable to consent, it is usually mandatory to have consent from (a) a legal guardian and (b) an expert on the condition that makes the human unable to consent. In NHP research, both conditions are usually absent: Not only there are no legal guardians whose task is to safeguard each NHP's individual interests, but independent experts in primate behaviour do not normally verify protocol suitability for each animal.

It is interesting to note that it is largely amongst primatologists interested in studying NHPs by themselves, and not as models for humans, that we find the use of deontological principles and guidelines—mostly the beneficence and non-maleficence principles. For example, guidelines on darting arboreal primates state that darting cannot occur if the animal is facing the shooter, since the chest, face, neck, shoulder, thorax and lumber region, head or abdomen are unsuitable target sites that might harm the animals [82,83]. Similarly, semi-arboreal NHPs can only be darted on the ground [84]. These might exclude some animals from the sample in the same way some human participants are excluded from biomedical research, if they are at significantly increased risk of being harmed from an intervention or procedure [57].

### ***3.2. Utilitarianism***

Utilitarianism is a consequentialist ethical position which asserts that the value of an action is determined by the utility of its consequences, i.e., the morally right act is the one whose consequences maximise some form of utility (e.g., pleasure, wealth, wellbeing), for the majority [85]. However, the magnitude of pleasure and pain for all affected should receive equal consideration [86,87]. For example, it is morally justified to donate blood, because even though the number of individuals harmed is greater than the number of individuals who benefit from this action, it is a small harm, offset by a major gain.

The most popular utilitarian views maintain that all sentient beings are moral subjects and their interests should receive equal consideration when deciding what is the morally right act



[87]. NHPs are, beyond doubt, sentient beings. Hence, when using a utilitarian framework to evaluate the ethics of a biomedical procedure, the interests of NHPs that will be used as research subjects must receive the same consideration as the interests of those human beings who will benefit from the procedure. As a consequence, if the procedure is likely to cause serious harm (e.g., the death of NHP subjects) without bringing a substantial good (e.g., saving a greater number of human lives), it should not be conducted.

Importantly, the ethical rules and laws that guide animal experimentation rely heavily on utilitarianism [59,81,88]. Most legislation on the protection of animals used for scientific purposes—including the current European Directive 2010/63/EU [75] states that the potential benefit of each research project should be balanced against the likely harm inflicted on the animals.

In many cases, the funding agencies evaluate potential benefits, while animal care committees review proposals in terms of animal harms. These committees do not directly interact, arguably impeding efforts to compare potential harms and benefits.

Even when this is not the case, the weighting scale is often misused either in predicting the benefits of the experiments, or in calculating expected harms. Below, we provide evidence of this, as it is virtually impossible to accurately predict the benefits, and in the calculation of harms, many variables are commonly left aside.

### 3.2.1. Predicting Benefits from a Utilitarian Standpoint

NHPs are frequently used in drug trials which are often considered very promising [6]. However, retrospective examinations have demonstrated that the majority of those promising trials failed to translate to humans, or to produce the expected benefits [11], usually because of failures of safety or efficacy [89]. In fact, data from the Food and Drug Administration showed that 92% of drugs that succeed in preclinical tests fail to achieve their purpose within human clinical trials, and never reach the market [90]. These data were previously published in 2004, but more recent papers on the subject have demonstrated that there has not been a significant improvement: the success rate reportedly varies from 0.4% (in Alzheimer's trials [91]) to 20% [92].

One of us (Andrew Knight) has systematically evaluated the contribution of chimpanzee research to biomedical progress, showing that approximately half of all publications describing chimpanzee research identified in a large-scale study were never cited by any subsequent paper, in any field, thereby making little obvious contribution to the ongoing advancement of knowledge. Even those chimpanzee studies that were cited by subsequent medical literature

rarely made significant contributions to the development of therapeutic methods with significant potential for aiding human patients [9]. Bailey [10] also evaluated the role of chimpanzees in AIDS vaccine research, concluding that claims that chimpanzees have played a critical role in basic understanding of HIV-1 [93] were overstated.

More recently, Bailey and Taylor evaluated the contribution of NHPs to neuroscience research, demonstrating that there is a lack of robust evidence to support claims that NHPs are relevant and beneficial to human medical progress. These authors also concluded that human research methods, like functional magnetic resonance imaging with electrocorticography, are being simultaneously used in humans and NHPs for the same purpose, which, in their opinion, makes these NHP studies redundant [12]. Garner [89], on the other hand, maintains that the reason animal studies produce such poor results is the way they are performed, instead of the limitations of animal models.

The fact that one can frequently only evaluate the benefits achieved retrospectively markedly limits the suitability of utilitarianism in assisting an ethics committee to make an informed decision about whether a procedure should be permitted.

It is also important to mention the use of NHPs in experiments that will never reach human trials, due to ethical and legal limitations. Such is the case for experiments using NHP embryos or cloning experiments, which use NHPs due to their similarities with humans, although the use of humans in such experiments would be strictly forbidden [94]. Hence, potential benefits for human patients are absent or severely limited.

When predicting the potential benefits of a biomedical research project, all of humanity is usually considered to be potential beneficiaries. However, that is rarely if ever the case. According to the World Health Organization, approximately one-third of the people living in developing countries are unable to receive or purchase essential medicines on a regular basis [95].

Finally, all basic research produces knowledge, which is in itself a benefit to humankind, since scientific knowledge has cultural value in itself. Nonetheless, this benefit is hard to quantify, or to balance against concrete and substantial costs.

### 3.2.2. Assessing and Predicting Harms from a Utilitarian Standpoint

While addressing the harms inflicted on non-human animals, including NHPs, researchers tend to focus on the severity of the procedures described in the experimental protocols and overlook other harms. In fact, European Directive 2010/63/UE, with the aim of regulating the level of severity inflicted on laboratory animals, includes an annex on the severity classification

of procedures [75]. This may reinforce the propensity to disregard other sources of pain and distress.

Unlike humans, NHPs cannot be informed about their procedures—hence, even a painless procedure like an MRI can be terrifying for a naïve NHP [46,76]. To always classify this procedure as “mild”, in accordance with current European legislation, ignores subjective experiences, such as fear, that might vary individually.

Wild-caught NHPs also experience anxiety and pain during capture, in holding facilities, and often lengthy transportation and confinement, whereas laboratory-bred NHPs may undergo suffering during breeding, and from maternal separation, potentially much earlier than would occur in the wild [96,97]. It is noteworthy to mention that due to the intense stress caused by wild capture, the UK banned the wild capture of primates for their use in research in 1996. Similarly, European Directive 2010/63/EU states that only the offspring of wild-caught NHPs can be used in research experiments.

NHPs who live under laboratory confinement conditions may experience pain and distress not only during procedures, but also during many other situations that are not normally considered when evaluating the harms and benefits of the research. Self-injurious behaviour is an obvious sign of stress that has been extensively described in NHPs living in laboratories (for a review, see Reference [98]). Similarly, floating limb syndrome, which can be defined as raising the arms or legs without an obvious function, is a readily identifiable stress-related behaviour [99]. Another easily recognisable sign of stress in NHPs is the freezing response. In both humans and NHPs, this response is a common and immediate response to threat situations that allows the individual to evaluate the danger and decide how to deal with it [100]. When there is a dysregulation in fear response (e.g., post-traumatic stress disorder—PTSD), this behaviour may emerge in non-threatening or mildly threatening situations and may last for prolonged periods [100]. Hence, inappropriate freezing behaviour is a signal of fear and anxiety that researchers should not ignore, regardless of the stimulus. Some types of behaviour, such as a high frequency of self-grooming, are stereotypic abnormal behaviours in some species but not in others [100]. However, checking species-specific ethograms and normal activity time budgets could help to identify such abnormal behaviours.

Species ethograms can also be helpful in identifying the naturally occurring behavioural repertoire. In laboratory housing, most NHPs face restrictions on performing certain natural behaviour patterns. This is not usually considered when assessing harms. The same may occur when an NHP witnesses the harming or killing of peers [96]. Additionally, experiencing stress, especially at an early age, impacts the NHP immune system and brain structures [101,102].

These long-term stress-related harms are not normally considered when assessing animal welfare impacts and might even reduce the suitability of some NHPs as models for humans [101].

Facial expressions could be an important tool to understand NHP emotional states, since they often convey emotion or pain in many different NHP species (for reviews, see References [32,103,104]). In the case of chimpanzees, for example, the expression of a full closed grin as described by Goodall [27] is reliably associated with fear, distress, and painful contexts [32,103]. Similarly, in rhesus monkeys, a grin signals fear or submission [26]. In recent years, the facial action coding system (FACS) developed by Ekman and Friesen [105] has been adapted to several NHP species, like chimpanzees [106], rhesus monkeys [107], gibbons [108], and orangutans [109]. This tool could help researchers to more objectively assess NHP emotions.

With the help of such tools, it would become easier to evaluate which procedures should be prohibited or modified in order to spare NHPs from severe pain or stress. Their use has been suggested for NHPs [46,61], but has not yet been widely implemented [81].

#### **4. Ethical limitations of 3Rs Principles**

Current policies underpinning animal experimentation follow the 3Rs principles, first described by Russell and Burch [110]. These principles assert that whenever possible, animal models should be replaced with alternative methods; the number of animals used in experiments should be reduced to a minimum; and their suffering should, whenever possible, be ameliorated, e.g., through humane endpoints, less invasive procedures, and the use of anaesthesia (refinement).

Replacement is the first and, in our view, the most important of the 3Rs. Its achievement in a particular case makes implementation of additional Rs unnecessary. However, replacement is often grounded in the unverified assumption that animals are good models for human diseases—an assumption that is increasingly challenged by empirical evidence (for a review, see [111]). To gain regulatory acceptance and/or be funded, alternative methods often need to demonstrate that they can provide equivalent or superior data to those obtained through animal testing, even when the current animal model results are variable rather than consistent, and even when these models have often failed to reliably predict human responses to drugs [112]. This status quo approach delays the development of promising non-animal methods in toxicity and drug testing and diverts biomedical research away from non-animal methods.

These 3Rs principles underpin virtually all legislation and guidelines concerning the use of animals in scientific procedures. However, they do not offer a philosophically consistent ethical framework and are insufficient to address ethical concerns regarding NHP use within biomedical research.

The 3R policies comply—to some extent—with utilitarianism, since reduction and refinement are tools used to try to minimise the total amount of harm inflicted. However, they do not provide tools to predict benefits, or the extent of long-term harm, which makes them insufficient to fulfil the requirements of utilitarian analysis.

Whilst public health advancement might be a justifiable goal, from a utilitarian standpoint, the pursuit of biomedical NHP research (that might provide only modest benefit) might not be justifiable. From a deontological point of view, the 3Rs are largely irrelevant, since they do not prevent the research subjects from being used as means to an end. Additionally, the 3Rs do not comply with principles of autonomy or justice, which are crucial within the deontological approach prescribed by Beauchamp and Childress [58].

## **5. Societal Determination of Ethical Frameworks**

Whenever animal-based research is the topic of discussion, the balance between competing perspectives is often decided at the societal level, and the prevailing culture enables or proscribes a certain type of scientific activity [113].

When it comes to science, people tend to support animal experiments according to utilitarian principles, i.e., people consider the potential benefits for humanity when assessing their level of support for certain research [15,48,113–115]. However, when it comes to animals that people consider companions, such as domesticated dogs, the number of people who support their use in scientific research decreases dramatically, regardless of the perceived potential benefits for humankind [48]. With these animals, people shift their ethical paradigm, applying the beneficence and non-maleficence principles.

The emergence of ethical decisions is influenced by the feeling of discomfort that most people experience when confronted with the suffering of others, and their own sense of wellbeing and fulfilment when contributing to the alleviation of pain or the promotion of happiness [116,117]. However, these decisions rely on available information about the phenomenological experiences of others. Closeness, familiarity, and knowledge of animals, including NHPs, have all been variables linked to increased empathy for animals [118] and less tolerance for animal use in invasive or harmful scientific research [113].

People are more willing to accept research on nonhuman animals, including NHPs, if they believe animals are comfortable and well cared for, and in the mid-20th century, according to the National Opinion Research Centre, 75% of the public believed that medical schools treated laboratory animals as well as individual owners would [114]. Most owners consider their pets as individuals with intrinsic value, and veterinary clinical research conducted on pet dogs follows deontological principles similar to the ones used in human clinical research [119].

The way animal husbandry is portrayed, as well as the level of familiarity people have with different species, are thus critical features of engaging society with either utilitarian or deontological ethical frameworks. Accurate portrayal of the actual state of both variables, as well as a realistic portrayal of human healthcare benefits that arise from animal research, would, in our opinion, lead to stronger support for application of the deontological framework.

## **6. Ethical Research with NHPs**

There is no robust evidence that we need NHPs to model specific human diseases [9–11]; therefore, there is no overwhelming moral or scientific reason to confine NHPs within laboratories, to be invasively used as defective models for human disorders. In fact, Garner and colleagues recently [89,120] suggested that in order for biomedical research using non-human animals to be more effective, they should be treated as patients. We agree with this view but emphasise that this is not possible for animals confined in a laboratory.

### ***6.1. Ethical Research with Possible Healthcare Applications***

Disorders that affect humans and NHPs should ideally be studied using NHPs who suffer naturally from the disorder concerned, either in wild populations, or in captive NHPs who need treatment.

In 1966, Jane Goodall witnessed a polio outbreak in wild chimpanzees living at Gombe Stream National Park (Tanzania). In some individuals, the subsequent disability was so severe that some animals were euthanized [121]. Instead of infecting healthy laboratory animals with polio, these wild chimpanzees who succumbed to polio from natural causes could theoretically have been studied to understand polio. The knowledge acquired from these studies would have been useful for science in general, and for infected chimpanzees specifically—hence upholding the justice principle. It might or might not have been useful for humans but, given the more natural induction and progression of the disease, it could have been more useful than similar research performed on laboratory chimpanzees. Although the laboratory environment allows

for the control of possibly confounding variables, and manipulation of the exact time of infection, this level of control and information is rarely possible with human patients. Wild animals that naturally acquire a disease occurring in humans and other species can be a better model than laboratory animals, since—just like human patients—they are living in a complex environment where social and natural variables can modulate disease progression. In human patients, it is very hard or even impossible to determine the exact time of infection and what other variables (e.g., inadvertent exposure to external viruses) could interfere with disease progression and/or clinical trial results. Even researchers that support the use of animals as models for human disorders acknowledge that standardisation of too many variables in the laboratory can be a limitation, rather than a strength [89].

There are NHPs previously used by industries (e.g., entertainment, biomedical research) and subsequently suffering from psychological and behavioural disorders, for whom psychiatric/psychological treatment is not only appropriate, but also a moral imperative [122–124]. Using these animals as research patients for PTSD, for example, could benefit both science and these particular animals. Again, the data obtained might or might not be useful for human healthcare, but the results obtained from laboratory animals would not necessarily be more useful.

Epidemiological studies with wild populations can also be conducted with minimal disturbance of the animals [125,126], hence respecting the autonomy principle.

## **6.2. Basic Ethical Research**

Some may argue that NHP research facilities are useful for purposes other than medical research. This is the case for basic research, which, according to the Frascati Manual [127], is “experimental or theoretical work undertaken primarily to acquire new knowledge of the underlying foundation of phenomena and observable facts, without any particular application or use in view” [127]. The majority of such basic research is laboratory-based.

However, a large amount of such research can be conducted in non-invasive field studies, using wild populations—hence respecting the individuals as subjects with inherent value. For example, Kano and colleagues used a laboratory apparatus to study differences in gaze behaviour in chimpanzees and bonobos, concluding that bonobos pay more attention to the eyes and face of other individuals [128]. On the other hand, Fröhlich and colleagues reached the exact same conclusion by observing communicative interactions in mother-infant dyads of wild populations [129]. Similarly, Fujita and colleagues created an experimental laboratory procedure to study capuchin monkeys’ deceptive behaviours [130], while others [31] were able

to study tactical deception of the same species in the wild, gathering more robust and reliable data on the subject. Another good example which has been widely studied is chimpanzee communication: while in captivity, only 31 gestures were described [131], observations in the wild raised this number to 66 distinct gesture types to communicate at least 19 different messages [33]. In fact, most of what we know about behaviour and the ecology of NHPs has come from long-term field studies [17,27,132–134], and recent field studies continue to amaze us by revealing new species [135], as well as unexpected behaviour from well-known species, such as bonobo hunting [136], and some behaviours that might be ritual practices amongst wild chimpanzees [137,138].

These studies have been conducted respecting the beneficence and non-maleficence principles, and even the principle of justice, since the knowledge obtained from those data, some of it also presented as documentaries and in other forms for widespread media dissemination, raises awareness of animal emotion and cognition [139], potentially increasing empathy towards these NHPs, and ultimately increasing the impetus for their conservation.

However, there are research questions, either in safety and efficacy testing or fundamental research, where it is not possible to obtain knowledge using only observational techniques (e.g., genetics, neuroscience). However, there are ways of continuing this research without overlooking ethical constraints.

In questionable situations, it is always pertinent to ask whether the knowledge acquired through the suffering of other animals complies with the bioethical principles described by Beauchamp and Childress [58]. In the literature, we can find examples of basic research with non-human animals that fulfil these principles. Berns and colleagues [140] used positive reinforcement to train dogs to stay still within a functional MRI device. The dogs were unrestrained and free to leave the device at all times, including during training sessions (autonomy principle). Without harm or distress, much fundamental knowledge on the canine brain was obtained (non-maleficence principle), which may ultimately benefit the wider canine population (principle of justice). The researchers would gradually play louder sounds in the surrounding environment so that animals would not get startled by MRI sounds (principle of non-maleficence). This innovative method has been providing exciting insights into the canine brain [141–143], and a similar technique could be used to study NHPs living under semi-natural conditions, replacing neuroscience NHP laboratories where even the least invasive techniques [144] require temporarily restraining fearful animals. Some NHPs share habitat with human beings (e.g., rhesus monkeys in India or Nepal) and sometimes even enter and explore human homes, which should make it possible to conduct experiments with these animals similar to



those described above with dogs. Some NHPs species are particularly harmless and cooperative (e.g., marmosets or capuchin monkeys). Individuals from those species who are held captive for other reasons (e.g., rescued animals living in sanctuaries) could also be enrolled in experiments similar to the ones conducted by Bern and colleagues on dogs [140–143]. Even potentially dangerous NHPs, such as chimpanzees, can participate in experiments consensually, in the same way human participants do [41].

## 7. Conclusions

In light of the current knowledge, the use of NHPs in basic research warrants something of a paradigm shift. We propose that basic research with NHPs should continue only if carried out under the same ethical deontological criteria that guide basic research with human beings.

Whenever non-invasive basic research protocols require the use of NHPs, the participants should be recruited from sanctuaries or similar facilities. Local legal guardians of NHPs should evaluate the procedures to verify whether the principles of autonomy, beneficence, non-maleficence and justice, as defined by Beauchamp and Childress [58], have been fully incorporated. That being the case, the legal guardian would provide the necessary informed consent.

By complying with such standards, we would not only grant other primates a level of respect and protection consistent with that we provide to members of our own species, but we would also be encouraging researchers to develop better research protocols and higher standards for captive management, which could, in turn, result in improvements in data quality, and in the reliability of some research results.

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## **CHAPTER 8**

### **Discussion**

## Chapter 8. Discussion

Animal models are widely used in biomedical research, namely in mental disorders research. Their benefits for healthcare improvement are disputed and seldom empirically evaluated. Nevertheless, this assessment is important, because if we do not empirically assess the contribution of animal models to current knowledge of human disorders, we are merely assuming the benefits of these models, which might lead to misuse of limited funding, approval of ethically questionable protocols, and more importantly, negative impacts on patient care.

Major Depressive Disorder (MDD) is the most severe depression type, affecting more than 300 million people worldwide (Global Burden of Disease 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). Even though thousands of animals are used in an attempt to gain knowledge of MDD aetiology, pathogenesis and treatment, to my knowledge, this thesis provides the first empirical data evaluating the contribution of animal models to current knowledge of MDD.

The citation analysis conducted on this subject (Chapters 3, 4, and 5) reveals that data obtained using animal models is largely invisible to, or ignored by, the human medical research community. This trend is consistent with that observed for other mental disorders such as Attention Deficit Hyperactivity Disorder – ADHD (Chapter 2) and eating disorders (Shapiro, 1998).

In this thesis (Chapter 4), I also compared the effectiveness of animal models with other indirect research approaches (human based *in vitro* and *in silico*) in reaching the human biomedical research community. *In vitro* and *in silico* studies are more cited in human medical papers focused on MDD, than papers using primates as models for MDD. Interestingly, animal research papers do not cite substantially *in vitro* or *in silico* papers (Chapter 6) challenging the view that *in vitro* and *in silico* are simple models useful as a step prior to animal experiments aiming to reduce or refine the number of animals required for biomedical advancements.

The last chapter of this thesis (Chapter 7) explores the ethical and scientific pitfalls of biomedical research using non-human primates (NHP) as models for human disorders, and proposes a paradigm change similar to the one that currently regulates research with human beings. Even though the chapter is limited to primates, I believe that the paradigm change we propose should apply, at least to a certain extent, to other species currently used as models for biomedical research.

In the following discussion, I address the strengths and limitations of the chosen methods, discuss the key findings and point future directions.

## **Strength and limitations of chosen methods**

### ***Citation analysis (Chapters 2 to 6)***

As explained in the introduction of this thesis, conducting a citation analysis means investigating how many citations a paper receives and by what sort of papers (Garfield & Merton, 1979).

Citation analysis is a robust method for bibliographic impact assessment (Huang et al., 2019); however, it is not flawless (Huang et al., 2019; Ioannidis, 2015). Its main limitations are the likelihood of missing relevant papers within search engines due to labelling errors or language barriers and the exclusion of unpublished materials such as negative outcomes, unpublished data from pharmaceutical trials or manuscripts not indexed in big search engines (e.g., PhD or Masters theses). Other common sources of bias in citation analysis are self-citations (Ioannidis, 2015), which we did not exclude, and overestimation of the importance of some papers (Ioannidis, 2015), which we controlled via systematic qualitative analysis of citations in two of the published papers of this thesis (Chapters 2 and 4).

The limitations mentioned above can be a serious problem in other types of bibliographic analysis (e.g., assessing the impact of a research author), but here the majority of these limitations are either trivial (as in the case of self-citations, which did not change the trends found), or strengthen our results, as in the case of the omission of grey literature. Had we included grey literature (i.e., hard to locate papers), the average number of citations received by animal papers would probably decrease even more since the papers only found in grey literature were probably cited very few times. This happens because unpublished or not-indexed data is only available for few researchers, usually colleagues working in the same research field. It is also reasonable to assume that unpublished animal data would be more easily available to other animal researchers, than to researchers working with humans.

In this thesis, there was an additional limitation: access to SCOPUS – the largest citation database, covering nearly 36,377 titles from approximately 11,678 publishers – was only possible during the final part of data collection presented in Chapter 3. Therefore, it is realistic to assume that some relevant citations may have not been accessed in the studies presented in Chapters 2, 4, 5 and 6.



### ***Systematic qualitative analysis of citations (Chapters 2 and 4)***

Our systematic qualitative analysis of citations involved having two independent raters reading every human paper on the target disorder (ADHD or MDD) that cited the animal research papers identified in the citation analysis. Each rater assigned each citation to a discrete category of relevance (redundant, minor relevance, relevant for the hypothesis, relevant for methods). The categories were defined prospectively and all disagreements between raters were solved via discussion and consensus. The systematic qualitative analysis of citations was a method refined by myself and a co-author of Chapter 2 (Luísa Bastos) from the qualitative analysis found in previous citation analysis papers (e.g., Knight, 2007, Lindl & Voelkel, 2011) in an attempt to create an objective tool (hence, the use of two different raters) that could be used to evaluate the relevance of papers to subsequent papers that cited them.

The majority of citation analysis aiming to evaluate the contribution of animal models to biomedical progress devote a substantial part of the discussion to a qualitative analysis of relevant papers, but this assessment is usually made only by the author of the study (e.g., Knight, 2007). This may lead to subject bias. We were hoping that the systematic qualitative analysis of citation could help increase the objectivity. Unfortunately, the initial assessment made by the raters was often divergent, meaning that this tool can be useful to understand interrater reliability, but may not be enough to attain perfect consistency. Two different people assessing qualitative criteria inevitably leads to differences in the initial assessment, but it is interesting to note that it was possible to reach a consensus in all cases, which means that the initial divergences force the raters to consider other perspectives, which in the end reduces bias. In the future, it would be interesting to apply this methodology with more than two raters. This tool was developed for the study described in Chapter 2, and was used again for the study described in Chapter 4.

### ***Comparison between research methods (Chapters 4 and 6)***

A direct comparison of results consists in using the same method to compare outcomes. It is routinely used within biomedical research, for example, to evaluate treatment outcomes retrospectively (e.g., within randomized controlled trials, where the results of test and control groups are compared).

Here, we performed citation analysis in papers using different research approaches (*in silico*, *in vitro* and NHP in Chapter 4, as well as *in silico* and *in vitro* in Chapter 6), and compared the citations received by each research approach in terms of frequency (total number of citations received) and patterns (which papers cited the analysed papers).

Within the context of this thesis, the biggest strength of comparing the frequency and patterns of citations different research methods receive is that it allows one to compare the effectiveness of different alternative research approaches in informing medical research community dedicated to MDD research and treatment. This evaluation can be very useful in informing the funding bodies about the most promising methods. It is desirable that similar analyses be conducted for other disorders.

The major limitation found within the comparison between different methods in this thesis was the small number of papers located, hence submitted to citation analysis. This led to lack of statistically significant results even when there were observable practical differences between results (Chapter 4). In time, this limitation will disappear for, as *in silico* and *in vitro* techniques evolve, the number of published papers reliant on those techniques increases.

## **Discussion of findings**

### ***The poor contribution of animal models to MDD research***

The first conclusion that can be drawn from this thesis is that the contribution of animal models to MDD research has been poor, questioning the need of this so-called “necessary evil”. Chapters 3 to 5 demonstrate this poor contribution through robust empirical evidence.

MDD is a complex disorder and its aetiology is still not fully understood. Nonetheless, there is a large consensus that both biological and environmental factors contribute to its aetiology (Mandelli & Serretti, 2013). The extent of biological and environmental variables that seem to play a role in MDD aetiology and pathogenesis probably contribute to the low success rates of animal models in informing clinical research. Animal models are mainly used in an attempt to understand the physiology and biological mechanisms of the disorders. However, that is of very limited utility since the biological factors involved in MDD are diverse, ranging from genes, neurotransmitters and hormones to inflammation, and may be caused by environmental factors, impossible to account for in animal models (for a review on the biological and environmental causes of MDD and their relation, see Chapter 3). In humans, the environmental factors related to MDD are stressful life events (that may be very diverse), sexual abuse, low educational attainment and personality traits. Even when animals are submitted to chronic or acute stress protocols in an attempt to model the aetiology of MDD, it is excessive to assume that these protocols will mimic the environmental factors of MDD, namely because in humans they may be very individualized.

Humans are, without a shadow of doubt, the best “models” to gain understanding of MDD. Technological advances such as functional magnetic resonance imaging (fMRI), single-photon emission computed tomography (SPECT) or positron emission tomography (PET) scans allows us to compare in a controllable but non-invasive setting the changes between MDD patients and healthy controls’ brains in terms of physiological and metabolic characterization of brain tissue. These techniques also allow us to see the human brain functioning and recording its activity (Tsougos et al., 2019). Similarly, blood or urine samples can help understand the genetics and biochemistry behind MDD (e.g., Shadrina et al., 2018).

The systems biology approach – a strategy that integrates human data at several levels (molecular, cellular, tissue, organ, patient, population) (Langley et al., 2014) – is also an available and valuable tool to understand aetiology and pathogenesis of MDD.

Longitudinal studies (i.e., studies that observe the same people over extensive periods of time, such as the one conducted by Tao and colleagues (2019) to investigate what causes some MDD patients the development of Alzheimer Disease), are very useful but very scarce, mainly due to several constraints. Research focused on human beings is usually expensive and time-consuming (especially in longitudinal studies). It can also be ethically contentious (e.g., randomized control drug trials). As a result, early stages of research rely on indirect methods, mainly animal models. There are several animal models for MDD, but they all have severe limitations (for a review, see Akil et al., 2018). Within MDD research, animal models are used in an attempt to gain knowledge on MDD biological pathways (e.g., Gong et al., 2019), to test environmental impact on depression (e.g., Mehta-Raghavan et al., 2016), and to test treatments, mainly antidepressants (e.g., Nguyen et al., 2018).

There are different antidepressant drugs as well as non-pharmacological treatments for MDD (for a review of the available treatments see Pandarakalam, 2018), still almost half of the patients do not reach full remission (Novick et al., 2017) even after trying different courses of treatment (Kraus et al., 2019). In recent years, several non-pharmacological human-specific treatments have proven at least as effective as psychopharmacological drugs within MDD (e.g., Amick et al., 2015; Knapen et al., 2015). Obviously, some of them may also be demonstrated in animals (e.g., the link between exercise and positive mood), but this demonstration is not a necessity for human research. A similar example are the famous experiments aiming to explore the effect of maternal deprivation in rhesus monkeys performed by Harlow and Harlow (1965). These experiments gave us knowledge about rhesus monkeys behaviour but did not add significant knowledge to that obtained in previous studies on human beings by John Bowlby and Mary Ainsworth starting in the 1950s. These studies were the basis of Attachment Theory,

one of the most important theoretical concepts of clinical psychology (for a review on these authors work see Ainsworth et al., 1978, and Bowlby, 1969).

### ***The poor contribution of animal models to mental disorder research***

MDD is one amongst 200 mental disorders (American Psychiatric Association – APA, 2013). However, it is reasonable to infer that the poor contribution of animal models to our current knowledge of MDD might also apply to at least some other mental disorders, since they all have human-specific symptoms, multifactorial origin and vary greatly, not only in terms of available treatments, but also treatment response from one patient to another.

The data on ADHD (Chapter 2 and 5) also demonstrates that the contribution of animal models for current knowledge on this disorder has been poor. MDD's and ADHD's findings are in agreement with previous research within the mental disorders' research field (Shapiro, 1998). This further supports the assertion that animal models might be unsuitable for biomedical research on mental disorders.

Even though the claim that animal models are unsuitable for biomedical research on mental disorders may be disputed, the lack of effectiveness of animal models to investigate mental disorder research is unanimously recognized. Different authors offer different possible solutions to overcome this limitation, such as: a) switching to different animal models (Eaton & Wishart, 2017); b) using different animal models of depression, as well as human patient data, to identify which genes and brain circuits are dysfunctional (Akil et al., 2018); c) overcoming the biological differences between species (Hodge et al., 2019); d) improving the methodological quality of the animal studies to make them more “human-like” (e.g. Richter-Levin et al., 2019); e) changing the way we categorize and study mental disorders (Söderlund & Lindskog, 2018); f) establishing protocols for systematic reviews (Bannach-Brown et al., 2016); g) treating animals as patients instead of models (Bradshaw et al., 2008); h) using humans or human-based approaches (Papassotiropoulos & de Quervain, 2015).

Interestingly, the majority of psychologists (who work with patients who suffer from the disorders investigated using animal models) are either unaware of animal experiments for mental disorder research, or opposed to them (Compton et al., 2019; Shapiro, 1998). In 1996, Plous conducted a survey to understand psychologists' attitudes toward the use of animals in psychological research and education and found that 92.2% of psychologists who were mental health workers rarely, never, or only occasionally used findings from psychological research on animals (Shapiro, 1997). A recent study by Crompton et al. (2019) showed that psychology students were more likely to approve invasive research protocols with animals outside their

fields of expertise than within direct psychology research. The latest protocols were considered not important by the majority of the students.

### ***Human-based research: the way forward***

Results presented in Chapter 4 suggest that human-based *in vitro* and *in silico* studies are more effectively informing human medical research community than primate models, at least when evaluating studies on MDD. This is also in agreement with similar studies from other research fields. For example, a recent citation analysis conducted by Adnan & Ullah (2018) revealed that, within endodontic research field, papers describing *in vitro* studies received the highest number of citations, more than review papers or animal research papers. Also, Passini and colleagues (2017) reported that *in silico* models demonstrate higher accuracy than animal models to predict the cardiotoxicity of drugs. Similarly, an increasing number of *in vitro* tests have shown better results than animal models (for a review see Passier et al., 2017, or Ronaldson-Bouchard & Vunjak-Novakovic, 2018). Such is the case of the 3T3 NRU-PT *in vitro* test, which is the only test with 100% sensitivity to phototoxicity (Ceridono et al., 2012). Also, induced pluripotent stem cells (iPSCs) are already being used to directly inform clinical trials (Mullard, 2015).

Despite the examples given above, amongst others, the current paradigm for biomedical research and testing postulates that human-based approaches (like *in vitro* and *in silico*) should be used as a first step in research before conducting tests in animal models (Taylor, 2019) as animals are considered “gate keepers” (Langley, 2014). However, at least regarding MDD research, this formula is not actually being followed: not only clinical papers tend to cite directly *in silico* and *in vitro* papers (as presented in Chapter 4 and 6), but also the amount of citations that *in silico* and *in vitro* papers receive by subsequent animal papers is lower than would be expected if *in silico* and *in vitro* experiments are conducted as a first step in biomedical research with the aim of informing animal studies (Chapter 6).

Human-based approaches such as *in vitro*, *in silico* or a combination of both are a growing and promising field, leading several authors to advocate that a combination of both should totally replace animal experiments within biomedical research (e.g., Archibald et al., 2018; Papassotiropoulos & de Quervain, 2015; Taylor, 2019). Some go even further and suggest the current 3Rs – replacement, reduction, refinement – should be replaced by new 3Rs – replacement, research (human-based for safety purposes) and relevance (for human healthcare) (Herrmann et al., 2019).

### *The controversy around animal experiments*

The results found in this thesis are in agreement with previous results on the contribution of animal models to other biomedical fields (e.g., Bailey & Taylor, 2016; Dagg & Seidle, 2004; Lindl & Voelkel, 2011; Perel et al., 2007).

As evidence accumulates, it becomes obvious that the assumption of animals as the gold standard models for biomedical research has been challenged. More and more studies using evidence-based methodologies have demonstrated that animal models have made a poor contribution to medical advances. This justifies a paradigm shift within biomedical research, not only due to consideration for the animals used, but also because the current paradigm may compromise human patients' healthcare.

It is interesting to verify the resistance of researchers who use animals as models for human disorders, to changing current practices. According to Frank (2005), this phenomenon happens because animal experimentation suffers from technological lock-in, i.e., it is perpetuated because of resistance to change. Pappalardo and colleagues (2018) also note that compared to others, the pharmaceutical industry is historically slow in adopting technological innovation, which enforces Frank's (2005) view.

Surveys demonstrate that people tend to support animal experiments when they perceive the experiments will benefit human healthcare (Ormandy & Schuppli, 2014). This view is also visible in the distribution of scientific funds, as well as in the likelihood of publishing papers, which may pressure researchers to overestimate the applicability of their data, hence reinforcing the technological lock-in effect.

Basic research (i.e., research conducted with the sole or primary purpose of acquiring scientific knowledge, Organisation for Economic Co-operation and Development – OECD, 2015) is the type of research that consumes more animals. Just within European Union (EU) it is responsible for 45% of total animal use (European Commission – EC, 2020). It is also one of the few research areas where the utility of animal research is undeniable because ultimately every single well-conducted experiment may produce new knowledge, and scientific knowledge should be valuable in itself for society as part of its development and culture, regardless of its applicability in whatever field (Bunge, 1997). However, for most of us, scientific knowledge should not be acquired at any cost.

There are many ways to conduct basic research in animals without compromising their wellbeing. Taking as an example the previously mentioned Harlow's experiments (1965), the fact that attachment is a trait that humans share with other primates and the traits and ontogeny

of primate's attachment could have been easily discerned without traumatic experiments, as did Kim Bard and her colleagues using captive chimpanzees (Bard, 1996; Bard, 2002; van IJzendoorn et al., 2009). Many other examples can be found in Chapter 7, where it is proposed that the deontological framework that guides research with human beings should be applied to NHPs. When doing so, both science and animals benefit. If this were the case, a big proportion of invasive animal research would be replaced by other approaches. Certainly, there are layers of knowledge that one cannot access without harming animals, and those should be the cases for ethical committees to evaluate.

Hence, the ethical discussion regarding the use of animals in invasive procedures should move from biomedical research to basic research where it comes down to one ethical dilemma: what do we, as a society, value the most – scientific knowledge, or animals' lives and wellbeing?

## **Future directions**

In the future, it would be interesting to conduct similar studies on other disorders. In fact, encouraging citation analysis within biomedical research using animal models, in the same areas in which systematic reviews are currently focused (e.g., Pound & Nicol, 2018) could help prevent unnecessary animal studies, instead of just refining them.

It would also be exciting to use citation analysis in combination with systematic reviews, for example, to verify if better designed studies receive more citations than those with methodological flaws. Similarly, it is worth verifying if papers resorting to contentious procedures, such as the forced swim test (Can et al., 2012), receive a high number of citations that might justify their use.

It would also be valuable to compare the effectiveness of different research approaches as made in Chapter 5. *In vitro* and *in silico* approaches are evidencing an exponential growth, which makes them good candidates to elucidate more clinical research, and this comparison may inform stakeholders (e.g., funding agencies, editors) about where to invest.

There is a pressing need to verify if the contribution of *in vitro* and *in silico* for animal studies are as low in other areas (e.g., animal drug trials) as they are within MDD research. If that is the case, then current legislation is not being properly implemented and should be enforced. Within the EU, the legislation that regulates the use of animals in research is Directive 2010/63/EU. It clearly states, not only that the use of live animals should be avoided whenever there is a valid alternative, but also that the number of animals used should be reduced to a

minimum. If animal researchers are not aware of the information gathered through *in vitro* and *in silico*, they are failing, even though inadvertently, this required legal obligation.

Overall, with this thesis, I hope to inform all key stakeholders, including editors, funding agencies, ethics committees, researchers and citizens, about the actual contribution of animal models to biomedical research within mental disorders, contributing toward a paradigm change within biomedical science – in favour of more human-based research. I am also hoping to contribute to the urgent discussion on whether it is legitimate or not to use animals in scientific procedures, in the way they are currently used. As demonstrated in Chapter 7, current guidelines and legislation governing animal experimentation rely on a utilitarianism ethical framework, while the guidelines and legislation governing research with human beings rely on a deontological ethical framework. I propose a paradigm change to the current processes, i.e., to use similar rules regardless of whether the research subject is a human or non-human animal. I believe by making this switch, both science and animals would benefit (see more details in Chapter 7), but perhaps more importantly, human health and humankind would benefit too.

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