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Animal Models in Pharmacology: A Brief History Awarding the Nobel Prizes for Physiology or Medicine

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Abstract
Background: The Nobel Prize of Physiology or Medicine (NPPM) has recognized the work of 222 scientists from different nationalities, from 1901 until 2020. From the total, 186 award researchers used animal models in their projects, and 21 were attributed to scientists and projects directly related to Pharmacology. In the most recent years, genetics is a dominant scientific area, while at the beginning of the 20th century, most of the studies were more related to anatomy, cytology, and physiology. ***Summary:*** Mammalian models were used in 144 NPPM projects, being rodents the most used group of species. Moreover, 92 researchers included domestic species in their work. The criteria used to choose the species, the number of animals used and the experimental protocol is always debatable and dependent on the scientific area of the study; however, the 3R's principle can be applied to most scientific fields. Independently of the species, the animal model can be classified in different types and criteria, depending on their ecology, genetics, and mode of action. ***Key-Messages:*** The use of animal models in NPPM awarded projects, namely in Pharmacology, illustrates their importance, need and benefit to improve scientific knowledge and create solutions. In the future, with the contribute of technology, it might be possible to refine the use of animal models in pharmacology studies.

Introduction

Alfred Nobel was a chemist, inventor, entrepreneur, and industrialist of the 19th century, being one of the most notable men of his time. He was born in Stockholm in 1833 and one of his most recognized achievements was the use of nitroglycerin as an explosive and the way to control it. Before his death, he left virtually all his fortune to establish prizes for people from different nationalities who made the most compelling achievement for the global benefit, in the fields of chemistry, physics, physiology or medicine, literature, and peace among nations [1, 2].

According to his words, the Nobel Prize of Physiology or Medicine (NPPM) should be attributed to "the person who shall have made the most important discovery with-

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in the domain of physiology or medicine." Until today, 222 scientists awarded the NPPM from 1901 to 2020 [3]. According to the Foundation for Biomedical Research, 186 award researchers (from the 222 total) used animal models in their projects [4]. From the total of NPPM awards, 23 were attributed to scientists and projects directly related to Pharmacology and the use and test of different drugs and chemicals in order to treat diseases, infectious agents, and physical conditions [4].

Animal models are based on the principle of comparative medicine that animals share physiological, pathological, behavioral, or many more other characteristics with humans. Historically, it is possible to say that the use of animals for health purposes started in ancient Greece, >2,000 years ago, when anatomy and physiology were born as scientific fields [5].

The use of animal models in the projects recognized by the NPPM is more than illustrative of the importance and need of them to improve scientific knowledge. In this way, this review aims to analyze the use of animal models as essential tools for scientific development, especially in pharmacology, using the NPPM projects as examples of worldwide recognized scientific improvements considerably depends on those models.

Diversity in Animal Modeling: Selected Species and Scientific Fields

As mentioned above, 186 award researchers used animal models in their projects (Table 1). For instance, Emil Adolf von Behring, the 1st NPPM winner, in 1901, used horses, rabbits, and guinea pigs in order to discover a diphtheria treatment. In the same way, the 2020 winners, Harvey J. Alter, Michael Houghton, and Charles M. Rice choose the chimpanzee as a model to study the hepatitis C. Furthermore, most of the awarded discoveries required >1 species as an animal model, in different parts of their studies. In fact, 87 of the prized researchers needed >1 animal species in their award projects [4].

Almost all the medical and biological fields require the use of animal models. Over time, the 186 NPPM winners that used animal models in their research came from distinct areas of Physiology and Medicine. In the most recent years, genetics is a dominant scientific area, with a lot of prizes attributed in this area, as the discovery of a mechanism that allows mature cells to become pluripotent, by John B. Gurdon and Shinya Yamanaka, awarded in 2012 [102]. In contrast, at the beginning of the 20th century, the majority of the studies were more related to understand the structure and function of different cells, tissues, organs, and systems. For example, Henry H. Dale and Otto Loewi won the NPPM prize in 1936 for describing the chemical transmission of nerve impulses, using animal models from 4 different taxonomic classes (cats, frogs, birds, and reptiles) [28]. Microbiology, immunology, radiology, biochemistry, and pharmacology represent other scientific areas where animal models are frequently used, also in studies developed by NPPM winners.

The species of animals used as models in projects recognized by the NPPM are very diverse. Considering the taxonomic classes, the mammalian class is the most used, since 144 recognized projects and winners used mammalian species in some part of their work, followed by the birds, used in 35 discoveries (Fig. 1, 2). However, among the years, due to public opinion and animal welfare concerns, more primitive tend to use, as insects and nematodes, comparing to mammalian species. Nevertheless, in some studies and scientific fields, mammals, including primates, are needed to reach the proper conclusions (Fig. 1) [115].

Regarding the use of mammalian species in NPPM recognized works, the order Rodentia, which includes rats, mice, and guinea pigs, is the most used as an animal model. In detail, 86 projects used rodents, 29 used dogs, and 27 required rabbits, being those the most common mammals used (Fig. 3). Considering the use of domestic species (namely dogs, cats, ruminants, horses, chickens, and turkeys), 92 of the researchers used at least one domestic animal model in their works (Table 1).

The use of animal models in different scientific areas has always been a topic of debate in multiple conferences, interviews, and discussions. In the same way, the criteria used to choose the species, the number of animals, used and the experimental protocol is always debatable [116]. Therefore, it can be challenging to understand what criteria are used by recognized scientists for choosing a species instead of others as animal model. August Krogh, who was also a NPPM winner, was one of the 1st scientists to argue that despite the number of animals used and the research conditions, there is always the "most convenient species" to study a particular biology issue. Currently, concrete criteria are suggested in the literature mostly based on empirical data and ethical arguments. Very recently, in 2020, Dietrich et al. [117] established a total of 20 criteria, divided into 5 clusters. Some of them include, for instance, the ease of supply, the financial considerations, and the cultural attributes, that can contribute to the use of rodents. On the other

Table 1. NPPMs attributed to researchers that used animal models from 1901 to 2020 [6–114]

Year	Researcher	Animals used	Subject	Ref.
1901	Emil Adolf von Behring	Guinea pig, horse, and rabbit	Development of diphtheria antiserum*	[6]
1902	Ronald Ross	Pigeon	Malaria life cycle	[7]
1904	Ivan Petrovich Pavlov	Dog	Animal responses to various stimuli	[8]
1905	Robert Koch	Cow, sheep, rabbit, and mouse	Pathogenesis of tuberculosis*	[9]
1906	Camillo Golgi and Santiago Ramón y Cajal	Dog and horse	Characterization of the central nervous system	[10]
1907	Charles Louis Alphonse Laveran	Bird	Protozoa as cause of disease	[11]
1908	Ilya Ilyich Mechnikov and Paul Ehrlich	Bird, fish, and guinea pig	Immune reactions and functions of phagocytes	[12]
1910	Albrecht Kossel	Bird	Cell chemistry through work on proteins, including nuclear substances	[13]
1912	Alexis Carrel	Dog and cat	Surgical advances in the suture and grafting of blood vessels	[14]
1913	Charles Robert Richet	Dog and rabbit	Mechanisms of anaphylaxis*	[15]
1919	Jules Bordet	Guinea pig, horse, and rabbit	Mechanisms of immunity	[16]
1920	August Steenberg Krogh	Frog	Capillary motor regulating mechanism	[17]
1922	Archibald Vivian Hill	Frog	The production of heat in the muscle	[18]
1923	Frederick Grant Banting And John James Richard Macleod	Dog, rabbit, and fish	Insulin and mechanism of diabetes*	[19, 20]
1924	Willem Einthoven	Dog	Mechanism of the electrocardiogram	[21]
1928	Charles Jules Henri Nicolle	Monkey, guinea pig, rat, and mouse	Pathogenesis of typhus	[22]
1929	Christiaan Eijkman	Chicken	Antineuritic and growth stimulating vitamins*	[23]
1929	Sir Frederick Gowland Hopkins	Chicken		
1932	Sir Charles Scott Sherrington	Dog and cat	Function of neurons	[24]
1932	Edgar Douglas Adrian	Dog and cat		[25]
1934	George Hoyt Whipple, George Richards Minot, and William Parry Murphy	Dog	Liver therapy for anemia*	[26]
1935	Hans Spemann	Newt and frog	Organizer effect in embryonic development	[27]
1936	Sir Henry Hallett Dale and Otto Loewi	Cat, frog, bird, and reptile	Chemical transmission of nerve impulses	[28]
1938	Corneille Jean François Heymans	Dog	The sinus and aortic mechanisms in regulation of respiration	[29]
1939	Gerhard Domagk	Mouse and rabbit	Antibacterial effects of prontosil*	[30]
1943	Henrik Carl Peter Dam	Rat, dog, chick, and mouse	Function of vitamin K	[31]
1943	Edward Adelbert Doisy	Rat, dog, chick, and mouse	Function of vitamin K*	[32]
1944	Joseph Erlanger and Herbert Spencer Gasser	Cat	Specific functions of nerve cells	[33]
1945	Sir Alexander Fleming, Ernst Boris Chain and Sir Howard Walter Florey	Mouse	Penicillin and its curative effect in various infectious diseases*	[34]
1947	Carl Ferdinand Cori and Gerty Theresa Cori, née Radnitz	Frog, toad, and dog	Catalytic conversion glycogen	[35]

Table 1 (continued)

Year	Researcher	Animals used	Subject	Ref.
1947	Bernardo Alberto Houssay	Frog, toad, and dog	The pituitary role in sugar metabolism	[36]
1949	Walter Rudolf Hess	Cat	Functional organization of the brain as a coordinator of internal organs	[37]
1949	Antonio Caetano de Abreu Freire Egas Moniz	Cat	Therapeutic value of leucotomy in certain psychoses	[38]
1950	Edward Calvin Kendall, Tadeus Reichstein, and Philip Showalter Hench	Cow	Anti-arthritic role of adrenal hormones	[39]
1951	Max Theiler	Monkey and mouse	Yellow fever vaccine*	[40]
1952	Selman Abraham Waksman	Guinea pig	Streptomycin, the 1st effective antibiotic against tuberculosis*	[41]
1953	Hans Adolf Krebs	Pigeon	The citric acid cycle	[42]
1953	Fritz Albert Lipmann	Pigeon	Co-enzyme A and its importance in intermediary metabolism	[43]
1954	John Franklin Enders, Thomas Huckle Weller, and Frederick Chapman Robbins	Monkey and mouse	Culture of poliovirus	[44]
1955	Axel Hugo Theodor Theorell	Horse	Nature and mode of action of oxidation enzymes	[45]
1957	Daniel Bovet	Dog and rabbit	Synthetic compounds production and action on the vascular system and muscles	[46]
1960	Sir Frank Macfarlane Burnet	Rabbit	Acquired immunological tolerance*	[47]
1960	Peter Brian Medawar	Rabbit	Acquired immunological tolerance*	
1961	Georg von Békésy	Guinea pig	Physical mechanism of stimulation within the cochlea	[48]
1963	Sir John Carew Eccles, Alan Lloyd Hodgkin, and Andrew Fielding Huxley	Cat, frog, squid, and crab	Ionic mechanisms involved in excitation and inhibition of the nerve cell membrane	[49]
1964	Konrad Bloch and Feodor Lynen	Rat	Regulation of cholesterol and fatty acid metabolism	[50]
1966	Peyton Rous	Rat, rabbit, and hen	Tumor-inducing viruses	[51]
1966	Charles Brenton Huggins	Rat, rabbit, and hen	Hormonal treatment of prostatic cancer*	[52]
1967	Ragnar Granit, Haldan Keffer Hartline, and George Wald	Chicken, rabbit, fish, and crab	Primary physiological and chemical processes of vision	[53]
1968	Robert W. Holley	Rat	Interpretation of the genetic code and its function in protein synthesis	[54]
1968	Har Gobind Khorana	Rat		
1968	Marshall W. Nirenberg	Guinea pig		
1970	Sir Bernard Katz, Ulf von Euler, and Julius Axelrod	Cat and rat	Mechanism of storage and release of nerve transmitters	[55]
1971	Earl W. Sutherland, Jr	Mammalian liver	Mechanism of the actions of hormones	[56]
1972	Gerald M. Edelman and Rodney R. Porter	Guinea pig and rabbit	Chemical structure of antibodies	[57]
1973	Karl von Frisch, Konrad Lorenz, and Nikolaas Tinbergen	Bee, bird, and fish	Organization of social and behavior patterns in animals	[58]
1974	Albert Claude, Christian de Duve, and George E. Palade	Chicken, guinea pig, and rat	Structural and functional organization of cells	[59]

Table 1 (continued)

Year	Researcher	Animals used	Subject	Ref.
1975	David Baltimore, Renato Dulbecco, and Howard Martin Temin	Monkey, horse, chicken, and mouse	Interaction between tumor viruses and genetic material	[60]
1976	Baruch S. Blumberg and D. Carleton Gajdusek	Chimpanzee	New mechanisms for the origin and dissemination of infectious diseases	[61]
1977	Roger Guillemin and Andrew V. Schally	Sheep and pig	Peptide hormone production of the brain	[62]
1977	Rosalyn Yalow	Sheep and pig	Radioimmunoassays of peptide hormones	[63]
1979	Allan M. Cormack and Godfrey N. Hounsfield	Pig	Computer assisted tomography (CAT scan)	[64]
1980	Baruj Benacerraf, Jean Dausset, and George D. Snell	Mouse and guinea pig	Histocompatibility antigens and mechanism of action	[65]
1981	Roger W. Sperry	Cat and monkey	Functional specialization of the cerebral hemispheres	[66]
1981	David H. Hubel and Torsten N. Wiesel	Cat and monkey	Information processing in the visual system	[67]
1982	Sune K. Bergström, Bengt I. Samuelsson, and John R. Vane	Rat, rabbit, and guinea pig	Prostaglandins*	[68]
1984	Niels K. Jerne, Georges J.F. Köhler, and César Milstein	Mouse	Techniques of monoclonal antibody formation	[69]
1985	Michael S. Brown and Joseph L. Goldstein	Rats	Regulation of cholesterol metabolism	[70]
1986	Stanley Cohen and Rita Levi-Montalcini	Mouse, chick, and snake	Nerve growth factor and epidermal growth factor	[71]
1987	Susumu Tonegawa	Mouse embryo	Genetic principle for generation of antibody diversity	[72]
1988	Sir James W. Black	Guinea pig, cat, dog, and rat	Important principles for drug treatment*	[73]
1988	Gertrude B. Elion	Mouse, dog, rabbit, and monkey	Important principles for drug treatment*	[74]
1988	George H. Hitchings	Mouse, rat, and dog		
1989	Harold E. Varmus	Chicken	Cellular origin of retroviral oncogenes	[75]
1989	J. Michael Bishop	Chicken		[76]
1990	Joseph E. Murray	Dog	Organ transplantation techniques	[77]
1990	E. Donnall Thomas	Dog		
1991	Erwin Neher and Bert Sakmann	Frog	Chemical communication between cells	[78]
1992	Edmond H. Fischer	Rabbit	Reversible protein phosphorylation as a Regulatory mechanism	[79]
1992	Edwin G. Krebs	Rabbit and rat		
1993	Richard J. Roberts	Rat	Split genes	[80]
1993	Phillip A. Sharp	Mouse		
1994	Alfred G. Gilman	Rat, cow, rabbit, and turkey	G-proteins and the role of these in signal transduction in cells	[81]
1994	Martin Rodbell	Rat, guinea pig, and turkey		
1995	Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric F. Wieschaus	Fruit fly	Genetic control of early embryonic development	[82]
1996	Peter C. Doherty and Rolf M. Zinkernagel	Mouse	Recognition of virus-infected cells by the immune system	[83]

Table 1 (continued)

Year	Researcher	Animals used	Subject	Ref.
1997	Stanley B. Prusiner	Mouse and hamster	Prions, a new biological principle of infection	[84]
1998	Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad	Rabbit	Regulation of blood pressure with nitric oxide*	[85]
1999	Günter Blobel	Mouse, rat, and dog	Intrinsic protein signals that govern their transport and localization in the cell	[86]
2000	Arvid Carlsson, Paul Greengard, and Eric R. Kandel	Sea slug and mouse	Signal transduction in the nervous system	[87]
2001	Leland H. Hartwell	Sea urchin and frog	Key regulators of the cell cycle	[88]
2001	Tim Hunt	Sea urchin, frog, rabbit, xenopus, and clam		
2001	Sir Paul M. Nurse	Sea urchin and frog		
2002	H. Robert Horvitz, Sydney Brenner, and John E. Sulston	Nematode	Genetic regulation of organ development and programmed cell death	[89]
2003	Paul C. Lauterbur and Sir Peter Mansfield	Clam, mouse, dog, rat, chimpanzee, pig, rabbit, and frog	Magnetic resonance imaging (MRI)	[90]
2004	Richard Axel	Mouse and fruit fly	Odorant receptors and the organization of the olfactory system	[91]
2004	Linda B. Buck	Mouse		
2005	Barry J. Marshall	Piglet	*Helicobacter pylori* and its role in gastritis and peptic ulcer disease	[92]
2006	Andrew Z. Fire and Craig C. Mello	Nematode roundworm	RNA interference – gene silencing by double-stranded RNA	[93]
2007	Mario R. Capecchi	Mouse	Gene modifications by the use of embryonic stem cells	[94]
2007	Sir Martin J. Evans	Mouse and chicken		
2007	Oliver Smithies	Mouse		
2008	Harald zur Hausen	Hamster, mouse, and cow	Human papilloma viruses as a cause of cervical cancer	[95]
2008	Françoise Barré-Sinoussi and Luc Montagnier	Monkey, chimpanzee, and mouse	Human immunodeficiency virus	[96, 97]
2009	Carol W. Greider	Protozoan, mouse, and frog	Telomeres and the enzyme telomerase	[98]
2009	Elizabeth H. Blackburn	Protozoan and mouse		
2009	Jack W. Szostak	Protozoan		
2010	Robert G. Edwards	Rabbit	In vitro fertilization	[99]
2011	Bruce A. Beutler	Mouse	Activation of innate immunity	[100]
2011	Jules A. Hoffmann	Fly	Activation of innate immunity	
2011	Ralph M. Steinman	Mouse	Dendritic cell and its role in adaptive immunity	[101]
2012	John B. Gurdon and Shinya Yamanaka	Frog and mouse	Reprograming mature cells to become pluripotent	[102]
2013	James E. Rothman and Thomas C. Südhof	Mouse and hamsters	Vesicle traffic, a major transport system in our cells	[103]
2014	John O'Keefe and May-Britt & Edvard I. Moser	Rat	Cells that constitute a positioning system in the brain	[104]

Table 1 (continued)

Year	Researcher	Animals used	Subject	Ref.
2015	William C. Campbell and Satoshi Ōmura	Mouse, dog, sheep, cattle, chicken, and monkey	Therapy against infections caused by roundworm parasites*	[105]
2015	Youyou Tu	Mouse, dogs, sheep, cattle, chicken, and monkey	Therapy against Malaria*	[106]
2016	Yoshinori Ohsumi	Mouse	Mechanisms for autophagy	[107]
2017	Michael Rosbash, Jeffrey C. Hall, and Michael W. Young	Fruit fly	Molecular mechanisms controlling the circadian rhythm	[108, 109]
2018	Dr. Tasuku Honjo and James P. Allison	Mouse	Cancer therapy via inhibition of negative immune regulation*	[110, 111]
2019	William G. Kaelin, Jr., Gregg L. Semenza, and Peter J. Ratcliffe	Mouse	How cells adapt to changing oxygen availability	[112]
2020	Harvey J. Alter, Michael Houghton, and Charles M. Rice	Chimpanzee	Hepatitis C virus	[113, 114]

Adapted from Foundation for Biomedical Research [3, 4]. NPPM, Nobel Prize of Physiology or Medicine. * Project related to pharmacology.

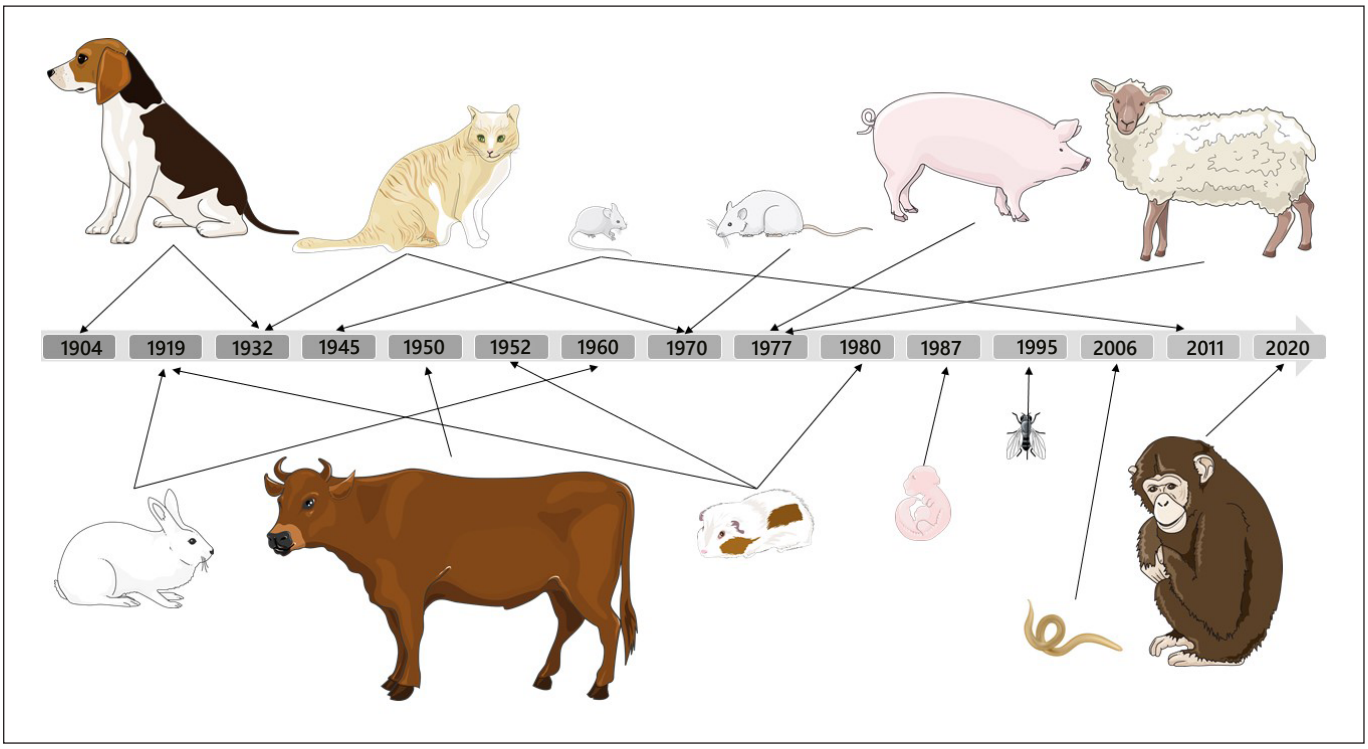

Fig. 1. Timeline illustrating some animal species used as models in NPPMs. NPPM, Nobel Prize of Physiology or Medicine.

hand, the researcher risks can be an explanation for the use of domestic species, and the comparative potential has a considerable impact on the choice of primates as animal models.

Moreover, some authors argue that the model choice depends on the biological area of the study and on the phase of the biological process. For example, in teratogenic studies, it is crucial to understand that embryonic

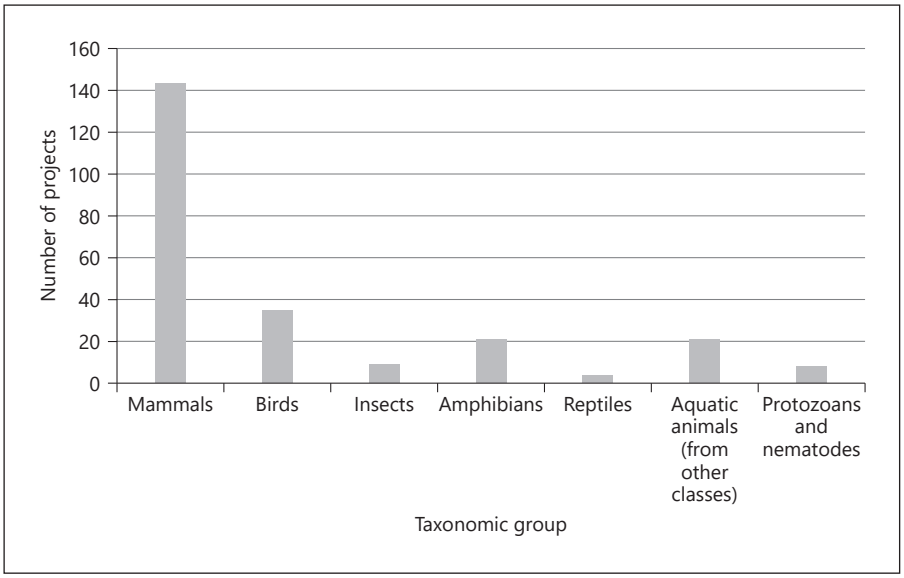

Fig. 2. Groups of animals used as models in different NPPMs. NPPM, Nobel Prize of Physiology or Medicine.

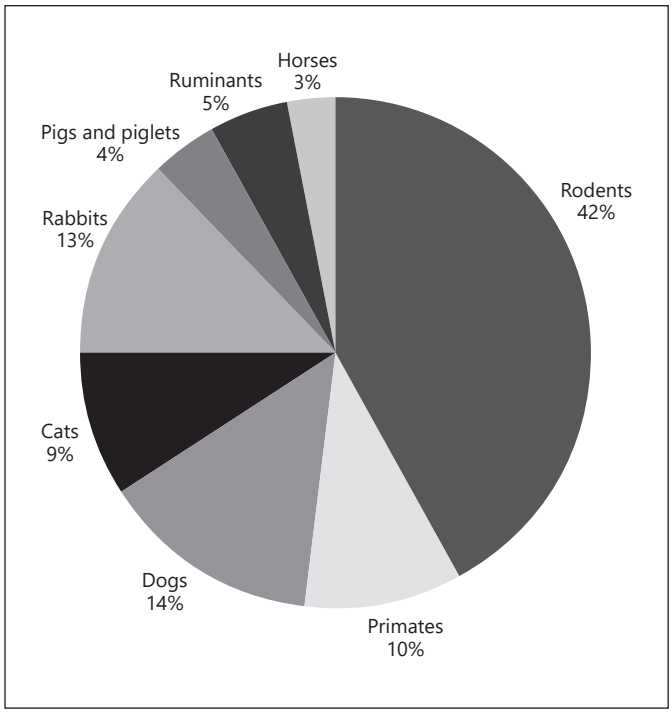

Fig. 3. Proportion of mammalian species used as animal models in NPPMs. NPPM, Nobel Prize of Physiology or Medicine.

development can be very distinctive among taxonomic groups and different phases of the development have distinct ideal animal models. In fact, invertebrates (as *Caenorhabditis elegans* and *Drosophila melanogaster*) are adequate for screening simple mechanisms in the early periods of the reproductive cycle, while mammalians, as rodents and rabbits, are phylogenetically closer to humans, allowing the possibility of taking better extrapolations and conclusions [118].

However, when choosing an animal model, it is not only essential to select the appropriate species but also the method to simulate a specific scenario. Thus, different types of animal models are usually defined in the literature and classified according to distinct criteria. Regarding their ecology or microecology, the models can be gnotobiotics, where the microbiota is totally known, germ free, where there is no microorganisms present, specific germ free, which means that the organism is free from specific bacteria or pathogens, or conventional, which is an animal with a normal microbiota [119]. On the other hand, according to genetics, they can be classified as outbred, defined as a closed population that are bred to maintain maximal heterozygosity, inbred, individuals of a particular species which are nearly identical to each other in genotype due to long inbreeding in order to preserve a characteristic, and transgenic, created by manipulating and adding a specific genetic material [120]. Finally, they can be categorized according to their mode of action. One of them is the induced model, where the studied condition is experimentally created in the animal and can be either through the exposition to chemical, biological or physical agents or surgically induced. For instance, the use of alloxan to kill the beta cells to study *diabetes mellitus* or the intestine reduction surgery to create a short-

bowel syndrome model represent, respectively, examples of the 2 types of induced model [121, 122]. Furthermore, there is the negative model, which is referred to a species, strain or breed that does not develop a certain disease following an experimental treatment. This model is frequently used to study infectious diseases, which are often restricted to a limited number of susceptible species, being innocuous to the negative model [123]. Moreover, the orphan model is used to study a condition that occurs naturally in a nonhuman species but has not yet been described in humans. Finally, the spontaneous model is a mutant, a natural organism with a genetic variant, being the athymic nude mouse an example of this model, used to study heterotransplanted tumors and allowing the 1st description of natural killer cells [124].

In oncology, besides using induced models, by promoting carcinogenesis physically, chemically, biological, or hormonally, it's common to use models for cellular implantation. In this case, 4 different models using mice are frequently used (syngeneic, ectopic, orthotopic, and Hollow Fiber Assay [HFA]), all of them with advantages and disadvantages, following described. The syngeneic model is necessary to study anticancer drugs that interfere with the immune system, but it can often reduce the number of cell lines available. Even though the ectopic model promotes fast growth of the tumor, it does not allow the study of the interaction between the tumor and the primary tissue and metastasis. On the other hand, the orthotopic model permits a fast growth of the tumor, allows its study in natural microenvironment and possible metastasis. However, it usually is expensive and technically complex. Finally, although the HFA does not promote the microenvironmental interactions and is also complicated and costly, it has minimal effects on animal welfare, reduces the number of animals used and promotes fast results [125].

Animal Use in Pharmacology NPPM-Awarded Studies

From the total of NPPM awards, 30 are related to different areas of therapy. Concretely, as mentioned above, 21 were attributed to scientist and projects directly related to pharmacology, as the use and test of various drugs and chemicals in order to treat diseases, infectious agents, and physical conditions [4]. From those 21 pharmacology projects, 17 used animal models and are properly marked in Table 1. In the following paragraphs, we detail some of them, all very relevant on our 21st century global society.

In 1905, Robert Koch won the NPPM for his tuberculosis studies, also presenting tuberculin as the 1st possible cure for tuberculosis. Before his studies on people with *lupus vulgaris*, a form of skin tuberculosis, performing skin injections of tuberculin and destroying the infected tissue, he also used cow, sheep, mouse, rabbit, and other species in different parts of his study to test his hypothesis [9, 126].

Frederick G. Banting and John J. R. Macleod were awarded in 1923 for their discovery of insulin, as a substance and the possibility of its use in diabetic patients. At 1st, dogs were used as an animal model. They created an extract from different dogs' pancreas and injected in a dog whose pancreas was surgically removed. Consequently, they observed a change in the dog's movements and behavior due to the dramatic decrease in their sugar levels [20, 127]. Nowadays, *diabetes mellitus* still requires animal models to be studied, especially rodents. To create an animal model of diabetes type I, the deficiency in insulin production is achieved by distinct mechanisms, from chemical ablation of the beta cells by streptozotocin to breeding rodents that spontaneously develop autoimmune diabetes, depending on the type of model, as mentioned above. Regarding animal models of type II diabetes, they tend to include models of beta cell failure or for insulin resistance. Additionally, many of them are obese, reflecting the human condition where obesity is closely linked to type II diabetes [127].

In 1939, Gerhard Domagk was recognized for his discovery of protosil. Domagk found, in 1932, that protosil was efficient in treating mice that were injected with a lethal dose of streptococci, and, 2 years later, was able to attribute that efficiency to sulfanilamide, as an active principle. Also concerning antibiotic therapy, Selman A. Waksman, in 1952, improved the Koch discoveries, mentioned above, to another level, by discovering that *Streptomyces griseus* suppressed the growth of tubercle bacteria. Its active principle, streptomycin, was also isolated after using guinea pigs as a model. Furthermore, Alexander Fleming, Ernst Boris Chain, and Howard Walter Florey were also recognized in this area of therapy, in 1945, for discovering penicillin and its potential as a treatment for multiple infectious diseases. After extracting the active principle from *Penicillium rubrum* and testing it in several concentrations in vitro *Staphylococci*, they needed to prove its efficacy in vivo, using mice [34, 128].

In 1988, Sir James W. Black, Gertrude B. Elion, and George H. Hitchings were awarded for their research on antimetabolites of nucleic acid purines, leading to the development of a variety of drugs for the treatment of dif-

ferent diseases. Thus, allopurinol is now used to treat gout and hyperuricemia, acyclovir is an antiviral drug that treats herpes virus infections, and azathioprine can be used to many immunological conditions and, for instance, to prevent organ transplant rejection. All of them, were frequently used in human and veterinary medicine nowadays and were previously tested in mice, rats, dogs, and small primates [74].

Finally, and more recently, in 2018, James P. Allison and Tasuku Honjo were recognized for their discovery of inhibition of negative immune regulation as a possibility of cancer therapy. Both of them identified proteins, with different modes of action, that release or activate our immune cells to attack tumors and both used mice as animal models in order to create a new oncologic therapy that proved to be effective [111].

Animal Models: Social and Legal Evolution

Historically, the use of animal models and the way they were used suffered remarkable changes associated with the concern to base these procedures on the current legal directives. From the Antiquity to the Renaissance, due to the taboos and religious issues on the dissections of humans, recognized physicians and scientists performed "vivisections," which is the exploratory surgery of live animals, without almost no social opposition or institutional regulations. Before the end of 17th century, there was already some opposition to vivisection. However, many scientists justified that their scientific undertakings were not cruel, basing themselves in the description of animals as "machine-like" by Renée Descartes. In 18th century, anthropocentric views on human duties to animals and philosophy question on their sensibility and suffering began questioning vivisections. Only in the 2nd half of the 19th century, due to a major medicine revolution, the use of animals in experiments began to require formal justifications and regulations. The publication of the *Handbook for the Physiological Laboratory* (1873) and the creation of *Victoria Street Society for the Protection of Animals Liable to Vivisection* (1875), later known as National Anti-Vivisection Society, illustrate those mental changes. In the 20th century, the concept of "animal ethics" emerged as a new field of bioethical studies and diverse ethical views on animals and of our duties toward them. However, public debate on animal research became polarized between animal rights activists and animal research advocates [129, 130].

The 3R's principles, established by Russell & Burch in 1959, were created to plan scientific studies that require animal models and establish criteria for them. In a resume, the 3R's are referring to Replacement, Reduction, and Refinement. In other words, to replace the protected and more intellectually complex species to less sentient forms of life, cells, tissues, or computer models, if it is possible. To reduce the number of individuals used as much as possible to extract the necessary conclusions. Finally, to refine the procedures, ensuring the proper and ethical use of the animals and taking their health and welfare into account is essential [131, 132].

In 1999, in the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, the Declaration of Bologna was signed and reaffirmed that "*humane science is a prerequisite for good science, and is best achieved in relation to laboratory animal procedures by the vigorous promotion and application of the 3R's*." Nowadays, animal research is developed in compliance with regulatory requirements which cover the inspection and licensing of animal sites, the training and competence of all the people involved in their manipulation and husbandry, and the mandatory authorization of every project by a competent authority upon ethical evaluation by an Animal Ethics Committee. The European Directive 2010/63/EU has set the regulatory framework for all animal research [133]. Moreover, the ARRIVE (Animal Research: Reporting in vivo Experiments) guidelines, established in 2010 and revised very recently, in 2020, provides practical and detailed instructions on the design of a research project, considering the sample size, husbandry, statistical analysis, and all the necessary aspects to plan a project. These guidelines are applied to all areas of research involving living animals from *Drosophila* or *Caenorhabditis elegans* to mammalian species [134].

Conclusion

We believe that the NPPM-awarded projects illustrate how animal models are essential for the advances in different subjects of biology and medicine, as in pharmacology or, more concretely, in antibiotherapy, endocrinotherapy, immunotherapy, and cancer therapy. However, we also consider that criteria for the use of animal models must be taken into account and applied in a practical context, according to the study area, species used, and selected type of model. Russel & Burch 3R's criteria represent an example of simple, concise, and easy to remember criteria applicable to a variety of studies.

In the future, we believe that animal models will become more specific and informative to the different stud-

ies, as they are continuously becoming in the past 100 years. Concretely in Pharmacology, the use of mathematical models and computer science will possibly allow the real time monitoring of the effect of an active principle tested on a live model, perhaps allowing the scientist to have complete and accurate results with a less quantity of animals used and minimizing the welfare concerns.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Catarina V. Jota Baptista wrote the paper. Ana I. Faustino-Rocha and Paula A. Oliveira supported the 1st author, correcting and revising the paper.

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