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Abstract	The use of biotechnologies has dramatically increased in recent years, radically changing our relationship with animals and their use in several fields, from food industry to scientific research. Provided here is an overview of those biotechnologies having the most relevant impact on our approach to animals, and a discussion of their implications in bioethical terms as well as their scientific and general advantages <i>versus</i> their limitations. This chapter concentrates on biotechnologies applied to biomedical science, providing a large range of examples particularly focused on genetic modifications of laboratory animals.	
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Biotechnologies and Animals: The Impact of Genetic Engineering on Human-Animal Relationships

Susanna Pietropaolo

1 Introduction

In recent years, a wide range of biotechnologies involving living animals has been developed in several fields. Among them, the manipulations of animal genomes for biomedical research are probably the most relevant ones, and have critical ethical implications. Indeed, procedures such as selective breeding of animals for specific traits or cloning of individuals have a strong impact not only on our society, but on our general approach to animals. On the one hand, negative terms like “eugenics” have often been associated with these types of manipulations and are often interpreted by the general audience as an aberrant use of human “supremacy” over the other animals. On the other hand, these and other genetic manipulations have enhanced enormously our knowledge of biological systems, including our understanding of the genetics of animal and

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human behaviour, as well as of several human pathologies, an issue of obvious primordial relevance.

Gene-targeting techniques, for instance, have been fundamental in understanding the basic role of a plethora of genes, and in designing genetic animal models for a myriad of human pathologies. These animal models obtained through biotechnological interventions have been and still are a necessary step for pre-clinical research, thus allowing the investigation of molecular etiopathological mechanisms and the design of novel therapies for subsequent clinical research. At the same time, the use of genetically modified animals has promoted the human view of animals as “tools” to improve the quality of life of human beings. The spread of research involving mutant mice has been so massive, in both research and industrial laboratories, that it may have somehow made us forget their original nature as living beings, aggravating the mutant mice’s exploitation by humans. According to some authors (Ryder 1975), this approach could be considered as a form of speciesism, inducing a sort of discrimination similar to racism. Genetic manipulations are also associated with litter culling and selective killing of individuals that do not carry the mutation of interest or are not further necessary for experimental work; these actions can be regarded as intrinsically wrong, if animals are meant to have rights (Regan 1984).

These ethical implications of biotechnologies and their impact on human-animal relationships are common to the general use of animals by humans; in addition to these, some specific aspects relate to the application of biotechnologies only. For instance, introducing artificial genetic mutations in animals may have unexpected effects, as they do not undergo Darwinian natural selection, thus promoting the view of biotechnologies as a way of “messing up” nature. Furthermore, the development of biotechnologies has led to the discovery that sequences of the human genome can be found in other animals, thus potentially lending further support to the argument that sacrificing the non-human for the sake of the human-animal relationship should not be legitimate (Hoeyer and Koch 2006). Indeed, biotechnologies applied to animals are often beneficial to humans and their research, but may have deleterious effects on animals’ well-being, inducing pathological phenotypes of various degrees of severity. The concept of “deleterious phenotypes”—though mostly considered

in its physical implications, for example, reduced body growth or development—is indeed an important part of regular assessment of research projects by national ethical committees and requires specific legal authorisations for experimentation. Nonetheless, these genetically induced deleterious phenotypes often include conditions of emotional suffering, and/or behavioural alterations, as it typically occurs in the case of animal models of neuropsychiatric and neurological disorders. These studies are, therefore, characterised by the specific ethical dilemma of being based on the assumption that the animals can model human mental suffering (e.g., depression, anxiety, drug addiction) by sharing similar highly developed neuronal functions and dysfunctions (Cassaday 2015), and concomitantly harming these human-like functions for research purposes.

Biotechnologies involving animal genes have developed rapidly through technical advances that appear increasingly sophisticated: the use of optogenetic approaches or the most recent Crisp/CAS9 methods are excellent examples of this advancement. These tools are leading to discoveries that are dramatically improving our knowledge of human physio-pathology, discoveries that are especially evident in the last frontier of scientific research, that is, the understanding of human brain functioning and related dysfunctions. At the same time, these manipulations risk to exacerbate the view of animals as “tools”, enhancing the ability of humans to control animals’ behaviour and brain functionality and possibly suggesting an approach to laboratory animals as “robotic units” under a sort of human remote control.

The present chapter intends to highlight the conflicting ethical aspects of the use of biotechnologies in biomedical research on animals, with a focus on genetic manipulations in laboratory species. Examples of the most relevant technologies manipulating animal genomes will be introduced, with an extensive discussion of their major contradictory ethical implications and their impact on animal-human relationships. The effects of selected major applications of these biotechnologies on the way humans see animals will also be described, with an emphasis on biomedical research and neuroscience in particular. The chapter will conclude with a discussion of the strategies that may contribute to solve some of the major ethical problems related to the application of these technologies to animals, thus suggesting novel directions for future biomedical researchers.

2 Ethical Implications of Genetic Technologies in Biological Research on Animals

Rodents, that is, rats and mice, are the animals most widely used in biomedical research, mice being the species of choice for most studies based on genetic modifications. Overall, rodents provide a good trade-off between the complexity of physiological functions necessary to meet the scientific objectives of biomedical research and the need to minimise ethical concerns, excluding animals with higher phylogenetic positions and increased capacity of suffering, according to current legislation on animal experimentation and animal protection. Hence, the preferential use of rodents for biomedical technologies can be (maybe surprisingly) viewed as a strategy promoting replacement for the use of higher animals (Cassaday 2015), and therefore benefiting from the fulfilment of one of the 3R principles (i.e., Reduction, Refinement, Replacement; (Russell 1959)).

Nonetheless, this potential ethical advantage is counterbalanced by the impressive increase in the number of laboratory animals used in biomedical research stimulated by the rapid progress in the development of genetically modified mouse lines and the success of their use for research, thus violating the Reduction principle of the same 3R approach (Blakemore et al. 2012). A conflicting ethical nature therefore characterised already the choice of the species used for biotechnologies; such ethical conflict seems a constant characteristic of the applications of all major types of biotechnologies used for biomedical research on animals, that is, selective breeding or genetic engineering.

2.1 Selective Breeding

Selective breeding (also called artificial selection) is a process used by humans since ancient times; it is based on manipulations of animal breeding to selectively develop particular phenotypic traits by intercrossing individuals with the characteristics of interest across several generations. The result is a line of animals with established (selected) traits

that will be inherited by the next generations in a stable manner. The selective breeding approach has been applied in a wide variety of animal species, including farm, pet, and laboratory animals for several commercial, industrial, and scientific purposes and can focus on the selection of both physical and behavioural phenotypes. The ethical implications of selective breeding are multiple as are their effects on human-animal relationships.

The first feeling that selective breeding may induce in a layperson is an unpleasant association with a sort of “eugenic” approach: selective breeding, especially when applied to farm animals or pets, can be intuitively associated with the idea of breeding the best to the best, selecting for “superior” qualities, with considerable culling of the other individuals considered as “inferior”. Hence, selective breeding obviously brings together two concepts that are ethically difficult to accept by many, that is, the idea that a rational, planned killing of animals is acceptable and that humans have the right to improve the genetics made by nature for their own advantage. Furthermore, since such an advantage has immediate commercial implications, that is, increased sales of selected farm or pet animals, the ethical conflict generated by selective breeding becomes unacceptable to some.

This feeling is exacerbated by the fact that some forms of selective breeding seemed to be applied for the mere “pleasure” of humans, as in the case of dog species bred for traits that make them physically more attractive as companions but that are often accompanied by health problems (Nicholas et al. 2010), for example, the case of French bulldogs, with their particular facial “baby-like” characteristics, but also their severe cardiovascular and eye problems. In the case of companion or pet animals, selective breeding may then promote the human view of animals as either “toys” or objects with high commercial value (as the pedigree market confirms...) that can be collected by humans as status symbols (Redmalm 2014). Animals with extreme physical characteristics are then subjected to the whims of fashion, and may become highly popular among humans despite their serious health problems (Sandoe et al. 2017).

As shown by the example of dogs, the price paid at the animal welfare level can be high, and selective breeding to enhance some traits over others can induce not only severe physical health problems but also aberrant

behaviours. For example, it has been reported that roosters bred for fast growth or heavy muscles kill hens after mating, as a consequence of the alienation due to their inability to perform species-typical courtship rituals (Grandin 2005). Conversely, selective breeding can also have beneficial effects on animal welfare, for example, increasing the resistance/adaptability of animals to captivity or breeding conditions and reducing their levels of stress. These animal welfare benefits are in turn translated into advantages for humans, as reduced stress and increased adaptability are known to enhance the quality of the outcomes of animal breeding, for example, providing higher-quality meat or more reliable scientific results.

Selective breeding has also contributed to our understanding of the genetics of animal behaviour, through so-called psychogenetic selection. This is a type of selective breeding that has been widely applied to laboratory rodents; it is typically based on the genetic selection of performances in a particular behavioural test to create animal lines or strains that show consistent, usually divergent, patterns of behaviour over generations. Well-known examples of this approach are the Roman High- and Low-avoidance rat lines, bred since the 1960s (Bignami 1965; Broadhurst and Bignami 1965) according to their performance in the two-way active avoidance learning test. Low-avoidance rats show not only poor learning but also increased stress responses (e.g., freezing behaviour, ACTH, corticosterone, and prolactin secretion) and anxiety (Steimer and Driscoll 2003), and adopt a more passive (or reactive) coping style when confronted with a novel environment (Driscoll et al. 1998; Steimer and Driscoll 2005). In contrast, High-avoidance rats are less responsive to stress, and show little anxiety in novel situations and tend to be impulsive and novelty seekers (Steimer and Driscoll 2003). Indeed, an important implication of selected breeding studies is the discovery that psychogenetic selection also influences physiological characteristics, not only those more closely associated with certain behavioural responses, for example stress hormones, but also immune functions (Koolhaas 2008). Studies in laboratory mice have, for instance, demonstrated that differences in inherited behavioural traits can explain inter-individual differences in the propensity to develop tumours, through alterations in angiogenesis and the functionality of Natural Killer cells (Vegas et al. 2006). Hence,

psychogenetic selection has important implications for studying the relationship between personality traits and the susceptibility to stress and illness (McEwen and Stellar 1993).

Hence, psychogenetic selection provides a useful tool to study the biological bases of personality (Driscoll et al. 2010; Giorgi et al. 2007) and their implications in health and disease (McEwen and Stellar 1993). Selective breeding of laboratory animals has in fact critically contributed to research on animal personalities, a topic that is of great scientific relevance *per se* and that can somehow give back to animals a central and active role in the relationship with humans. In this regard, the application of psychogenetic breeding to study the complexity of animal personalities somehow puts humans back at an equal level of importance with the other animals, in contrast to the anthropocentric view commonly associated with animal biotechnologies. Indeed, studies of behavioural genetics have demonstrated that in animals, exactly as in humans, some patterns of individual phenotypic characteristics show high consistency over a lifetime, and can therefore be classified as temperaments or personalities, defining specific ways of coping with the surrounding environment and life events (Gosling 2001). Recognising in animals the presence of certain personalities equivalent to humans (e.g., categorised as “shy,” “aggressive,” “impulsive,” “anxious”) can therefore improve our consideration of animal dignity and of their rights as complex well-beings, thus enriching our relationship with them.

Finally, selective breeding can be a powerful tool to investigate important processes and major biological phenomena involving animals. It is interesting to remark that the term “selective breeding” was coined by Charles Darwin (Darwin 1859), who was interested in “artificial selection” as an illustration of his proposed wider theory on natural selection. Darwin noted that many domesticated animals (as well as plants) had special properties that were developed intentionally by humans through the selection of desirable *versus* undesirable characteristics (Chapters IV and VI of “On the Origin of Species”). Darwin argued that the fact that humans with their weaknesses can achieve so much by artificial selection can only further support the existence of the more powerful process of natural selection and provide evidence supporting the evolutionary theory.

2.2 Genetic Engineering: Transgenesis, Gene-targeting, and CRISPR/Cas9

Genetic engineering includes all techniques leading to genetically modified organisms, that is, organisms in which the genome has been modified in a way that cannot occur naturally through mating and/or natural recombination. Originally, transgenic mammals, most commonly including mice, rabbits, pigs, goats, and cattle, were created through a technique called pronuclear microinjection (Kumar et al. 2009). During this process, a transgene—a linear DNA construct containing the gene of interest coupled to its regulatory elements—was directly transferred into the pronucleus of a single-celled zygote. Because transgene integration is a random event, and the insertion site of the transgene may interfere with critical genes, the embryo may fail to normally develop. Even if the transgene successfully integrates into genomic DNA, its expression may be more or less successful depending on the location of integration. With approximately 15% of microinjected livestock embryos resulting in live offspring, and a success rate of less than 1% of those embryos resulting in transgenic offspring, it is evident that transgenic animal production requires a high number of animals and a massive culling of non-transgenic offspring, with all the related ethical implications discussed above.

The use of large numbers of animals has been partially reduced by the introduction of other methods for creating transgenic mammals, that is, through embryonic stem cells (ESCs) or somatic cell nuclear transfer (SCNT) cloning techniques (Capecchi 1990; Capecchi 1989; Thomas and Capecchi 1987). Somatic stem or stem-like cells grown in vitro can be transfected with a transgene taking advantage of the natural homologous recombination process between the mutated DNA sequence and the cognate DNA sequence of the recipient cell. Subsequently, by including a selective marker on the construct (e.g., antibiotic resistance), selection can be applied to eliminate the non-transgenic cells. Additionally, populations of cells containing the transgene can be screened for colonies with a specific site of integration, if the goal is to disrupt a target gene through the insertion of the transgene. Mouse ESCs are then used to generate transgenic animals by injecting the modified cells into a blastocyst to form a chimera.

Additional gene editing tools have been further developed, more recently. The clustered regularly interspaced short palindromic repeat (CRISPR)-associated nuclease Cas9 (CRISPR/Cas9) system was developed from a bacterial defence mechanism, whereby the Cas9 endonuclease is guided to a specific region in dsDNA by small guide RNAs (gRNA), resulting in cleavage at that site (Vesikansa 2018). If a transgene is injected along with the gRNA and mRNA for Cas9, homologous recombination can occur, effectively swapping out a region of genomic DNA for the construct. This has the potential to make allelic changes without relying on selective breeding, which can affect closely linked genes that carry desirable traits, or replacing a mutated gene sequence for a functional sequence, or vice versa. It is now possible to make down-to-single-nucleotide changes, whether via insertion, deletion, or substitution (Vesikansa 2018).

These major improvements in efficiency in creating genetically modified organisms have markedly contributed to reduce the costs of these technologies in terms of animal lives: fewer embryos are now needed to create live, transgenic offspring, which results in a reduction in the number of egg donors—usually, super-ovulated females need to be sacrificed to collect the ova for SCNT or zygotes, if naturally mated. Furthermore, by increasing the success rate of live-born transgenic offspring, fewer animals are sacrificed. This has an obvious ethical benefit and positive impact on animal welfare, addressing at least two of the 3R principles: although suitable replacements are not available in this case, technological advancements have indeed refined the methods and reduced the number of donor and recipient animals.

The gene-targeting techniques have enabled researchers to conduct site-specific modifications to the genome and knock-out/-in specific genes (Capecchi 1989). Indeed, the immense power of these biotechnologies is that the investigator chooses both which genes to modify and how to modify them, keeping virtually complete control over the way in which the gene's DNA sequence or surrounding regulatory elements are modified (Capecchi 2000). This technology, therefore, permits evaluating deliberately selected gene functions in the intact animal and a systematic dissection of the most complex biological processes. Because nearly all biological phenomena are mediated or influenced by genes, this

technology has had an enormous impact on all areas of biological research in animals, including the study of cancer, immunology, neurobiology, and genetic diseases.

It is therefore not surprising that biotechnologies for the creation of genetically modified organisms have raised so many ethical concerns and have had a strong influence on the human-animal relationship. Firstly, the idea of “playing god” is more easily associated with these genetic modifications than with others, as they allow humans to create “new” animals, at least from the genetic point of view, rather than only selecting some of them, as in the case of selective breeding. This ethical concern is therefore associated with the philosophical acceptability of changing “the nature” of animals and morally accepting the supremacy of human over animal life. According to some authors, in order to consider the nature of an animal, we must take into account the Aristotelian concept of *telos* which describes the essence and purpose of a creature (Rollin 2003). It has been argued that altering the animals’ *telos* is morally permissible, as long as we are careful to accommodate the animals’ interests (Rollin 2003). According to other authors, animal interests and welfare may not be the only factors to be taken into consideration when establishing ethical limits to biotechnologies. For example, some authors have argued that genetic engineering requires us to expand our existing notions of animal ethics to include concepts of animal dignity, integrity (Ortiz 2004), and the intrinsic value of animals (Verhoog 1992). In particular, it should be considered that animals have an intrinsic value, which is separate from their value to humans. This approach is well reflected by the results of several public opinion surveys, where participants often state to be concerned about the nature of animals and worried about how it can be negatively affected by bioengineering (Macnaghten 2004). Another view suggested by other authors argues that genetic engineering may damage the human-animal relationship because of the increasingly imbalanced distribution of power between humans and animals (Schicktanz 2006). This imbalance has been termed asymmetry and is raised as a concern in human-animal relationships together with the ambivalence towards animals (Schicktanz 2006).

It should be underlined that the ethical acceptability of gene-editing techniques is not independent of the type of animal species used for these

studies; for instance, the genetic engineering of mice, that is, pest animals, is often perceived by humans as more morally acceptable than the use of pigs, which is, in turn, perceived as more acceptable than that of primates. This approach can be itself criticised as a form of sentientism, which can be considered as discriminating as speciesism (Wurzel 2009), since judgements of sentience can be confounded by prejudice based on species, that is, the subjective human perception of intelligence of certain species over others.

Another major ethical concern generated by the use of genetically modified animals relies on its potential negative effects on animal welfare. These include both direct effects of the genetic manipulations on the mutated organisms or indirect effects due to interactions between the mutated organisms and others that are genetically intact. Examples of the most relevant direct effects include the possible presence of deleterious phenotypes resulting in severe health problems or behavioural alterations, as shown by animal models of human pathologies (an issue further discussed in depth in section 3.2). Such direct effects are mostly intentional, as they are necessary to reproduce the pathological conditions observed in patients. Nonetheless, accidental negative effects of genetic mutation have also been described, as in the case of the enhanced intermale aggression levels displayed by transgenic mice for interferon- β , due to the fact that the transgene integration caused a disruption (was it a deletion or a disruption because the transgene inserted in the middle of the gene?) in the gene encoding monoamine oxidase A (MAOA), an enzyme that degrades serotonin and norepinephrine (Cases et al. 1995). Hence, the indirect negative effects on animal welfare are mostly unintentional, and they should be considered somehow undesired side effects of an extensive use of genetically modifying technologies on animals. This is the case of the risk that genetically modified animals may carry, and therefore transfer, antibiotic resistance to other organisms (including humans through the food chain), a consequence of the use of antibiotics in the early stages of the genetic modification process; such risk appears minimal but has not been ruled out by the major regulatory institutions (Food Safety Department 2005).

Another example of indirect negative effect of genetically modified organisms is their potential threat to biodiversity. In the evolutionary

history of species, spontaneous mutations submit the organism to a period of adaptation to a new gene, and the transformation of a single element reflects on the group as a whole. In the case of human-induced mutations, an exogenous gene is inserted into a receptive organism, thus abruptly interfering with the original network of pre-existing genes, possibly altering epistatic relations and feedback mechanisms that in turn can induce other unintended alterations in the host genome (Grisolia 2005). As a consequence, the exogenous modification of an organism may reduce its adaptability to the surrounding environment, as the modification was not the result of a long evolutionary process, as in the case of naturally occurring mutations (Grisolia 2005). Furthermore, genetic mutations induced by humans could endanger biodiversity interfering with natural selection and evolutionary mechanisms, for example, impacting on genetic diversity and integrity of wild species.

Nonetheless, it can be argued that the threat to biodiversity is not an exclusive risk of genetically modified organisms, as it characterises all human activities (Grisolia 2005). Conversely, the risk is minimised by the strict control that is generally exerted over the spread of genetically modified species into the wild. Instead, it is evident how other human activities, such as extensive commercial fishing and farming, or industrial exploitation, have already induced dramatic changes in the natural co-existence of species and their biodiversity. Hence, some authors support the idea that the criticisms of the use of biotechnologies, considered as an insult to nature, would be better directed to these other human activities which do not lead to equivalent benefits (Grisolia 2005). Indeed, the advances allowed by the use of genetically modified animals are undoubtedly numerous and of immense importance. They do not concern only human health and knowledge, as in the case of biomedical research or the study of animal behaviour, but also animal welfare, as in the case of genetically modified animals created to directly improve the health of the animals themselves—for example, improving their resistance to disease. As farming practices get more and more intensive, concerns of disease prevalence and spread of zoonoses from livestock to animal handlers become increasingly important, as well as concerns about the potential increase in antibiotic resistance in bacteria pathogenic to humans or livestock induced by the widespread use of antibiotics. These legitimate

concerns can be addressed in part by using genetically modified animals with increased resistance to disease, which would decrease the spread of disease among animals and reduce the frequency of "jump-over events" towards humans, while simultaneously decreasing the need for antibiotics. These genetically modified animals not only allow limiting the spread of diseases, for example, types of viral flu that can cause serious illness in both intensively farmed animals and humans, but they also improve animal welfare indirectly by avoiding the need for mass culling of cohoused animals, which is sometimes the only viable way to prevent epidemic disease transmission.

Finally, the same biodiversity that is supposedly threatened by genetic engineering could instead be preserved by these biotechnologies, as demonstrated by their applications in conservation programmes on endangered species. An example of this approach is provided by the application of cloning techniques to endangered or even extinct species (Lee 2001). In a more long-term view, gene-editing technologies including CRISPR could be used by ecologists to splice in a DNA sequence designed to handicap an invasive species, or to help a native species adapt to a changing climate, thus solving several problems for humans (also avoiding the environmental impact of pesticides or other invasive interventions) and concomitantly rescuing the balance among animal species (Redford et al. 2013). These latter applications of biotechnologies to "change" wildlife are obviously the subject of intense discussion (Redford et al. 2013), as they induce contradictory feelings among scientists and laypeople alike (Kohl et al. 2019; Thomas et al. 2013); nonetheless, it is undeniable that genetic technologies are likely to deeply transform the prospects for maintaining biodiversity into the future.

2.3 Optogenetics

The optogenetic approach, though part of genetic engineering technologies, can be associated with some specific ethical concerns regarding animal-human relationships that deserve a separate discussion. Optogenetics is an approach recently brought to neurobiology, combining both high-speed response and genetic targeting, based on a family of

fast light-responsive microbial opsins including halorhodopsins (e.g., NpHR) and channelrhodopsins (e.g., ChR2) (for review, see (Zhang et al. 2011)). These microbial opsins are single component transmembrane conductance regulators encompassing light sensitivity and fast membrane potential control within a single open reading frame, which can be used to achieve fast bidirectional control of specific cell types in freely moving animals (Airan et al. 2007; Arenkiel et al. 2007; Gradinaru et al. 2007). Optogenetic approaches in rodents have allowed investigating in depth the brain circuits involved in the physiological control of a variety of behaviours, as well as in the pathological development of neurological or neuropsychiatric diseases. For example, behavioural deficits in psychiatric diseases such as autism and schizophrenia have been hypothesised to arise from elevations in the cellular balance of excitation and inhibition (E/I balance) within neural microcircuitry. This hypothesis has become susceptible to direct testing, thanks to optogenetic tools exploring the circuit physiology associated with cellular E/I balance in freely moving rodents (Yizhar et al. 2011). Indeed, optogenetics allows us to understand the real mechanisms underlying animal (including human) behaviours by assessing the effects of stimulation or inhibition of specific neuronal circuits through light-based manipulations.

On the one hand, optogenetics is expected to advance our knowledge of common substrates of human and other animals' behaviours, thus reducing the "anthropocentric" gap commonly induced by genetic engineering technologies. Optogenetic approaches could also contribute to reduce the notion of the uniqueness of human beings, further demonstrating that certain human psychiatric pathologies are "only" a result of dysfunctioning brain circuits and may be induced in animals as well. On the other hand, the impressive real-time control humans can deliberately exert on animals' behaviour through optogenetic techniques may promote a view of animals as "automatable" organisms that can be artificially animated, hiding their original nature as living beings with their own identity. This impression, which is mostly emotionally based and is obviously out of the scope of optogenetic studies, may find support through the viewing of the several videos available from scientific publications in which optogenetics is used and an impressively rapid and precise control of certain behaviours in laboratory mice is shown, for example, stopping

or promoting movement through the optogenetic modulation of motor control circuits (Aravanis et al. 2007), or eliciting predatory-like attacks upon both natural and artificial prey by optogenetic stimulation of the central amygdala (Han et al. 2017). The viewing of these filmed experiments provides a direct experience of the power of optogenetic tools, and it may leave the viewer with the unpleasant feeling of witnessing an animal being under human remote control. Therefore, if we consider the ethical discussion on the *telos* and animal nature mentioned earlier (Rollin 2003), it could be argued that optogenetics is less acceptable morally than other gene-targeting techniques because it may affect not only the animal “essence” but also its “interests”. Furthermore, presently we do not have the slightest idea of the short- and long-term effects that inducing certain behaviours through optogenetic control may have on animal welfare: does a mouse who was induced to attack or to move by optogenetic control suffer consequences, for example in terms of stress or anxiety levels? This is an issue not specifically addressed in the present chapter and one that may be interesting to investigate in the future.

3 Implications of Genetic Technologies in Animal Biomedical Research: How Can They Change the Human View of Animals?

The ideas and concepts discussed so far have highlighted the complexity of the ethical implications of the different types of genetic engineering approaches to research on animals. Some of these biotechnologies have induced a profound change in the way humans view other animal species, both in a general and specific way. The general implications of biotechnologies have already been discussed in the previous sections; issues that are more specific to biomedical research and that may result in the emergence of novel ways of considering animals are now introduced. On the one hand, all examples described below are problematic from an ethical point of view, starting from a basic conflictual assumption: an animal is similar enough to humans to justify its experimental use for human

medical needs, but is sufficiently different in sentience and capacity for suffering for allowing the necessary experimental procedures (Cassaday 2015). On the other hand, it may be argued that all these applications of biotechnologies comply with the ethical guidelines on medical research arising from the 1947 Nuremberg Code, requiring that experiments be based on results of animal experiments to minimise unnecessary human suffering (Cassaday 2015). The need for a balance between these ethical conflicts seems to point in favour of animal medical research, as suggested by public surveys reporting that applications of genetic engineering to animals are generally better perceived and judged as more morally acceptable when their purpose concerns biomedical research rather than other fields, for example, food production (Schuppli and Weary 2010).

3.1 Animals as “Bioreactors” or Xenotransplant Donors

An important application of transgenic technology is the use of genetically modified animals as “bioreactors” to produce human pharmaceuticals. Transgenic goats, sheep, rabbits, and hen eggs have all been used to produce large quantities of pharmaceuticals for human use where expression of biologically active proteins in recombinant bacterial or yeast systems has failed. The human antithrombin III, an anticoagulant plasma protein, produced in the milk of transgenic goats was the first animal product approved for human use (Edmunds et al. 1998). This plasma protein must be purified from human blood and administered to patients suffering from antithrombin deficiency, but the yield of human plasma-derived antithrombin is low, creating a serious supply problem. Therefore, transgenic goats secreting human antithrombin in their milk provide an example of a cost-effective way to produce a necessary biopharmaceutical. Similarly, vaccines produced in the animal milk could be distributed cheaply and made more accessible to people around the world, avoiding the problematic issues related to their production, transportation, and injection (Hug 2008). Milk is not the only product used to make biopharmaceuticals, as transgenic mammals are not the only ones that can be used as bioreactors: for example, hen eggs have been used to produce

recombinant proteins in the albumin, for example, human interferons (Rapp et al. 2003) and monoclonal antibodies (Zhu et al. 2005), potentially providing a method for producing large quantities of proteins for vaccine use.

The use of animals as bioreactors consists of taking advantage of an animal's "natural" function, that is, producing milk or eggs, to provide a beneficial product to humans. Strictly speaking, the ethical implications of genetic engineering to produce animals that can be used as bioreactors do not appear to be different from those related to the exploitation of animals for the same products for food through classical "non-genetic" interventions. As in the case of the classical use of animals for meat production, the goal of genetic engineering can also be to produce animals that are *themselves* the beneficial product to be exploited by humans. The use of transgenic animals as organ donors for xenotransplantation represents a good example of this approach.

It is a well-known fact that the demand for human organs for transplant far outweighs the supply and certain animals, for example pigs, can serve as a pool for xenograft organs for humans, though differences in cell-surface antigens between the two species pose a great risk of rejection. Hence, engineering pigs by knocking out the enzymes producing some of these animal-specific antigens, for example, α -1,3-galactose, and/or inducing the transgenic expression of human surface antigens, can markedly increase the viability of xenografts (Ramsoondar et al. 2003). The ultimate goal of these manipulations would be the production of genetically modified pigs that would function as a universal donor, one not requiring the long-term use of anti-rejection immunosuppressive drugs. Nonetheless, at this stage we cannot know whether creating a single line of animals carrying several modifications may cause deleterious effects, compromising the health of animals and/or the quality of the tissue to be transplanted (Garas et al. 2015).

As mentioned before, the ethical implications of the use of genetically modified animals as bioreactors or xenotransplant donors should not differ fundamentally from those affecting the exploitation of genetically non-modified animals for food use, since in both cases animals are bred for the human use of their products (e.g., milk or eggs) or killed for their organs or meat. Yet, there is a somehow relevant emotional implication

that additionally affects the use of these genetic biotechnologies in biomedical research: it regards the creation of organisms that “mix” animal and human characteristics and that, as in the case of xenotransplants, can lead to humans “consisting of other animals’ parts”. This emotional concern seems therefore related more to the preoccupation about altering human integrity than about changing the nature of the other animals. This view could be criticised in that human “uniqueness” or integrity are concepts that lack a univocal interpretation; one could argue, for instance, that human beings are already a mixture of other animals, as they are the result of species evolution. Thus, exaggerating the importance of human integrity may be interpreted as the expression of an anthropocentric view.

3.2 Animals as Models of Human Pathologies

One of the most important and promising uses of genetically modified animals is to create models to study human pathologies. As genetic factors playing a role in a variety of pathologies are continuously discovered, genetic mutations have been implemented in several species in order to understand the molecular mechanisms involved in the etiopathology of human diseases as well as to identify novel therapies. A large variety of pathologies have been investigated through genetic animal models, especially mammals, including cancer and immune and cardiovascular pathologies, only to mention some major examples. While virtually anyone would applaud the aim of relieving human suffering, the moral acceptability of the use of non-human animals in reaching this aim can be a matter of controversy. As addressed earlier in this chapter, the use of animal models to study human pathologies could be considered contradictory precisely because we consider the use of human beings in such experiments morally impermissible. If the use of animal models is morally justified, there must be a relevant difference between human beings and the animals used in these experiments that justifies the differential treatment. Yet, the larger the difference between animals and humans, the less will be the validity of animals as good models for human conditions. According to some authors, this logical, philosophical, and ethical dilemma affects the use of animals in biomedical research, and becomes

especially relevant when we consider neuroscientific and neurobehavioural studies (de Castro and Olsson 2015).

Firstly, it is the type of disease studied in neuroscience that may enhance the relevance of ethical concerns on the use of animals in this field of biomedical research, as these pathologies include cognitive and emotional alterations that we know have a severe impact on the life quality of an individual. According to some authors, if behavioural neuroscience demonstrates that animals have cognitive abilities, emotional feelings, and social fear, we cannot treat them at the same time as if they are not conscious (Bovenkerk and Kaldewaij 2015). Furthermore, when we study neurobehavioural disorders we create negative experiences in the model animals, and we should consider the possibility that they may be even more overwhelmed by these states than us (Bovenkerk and Kaldewaij 2015). While many of these considerations may apply to other areas of biomedical research, these issues seem to become particularly relevant when sentience is the direct object of study, as in the case of neurobehavioural research on animal models (Cassaday 2015). This problem is exacerbated in the case of disorders that might have been avoided, as such situations are accompanied by the so-called human culpability factor, that is, a specific ethical concern affecting the use of animal research to alleviate human suffering that could have been avoided through behavioural change. This is the case of disorders that are often perceived as self-inflicted problems, such as drug addiction, obesity, alcohol consumption, or even stress-induced pathologies (e.g., (Lund et al. 2014)). This argument has overlooked important factors, including the genetic bases of these pathologies as well as the fact that several environmental factors, that is, low-educational levels or adverse rearing environment can be crucial in these pathologies and are not exclusively the product of a deliberate choice of the individual.

Secondly, it is the subject of neuroscience research, the brain, which may exacerbate the ethical concerns on the use of genetically modified animals in this biomedical domain. Experimental interventions affecting the central nervous system are indeed perceived as more ethically dubious than studies on other organs, maybe because the brain is indeed more identifiable with the human sense of self while its manipulation is more easily associated with deep and severe suffering; nevertheless, this is, once

again, quite an irrational form of criticism to neuroscientific research since biomedical research targeting other systems may result in suffering and distress just as, or even more than, neuroscientific studies. Therefore, ethical reflections and related guidelines should be general and not specific to the organ system or behavioural domain, as already suggested (Cassaday 2015).

4 Novel Directions for Future Researchers: Back to the Animal

This chapter has provided so far an overview of the major ethical concerns related to the use of genetic engineering in animal research and its consequences on the general view we have of the other animals surrounding us. I have underlined the fact that the most rational and scientific-based concern among those described here is the ethical one focusing on the impact of genetic technologies on animal welfare, while the other arguments are mainly psychologically or culturally based. Therefore, I firmly believe that the efforts of future researchers should focus on this concern, and that their investigations should bring back to the fore the central role that the animal has.

One of the major criticisms raised against the use of biotechnologies is that it induces a troubling shift in all human-animal relationships, as “it increasingly makes us neglect the living being behind the animal machine responsible for producing desired molecular products,” and, instead, it advances our self-understanding as “creator” or “designer” (Schicktanz 2006). This imbalanced human-animal relationship could then be counterbalanced by research considering the centrality of animals as living beings, that is, taking into account the complexity of animal welfare/biology and the role of non-genetic factors in modulating animal health. As anthropomorphism provides an unreliable basis from which to gauge animal welfare, research on laboratory animal behaviour is essential to an understanding of the normal behavioural repertoire (Barnard 2007), and in turn provides evidence for evolutionarily salient welfare (Ohl and van der Staay 2012).

Increasing animal welfare, in turn, enhances the validity of genetically modified animals for all human uses, especially as animal models of pathologies. Improving the scientific validity of animal models also enhances their moral acceptability, thus ameliorating the ethical costs-benefits balance related to the use of animals in biomedical research and, therefore, the quality of human-animal relationships. Biomedical research on genetically engineered animals is also often criticised because of the lack of consistent, replicable results within and between laboratories. Thus, the ethical acceptability of this research could benefit from (i) ameliorating the quality of the studies on genetic animal models, including the evaluation of factors that may influence their validity, for example, environmental influences and genetic background of the mutations, and (ii) promoting dissemination of the scientific results obtained from multiple laboratories, and (iii) encouraging data sharing and the publication of negative findings (de Castro and Olsson 2015).

4.1 The Importance of Behavioural Analysis and Ethological Approach

Behavioural analysis is of primordial importance in the case of genetically modified animals, even when behavioural modifications are not the ultimate goal of the genetic modifications. In that case, a behavioural evaluation is necessary to identify welfare problems that may be induced by genetic manipulations, such as the presence of self-injurious or stereotypic home-cage behaviours, or of cognitive, motor, or emotional alterations. The evaluation of animal welfare can particularly benefit from the recent advances in behavioural neuroscience allowing to measure subjective experiences. Two relevant examples of these advances are the use of cognitive bias to assess animal welfare in several species, including laboratory rodents (Burman and Mendl 2018; Paul et al. 2020; Trimmer et al. 2013), and the development of a face expression scale to assess pain in mice (Matsumiya et al. 2012; Mogil et al. 2020; Sotocinal et al. 2011). These approaches may have considerable potential to help quantify subjective harm in genetically modified animals, and could complement the

more classical measurements of animal health to refine the management of ethical issues in animal research.

An extensive behavioural analysis is also needed in the case of genetic animal models of human neuropsychiatric or neurological pathologies, involving behavioural abnormalities (Branchi and Ricceri 2002). Too often the testing of these animal models is limited to the behaviour of interest, that is, the one typically altered in the considered pathology. As a consequence, other behavioural changes that may be induced by the genetic mutation are overlooked and could instead have a critical role in confounding the desired behavioural phenotype (Crawley 2007). Similarly, multiple tests assessing the same behaviours should be conducted to confirm the robustness (and therefore the replicability) of the behavioural alterations of a genetically modified animal model (Crawley 1999). Incomplete or inappropriate behaviour analysis can indeed explain a major part of the discrepancies affecting much of the available scientific literature on the behavioural validity of certain genetic mouse models used in neuroscience research. In general, we witness an impressive increase in the number of studies using genetic mouse models of neuropathologies, exacerbating the competition among research groups and pushing further the commercial interests of pharmaceutical companies. As a consequence of this rush, we may note a dangerous tendency of some neuroscientists to draw a straight line to directly connect genes to a behaviour/pathology, sometimes totally overlooking the main element that stands in between, that is, the animal. Hence, drawing the attention back to the animal with its biology and ethology can only be beneficial for animals, humans, and biomedical research.

4.2 Not Only Genes: The Importance of the Environment

Focusing the attention of researchers on the animal should not only regard its behaviour, but also the other factors that characterise the animal as an organism. The single-minded search for genetic causal factors may sometimes lead scientists to almost forget that the genetically modified animals they use have not only genetic mutations, but also a sex, an

age, a genetic background of their own, and an environment surrounding them. As a consequence, until recent guidelines were developed (Crusio et al. 2009), it was not unusual to find scientific articles, even published in high-ranked journals, presenting data from a transgenic mouse line without mentioning whether they were obtained from male or female subjects, or describing the animals' rearing conditions.

Multiple experiments have demonstrated that the impact of genetic mutations can indeed be strongly modulated by the strain used as genetic background, by the sex of the subjects, and their environmental conditions (Crusio 1996, 2004). For example, being reared by more than one mother, a procedure that is not uncommon in many laboratory animal facilities can induce profound effects on brain and behavioural in laboratory mice (Branchi 2009) and even eliminate the pathological phenotypes of some genetic mouse models (Oddi et al. 2015). The inclusion of environmental factors is highly important not only to assess the validity of genetic animal models of human pathologies, but also to investigate more in depth the complex processes involved in the behavioural genetics of both animals and humans. For example, many of the selective breeding experiments as well as the personality studies mentioned in section 2 have shown the importance of gene-environment interactions in establishing the divergent behavioural patterns (Steimer et al. 1998), depending also on the specific life phases of an animal (Steimer and Driscoll 2003).

5 Concluding Remarks

While until recently the main limits to genetic engineering were technical, that is, what is possible to do, they have now become mainly ethical, that is, what is acceptable to do (Lassen et al. 2006). These ethical limitations should be drawn avoiding as much as possible arguments based on irrationality or built upon an unspecified technological scepticism or cultural criticism. They should instead focus on the understanding of animal biology alone and within the context of its relationship with humans.

An animal's nature is not a fixed entity, but a snapshot of a constantly dynamic, developing process of evolution (Rollin 2003). There is nothing wrong in itself with humans participating in that process, as they have

done with domestication. It is estimated that 40% of flowering plants and 70% of grasses represent new species created by humans through hybridisation, cultivation, and other means of artificial selection. Hence, we could reasonably argue that there is nothing intrinsically wrong with genetically engineering animals. Biotechnologies as such are indeed neither good nor bad, and they have the potential to alleviate or aggravate the impact of human control over animals and the environment, to improve animal and human health or to endanger their welfare, to promote biodiversity or to put it at risk.

The challenge is thus to develop and manage biotechnologies for the benefit of humankind and the environment. To this end, an interdisciplinary approach is necessary, requiring the collaborative work of scientists from several research fields, for example, conservationists, veterinaries, neuroscientists, ethologists, ecologists, and geneticists. These scientific efforts should then be taken into account by the work of legislative organisms and biotechnological industries. Ethical measures and safety monitoring should have their foundations in scientific and not in ideological reasoning. Emotional and pessimistic approaches towards innovative technologies should be avoided as much as possible in the future, as they can induce us to miss important opportunities, modify the perception of real risks and prevent efficient measures against them (Ciliberti and Molinelli 2005).

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Author Queries

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Queries	Details Required	Author's Response
AU1	Please check if the edit in the chapter title is okay.	
AU2	Please check if the edit in the sentence “Thus, the ethical acceptability of this research...” is okay.	