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# Biotechnologies and Animals: The Impact of Genetic Engineering on Human-Animal Relationships

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Abstract	<p>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.</p>	
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# Biotechnologies and Animals: The Impact of Genetic Engineering on Human-Animal Relationships

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## 1 Introduction

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

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

40 These ethical implications of biotechnologies and their impact on  
41 human-animal relationships are common to the general use of animals by  
42 humans; in addition to these, some specific aspects relate to the applica-  
43 tion of biotechnologies only. For instance, introducing artificial genetic  
44 mutations in animals may have unexpected effects, as they do not undergo  
45 Darwinian natural selection, thus promoting the view of biotechnologies  
46 as a way of “messing up” nature. Furthermore, the development of bio-  
47 technologies has led to the discovery that sequences of the human genome  
48 can be found in other animals, thus potentially lending further support  
49 to the argument that sacrificing the non-human for the sake of the  
50 human-animal relationship should not be legitimate (Hoeyer and Koch  
51 2006). Indeed, biotechnologies applied to animals are often beneficial to  
52 humans and their research, but may have deleterious effects on animals’  
53 well-being, inducing pathological phenotypes of various degrees of sever-  
54 ity. 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 inter-crossing 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

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

169 Selective breeding has also contributed to our understanding of the  
170 genetics of animal behaviour, through so-called psychogenetic selection.  
171 This is a type of selective breeding that has been widely applied to labora-  
172 tory rodents; it is typically based on the genetic selection of performances  
173 in a particular behavioural test to create animal lines or strains that show  
174 consistent, usually divergent, patterns of behaviour over generations.  
175 Well-known examples of this approach are the Roman High- and Low-  
176 avoidance rat lines, bred since the 1960s (Bignami 1965; Broadhurst and  
177 Bignami 1965) according to their performance in the two-way active  
178 avoidance learning test. Low-avoidance rats show not only poor learning  
179 but also increased stress responses (e.g., freezing behaviour, ACTH, cor-  
180 ticosterone, and prolactin secretion) and anxiety (Steimer and Driscoll  
181 2003), and adopt a more passive (or reactive) coping style when con-  
182 fronted with a novel environment (Driscoll et al. 1998; Steimer and  
183 Driscoll 2005). In contrast, High-avoidance rats are less responsive to  
184 stress, and show little anxiety in novel situations and tend to be impulsive  
185 and novelty seekers (Steimer and Driscoll 2003). Indeed, an important  
186 implication of selected breeding studies is the discovery that psychoge-  
187 netic selection also influences physiological characteristics, not only those  
188 more closely associated with certain behavioural responses, for example  
189 stress hormones, but also immune functions (Koolhaas 2008). Studies in  
190 laboratory mice have, for instance, demonstrated that differences in  
191 inherited behavioural traits can explain inter-individual differences in the  
192 propensity to develop tumours, through alterations in angiogenesis and  
193 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.

229 **2.2 Genetic Engineering: Transgenesis,**  
230 **Gene-targeting, and CRISPR/Cas9**

231 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.

249 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

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

303 It is therefore not surprising that biotechnologies for the creation of  
304 genetically modified organisms have raised so many ethical concerns and  
305 have had a strong influence on the human-animal relationship. Firstly,  
306 the idea of “playing god” is more easily associated with these genetic  
307 modifications than with others, as they allow humans to create “new”  
308 animals, at least from the genetic point of view, rather than only selecting  
309 some of them, as in the case of selective breeding. This ethical concern is  
310 therefore associated with the philosophical acceptability of changing “the  
311 nature” of animals and morally accepting the supremacy of human over  
312 animal life. According to some authors, in order to consider the nature of  
313 an animal, we must take into account the Aristotelian concept of *telos*  
314 which describes the essence and purpose of a creature (Rollin 2003). It  
315 has been argued that altering the animals’ *telos* is morally permissible, as  
316 long as we are careful to accommodate the animals’ interests (Rollin  
317 2003). According to other authors, animal interests and welfare may not  
318 be the only factors to be taken into consideration when establishing ethi-  
319 cal limits to biotechnologies. For example, some authors have argued that  
320 genetic engineering requires us to expand our existing notions of animal  
321 ethics to include concepts of animal dignity, integrity (Ortiz 2004), and  
322 the intrinsic value of animals (Verhoog 1992). In particular, it should be  
323 considered that animals have an intrinsic value, which is separate from  
324 their value to humans. This approach is well reflected by the results of  
325 several public opinion surveys, where participants often state to be con-  
326 cerned about the nature of animals and worried about how it can be  
327 negatively affected by bioengineering (Macnaghten 2004). Another view  
328 suggested by other authors argues that genetic engineering may damage  
329 the human-animal relationship because of the increasingly imbalanced  
330 distribution of power between humans and animals (Schicktanz 2006).  
331 This imbalance has been termed asymmetry and is raised as a concern in  
332 human-animal relationships together with the ambivalence towards ani-  
333 mals (Schicktanz 2006).

334 It should be underlined that the ethical acceptability of gene-editing  
335 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 (Wurbel 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- $\beta$ , 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

372 history of species, spontaneous mutations submit the organism to a  
373 period of adaptation to a new gene, and the transformation of a single  
374 element reflects on the group as a whole. In the case of human-induced  
375 mutations, an exogenous gene is inserted into a receptive organism, thus  
376 abruptly interfering with the original network of pre-existing genes, pos-  
377 sibly altering epistatic relations and feedback mechanisms that in turn  
378 can induce other unintended alterations in the host genome (Grisolia  
379 2005). As a consequence, the exogenous modification of an organism  
380 may reduce its adaptability to the surrounding environment, as the mod-  
381 ification was not the result of a long evolutionary process, as in the case  
382 of naturally occurring mutations (Grisolia 2005). Furthermore, genetic  
383 mutations induced by humans could endanger biodiversity interfering  
384 with natural selection and evolutionary mechanisms, for example,  
385 impacting on genetic diversity and integrity of wild species.

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

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

461 On the one hand, optogenetics is expected to advance our knowledge  
462 of common substrates of human and other animals' behaviours, thus  
463 reducing the "anthropocentric" gap commonly induced by genetic engi-  
464 neering technologies. Optogenetic approaches could also contribute to  
465 reduce the notion of the uniqueness of human beings, further demon-  
466 strating that certain human psychiatric pathologies are "only" a result of  
467 dysfunctioning brain circuits and may be induced in animals as well. On  
468 the other hand, the impressive real-time control humans can deliberately  
469 exert on animals' behaviour through optogenetic techniques may pro-  
470 mote a view of animals as "automatable" organisms that can be artificially  
471 animated, hiding their original nature as living beings with their own  
472 identity. This impression, which is mostly emotionally based and is obvi-  
473 ously out of the scope of optogenetic studies, may find support through  
474 the viewing of the several videos available from scientific publications in  
475 which optogenetics is used and an impressively rapid and precise control  
476 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

509 medical needs, but is sufficiently different in sentience and capacity for  
510 suffering for allowing the necessary experimental procedures (Cassaday  
511 2015). On the other hand, it may be argued that all these applications of  
512 biotechnologies comply with the ethical guidelines on medical research  
513 arising from the 1947 Nuremberg Code, requiring that experiments be  
514 based on results of animal experiments to minimise unnecessary human  
515 suffering (Cassaday 2015). The need for a balance between these ethical  
516 conflicts seems to point in favour of animal medical research, as sug-  
517 gested by public surveys reporting that applications of genetic engineer-  
518 ing to animals are generally better perceived and judged as more morally  
519 acceptable when their purpose concerns biomedical research rather than  
520 other fields, for example, food production (Schuppli and Weary 2010).

### 521 3.1 Animals as “Bioreactors” or 522 Xenotransplant Donors

523 An important application of transgenic technology is the use of geneti-  
524 cally modified animals as “bioreactors” to produce human pharmaceuti-  
525 cals. Transgenic goats, sheep, rabbits, and hen eggs have all been used to  
526 produce large quantities of pharmaceuticals for human use where expres-  
527 sion of biologically active proteins in recombinant bacterial or yeast sys-  
528 tems has failed. The human antithrombin III, an anticoagulant plasma  
529 protein, produced in the milk of transgenic goats was the first animal  
530 product approved for human use (Edmunds et al. 1998). This plasma  
531 protein must be purified from human blood and administered to patients  
532 suffering from antithrombin deficiency, but the yield of human plasma-  
533 derived antithrombin is low, creating a serious supply problem. Therefore,  
534 transgenic goats secreting human antithrombin in their milk provide an  
535 example of a cost-effective way to produce a necessary biopharmaceuti-  
536 cal. Similarly, vaccines produced in the animal milk could be distributed  
537 cheaply and made more accessible to people around the world, avoiding  
538 the problematic issues related to their production, transportation, and  
539 injection (Hug 2008). Milk is not the only product used to make bio-  
540 pharmaceuticals, as transgenic mammals are not the only ones that can  
541 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,  $\alpha$ -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

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

### 589 **3.2 Animals as Models of Human Pathologies**

590 One of the most important and promising uses of genetically modified  
591 animals is to create models to study human pathologies. As genetic factors  
592 playing a role in a variety of pathologies are continuously discovered,  
593 genetic mutations have been implemented in several species in order to  
594 understand the molecular mechanisms involved in the etiopathology of  
595 human diseases as well as to identify novel therapies. A large variety of  
596 pathologies have been investigated through genetic animal models, especially  
597 mammals, including cancer and immune and cardiovascular  
598 pathologies, only to mention some major examples. While virtually anyone  
599 would applaud the aim of relieving human suffering, the moral acceptability  
600 of the use of non-human animals in reaching this aim can be a matter of  
601 controversy. As addressed earlier in this chapter, the use of animal models  
602 to study human pathologies could be considered contradictory precisely  
603 because we consider the use of human beings in such experiments morally  
604 impermissible. If the use of animal models is morally justified, there must  
605 be a relevant difference between human beings and the animals used in  
606 these experiments that justifies the differential treatment. Yet, the larger  
607 the difference between animals and humans, the less will be the validity  
608 of animals as good models for human conditions. According to some authors,  
609 this logical, philosophical, and ethical dilemma affects the use of animals  
610 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

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

#### 653 **4 Novel Directions for Future Researchers:** 654 **Back to the Animal**

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

665 One of the major criticisms raised against the use of biotechnologies is  
666 that it induces a troubling shift in all human-animal relationships, as “it  
667 increasingly makes us neglect the living being behind the animal machine  
668 responsible for producing desired molecular products,” and, instead, it  
669 advances our self-understanding as “creator” or “designer” (Schicktanz  
670 2006). This imbalanced human-animal relationship could then be coun-  
671 terbalanced by research considering the centrality of animals as living  
672 beings, that is, taking into account the complexity of animal welfare/  
673 biology and the role of non-genetic factors in modulating animal health.  
674 As anthropomorphism provides an unreliable basis from which to gauge  
675 animal welfare, research on laboratory animal behaviour is essential to an  
676 understanding of the normal behavioural repertoire (Barnard 2007), and  
677 in turn provides evidence for evolutionarily salient welfare (Ohl and van  
678 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

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

713 An extensive behavioural analysis is also needed in the case of genetic  
714 animal models of human neuropsychiatric or neurological pathologies,  
715 involving behavioural abnormalities (Branchi and Ricceri 2002). Too  
716 often the testing of these animal models is limited to the behaviour of  
717 interest, that is, the one typically altered in the considered pathology. As  
718 a consequence, other behavioural changes that may be induced by the  
719 genetic mutation are overlooked and could instead have a critical role in  
720 confounding the desired behavioural phenotype (Crawley 2007).  
721 Similarly, multiple tests assessing the same behaviours should be con-  
722 ducted to confirm the robustness (and therefore the replicability) of the  
723 behavioural alterations of a genetically modified animal model (Crawley  
724 1999). Incomplete or inappropriate behaviour analysis can indeed explain  
725 a major part of the discrepancies affecting much of the available scientific  
726 literature on the behavioural validity of certain genetic mouse models  
727 used in neuroscience research. In general, we witness an impressive  
728 increase in the number of studies using genetic mouse models of neuro-  
729 pathologies, exacerbating the competition among research groups and  
730 pushing further the commercial interests of pharmaceutical companies.  
731 As a consequence of this rush, we may note a dangerous tendency of  
732 some neuroscientists to draw a straight line to directly connect genes to a  
733 behaviour/pathology, sometimes totally overlooking the main element  
734 that stands in between, that is, the animal. Hence, drawing the attention  
735 back to the animal with its biology and ethology can only be beneficial  
736 for animals, humans, and biomedical research.

## 737 **4.2 Not Only Genes: The Importance** 738 **of the Environment**

739 Focusing the attention of researchers on the animal should not only  
740 regard its behaviour, but also the other factors that characterise the ani-  
741 mal as an organism. The single-minded search for genetic causal factors  
742 may sometimes lead scientists to almost forget that the genetically modi-  
743 fied 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

777 done with domestication. It is estimated that 40% of flowering plants  
778 and 70% of grasses represent new species created by humans through  
779 hybridisation, cultivation, and other means of artificial selection. Hence,  
780 we could reasonably argue that there is nothing intrinsically wrong with  
781 genetically engineering animals. Biotechnologies as such are indeed nei-  
782 ther good nor bad, and they have the potential to alleviate or aggravate  
783 the impact of human control over animals and the environment, to  
784 improve animal and human health or to endanger their welfare, to pro-  
785 mote biodiversity or to put it at risk.

786 The challenge is thus to develop and manage biotechnologies for the  
787 benefit of humankind and the environment. To this end, an interdis-  
788 ciplinary approach is necessary, requiring the collaborative work of scien-  
789 tists from several research fields, for example, conservationists, veterinaries,  
790 neuroscientists, ethologists, ecologists, and geneticists. These scientific  
791 efforts should then be taken into account by the work of legislative organ-  
792 isms and biotechnological industries. Ethical measures and safety moni-  
793 toring should have their foundations in scientific and not in ideological  
794 reasoning. Emotional and pessimistic approaches towards innovative  
795 technologies should be avoided as much as possible in the future, as they  
796 can induce us to miss important opportunities, modify the perception of  
797 real risks and prevent efficient measures against them (Ciliberti and  
798 Molinelli 2005).

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# Author Queries

Chapter No.: 12      0005221702

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.	

Uncorrected Proof