REPLACEMENT IN THE SPIRIT OF THE 3RS PRINCIPLE

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In 1959 two British academics, Rex Burch e William Russell, published a book destined to become a milestone in the field animal experimentation: "The principles of humane experimental technique" (Russell & Burch, 1959). Burch and Russell were inspired and guided by Charles Hume's vision. Hume was at the time the President of the University Federation of Animal Welfare (UFAW) (a very active association in the field of animal welfare to this day), and was aware of a gap existing between scientific laboratory techniques and humanist values. Those humanist values were going to be instrumental in changing the attitudes towards experimental animals, considering these something more than just mere laboratory objects. Therefore, to bridge the gap, Hume thought it was necessary to introduce a new understanding of the experimental subjects, away from a strictly instrumental framework. The animals had to be intended as able to experience negative mental states, and a new methodological approach to laboratory techniques should have aimed at turning these negative states in at least neutral, if not positive ones.

As we all know now, this new methodological approach resulted in the introduction of the "3Rs principle", that is, "Replacement, "Reduction" and "Refinement". Therefore, when a researcher approaches a study utilising an animal model, he/she has to enquire firstly whether an alternative model to the use of a sentient animals can be used, then to consider whether to reduce the number of individuals utilised in his/her protocol, and finally to adopt all of the possible measures to avoid or minimise sufferance and pain. As said before, the final aim would be to alleviate, if not eliminate the negative mental states experienced by animals in research laboratories.

Replacement is the topic of the present report. In this contribution I will focus on some theoretical considerations concerning this particular concept.

Replacement as the first "R"

How Russell and Burch defined Replacement? They offered the following definition:

> "We shall use the term 'replacement technique' for any scientific method employing non-sentient material which may in the history of experimentation replace methods which use conscious living vertebrates. Among this non-sentient material, we include higher plants, microorganisms, and the more degenerate metazoan endoparasites, in which nervous and sensory systems are almost atrophied" (Russell & Burch, 1959 p. 69).

Replacement is the first "R" to be introduced by the two authors. As a matter of fact, nor Reduction, neither Refinement would be necessary where full Replacement is in place. It is also the most popular "R", because it is close to the point of view of animal rights movements, and is the easiest to communicate: as Olsson and colleagues pointed out, "not tested on animals" purveys a more convincing message than "tested on fewer aninals, but treated well" (Olsson *et al.*, 2012).

The "R" of Replacement is also the most controversial one, and can be potentially exploited by opposite camps in the debate on animal experiments. On one hand, the "3Rs principle" is sometimes considered as not working and being obsolete, because animals are still used in

research laboratories, and therefore no effective Replacement has been achieved; on the other, some researchers still consider the principle impossible to put into practice because "I still need my animal model". In both cases it is worth to cite Russell and Burch in their book:

> "Desirable as replacement is, it would be a mistake to put all our humanitarian eggs in this basket alone. The progress of replacement is gradual, not is it ever likely to absorb the whole of experimental biology" (p. 105).

This sentence could have been written today and it is a further proof of the current relevance of the principle. The message here is not to consider Replacement as detached from the other two "Rs", and to understand the principle as a unitary concept. However, Replacement generally has a prominent profile in EU Commission funding politics: although applicants are required to take the principle of the 3Rs in full consideration, is the "R" of Replacement that receives more specific funding.

The three "Rs" could give the impression to focus on two different ethical perspectives: Replacement focuses on no use, whereas Reduction and Refinement say that it is admissible to use animals, but under certain conditions. It is my opinion that this is not the way to correctly interpret the Russell and Burch's principle. The two authors did not want to write a book on animal ethics, but a methodological one. The question whether the use of animals in research laboratory is morally legitimate, and therefore if they be used or not, belongs to the "animal rights" line of arguments. The principle fully belongs to the "animal welfare" line of arguments. It is within this last perspective that the principle has to be taken into consideration and discussed and, eventually, challenged.

Partial Replacement?

What is "Partial Replacement"? Russell and Burch distinguish between "Relative Replacement" and "Absolute Replacement". For them "Relative Replacement" is when animals are still used, but not subjected to sufferance or distress, whereas in "Absolute Replacement" animals are not used at all. It is important to notice that, in its original meaning, Replacement is not indicated by the two authors as the elimination of the use of animals in experiments, but by the elimination of distress. In the case of "Absolute Replacement", no inhumanity is implied, simply because no animals are used. However, the use, for example, of complete anesthetized animals is indicated as a case of "Relative Replacement", provided the anesthesia is deep and running parallel to the course of the experiment.

In more recent times, Replacement has been defined in different ways: not using animals, or not using vertebrates, or using animals intended to be not sentient, or less sentient. For example, the "Guidelines for the care and use of mammals in neuroscience and behavioral research" of the Institute of Laboratory Animal Research defines Replacement as "use of non-animal systems or less-sentient animal species to partially or fully replace animals" (p. 10) (ILAR, 2003). One of the problems of such definition is the use of the term "less sentient". It could mean that the members of a particular species could be less capable to experience pain and/or distress than other species. However, the problem of how the define "sentience" in relation to, for example, behavioural biology is far from being solved (Vallortigara, 2017; *see also* Pollo & Vitale, 2019).

The NC3Rs (National Centre for the Replacement, Refinement and Reduction of Animals in Research), based in London, defines Partial Replacement (equivalent to the original Relative Replacement used by Russell and Burch) as the use of some animals that, based on current scientific thinking, are not considered capable of experiencing suffering. This includes

invertebrates such as *Drosophila*, nematode worms and social amoebae, and immature forms of vertebrates. Partial Replacement also includes the use of primary cells (and tissues) taken from animals killed solely for this purpose (i.e., not having been used in a scientific procedure that causes suffering (https://www.nc3rs.org.uk/the-3rs).

This means that the use of animals who are "less sentient" than the original ones is not considered in this case a form of Partial Replacement (e.g., a mouse instead of a monkey, or a fish instead of a mouse). The comparison of level of potential sufferance experienced by different species is also problematic in interpretative terms. We are still away from really understanding what sufferance means for different species (Dawkins, 2008; Borgi *et al.*, 2021).

The assumption is that more "complex" animals should be able to experience level of sufferance similar to humans. That would be in connection to their level of "sentience", which would be linked with a greater ability to remember and anticipate pain and distress, among other things. Is this really so? Do we really have robust data to support this assumption? This point of view is more in tune with what has been defined the "socio-zoological scale" (Arluke & Sanders 1996; see also Olsson *et al.*, 2012). It refers to what most of us, humans, perceive about the sufference of other species, rather than biological perspective. On top of the scale we put great apes, at the bottom invertebrates. This scale surely overlaps in some points with data collected by animal welfare scientists and cognitive scientists (see, for example, Mendl & Paul, 2004), but more research is still needed to better focus this scale on biological and evolutionary reality.

Replacement in the legislation

What are the references to Replacement and Partial Replacement in the European Directive 2010/63/EU, on the protection of animals used in scientific procedures? The principle is explicitly referred to in the normative text. For example, article 4 states:

> "Principle of replacement, reduction and refinement 1. Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure. 2. Member States shall ensure that the number of animals used in projects is reduced to a minimum without compromising the objectives of the project. 3. Member States shall ensure refinement of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum".

Furthermore, it is required from scientists, when deciding upon which procedure to use, to choose those that "involve animals with the lowest capacity to experience pain, suffering, and distress or lasting harm" (article 13.2) (Europe, 2010).

The Italian normative text on this regard, which is the application of the EU Directive into a national normative context, confirms this legal requirement (see, for example, article 13) (Italia, 2014). For what concerns Replacement, the article recommends to use procedures which implies the use of animals that show less capacity to experience pain, sufferance, distress or prolonged damage. Therefore, also here the normative wording does not seem to be in the spirit of the original idea by Russell and Burch, and points to the use of animals with less capacity to experience sufferance, a concept we saw before encountering some conceptual difficulties.

However, having said that, it is also expected that the understanding and application of the principle would change with the passing of time, from its original formulation, allowing for some new interpretations. The principle must also be understood as reflecting the state-of-the-art of animal welfare science.

Conclusions

The Istituto Superiore di Sanità has embraced the spirit of the "3Rs principle" when it comes to Replacement techniques, and the contributions presented in this volume are a demonstration of this attitude. There are two ways in which Replacement techniques can be thought and implemented: one-way is to pursue studies to improve *in vitro* and/or *in silico* methodologies, without reference to a particular experimental protocol; the other strategy is to device an alternative method with reference to a specific protocol or specific scientific question. The term "innovation" is crucial here. It represents a dynamic approach to experimental procedures, where old ways are revisited in the spirit of Replacement, or new methodologies that do not entail the use of animals are devised anew. However, most importantly, innovation in experimental procedures reflects the contemporary flavour that the "3Rs principle" continues to have since the year of its publication, 60 years ago (*see* also De Angelis *et al.*, 2019).

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