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Large Farm Animal Models of Human Neurobehavioral and Psychiatric Disorders: Methodological and Practical Considerations

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1 ANIMAL MODELS

In order to gain insight into human behavioral dysfunctions and neurologic and psychiatric disorders, it is of utmost importance that appropriate animal models are used in the behavioral neurosciences, such as neurobiology, biopsychology, neurology, and psychiatry. Animal model approaches have a long history in biomedical (Ericsson et al., 2013; Franco, 2013) and neuroscience research (Cowan et al., 2000), where the models have been developed to investigate brain–behavior relations. The aim has been to gain insight into human behavior and its underlying neuronal and neuroendocrinological processes, their role in neurological and neuropsychiatric diseases and their treatment.

Animal models in neuroscience research are mainly used for two purposes:

- for gaining insight in the processes and mechanisms underlying neurobehavioral disorder, which is primarily an area of basic research, but may also be a first step in translational research, and
- for identifying new therapeutic options and testing their efficacy and safety (van der Staay, 2006; van der Staay et al., 2009, 2014). For evaluating the safety and efficacy of interventions, translational research using (large) animal models alongside clinical trials is necessary (Drolet and Lorenzi, 2011).

In this chapter, we shall address the subject “large animal models.” First, the theoretical framework of performing animal experimental studies will be discussed and a definition of animal models will be given. Then, the position of (large) animal models in the translational chain will be discussed. Next, we direct our attention to selecting an appropriate animal model for addressing a scientific question. Some aspects of performing research using large animal models are challenging, compared to rodent models. We will address these challenges and their implications. Large model animal species need an adapted infrastructure for housing and testing. Also, we pay particular attention to methodological questions associated with the use of large animal models, such as considerations

about the experimental unit. The most efficient use of large animal models may imply multiple testing and multiple readouts to obtain a maximum of information. Similarly, reuse of animals within a study and/or reuse in successive (possibly unrelated studies) is discussed. Finally, the advantages and disadvantages of studies with large model animal species will be contrasted.

2 WHY ANIMAL EXPERIMENTAL STUDIES?

The possibilities to investigate the processes underlying behavioral dysfunctions and psychiatric disorders in the brain of humans are restricted, except when they are assessed in a clinical setting with patients as subjects. However, the extent and location of the damage [although emerging imaging techniques broaden the possibilities (Mier and Mier, 2015)], and its “history,” are often unclear, and the neurobiological variables associated with behavioral dysfunctions cannot be controlled sufficiently to achieve meaningful and interpretable results. Comparative approaches (Brenowitz and Zakon, 2015; Mehta and Gosling, 2008) relying on animal models could be used to answer questions about behavioral dysfunctions and their underlying neural substrate. Animals with a known and reproducible dysfunction or damage may help us to understand brain dysfunctions and their effects on behavior (Blanchard et al., 2013).

The comparative approach is based on the evolutionary theory and the assumption that fundamental aspects of the behavior of humans have a genetic basis with a common evolutionary trajectory that are shared with other species. A central intention of comparative behavioral sciences is to identify “(...) animal species with behavioral or psychological repertoires similar to humans so that the results of experiments with these animal models may throw light on seemingly related behavior in human beings” (Lickliter, 2004, pp. 27–28). Whereas animals are in most instances intended as a model for humans, one animal species may also serve as model organism for another species. Studies, however, which

explicitly compared behavior across species, are rare in neuroscience (Sharbaugh et al., 2003).

We define animal models of neurobehavioral disorders as follows:

An animal model with biological and/or clinical relevance in the behavioral neurosciences is a living organism used to study brain–behavior relations under controlled conditions, with the final goal to gain insight into, and to enable predictions about, these relations in humans and/or a species other than the one studied, or in the same species under conditions different from those under which the study was performed. *van der Staay, 2006, pp. 133–134*

3 ANIMAL MODELS IN BIOMEDICAL RESEARCH

Animal models are used during the entire cascade from proof of concept (POC) through efficacy testing, safety, teratology, and toxicology evaluations of candidate drugs to translational research to product-related research. POC, also called proof of principle (POP) is objective evidence supporting the functionality of a principle, concept, or theory and its potential for real-world application. In preclinical trials using appropriate animal models, the efficacy and safety of a drug is determined (Lalonde et al., 2007). Safety pharmacology aims to detect the liability of adverse effects, determine safety margin calculation, and monitor clinical safety (Pugsley et al., 2008). Teratology or developmental toxicology assesses agent-induced abnormal development (Vorhees, 1989), whereas toxicology is the study of the effects of (putative) poisonous chemicals or drugs on living beings. Finally, product-related research is performed during experimental research preceding and/or following the introduction of, for example, a therapeutic drug into the market. Its purpose is to fully tap the potential of a therapeutic substance at advanced stages in the clinical development and of the products that have already been launched onto the market (to our knowledge, this element of the cascade has not yet been addressed in scientific publications). All steps in the cascade of drug development are performed using animals as subjects, in particular rodent species. However, large animal models are increasingly being used and may change the cascade of animal experiments from basic research to research intended to develop new therapeutic principles.

4 CONCERNS ABOUT THE TRANSLATABILITY OF FINDINGS FROM ANIMAL EXPERIMENTAL STUDIES

The relevance of results from preclinical animal studies for developing new therapeutics and the translational value of preclinical animal studies has been criticized

(Plath et al., 2011; Pratt et al., 2012). Many putative new therapeutics turn out to be ineffective or not effective enough in clinical trials, whereas they showed good efficacy and safety in animal models (Gribkoff and Kaczmarek, in press; Macleod, 2011; van der Worp et al., 2010). Consequently, the translatability of result of animal studies to humans has been described as poor (Garner, 2014; Ioannidis, 2008; Macleod, 2011; van der Worp et al., 2010).

Many putative causes have been identified for the poor translatability of results from animal experimental studies (Sabroe et al., 2007), in particular ascribed to methodological flaws, such as underpowered studies, low group sizes, and/or lack of blinding, to name only a few (van der Worp et al., 2010). Awareness is increasing that animal studies can be flawed in multiple ways, and recommendations to increase the predictive validity of animal model-based research have been published (Ioannidis et al., 2014). Notably, it has hardly ever been questioned in this distending stream of critical reviews addressing lack of translatability, whether the appropriate model animal species have been used and whether a change of animal model species from rodents to large animals may improve translatability (presumably not in all, but probably in some areas of neuroscience research).

5 TRANSLATIONAL RESEARCH

Whereas research for identifying new therapeutic principles and putative therapeutics may be considered as basic research, using validated animal models to develop novel therapeutics may be considered translational research, where translation refers to the process in which knowledge generated in one area of research is applied in another area of research to advance goals in that area (Abernethy and Wheeler, 2011).

McGarland Rubio et al. defined translational research as follows:

Translational research fosters the multidirectional integration of basic research, patient-oriented research, and population-based research, with the long-term aim of improving the health of the public. T1 research expedites the movement between basic research and patient-oriented research that leads to new or improved scientific understanding or standards of care. T2 research facilitates the movement between patient-oriented research and population-based research that leads to better patient outcomes, the implementation of best practices, and improved health status in communities. T3 research promotes interaction between laboratory-based research and population-based research to stimulate a robust scientific understanding of human health and disease. *McGarland Rubio et al., 2010, p. 471*

The European Society for Translational Medicine (TM) defined translational medicine as “an interdisciplinary branch of the biomedical field supported by

three main pillars: benchside, bedside and community. The goal of TM is to combine disciplines, resources, expertise, and techniques within these pillars to promote enhancements in prevention, diagnosis, and therapies.” (Cohrs et al., 2015, p. 88).

Animal models are the first step in the translational continuum that has been described as consisting of three (Abernethy and Wheeler, 2011; Drolet and Lorenzi, 2011; McGartland Rubio et al., 2010), four (Lander and Atkinson-Grosjean, 2011), or five (Waldman and Terzic, 2010) distinct stages (Trochim et al., 2011). For our

purposes, the first translational stage(s) (T0 and T1) are of relevance. Fig. 3.1 depicts the chain of research from basic to translational. Nowadays, many if not most researchers using animal models in biomedical research denote their work as “translational.” The T0 stage (Waldman and Terzic, 2010) in Fig. 3.1 may cover this use of “translational.”

Translational animal models can be considered as a subset of the more general concept of “generalizability.” The “translational value” and “translational relevance” of an animal model, and the concept of “predictive validity” are connected, or may be conceived as basically

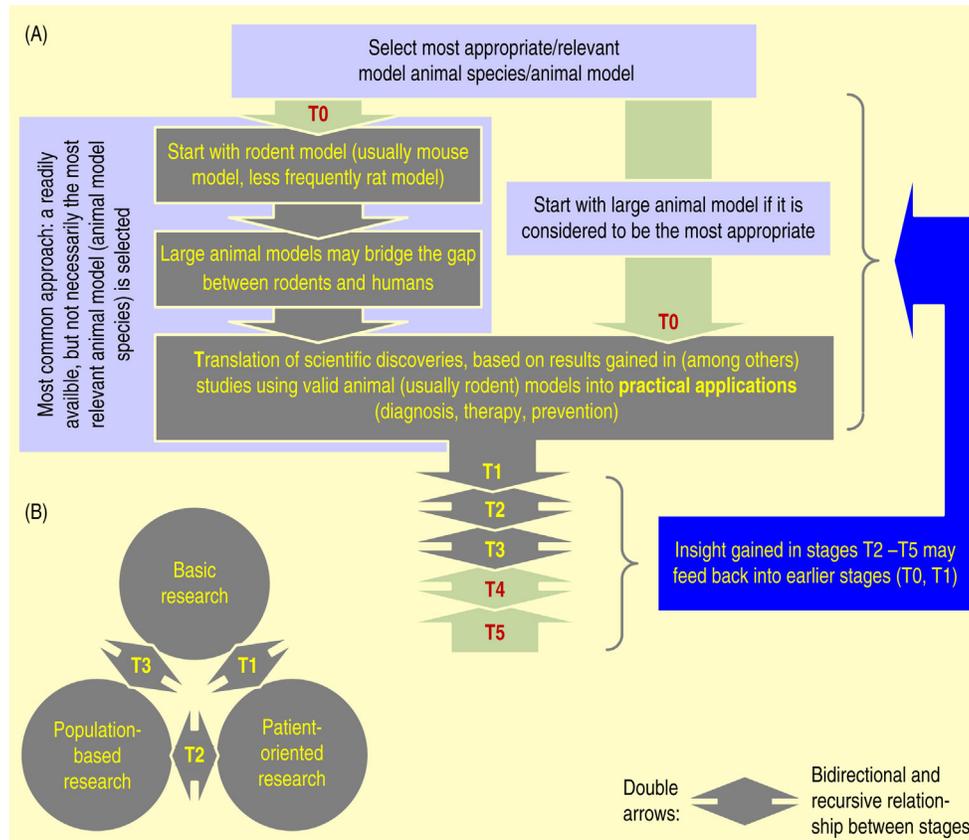


FIGURE 3.1 The position of animal models in the stages from basic (bench) to applied (bedside) translational research and the decision about the animal model species/animal model(s) to be used. Translational research distinguishes different stages, most commonly T1–T3 [panel (B) Drolet and Lorenzi, 2011; McGartland Rubio et al., 2010, p. 472], which may be considered as the basic concept. Others, however, distinguish more stages, for example, T1–T4 (Trochim et al., 2011), or T0–T5 (Waldman and Terzic, 2010). In all systems, the first stage (T1) or the first two stages (T0, T1) involve animal experimental research (in particular proof of concept research, Hostiuc et al., 2016). Proof of concept research may start using rodent experiments [panel (A) upper left] or may be performed using large animals, if the large animal model is considered as more appropriate/relevant, eventually completely skipping rodent experiments [panel (A) upper right]. T1 is “the transfer of new understandings of disease mechanisms gained in the laboratory into the development of new methods for diagnosis, therapy, and prevention and their first testing in humans” (Woolf, 2008, p. 211). Waldman and Terzic (2010) suggested to add the stages T0, preclinical research (in vitro research and in vivo animal model-based research), which may start as basic research, and T5, improving the wellness of populations by reforming suboptimal social structures. Basic research is performed for gaining scientific insight without the primary intention of practical application of the knowledge, whereas T0 already considers practical applicability. The bidirectional and recursive relationship between animal models, translation to applications, and reverse translation to animal models is indicated by the *double-headed arrows*. Also, insight gained in later stages may feed back to the first stage(s) of translational research (T0 or T1, depending on the classification of stages used). Stages T0, T4, and T5, which have been added to the original concept of three translational stages shown in panel B, are depicted as *green arrows* or *double arrows* in panel A. Source: Panel (A) modified from van der Staay, F.J., Arndt, S.S., Nordquist, R.E., 2014. *Developing mouse models of neurobehavioral disorders: when is a model a good model?* In: Pietropaolo, S., Sluyter, F., Crusio, W.E. (Eds.), *Behavioral Genetics of the Mouse*, vol. 2. Cambridge University Press, United Kingdom, pp. 3–17; panel (B) modified from McGartland Rubio, D., Schoenbaum, E.E., Lee, L.S., Scheingart, D.E., Marantz, P.R., Anderson, K.E., et al., 2010. *Defining translational research: implications for training*. *Acad. Med.* 85 (3), 470–475.

the same concept. “Translational,” however, has a clear focus on applicability (diagnosis, therapy, prevention). By comparing the effects of a new therapeutic with a compound with proven efficacy in the clinic, the predictive validity of an animal model can be determined experimentally. Likewise, the “translational value” or “translational relevance” of a (large) animal model is determined retrospectively: insights derived by using the animal model must successfully be translatable to practice.

Using models with proven construct validity may increase the probability of successful translation of animal research-based preclinical findings to clinical applications (Kimmelman and London, 2011; Pratt et al., 2012). The translatability can further be increased by using a broader range of relevant models (Pratt et al., 2012), for example, by using animals that show comorbidity (Libby, 2015), by applying a treatment regime that closely matches that of clinical practice (Green et al., 2011), and by applying appropriate behavioral tests that can distinguish between different behavioral domains and endophenotypes (Homberg, 2013). The “translational value” and “translational relevance” of large animal models largely depends on the degree to which they mimic the dysfunction/disease under study and the availability of reliable testing methods.

Translational research is bidirectional (Donaldson and Hen, 2015). It may start with clinical observations and characterizing disease-relevant endophenotypes in patients which are then reverse translated to animal models, that is, clinical findings inseminate basic research (Holschneider et al., 2011). This can be considered as a process of induction in the process of developing animal models (van der Staay, 2006). Sinha et al. (2011) assessed whether neuropharmacological findings in humans yield new insights, that is, whether they can be reverse translated to investigating underlying mechanisms in an appropriate animal model. Reverse translational approaches thus may contribute to conceiving and developing new animal models or to refining established ones. For developing translationally relevant animal models (Pratt et al., 2012), an intensive interaction of animal research scientists and clinical researchers is fundamental (Markou et al., 2009).

“The model’s circular structure suggests that research is a continuing cycle, and its bidirectional arrows emphasize that new knowledge and hypotheses are generated at each step. Some basic research and population-based research is translational, but neither type of research is by definition translational. In contrast, patient-oriented research fundamentally addresses issues that have the potential to translate to clinical practice and, therefore, affect health.” (McGartland Rubio et al., 2010, p. 472).

Animal models intended for use in basic research and animal models in translational research should,

in principle, fulfill the same criteria to be valid and relevant. However, translational animal models may need to fulfill additional criteria if they are applied in translational stage 1 (T1) (van der Staay et al., 2014). Whereas the key definitions per stage may differ for different classifications of the translational continuum, they all describe translational research as a process that moves from basic/preclinical research [T1 or T0/T1 that include(s) animal models] to the clinical application of the knowledge gained in animal experiment, and finally, to public health (Fig. 3.1). This process is bidirectional (Trochim et al., 2011) and in some instances, can be recursive.

6 CHOICE OF APPROPRIATE ANIMAL MODEL

In most instances, if authors reflect on the choice of an appropriate animal model, they are looking for models with rodents, and do not consider *nonrodent* species, that is, they usually mean *rodent model*, or *mouse model* when referring to *animal model*. Manger et al., 2008 discussed the consequences of the disproportionate use of rodent model, and particularly mouse models in basic neuroscience research. This extreme bias may hinder “(...) the discovery of novel aspects of brain structure and function that would be of importance in understanding both the evolution of the human brain and in selecting appropriate animal models for use in clinically related research.” (Manger et al., 2008, p. 2).

There has been a steady increase of using rodent species, mainly mice, whereas in nonrodent species, in this decade, the use of pigs has overtaken that of rabbits and dogs as model species in biomedical research (Ericsson et al., 2013; Table 3.1). Recently, the use of large animal models appears to increase owing to the development of genetically modified farm animals. For example, genetically modified pigs are being developed as models for human diseases, such as Alzheimer’s disease (Søndergaard and Herskin, 2012).

The reasons for sticking to rodent models, where nonrodent models might be more appropriate are mainly nonscientific:

- The researcher is mainly experienced with performing rodent studies. Rand (2008) cautions against selecting an animal model solely based on the familiarity of the researcher with a model, its availability and its costs.
- The question “which model species is the most suited for my research” very often is not even asked. This should, however, be one of the first questions when planning to perform animal experimental studies (Rand, 2008; von Rechenberg, 2012),

TABLE 3.1 Advantages and Disadvantages of the Reuse of Animals in Different Tests in the Same Study, Yielding Multiple Readouts From the Same Animals, or of the Same Animals in Successive, Unrelated Studies

Advantages	Disadvantages	
	Reuse of animals in one study (multiple tests and/or repeated testing)	Reuse of animals in successive, unrelated studies (which may comprise of one test or multiple tests, and eventually, repeated testing)
<i>Fewer animals are needed for research</i> (Reduction)	If nonrepresentative samples of the population are used, then this may affect the results of all experiments and/or tests with the same animals	If the samples are not representative for the population from which they were drawn, then this may affect all experiments/tests performed with the same animals
<i>Animals are handled and accustomed to being tested</i> ; less habituation or training trials are needed; animals are used to be handled and tested; lower stress level	Animals may not be “test naïve” in later tests or experiments, that is, previous testing may affect results of subsequent tests and/or experiments; excessive reuse of animals may compromise their welfare (depending on the degree of discomfort induced by the testing methods used, Festing et al., 1998)	Excessive reuse of animals may compromise their welfare (depending on the degree of discomfort induced by the testing methods used, Festing et al., 1998); reuse may necessitate transportation, mixing (introduction into a new group due to random assignment to treatment conditions), housing in an unfamiliar animal room
<i>Animals are test experienced</i> . Successive testing provides opportunities to adapt to different environmental demands and challenges; behavioral consistency may increase with age, due to experience with testing (Bell et al., 2009); less likely “Casper Hauser” individuals with unchallenging life (van der Staay et al., 2010)	Animals may not be “test naïve” in later tests or experiments; altered behavioral baseline; prior testing may alter effects of treatment (Holmes et al., 2001b); this may complicate comparison with studies that used test naïve animals; previous testing may interfere with subsequent testing (proactive interference)	Animals may not be “test naïve” in later tests and/or experiments; prior testing may alter effects of treatment (Holmes et al., 2001b); this may complicate comparison with studies that used “test naïve” animals; previous testing may interfere with subsequent testing (proactive interference)
<i>Knowledge accumulates with each test/experiment</i> , providing a more complete picture, and allowing investigation of relationships between variables (readouts) (Reduction, Refinement)	Animals are older in successive tests (age cannot strictly be controlled); animals may reach “ceiling” performance level, reducing sensitivity to detect treatment effects; correction of <i>P</i> -values for multiple comparisons may reduce sensitivity to detect (subtle) effects	Animals are inevitably older in each successive study (age cannot strictly be controlled)
<i>Testing may act as enrichment</i>	Testing per se may overshadow the effects of experimental manipulations (Westlund, 2014a,b)	Testing per se may overshadow the effects of experimental manipulations (Westlund, 2014a,b)

where the appropriateness of the selected animal model species should be the first consideration ([Held, 1983](#)).

- Conservatism of rodent researchers may withhold them from considering alternative animal model species. In this connection, [Libby \(2015\)](#) coined the term *Murine “model” monotheism*, which is a nice label for the bias toward rodent models.
- Neglecting publications that used nonrodent animal models (perhaps best described as “reading bias”).
- In most research institutes, facilities other than those needed for housing and testing rodents are unavailable.

Instead, the selection of rodent and nonrodent species for preclinical assessment of, for example, the efficacy and/or safety of new putative therapeutics, should

always be based on scientific and ethical justifications. Animal models must fulfill a set of scientific criteria and should thoroughly be validated ([Belzung and Lemoine, 2011](#); [Markou et al., 2009](#); [van der Staay, 2006](#); [van der Staay et al., 2009, 2014](#)). Of course, these criteria are closely connected with the research question(s) that the animal study is supposed to answer. Besides this very specific consideration, general questions must be addressed to help finding the animal model best fitting one’s research question(s). This implies that the selection of an animal model species and the animal model should be considered on a case-by-case basis ([Colleton et al., 2016](#); [Rand, 2008](#)). Based on [Rand’s \(2008\)](#) listing of scientific and practical criteria, the following questions need to be answered before selecting an animal model and starting a study. The first questions are related to the experimental approach to be chosen:

- What is known about the problem under consideration (based on a thorough review of the scientific literature, for example, via a systematic review, [Hooijmans and Ritskes-Hoitinga, 2013](#), or via less time-consuming rapid reviews, [Featherstone et al., 2015](#))?
- Has the selected model been validated and is it deemed to be the most appropriate for answering the research question(s)? How closely, for example, does the animal replicate the human disease ([Søndergaard and Herskin, 2012](#))?
- What kind of model is it: does the model animal express naturally occurring or experimentally induced deficits (for different classifications of “animal models,” see [Rand, 2008](#); [van der Staay, 2006](#))?
- What are the ethical implications of this choice (similar considerations as, e.g., for stroke models in primates [Sughrue et al., 2009](#), may be relevant in other large animal model species)?
- How many animals are needed to answer the research question(s), that is, which group sizes are needed to ensure sufficient power?

Next, questions concerning availability, animal housing, and animal care must be answered, such as:

- Is the animal model species chosen readily available. In most instances, availability should not be a major problem in research using large farm animals.
- Can the animals be housed appropriately? This may be a concern in large animal research because they may need considerably more space and must be housed in stables/pens that fulfill their species-specific needs.
- Are the researchers and animal caretakers trained to work with large model animal species and provide special care, if necessary?

Finally, a number of specific questions must be answered:

- Do animals with genetic homogeneity or heterogeneity best serve the research question(s)? Most large model animal species are heterogeneous lines/breeds, that is, homogeneous (inbred) lines are not yet readily available. However, for most farm animal species, a large variety of breeds is available.
- Is the selected model animal species testable, both in terms of ease of handling, as well as in terms of availability of well-established and validated test methods and test equipment (see also Chapter 39)?
- Does the size of the animal facilitate taking samples from the animal (blood, hair, tissues)? Is it crucial that organ size is similar to that of humans?

- Should the animal be tested during ontogeny, (e.g., early postpartum, as juvenile), as adult, or during senescence. Whereas large animal species may be specially suited for pre-, peri-, and early postnatal experimental manipulations, they may be less suited for aging research, due to poor availability of aged subjects and the high life expectancy of many of these species.
- Recently, the “sex bias” in neurosciences and biomedical sciences in general attracts increasing attention ([Beery and Zucker, 2011](#); [Zakariaeiz et al., 2016](#)). It has to be decided whether males, females, or both sexes should be included. Due to the extra space, special equipment, well-trained personnel needed to perform experiments using large model animal species, inclusion of both sexes may overburden available resources.

7 WHERE IN THE PROCESS OF MODELING HUMAN DISEASES AND DEVELOPING PUTATIVE THERAPEUTICS HAVE LARGE ANIMAL MODELS BEEN USED?

Large animal models may be relevant for research that aims to improve production of animal-derived products (not considered further in this chapter), for animal health and welfare, and for translational biomedical research ([Gonzalez-Bulnes et al., 2016](#); [Ireland et al., 2008](#); [Koopmans and Schuurman, 2015](#)), that is, they can be considered as multiple purpose models. Large animal models are well accepted in the area of orthopedic and xenotransplantation research ([Cook et al., 2014](#); [Gregory et al., 2012](#); [Harding et al., 2013](#)). They are, however, only sporadically used in neuroscience, where they are mainly applied in stroke and traumatic brain injury studies ([Boltze et al., 2008](#); [Duhaime, 2006](#); [Margulies et al., 2015](#); [Mehra et al., 2012](#); [Platt et al., 2014](#); [Wells et al., 2012](#)).

Most of the efficacy, toxicology, teratology, and safety studies use rodents species as subjects. However, in particular in toxicology, teratology, and safety studies a considerable proportion of animals used are large animals (e.g., dogs and miniature pigs). In particular in safety pharmacology/toxicology/teratology studies, the minipig is gaining importance ([Bode et al., 2010](#); [Forster et al., 2010a,b,c](#); [Svendsen, 2006](#); [van der Laan et al., 2010](#)).

Other than in risk assessment studies, the use of large animal model species is still negligible in drug testing, be it in POC studies or in studies, which aim to test the efficacy of a new compound preclinically, compared with rodents.

8 WHICH MODEL ANIMAL SPECIES ARE CLASSIFIED AS LARGE IN SCIENTIFIC RESEARCH?

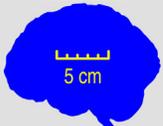
One may distinguish between “small (e.g., mouse, rat),” “midsized” (e.g., dove, chicken, rabbit, guinea pig, marmoset, cats) and “large” (e.g., pig, dog, rhesus monkey, baboon, chimpanzee) animals. However, Hagen et al. (2012) categorize companion animals, such as cats, dogs, rabbits, and ferrets, farm animals, such as pigs, goats, sheep, cows, and horses, chickens, ducks, goose, etc. as “large” animals, if compared with the most commonly used species in scientific research, mice and rats (Fig. 3.2).

The designation of specific species as “small,” “midsized,” or “large” animals depends in part on the system under study. Rodents are virtually always considered “small” animals, while dogs, sheep, swine, and primates

are typically considered “large” animals. Chickens and cats can be included in either group, depending on the specific system studied.

For purposes of brain research, it may be useful to ask which feature in humans one is attempting to reconstruct in a given model. In a study of experimentally induced brain injury one may, for example, try to model one or more of the following features: the mechanistic forces that caused the brain injury, the resultant morphological changes in the brain, the effects of age (maturation) and aging on these changes, and/or the effects of treatment with putative therapeutics on the development and final extent of the damage (Duhaime, 2006).

The size of the model animal species and of its brain may crucially determine the resolution at which these changes in the afflicted brain can be studied. Consequently, big is sometimes better. For example, bigger brains contain more tissue for analysis, nuclei and areas

(A)	(B)	Species	Approximate brain weight	Gyri and sulci	
		Human	 5 cm	1300–1400 g	Gyrencephalic
Large animal model species	Large animal model species	Cow		480 g	Gyrencephalic
		Sheep		175 g	Gyrencephalic
		Pig		80–180 g (large variation between breeds)	Gyrencephalic
		Dog		70–130 g (large variation between breeds)	Gyrencephalic
		Cat		30 g	Gyrencephalic
small*	mid-sized	Chicken		2.5–4.5 g (large variation between breeds)	Lissencephalic
	small*	Rat		2 g	Lissencephalic
		Mouse		0.5 g	Lissencephalic

^aNote: quail and pigeons may be considered as small model animal species

FIGURE 3.2 Classification of animal model species as large, midsized, or small (columns A and B), and weight (g) of the brain of adults of these species, from mice to humans. In pigs and dogs, the variability is high, due to selection on, among others, body size. Source: *Silhouette of brains of human, sheep, pig, dog, cat, rat, mouse, and chicken redrawn from photographs: Welker, W., Johnson, J. I., Noe, A., 1995. Comparative mammalian brain collections: major national resources for study of brain anatomy. The University of Wisconsin, Michigan State University, and the National Museum of Health and Medicine. Dept. of Physiology, University of Wisconsin. Available from: <http://www.worldcat.org/title/comparative-mammalian-brain-collections-major-national-resources-for-study-of-brain-anatomy-the-university-of-wisconsin-michigan-state-university-and-the-national-museum-of-health-and-medicine/oclc/37416838#details-allauthors>; brain of cow redrawn from photograph: Wouterlood, F., Voorn, P., 2010. The bos taurus brain. Department of Anatomy & Neurosciences, VUMC Amsterdam, Amsterdam, The Netherlands. Available from: http://www.anatomie-amsterdam.nl/sub_sites/kalfshersenen/start.htm. Modified from Gieling, E.T., Schuurman, T., Nordquist, R.E., van der Staay, F.J., 2011. The pig as a model animal for studying cognition and neurobehavioral disorders. *Curr. Top. Behav. Neurosci.* 7, 359–383.*

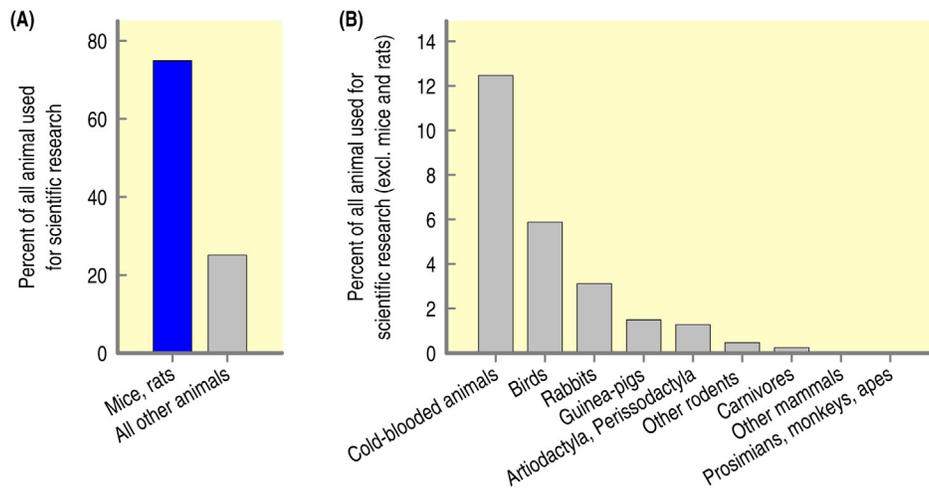


FIGURE 3.3 Animal use in the European Union in 2011. Panel (A) shows that the majority of studies are performed using mice and rats. In panel (B), “All other animals” from panel (A) is subdivided. Note that chickens are included in “Birds.” The Perissodactyla include horses, donkeys, and their crossbreds. The Artiodactyla include pigs, goats, sheep, and cattle (European Commission, 2013). Unfortunately, the different reporting schedules, classifications of model animal species, and inclusion/exclusion of model species preclude a one-to-one comparison between statistics about experimental animal use in the EU and the USA (USDA, 2015).

of interest are bigger, allowing more precise lesioning of target structures, local injections of test compounds via cannulas, electrophysiological stimulation or recordings in deep brain structures, or electroencephalography from the surface of the brain or skull.

The use statistics of different animal model species in scientific research in the European Union in 2011 (European Commission, 2013) are depicted in Fig. 3.3. Large animal use represents only a very low percentage of the total use of experimental animals.

9 WHICH TYPES OF (LARGE) ANIMAL MODELS ARE AVAILABLE?

A number of classifications of animal models have been proposed. These classifications typically distinguish between normal animals, animals with spontaneously occurring deficits, and animals with experimentally induced deficits (Gamzu, 1985; van der Staay, 2006), but other classifications have also been proposed (Rand, 2008). The classification can be applied to rodent and nonrodent (e.g., farm animal) models.

Normal subjects, that is, animals without observable (behavioral) deficit are useful for assessing the safety/toxicology risk of putative therapeutics (Dixit and Boelsterli, 2007), for assessing the putative abuse liability of a compound, and for investigating the neurobiological specificity of compounds and their mechanisms of action.

Spontaneous models are, for example, old animals, animals showing spontaneously and endogenously

occurring psychiatric or neurological conditions, spontaneously occurring mutations, genetic lines (inbred strains and their crossings), and lines resulting from selective breeding, and selected extremes from a particular animal population (Hudler, 2007; van der Staay, 2006).

Induced models are healthy animals in which the pathological condition is induced experimentally, for example, transgenic and knockout animals, selection lines resulting from selective breeding, animals with disruptions induced, for example, by stimulation with electric currents, pharmacological treatments, or by inducing hypoxia or anoxia. This class also includes animals with neuro- or immunotoxic, radiofrequency, cryogenic CNS-specific lesions, and lesions induced by aspiration or ablation (knife cuts). Finally, this class contains animals with experimentally induced cerebral ischemia or hemorrhagic stroke.

Rand (2008) in addition distinguishes negative models and orphan models. Negative models may be useful to investigate the mechanisms behind disease resistance because they are characterized by insusceptibility to disease or chemical stimulation. The opposite of negative models are orphan models. These animals show a disease/deficiency/dysfunction for which no correspondence has yet been described in humans. As soon as a similar disease has been identified in humans, an orphan model may become the basis of a spontaneously or naturally occurring, or an induced model (e.g., sheep suffering from scrapie, now may serve as model human spongiform encephalopathies).

A considerable number of rodent models have been developed for at least the first three classes. However,

they have not yet been established broadly in large model animal species.

Already 4.5 decades ago, [Douglas \(1972\)](#) summarized arguments why the pig may be a useful animal model species for human biomedical research, predominantly based on hematological and organ similarities between pigs and humans. However, he also noted that “Apart from the biological side, the pig must not be considered stupid. The pig can be used for the observation of many well-defined patterns of individual and group behavior (...)” ([Douglas, 1972](#), p. 232), a first hint that this species may also be useful in neuroscience research. Reasons why large animal models should be used are given by [Bähr and Wolf \(2012\)](#), [de Groot et al. \(2005\)](#), [Gieling et al. \(2011b\)](#), [Reynolds \(2009\)](#), and [Roberts et al. \(2009\)](#). They all agree that “(...) the validity of an animal model as a predictor of human response depends on how closely the model resembles humans for the specific characters being investigated.” ([Festing and Altman, 2002](#), p. 246).

10 SPECIAL ASPECTS IN USING LARGE FARM ANIMAL MODELS

Large animal models need more housing and testing space than rodent species ([Fig. 3.4](#), panel A). Also, test procedures and equipment must be adapted from rodent studies or must be developed and validated.

New equipment and new tools for large animal models are being developed. For example, stereotaxic atlantes and stereotaxic frames ([Marcilloux et al., 1989](#); [van Eerdenburg and Dierx, 2002](#)) are available for many large animal species. For about a decade, the stereotaxic, histology-based atlas by [Félix et al. \(1999\)](#) and the magnetic resonance imaging-based atlas by [Watanabe et al. \(2001\)](#) were the only source of stereotaxic coordinates of the pig brain. Recently, higher resolution three-dimensional representation of the pig brain have been published (Göttingen minipig, [Andersen et al., 2005](#); neonatal pig, [Conrad et al., 2014](#); commercial pig, [Saikali et al., 2010](#)). A stereotaxic atlas for the chicken brain has been published a decade ago ([Puelles et al., 2007](#)), whereas for sheep (and for a number of other species), three-dimensional representations became recently available online ([Johnson et al., 2016](#)).

For pigs, a large number of tests to assess emotion (reviewed by [Murphy et al., 2014](#)) and cognition (reviewed by [Gieling et al., 2011a](#)) have been developed. Similarly, an increasing repertoire of behavioral test is being developed for sheep (e.g., judgment bias, [Doyle et al., 2011](#); face and object discrimination learning, [Kendrick et al., 1996, 2001](#); spatial learning, [Lee et al., 2006](#)), although progress is slow. The repertoire of tests for assessing learning and memory in chicks is still limited, but expanding (e.g., the spatial holeboard task, [Nordquist et al., 2011](#); [Tahamtani et al., 2015](#); [Fig. 3.5](#), panel B).

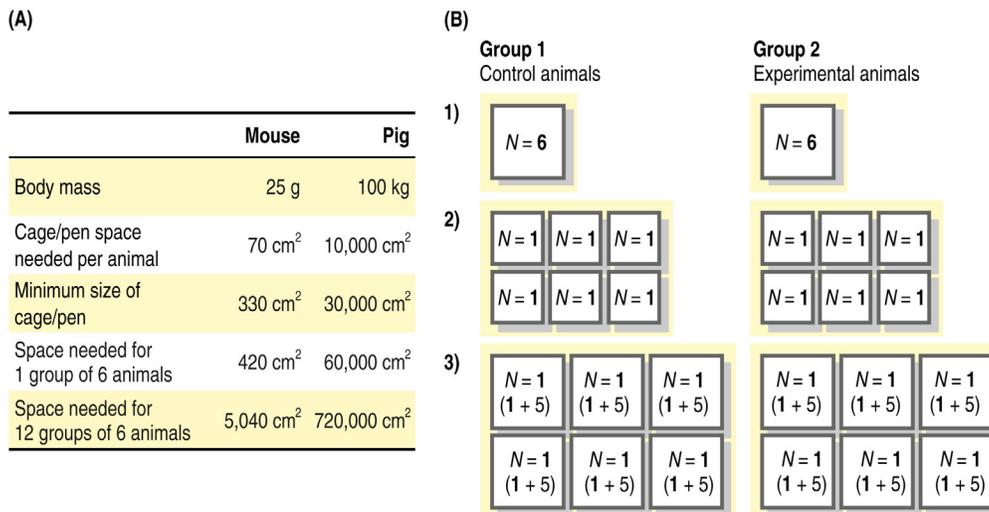


FIGURE 3.4 Space requirements and examples of different experimental setups of the same study. In panel (A), the space requirements for housing 6 mice or pigs individually or as group are tabulated in cm². In the example depicted in the panel (B), each of 12 animals is randomly assigned to one of two treatment groups (assuming that a group size of 6 animals is sufficient to address the scientific questions of the experiment). Although it cannot be excluded that cage is a confound variable that might codetermine or even cause differences between treatments in setup 1, the mere assumption leads to a huge increase in animals used, if a “state of the art” experimental setup (setup 3) is applied. Note, that the 6 pens per condition in setup 3 do not provide 100% identical environments for the 6 experimental animals per treatment group. Instead, strict standardization of the housing conditions (i.e., of the two cages in setup 1) could be applied, reducing the number of animals to 12. In social species, setup 1, but not setup 2 is an alternative for setup 3. Note: none of the setups is able to control all putative intervening variables. The number of control or experimental animals per cage/pen is printed bold (the *squares* representing cage/pen areas are on scale and correspond to the tabulated values; based on [Forbes et al., 2007](#)).

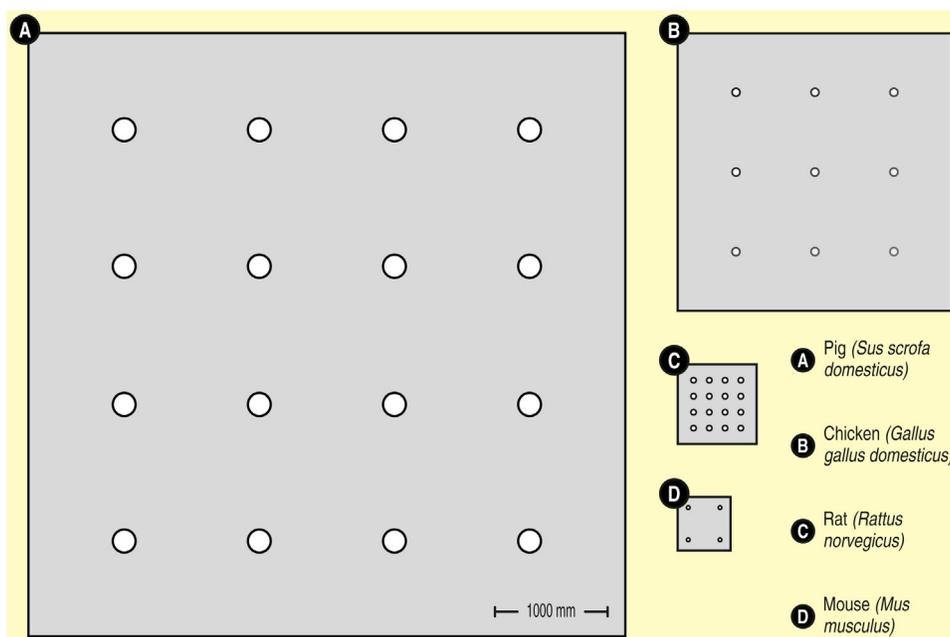


FIGURE 3.5 Comparison of holeboard arena's used to test spatial learning in two large animal model species [(A) pigs, Antonides et al., 2015a; Gieling et al., 2013; (B) chickens, Nordquist et al., 2011; Tahamtani et al., 2015] and two small animal model species [(C) rats, van der Staay, 1999; (D) mouse, Kuc et al., 2005].

Many of these tests have been developed for assessing cognitive functions in farm animal species, and have only sporadically been used in biomedical studies. They still need scientific validation but they provide a basis for testing learning and memory in disease models using large animal model species. However, it is obvious that additional tests must be developed to increase the usability and relevance of large animal models of neurobehavioral and psychiatric disorders.

The test equipment for large animals must fulfill a number of special requirements. First, obviously, it must fit the size of the animal species to be tested (Fig. 3.5). This may require a room which exceeds the size of a standard rodent laboratory. Second, the equipment must be stable enough to resist destruction by the tested animals. Our test apparatuses for pigs (Fijn et al., 2016; Gieling et al., 2014; Murphy et al., 2013; van Eck et al., 2016) were constructed by a stable builder according to our specifications, using standard material that normally is used to construct pig pens. Stable builders know how to construct pig-proof equipment.

Also, testing procedures and methods need to be suited for the species to be tested. For example, pigs must thoroughly be habituated to the test environment, the experimenter(s), and to being alone in the test apparatus (Gieling et al., 2011a; Murphy et al., 2014) (see also Chapter 39). The habituation and training period can be time consuming, before formal testing starts.

11 EXPERIMENTAL UNIT

Most animal experimental studies do not explicitly define the experimental unit. It needs to be stressed that *experimental unit* is not the same as *unit of analysis*. Depending on the experimental design used, there may be multiple units of analysis. Whereas in a basic single-level experiment the *experimental unit* would be considered the *unit of analysis*, a multilevel design may have more than one unit of measurement (Festing, 2006; Festing and Altman, 2002; Perrett, 2012).

An "experimental unit" is defined as the smallest division of the experimental material that allows any two experimental units to receive different treatments (Festing, 2006, 2011), or as Bate and Clark define it, "An experimental unit for a treatment factor is the smallest unit which a level of the treatment can be applied to." (Bate and Clark, 2014, p. 37). An experimental unit is one member or a set of animals that are initially similar on the measure(s) of interest, with each animal then subjected to one of several experimental treatments (Fig. 3.6, panels A–D). An experimental unit can also be a part of an animal (e.g., skin patches that undergo different treatments; Fig. 3.6, panel E). Each treatment represents one of a set of different experimental conditions. The effects of these different conditions are assessed experimentally.

For some, it must be possible to treat an animal independently from all other animals involved in the study *at any time*, that is, "The experimental unit may be defined

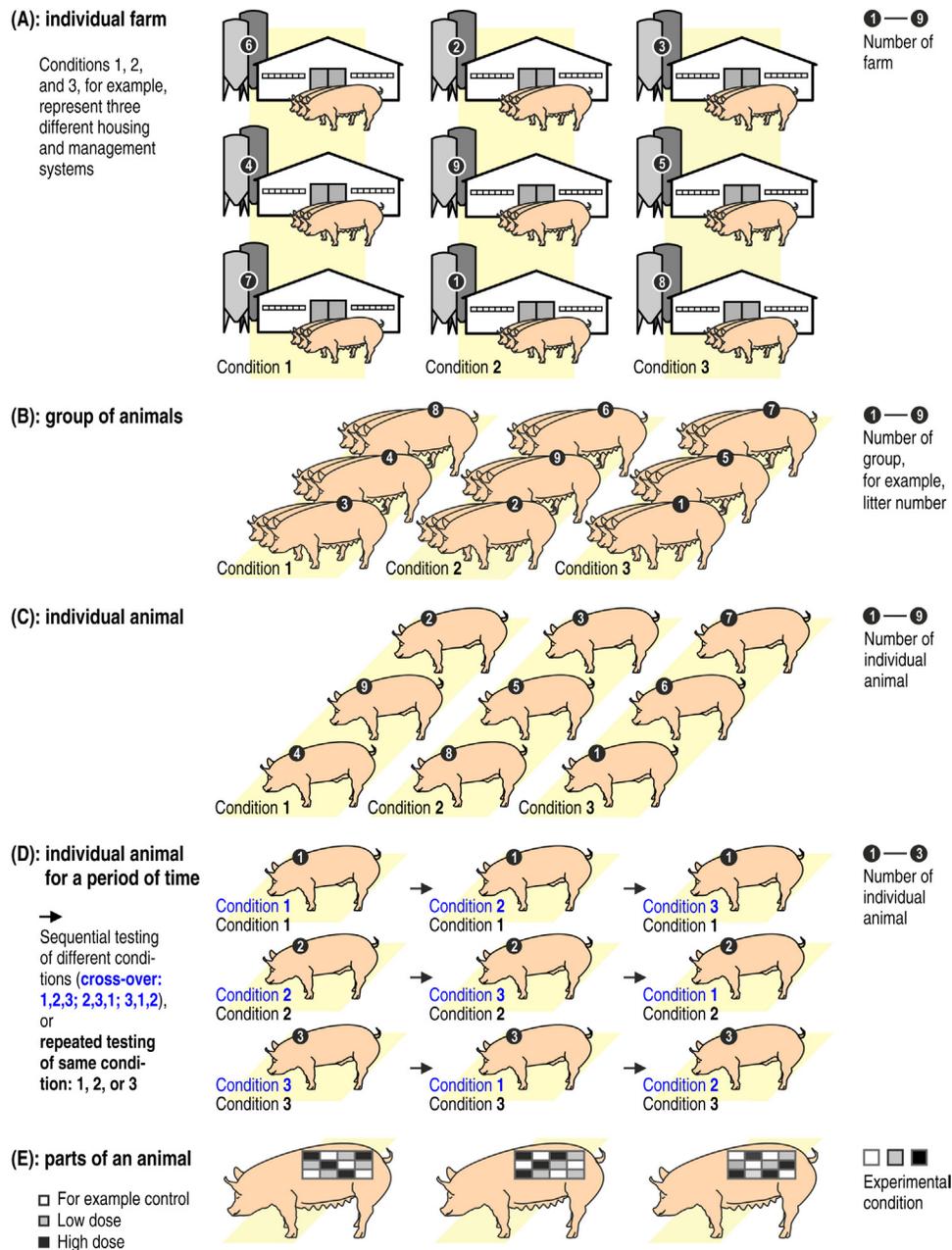


FIGURE 3.6 Experimental units in animal research. “Condition” refers to experimental condition (treatment or control). While it is unusual to depict pigs instead of rodents as experimental animals, studies with large animals may pose additional challenges that need to be addressed when designing and performing an experiment. Source: Modified from Gieling, E.T., Schuurman, T., Nordquist, R.E., van der Staay, F.J., 2011. The pig as a model animal for studying cognition and neurobehavioral disorders. *Curr. Top. Behav. Neurosci.* 7, 359–383, Fig. 4.

as the object independently treated in an experiment.” (Perrett, 2012, p. 3). For others, this may refer to random assignment or placement to one of the experimental conditions *at the start of an experiment*, before the animals undergo their assigned experimental manipulations. According to Cheng, following a definition of Cox from 1958 “In general, an experimental unit can be defined as the smallest division of the experimental material such

that different units may receive different treatments.” (Cheng, 2013, p. 1) (see Figs. 3.6 and 3.7 for schematic overviews of experimental units). Random assignment is the procedure by which animals are allotted to different treatment groups (treatment conditions), or to an untreated control group (control condition; note that some studies comprise more than one control group). It is assumed that as a result of random assignment to

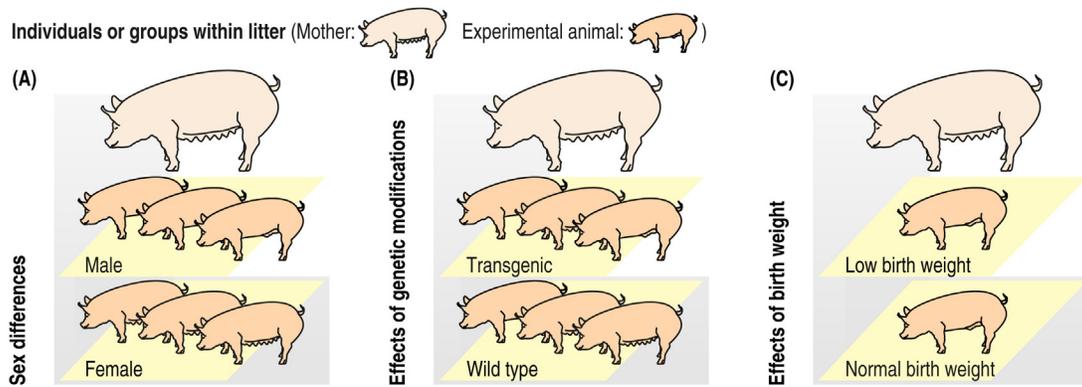


FIGURE 3.7 Examples of individuals or groups within litter (i.e., offspring of the same sow) are shown, where litter is the experimental unit. Instead of litter, the experimental unit may be pen, herd, compound in a zoo, etc. In (A) male and female pigs are selected from the same litter for assessing sex differences. In (B) transgenic and wild-type pigs are derived from the same litter to investigate the effects of genetic manipulations, whereas in (C) low birth weight (LBW) and normal birth weight (NBW) pigs are selected for investigating the effects of birth weight. In all examples, a study comprises a number of litters. Note that in these examples, random assignment of individuals to a group is not possible, but random selection of animals from a larger pool (e.g., selection of one or a few males in a litter from a larger number of male littermates) to a study is possible.

treatment conditions, the different treatment groups will be initially similar; differences between the treatment groups (conditions) are likely not due to differences between the groups that already existed before the start of the experimental manipulations. Once an individual has been assigned to a particular experimental condition, it may be impossible to treat that individual animal independently from the other animals in the same group during the term of the experiment. Due to space requirements and space restrictions, this is a problem inherent in studies using large animal model species.

Most large model animal species produce litters with multiple offspring. It is advisable to use this in designing experiments. There are two ways to assign littermates to experimental groups (conditions): either all littermates are assigned randomly to the same experimental group, a nested design, or the littermates are randomly assigned to different experimental groups, a randomized blocks design (Denenberg, 1984). In the nested design (Fig. 3.6, panel B and Fig. 3.7) litter must be considered as experimental unit because littermates are not independent. As Lazic and Essioux (2013) pointed out, litter effects are common and usually large. Consequently, replication of findings is difficult if this source of variation is ignored when analyzing the data and the translational value of the study may be low.

Mixed model analyses of variance may be used to account for nested effects. They have been developed to account for “nested (multiple observations within a single subject/animal in a given condition) and crossed (subjects/animals observed in multiple conditions) structure of the data” (Boisgontier and Cheval, 2016). Unfortunately, this statistical approach has not yet been fully appreciated in the neurosciences.

In the randomized block design of a one-factorial study consisting of an experimental and a control condition one might use pairs of littermates from a number of different litters equal to the number of animals per group used. Then, one sibling per pair is assigned randomly to the experimental group, the other to the control group. Fig. 3.7 (panels A–C) shows a different design in which a *group of littermates within litter* is assigned randomly to the experimental condition, and *another group of littermates within the same litter* to the control condition. In these designs, accounting of litter effects helps to obtain a better estimate of the effects of the experimental manipulation (Denenberg, 1984; Healy, 1972).

12 EXPERIMENTS USING SOCIAL ANIMALS REQUIRING GROUP HOUSING

Fig. 3.4 (panel B) shows a schematic overview of different experimental setups for studying the effects of experimental manipulations on social animals (here: pigs). Assuming that six animals per treatment condition are sufficient for sound statistical interference about the effects of the experimental intervention(s) and that a group size of six animals fulfills the need to live in a group, and strictly following the previously given definition of “experimental unit” (Festing, 2006), the state of the art setup of the study is depicted as setup 3. Note, that this setup needs 5 times more animals than the 2×6 animals assigned randomly to the treatments groups in setups 1 and 2. In setups 2 and 3, each of the 2×6 experimental animals can be treated independently. In large animal research, setup 3 may already exceed the possibilities of appropriate housing of the animals. Instead,

one may house each of the 2×6 animals individually in smaller pens (Fig. 3.4, panel B, setup 2), adhering to the postulation of independent treatment. However, still the space requirements for housing all animals are high (Fig. 3.4, panel A). In addition, welfare of the animals may be at stake because social animals should be housed in groups.

In setup 1, all animals of the same treatment group are housed in one pen. In this case it is impossible to treat each individual animal independently from the pen mates, that is, the other animals undergoing the same experimental intervention. Consequently, according to the definition, the pen is the experimental unit and it is impossible to perform proper statistics on the effects of the experimental manipulation(s), as $N = 1$. However, the animals are housed socially and not individually as in setup 2, and no surplus animals are used, as in setup 3.

We discuss the implications of the definition of experimental unit, of housing animals in groups, and of repeated testing and/or reuse of animals against the background of principles of two (Reduction, Refinement) of the 3Rs (the third one being Replacement: Russell and Burch, 1959).

What are the consequences for the majority of studies performed, in which animals are housed in groups? For example, pigs are group housed in a barren or an enriched pen to assess the effects of environmental enrichment (Bolhuis et al., 2013; Grimberg-Henrici et al., 2016). A strict definition of “experimental unit” may have major implications for studies with socially housed animals, and may lead to an undesirable increase in the number of animals used. It also may considerably increase the costs of a study, in particular if large animals are involved (e.g., pigs, sheep, cattle, horses), but also if rodents are kept under special condition, such as isolators or ventilated cages.

Finally, we address the question what the welfare consequences are of collecting a multitude of measures in the same animal, of testing the animal repeatedly in the same study, and of reusing the animal in subsequent (unrelated) studies.

13 PUTATIVE ADVANTAGES AND DISADVANTAGES OF GROUP HOUSING

Group housing and environmental enrichment are common measures to improve the welfare of social animals. Most large animal model species (in particular the farm animals, such as chickens, pigs, sheep) are social animals that live in groups/herds (Estevez et al., 2007). Compulsory group housing might reduce the number of experimental units (i.e., cages/pens) and may consequently increase the number of animals needed (even if a

part of the penmates are not tested). Individual housing of these animals with the aim to increase the number of experimental units, compromises their welfare (Fig. 3.4, panel B, setup 2).

When using social animals, it is mandatory to apply group housing from an animal welfare perspective. Some Ethics Committees routinely demand group housing, even in cases where individual housing would be more appropriate, for example, if highly aggressive male mice are used in a study, despite the fact that this practice may compromise the experimental results (Poole, 1997). Older, sexually mature minipig boars, for example, should be housed individually to prevent fierce fighting, whereas younger boars can be kept in groups (Ellegaard et al., 2010).

One of the disadvantages of group housing and testing more than one member of the group or all group member, effects of test order, have been documented by Kask et al. (2001) for rats and by Arndt et al. (2009), and Takao et al. (2016) for mice. In rats, cohort removal induced anxiogenic like effects (Kask et al., 2001). In the study by Takao, cohort removal induced an increase in body temperature, pain sensitivity, and anxiety-like behavior in mice. Cohort removal also increased the plasma corticosterone concentration in mice (Takao et al., 2016). Arndt et al. (2009) observed a within cage order effect on the hormonal stress response (corticosterone) in socially housed female C57BL/6 mice. Whether similar effects may occur in other group-housed animals species as well (Fig. 3.4, panel B, setup 1) needs to be investigated. A thorough habituation of group-housed animals to the test environment and to being tested alone might help to forestall test-order effects.

14 PRINCIPLES OF THE 3R— REPLACEMENT, REDUCTION, REFINEMENT

Replacement is the “the use of non-animal methods, such as cell cultures, human volunteers and computer modeling instead of animals to achieve a scientific aim.” (Richmond, 2000, p. 84). Under certain conditions, in vitro studies may replace in vivo studies, or insentient lower creatures may replace higher animals, that is, it should be obvious that we are not dealing with Replacement when discussing large animal models. Using, for example, pigs instead of dogs in regulatory toxicology studies cannot be seen as Replacement according to the 3Rs, as they do not replace or avoid animal use (Russell and Burch, 1959; Webster et al., 2010). However, many people have severe objections against using pet animals, such as dogs (Hasiwa et al., 2011) in scientific research laboratories. These objections may be less severe if pigs are used instead (Fig. 3.8).

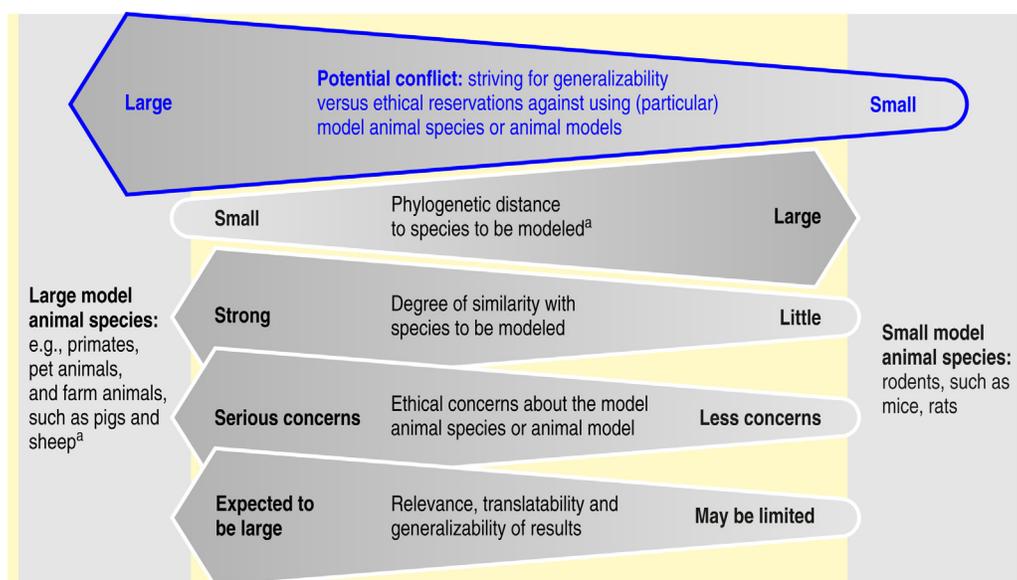


FIGURE 3.8 General characteristics of large animal models versus rodent models for studying human disease, area of potential conflict between the model animal species/animal model, and the expected degree of generalizability of results are compared. ^aNote that the phylogenetic distance between some large animal model species, such as domestic artiodactyls (which include cattle and pigs) and humans is larger than that between rodents and humans (Varga, 2012), whereas they may share more anatomical and physiological similarities with humans than rodents (Bähr and Wolf, 2012). Source: Modified from van der Staay, F.J., Arndt, S.S., Nordquist, R.E., 2009. Evaluation of animal models of neurobehavioral disorders. *Behav. Brain Funct.* 5, 11., Fig. 4.

Reduction is “Any approach in scientific research, product testing or education that leads directly or indirectly to a decrease in the number of animals used while meeting the scientific requirements.” (Consensus definition in: de Boo and Hendriksen, 2005, p. 376). Reduction thus refers to the minimum number of animals necessary to answer a scientific question. The National Centre for the Replacement, Refinement, and Reduction of Animals in Research adds to this definition methods that enable researchers to obtain comparable levels of information from fewer animals or to obtain *more information from the same number of animals*, thereby avoiding further animal use (Wellcom Trust, 2013).

Refinement is “Any approach which avoids or minimizes the actual or potential pain, distress and other adverse effects experienced at any time during the life of the animals involved, and which enhances their wellbeing” (Buchanan-Smith et al., 2005, p. 381). Remarkably, Refinement does not include the measures that improve the quality of experiments, which, by reducing the number of poor experiments, reduces the number of animals that is necessary for gaining solid scientific insight.

Note that the 3Rs are a somewhat one-sided view on the use of animals in scientific experiments (for critical notes about the 3Rs see Ibrahim, 2006). Refinement, for example, is considered exclusively in the context of pain, distress, adverse effects. However, Refinement of procedures and methods may yield clearer, replicable results which answer the research questions without the need

of performing an endless series of additional experiments. Using the most appropriate animal model species thus may in fact reduce the use of animals as well. Approaches to the evaluation and improvement of animal models have extensively been described (Belzung and Lemoine, 2011; van der Staay, 2006; van der Staay et al., 2009, 2010). Developing valid large animal models may contribute to reducing animal experimentation in the long run.

15 GETTING THE MOST OUT OF AN ANIMAL EXPERIMENTAL STUDY

Experiments should be designed in a way that a maximum of relevant information can be derived. This may have considerable implications for the study design, the number of animals used, and the statistical analysis of data. Repeated testing and/or reuse in related or unrelated experiments are means to increase the amount of information derived from the same animal, and to reduce the number of animals in experimental research. Repeated testing may also be applied for determining onset, progression of neurobehavioral dysfunctions, neurologic and psychiatric conditions, their duration, and their reversibility by treatment with putative therapeutics. Similarly, this testing schedule may be used for assessing onset, progression, duration, and reversibility of neurotoxic injury (Henck et al., 2016).

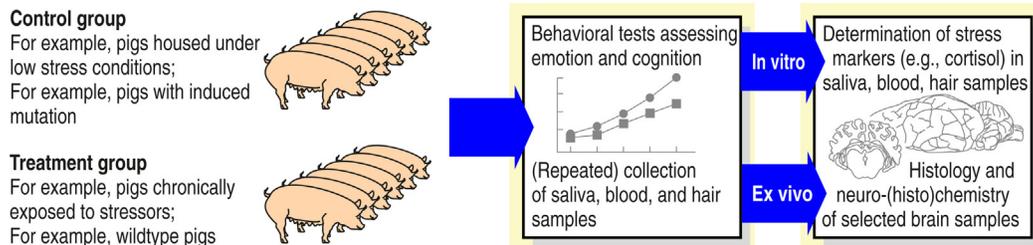


FIGURE 3.9 Example of multiple readouts (dependent variables) in a single animal experimental study. After chronic exposure to stressors, pigs are subjected to behavioral tests of emotion and cognition, each of which may reveal a large number of measures. In addition, saliva, blood, and hair samples are collected to determine stress markers *ex vivo*. The animals are sacrificed at the end of the study, and brain and organ samples are taken for a variety of analyses, for example, for assessing the correlation between stress markers and cognitive performance.

An advantage of measuring multiple variables is that more information can be gained from a study, without increasing the number of animals needed (see Fig. 3.9 for a hypothetical example, which, however, is inspired by Antonides et al., 2015a). The approach may be considered as contributing to Refinement and Reduction according to the principles of the 3Rs.

In an attempt to reduce the number of animals employed in experimental studies, one may decide to reuse animals. Reuse may refer to using animals in one particular study in successive experiments to address additional scientific questions within the study or in successive studies that may address scientific questions unrelated to those of the first study. The gain of information must be weighed ethically against an increase of discomfort that the animal may experience, as must be any reuse of the same animals in subsequent studies. Festing (1998) cautions against possible welfare consequences of the wish to reduce animal use, for example, through excessive reuse of animals. Such a practice may threaten the animal's welfare, depending on the discomfort caused by each of the tests employed. The reuse of animals has a number of advantages and disadvantages (Table 3.1).

16 NEED TO CORRECT FOR MULTIPLE COMPARISONS?

Without doubt, there is a higher probability of false positive results in studies with multiple dependent variables. On the statistical level, multiple read-out variables and multiple testing in the same study (Fig. 3.9) imply that the *P*-values must be corrected for multiple comparisons, that is, that the *P*-value for accepting the alternative hypothesis (incorrect rejection of the null hypothesis) becomes more stringent. Consequently, when performing a large number of statistical tests of significance, a correction for multiple testing (e.g., the Bonferroni correction for multiple testing) must be applied. This approach, however, leads to a loss of sensitivity (Benjamini et al., 2001; Storey, 2002). Therefore, a more sophisticated

approach, such as controlling the “false discovery rate” (FDR) is indicated (Benjamini and Yekutieli, 2001).

In studies with an exploratory character, all differences/correlations with associated probabilities <0.05 are considered, without making provisions for multiple testing through applying a Bonferroni correction or controlling the FDR (Bender and Lange, 2001; Sainani, 2009).

17 REPLICATION STUDIES

The risk of false positive results for the main question(s) of a study (e.g., does an experimental intervention affect working memory and/or reference memory performance in a cognitive holeboard task, Antonides et al., 2015a,b), is unaffected by analyzing additional variables obtained from the same subjects. These ancillary variables may provide valuable information about the study (Gaines Das, 2002) and thus may help to understand and discuss the results of a study. The additional variables measured might be treated as “exploratory.”

Subsequent replication studies must be performed to corroborate the effects found in the exploratory studies (van der Staay et al., 2010), that is, the repeatability and robustness of findings across studies should be investigated. One should consider all findings as tentative, until they have been corroborated in additional, independent studies (Feise, 2002; van der Staay et al., 2010). Replication studies help to determine the generalizability of previous findings (van der Staay et al., 2010). These studies should, however, not be exact replications. Instead, they should be extended replications (partial, systematic or differential, conceptual, or quasireplications; van der Staay et al., 2010). Extended replications are based on a wider notion of replication, namely the repetition of a test of a hypothesis or a result of earlier work with different methods (Schmidt, 2009).

In “partial” replications (slight) procedural modifications are introduced whereas all other aspects closely mimic the original study. “Conceptual replications”

investigate the same relationships/constructs as the original study, using different procedures. “Systematic or differential replications” induce variations in major independent variables, for example, in rearing, housing, and/or test conditions, or gender. This type of replication may even extend to seminatural or natural environments. In “quasireplications” species different from the one used in the original study are tested (Palmer, 2000). Quasireplications are often performed using large animal models to fill the gap between rodent studies and humans.

For example, in a study addressing the effects of different levels of environmental enrichment, all animals undergoing the same level of environmental complexity may be housed in the same cage/pen/enclosure. Under this condition, of course, the level of environmental enrichment cannot be manipulated per individual animal (Fig. 3.4, panel B). Comparing the effects of experimental manipulations in wildlife populations in different

territories or in groups of animals in different zoos suffers from the same restriction.

18 IDENTIFICATION OF POSSIBLE CONFOUNDS

“A primary function of research design is to maximize the validity of the conclusions (...), i.e. to minimize the number of alternative hypotheses that are consistent with the data.” (Altmann, 1974, p. 15). A main purpose of designing the experimental conditions is the identification and marginalization of the influence of confounding variables (MacVittie, 2014), for example, through a systematic inventory of the factors that affect an animal during its life that may affect its behavior in a study (Hendrick, 1991) (Fig. 3.10, panel A). Unfortunately, some of these putative confounds are not under control of the experimenter, and their effects may remain undetected

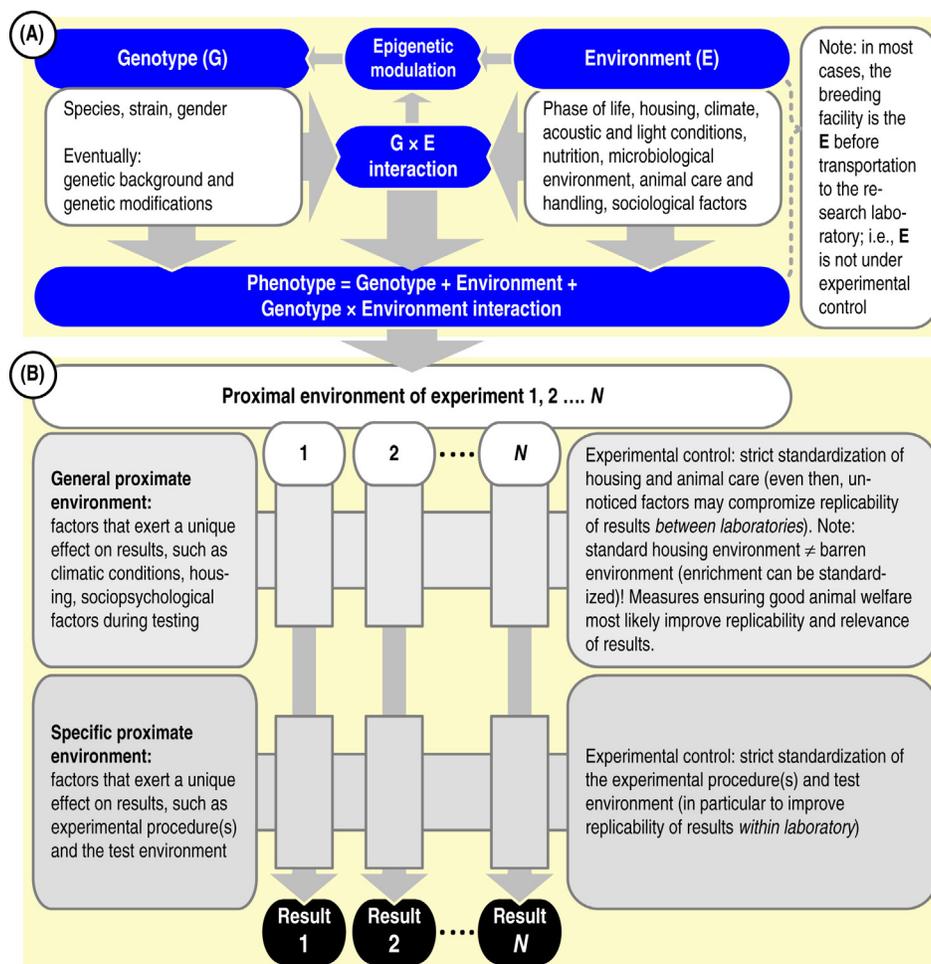


FIGURE 3.10 Factors affecting results of animal experimental studies, and how to control them. Variations in responses to experimental conditions are caused by the interplay between the phenotype and the effects of the response-eliciting proximate environment that in turn consists of the testing environment and testing procedures (Nomura et al., 2008; Russell and Burch, 1959). Source: Modified from van der Staay, F.J., Arndt, S.S., Nordquist, R.E., 2014. *Developing mouse models of neurobehavioral disorders: when is a model a good model?* In: Pietropaolo, S., Sluyter, F., Crusio, W.E. (Eds.), *Behavioral Genetics of the Mouse*, vol. 2. Cambridge University Press, United Kingdom, pp. 3–17.

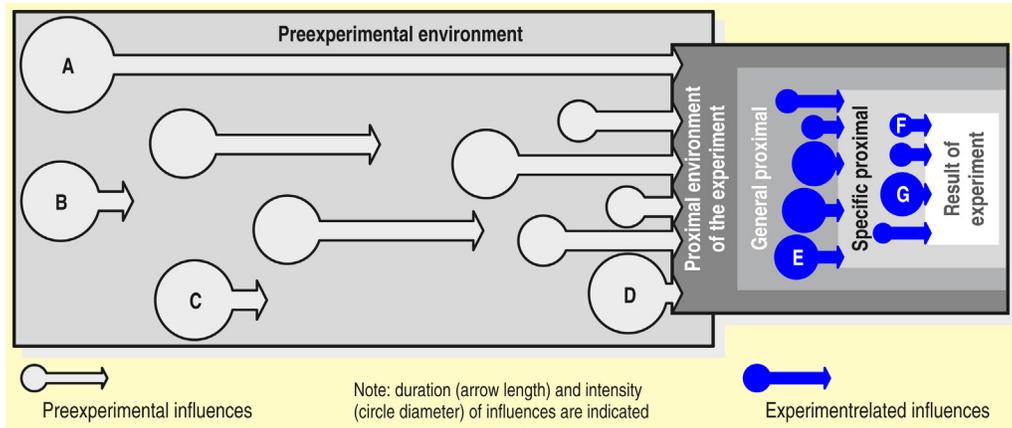


FIGURE 3.11 Factors affecting experimental results (Fig. 3.10B). Factors are, for example, (A) housing conditions and animal care routines, (B) maternal care, (C) weaning and mixing, (D) transport from breeder to animal laboratory, (E) construction clatter due to structural alteration works in the building housing the laboratory, (F) change of experimenter during testing, (G) order of testing animals housed in the same cage (Arndt et al., 2009; Takao et al., 2016). Note that, although a factor may have affected the experimental animals during a narrow time period, its effects may extend into the testing phase.

or defy statistical control, that is, their contribution to test results cannot properly be estimated.

Other factors are part of the proximate experimental environment (Gáspár et al., 1991; Fig. 3.10, panel B). Already more than half a century ago, Russell and Burch (1959) directed attention to the role of the testing environment and testing conditions, using the concept called “dramatype.” More recently, these thoughts were adopted in the elaboration of the concept of “response action pattern” by Nomura et al. (2008).

Both concepts—“dramatype” and “response action pattern”—are poorly defined, nor have they broadly been adopted by animal behavioral scientists. According to Hino (2004), they stand for how the animal presents itself in the experiment as evidenced by the test results derived from it. Nevertheless, this concept has focused attention on the *general* and *specific proximate environment* of an experiment (Fig. 3.10, panels A and B, and Fig. 3.11).

In brief, both concepts act on the assumption that the variations in responses to experimental conditions are caused by the interplay between the phenotype and the effects of the response-eliciting proximate environment that in turn consists of the testing environment and testing procedures (Nomura et al., 2008; Russell and Burch, 1959). Since even slight variations in the proximate environment may affect the results of an experiment, it is important to identify, and eventually control these factors (Fox, 1986; Russell and Burch, 1959; van der Staay et al., 2010, 2014). Schellinck et al., 2010 summarized the potential confounding factors in mouse studies. Many of these factors may also act as confounds in animal studies using other species than the mouse. Recently, Nevalainen (2014) directed attention to the effects of laboratory animal husbandry praxis as an integral

part of the experimental design. It can cause major interference with the results, whereas these influences can easily be overlooked (Fig. 3.11). If these confounds are undetected but relevant for the test scores obtained, then only robust effects may be detectable (van der Staay et al., 2010), or the study may yield false positive or false negative results.

The genetic makeup of the experimental animal is relatively stable and thus controllable; in particular if animals with a defined genotype are used, such as inbred strains or the first filial generation (F1) form crossings between inbred strains. However, even then they may be subject to epigenetic modulation. The environment is less well controllable and subject to change (Hino, 2004).

For studies using large animal model species, availability of inbred strains is still extremely limited (Fang et al., 2012; Meurens et al., 2012). Developing inbred strains in farm animals, that is, strains established by brother by sister mating for 20 or more consecutive generations (Staats, 1976) can be a long-standing undertaking, in particular due to the slow succession of generations in many large species (Bähr and Wolf, 2012).

19 EFFECTS OF OBESITY ON EXPERIMENTAL RESULTS

A putative intervening variable in animal experimental research, that has been neglected, is obesity in the experimental animals. Martin et al. (2010) discussed the effects of obesity in laboratory rodents on the results of scientific experiments. Instead of moving around and foraging during the major part of the period of wakefulness, rodents in the laboratory live in a very restricted area, with food and water available *ad libitum*. Under



FIGURE 3.12 Minipigs are prone to becoming obese. The left panel shows a lean and an obese minipig side by side. The right panel shows group housing of Göttingen minipigs. Source: Photographs courtesy Ellegaard Göttingen Minipigs, DK.

these conditions, they are overweight to obese. A direct consequence is that laboratory rodents may develop insulin resistance, hypertension, and that their life expectancy is reduced (Gibbs and Smith, 2016), compared with animals living in a larger enriched cage, fed a restricted diet where food is provided intermittently. Restricted feeding retards the development of neurological disorders (e.g., Parkinson’s and Huntington’s disease), compared with overfed conspecifics. Also, the brains of overfed rodents may be more vulnerable to stroke and traumatic brain injury. All these factors may affect the outcome of drug testing and may contribute to a lack or translatability of results to humans (Martin et al., 2010) and consequently, overfeeding and obesity should be avoided.

In particular commercial pigs and minipigs (Fig. 3.12) are also prone to obesity if fed *ad libitum* (making them interesting animal models in obesity research, Johansen et al., 2001; Koopmans and Schuurman, 2015). Even more pronounced this is also true for the parental lines of broiler chickens. To control their weight gain and to avoid obesity-associated problems, these birds are kept on a strict and severe diet. Until they reach adulthood, they are fed $\leq 50\%$, then approximately 90% of the quantity that they would consume if feed were available *ad libitum* [broiler breeder may be interesting for studying (abnormal) regulation of feed intake, Buzala et al., 2015; D’Eath et al., 2009; Richards et al., 2010]. This may lead to similar effects as discussed for rodent studies. Therefore controlled restricted feeding to prevent obesity is indicated in minipigs (Bollen et al., 2005; Boonen et al., 2014). Restricted feeding can induce a chronic sensation of hunger that may reduce welfare, but not health (Bollen and Ritskes-Hoitinga, 2007). However, the sensation of hunger per se does not impair welfare: it is a natural motivational state that triggers foraging and eating behavior (Tolkamp and D’Eath, 2016). Therefore contrary to the Farm Animal Welfare Council (FAWC, 2103) that defined welfare as, among others, “freedom from hunger and

thirst”, we assume that animal welfare is not at stake as long as an animal can adequately react to hunger, thirst, or incorrect food (Ohl and van der Staay, 2012).

20 TESTING UNDER UNIFORM CONDITIONS IN THE LABORATORY VERSUS TESTING IN A HETEROGENEOUS ENVIRONMENT, SUCH AS A FARM

If experiments are not highly standardized, environmental influences, in particular the proximal environment of the experiment, are probably the major source of variation between experiments (Nomura et al., 2008). This may reduce the replicability of results (van der Staay et al., 2010) *within* and *between* laboratories (Fig. 3.13).

The reason why setup 1 in Fig. 3.4, panel B is not considered appropriate is that pen or cage may act as confound. “For example, if one cage held all the control animals and another all the animals treated with some test substance and a difference was found between the means of the two cages for some character of interest, this might be due to the effect of the treatment, or it might be because the animals in one cage had been fighting, or had a sub-clinical infection not found in the other cage. In such cases any treatment effect is “confounded” or inextricably mixed with an environmental effect.” (Festing, 2011, p. 3). Unfortunately, this is a procedure that is used in a large proportion of animal experimental studies, either because this is common use in a laboratory [especially in research using (mutant) mice as subjects and in research in which rodents are kept in ventilated cages], or because of space restrictions, for example, in biomedical studies using large animals, such as farm animals.

When deciding which experimental setup to choose—more pens with fewer animals or fewer pens with more animals, or many pens with many animals of which only

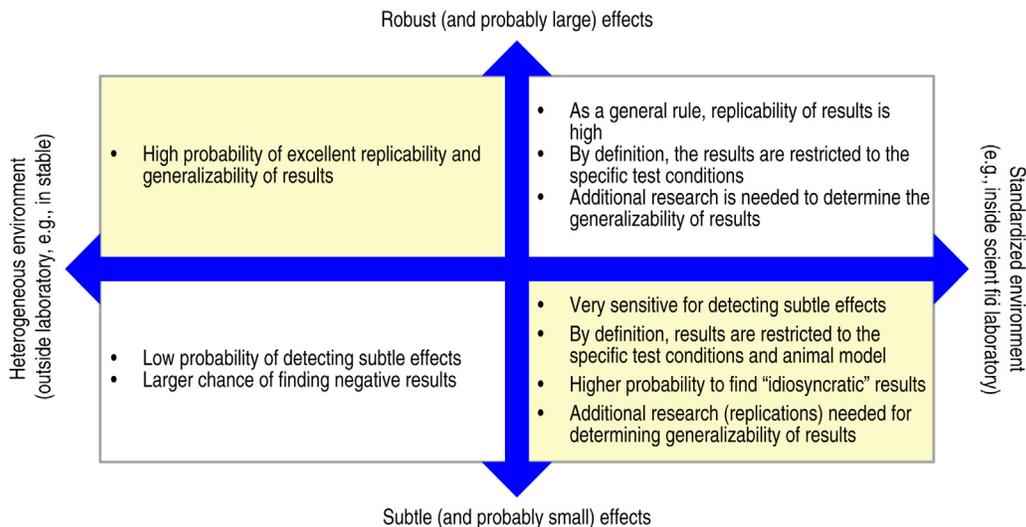


FIGURE 3.13 Consequences of strict standardization versus heterogenization of the experimental environment for the detection of effects of experimental manipulations. Highly standardized conditions can be realized in a laboratory setting, whereas, for example, experiments on farm are more likely performed under heterogeneous (and less well controllable) environmental conditions.

one serves as experimental animal (Fig. 3.4, panel B)—two sources of (uncontrolled) variation may affect the results:

- 1 between pen variation (Demétrio et al., 2013), which may be experimentally controlled by strict standardization of housing and testing conditions, and
- 2 between animal within-pen variation (Demétrio et al., 2013), which may be controlled by random assignment or random matched assignment of animals to the pens/conditions, on characteristics that might imbalance the groups, such as the weight of the animals, age (which often correlates with weight), or the sex ratio in the group.

Consequently, experimental control through strict standardization (van der Staay, 2006; van der Staay and Steckler, 2002; van der Staay et al., 2009, 2010) may increase the confidence in the conclusions drawn from an experiment. Unfortunately, this problem is more complex and the setup of an experiment depends on whether the expected effects are large or small, and on the desired degree of generalizability of results (Fig. 3.13).

21 TRAINING AND TESTING MAY ACT AS ENVIRONMENTAL ENRICHMENT

Coleman et al., 2013 distinguish between different classes of enrichment, among them social, physical, food, sensory, and occupational enrichment, the latter including mental activity and training). If, for example, training and testing act as (cognitive) enrichment, they may overshadow the effects of experimental manipulations, such as housing in barren versus enriched environments

(Grimberg-Henrici et al., 2016; Westlund, 2014a,b). Cognitive enrichment may improve animal welfare (Boissy and Lee, 2014; Boissy et al., 2007; Špinka and Wemelsfelder, 2011). It may also ameliorate disease-associated cognitive deficits, similar to the effects of environmental enrichment. More research is needed to learn about the effects of (extended) behavioral training and testing. To circumvent this putative confound in large animal models of neurobehavioral deficiencies, new, short-lasting cognitive tests are urgently needed, that do not necessitate extended training before testing can start (Roelofs et al., 2016).

22 MODELING EARLY LIVE EVENTS THAT AFFECT SUBSEQUENT DEVELOPMENT

Large animal models may be especially suited for investigating long-term effects of pre-, peri-, and early postnatal adverse events on functioning later in life, for example, events that might adversely affect (brain) development (see also Chapter 39), which is already vulnerable during gestation. The gestation length is approximately 21 days in rats, varies between 142 and 152 days in sheep, is approximately 115 days in pigs, and 280 days in humans (Fig. 3.14). Moreover, within the framework of neurobiological investigations on factors affecting early brain development, rodents deviate more from humans than, for example, pigs and sheep. If a study aims to investigate the effects of putative adverse factors on brain development during its most vulnerable period, the brain growth spurt, then pigs are especially suited as model for humans. The growth spurt in pigs starts prenatally and peaks around birth, similar

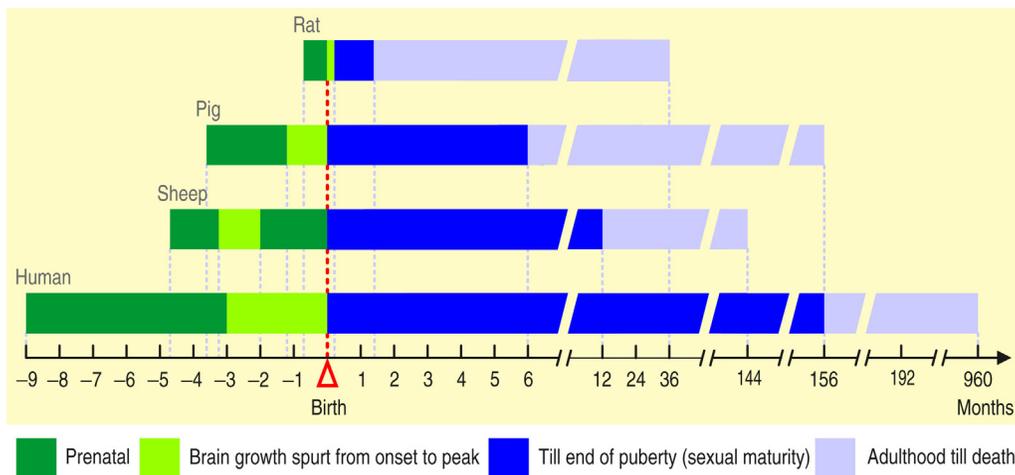


FIGURE 3.14 Brain growth spurt in rats, sheep, pigs and humans. Whereas the brain growth spurt in rats occurs after birth, it starts before birth in sheep, pigs and humans, and pigs. Note that the timing of events between conception and birth within species is quite invariable, but may vary considerably between breeds and individuals within breeds of pigs and sheep postnatally. Also in humans, the variability is large postnatally. Rats can be considered as postnatal brain developers, sheep are prenatal brain developers, whereas pigs and humans might be categorized as perinatal brain developers, with the strongest brain growth spurt peaking around birth (Dobbing and Sands, 1979). Note that for proliferation, synaptogenesis, subplate neurons and myelination of rats, sheep and humans, a similar overview is given by Yager (2004, Fig. 3.1, p. 33). The natural life expectancies of pigs and sheep have not systematically been documented and are therefore estimates primarily based on anecdotal evidence.

to that in humans. On the other hand, the growth spurt in sheep starts and peaks much earlier during pregnancy, and in rodent species, such as rats it starts after birth (Dobbing and Sands, 1979). Using more advanced techniques, this proposed timing of events in brain development seems generally to be corroborated (Clancy et al., 2001). Clancy et al., 2007 created a website that enables fast comparison of neurodevelopmental stages in a number of mammalian species (unfortunately, chickens and pigs are not included).

23 BRAIN INFARCTION, HEMORRHAGE, TRAUMATIC BRAIN INJURY

Large animal models have proven relevance in research on brain infarction, hemorrhage, and traumatic brain injury. Cai and Wang (2016), Mehra et al. (2012), and Mergenthaler and Meisel (2012) compared the advantages and disadvantages of using rodent versus large animal models for studying stroke. Duhaime (2006) performed a similar comparison for models of traumatic brain injury. Regional imaging techniques, such as nuclear magnetic resonance spectroscopy and imaging, and functional imaging, are easier to perform in large gyrencephalic animals, that is, in animals with a cerebral cortex that has convolutions (gyri). Also, advanced physiological monitoring can repeatedly and simultaneously be performed, eventually supplemented with neurological examinations, neurobehavioral tests, and neurochemical and neuropathological analyses (Traystman, 2003). Many techniques to induce infarction

[e.g., middle cerebral artery occlusion (MCAO), Platt et al., 2014], hemorrhage (James et al., 2008), and traumatic brain damage (Margulies et al., 2015) have been developed. Large animal models may be especially suited to assess the effects of early (neonatal) stroke (Duhaime, 2006) and traumatic head injury (Friess et al., 2007, 2009).

24 AGING AND AGING-RELATED DISEASES

Aging research using large (farm) animal species may not easily be realized. For farm animal species, the anticipated average life is unknown, and estimates are based on anecdotal evidence at the best (Fig. 3.14). Aged pet animals could provide an alternative. With their owners consent, these animals could be used in noninvasive and nonaversive studies to assess naturally occurring age-related and/or disease (e.g., beta amyloid pathogenesis)-related neurobehavioral dysfunctions and putative therapeutic options to treat them (e.g., dogs, Martin et al., 2011).

Combined with an *Animal donor codicil* (Faculty Veterinary Science, University Utrecht, 2016), this approach may even make postmortem examinations possible. This approach, arguably, would only be applicable for a restricted number of research questions because it largely depends on the cases presented in a veterinary practice, the willingness of the pet owner to allow scientific investigations on his/her animal, the existence of a signed codicil, and timely offering of the dead animal to the research institution. Postmortem MRI

and other suitable techniques may then provide insight into the brain tissue integrity (Dawe et al., 2016). This approach may allow to investigate the effects of sex, age, strain, and comorbidities, to name a few. Although this approach can be considered as “Reduction” in the spirit of the 3Rs, as it spares laboratory animal use (Russell and Burch, 1959), many putative intervening variables cannot properly be controlled (Fig. 3.11).

25 TRANSGENIC LARGE ANIMAL MODELS

The development of techniques for experimentally manipulating genes gave a boost to the use of mice in biomedical and translational research. Newly developed and validated techniques for manipulating genes in large animal model species (Aigner et al., 2010; Bähr and Wolf, 2012; Klymiuk et al., 2012), in particular swine, could stand at the cradle of developing sophisticated, translatable large animal models of human disease. Transgenic pig models of neurodegenerative diseases, such as Huntington’s disease (Baxa et al., 2013), Parkinson’s disease, and Alzheimer’s disease are under development or have been developed (Holm et al., 2016). The development of inducible gene expression in large model animal species is a further milestone for deriving at relevant and translational biomedical models (Klymiuk et al., 2012) of human diseases.

26 DISCUSSION

26.1 State of Large Animal Model Development and Research

Appropriate animal experimental studies can contribute to unraveling mechanisms and processes underlying human diseases, and to detecting and developing putative therapeutics for the treatment of these diseases. Recently, the lack of translatability of animal experimental studies to humans has severely been criticized. “(...) livestock models have been underutilized in translational research. This has been partially due to the slow realization of their advantages and value and to the perceived expense and difficulty of using livestock models.” (Roth and Tuggle, 2015, p. 5).

Can large animal models fill the gap between rodent studies and clinical testing in humans? For example, in stroke research, lissencephalic rodents are generally used, rather than gyrencephalic species (Fig. 3.2). The brains of gyrencephalic species show a greater resemblance with the human brain (Gribkoff and Kaczmarek, in press).

We are just beginning to appreciate the value of large animal model species for biomedical research and for

gaining insight into disease processes and their cure. The available knowledge about a putative animal model species is a determining factor in the choice of using this species (Clancy et al., 2007). Also, available information that helps to closely compare results between species (preferentially including humans) affects the selection of an animal model species. Comparative studies provide valuable information to close this gap (Clancy et al., 2007; de Vere and Kuczaj II, 2016; MacLean et al., 2012; Workman et al., 2013) and facilitate interpretation and generalization of results.

26.2 Factors Specifically Associated With Large Animal Models

False positive or negative findings may be caused by use of an inappropriate animal model (Belzung and Lemoine, 2011; van der Staay, 2006; van der Staay et al., 2009), an inappropriate experimental design, and/or inappropriate statistical analyses. In principle, the criteria with respect to the reliability and validity of rodent models also apply to large animal models. However, researchers using large animal models are confronted with practical questions, which are related to the specific infrastructure needed, such as space for housing and testing the animals. These practical problems and their solutions may also raise methodological/scientific questions, such as about the experimental unit, the reuse of animals in order to reduce the number of animals needed and/or to collect as much information as possible from a study.

Provided an appropriate animal model was used and effects of an experimental intervention were demonstrated, then replicability of result may be compromised by differences in the proximal experimental conditions in subsequent studies (van der Staay et al., 2010). We have proposed a replication strategy to corroborate (and extend) experimental findings (van der Staay et al., 2010), depending on the type of replication (Nuzzo, 2014).

In this chapter, we focused on methodological and practical considerations with respect to the development and application of large animal models for biomedical research and for human neurobehavioral and psychiatric disorders. A number of these considerations may also be relevant for rodent models, and as such are not specific and unique for large animal models. However, because large animal models need an infrastructure for performing studies that cannot be delivered by the standard rodent housing and testing facilities, many of the points raised in this chapter are of special relevance for studies using large animal model species.

26.3 Small Sample Sizes and Reuse of Animals

Owing to the infrastructure needed and higher costs, group sizes in experiments using large animal models are generally small. Reduction of animal use is one

of the principles of the 3Rs (Russell and Burch, 1959). However, these principles are not objectives in itself, but directed by the goal of the experiment. This set of principles should not restrict the validity and generalizability of results by, for example, reducing the number of subjects per treatment group to such an extent that an experiment becomes underpowered (Button et al., 2013). Such underpowered experiments, that is, experiments designed with a too small number of animals, run an increased risk of failing to detect an effect of the treatment (false negative result) or to detect an effect that essentially is a chance finding (false positive result) (Eisen et al., 2014). Consequently, the animals in underpowered experiments have been used/sacrificed unnecessarily. This type of studies thus only contributes to increasing the number of subjects needed to answer a question scientifically because additional (and better) experiments with sufficient statistical power will be required.

On the other hand, reuse of animals reduces the number of animals for animal experimental studies. Repeated testing within a study can be considered as a special variant of reuse of animals. This approach introduces an extra dimension, namely within subject comparisons, sometimes on a timeline (e.g., progress of learning across training sessions; changes due to aging processes, due to therapeutic interventions, etc.).

26.4 Practical and Methodological Consequences of Mandatory Group Housing

Farm animals, such as chickens, sheep, goats, and pigs are social, living in groups, often with a well-established hierarchy (Estevez et al., 2007). Testing of group-housed animals consists of separating one group member at the time from the group, and returning it to the group after testing. It has been shown in group-housed mice, that sequential removal of an individual for testing and reintroduction into the group may have adverse effects, such as stress or anxiety (Arndt et al., 2009; Takao et al., 2016). Similar effects might be expected to occur in large social, group-housed farm animals, such as sheep, goats, and pigs (although systematic studies about this topic in using farm animals are still missing). Minimizing the effects of testing outside the group may necessitate extensive habituation of the animals to the housing conditions, the experimenters, the testing environment and the test procedures (Antonides et al., 2015a). Some of these factors are depicted under “general proximal environment and “specific proximal environment” in Fig. 3.10, panel B).

26.5 Multiple Readout Parameters

For a better understanding of an animal’s behavior in a test, but also to derive maximal advantage of experiments with large animal species, we may need to

consider multiple variables, which may represent multiple underlying mechanisms, such as learning, memory, motivation, and sensorimotor capacities (Kapadia et al., 2016; van der Staay et al., 2012). Many behavioral tests yield more than one read-out parameter. For example, holeboard-type tasks may provide dependent parameters that reflect different components of spatial memory, such as working and reference memory, or the food search strategy adopted, the animal’s motor abilities and their motivation (van der Staay et al., 2012). More than one readout parameter may necessitate correction for multiple measurements, that is, the *P*-values may be corrected to more stringent testing of effects.

26.6 Advantages and Disadvantages of Using Large Animal Models

Using large animal models has a number of advantages and disadvantages both at the scientific and the practical level (Rand, 2008). It should be noted, that the subsequent lists of advantages and disadvantages are not exhaustive. Depending on future developments, more items may be listed or items may be delisted because problems may be solved.

Advantages of the use of farm animals as model species:

- Larger farm animal species (e.g., pig, sheep) show a closer resemblance to humans (size matters) with presumably higher translational value than the usual rodent models, although the phylogenetic distance between large animal model, such as domestic artiodactyls (which includes cattle and pigs) and humans is larger than that between rodents and humans (Varga, 2012) (Fig. 3.8). This also holds true for the phylogenetic distance between Galliformes (e.g., chickens, junglefowl, turkeys, quails, and pheasants) and humans (Maximino et al., 2015). However, large model animal species may be more similar to humans than rodents in many respects (e.g., physiology, anatomy) (Gieling et al., 2011b). Mammalian large model species may more faithfully replicate human disease than do rodent species (Pinnapureddy et al., 2015).
- Many large animal model species have larger, *gyrencephalic* brains, in which the cerebral cortex has convolutions formed by gyri and sulci, than the commonly used rodent species, which have *lissencephalic brains*, in which the cerebral cortex is smooth, without gyri and sulci (Figs. 3.2 and 3.8). Gyrencephalic brains are generally larger than lissencephalic brains and thus provide higher resolution images using, for example, imaging techniques, such as MR, positron emission tomography, and CT scanners (Mehra et al., 2012).

- Large farm animal models may serve as “second species” in preclinical drug development, in particular in risk assessment studies (Hasiwa et al., 2011), filling the gap between rodents and humans. Minipigs, for example, may replace dogs as frequently used species in risk assessment studies (e.g., safety pharmacology, toxicology, Bode et al., 2010; Forster et al., 2010c; Swindle et al., 2012; Vamathevan et al., 2013; van der Laan et al., 2010). As far as prenatal development is concerned, chickens may also be useful for safety studies (Bjørnstad et al., 2015).
 - Large animals may be used in comparative approaches to identify and characterize differences and similarities between various species. “The standard model species represent a vanishingly small percentage of the total biological diversity.” (Brenowitz and Zakon, 2015, p. 273). “Convergence on selected model species often carries an implicit assumption that mechanisms observed in one species are characteristic of all related species. A focus on any single species, however, fails to encompass the diversity of mechanistic adaptations present in even closely related species that differ behaviorally.” (Brenowitz and Zakon, 2015, p. 274).
 - The number of behavioral tests for assessing emotion and cognition in a number of farm animal species is increasing (Gielsing et al., 2011a; Kornum and Knudsen, 2011; Murphy et al., 2014).
 - Generalizability and translatability of results may increase through replication studies with other species than the rodent species that most likely were used previously (van der Staay et al., 2010).
 - Nearly unrestricted availability of a large number of species/strains from controlled suppliers and well-defined sources (e.g., for Göttingen minipigs see Simianer and Köhn, 2010).
 - Unlike rodent studies in which experiments are short lasted because of the short life expectancy, experiments with large animal models can be long lasting. This allows performance of longitudinal studies in, for example, the area of gene therapy (Casal and Haskins, 2006).
 - Large animal model species, such as chickens (approximately 275 eggs per layer hen per year) and pigs (approximately 30 piglets per sow per year) produce many progenies.
 - Special miniature pig and commercial pig breeds are available for biomedical research (Smith and Swindle, 2006) with known/controlled health status [e.g., conventional, specific pathogen free, or gnotobiotic, that is, germ-free or formerly germ-free animal with fully defined composition of its associated microbial flora (Miniats and Jol, 1978)].
 - Higher level of acceptance of general public—in particular if farm animals are used as large animal model instead of primates or pet animals, such as cats and dogs (Hagen et al., 2012; Ormandy and Schuppli, 2014; van der Staay et al., 2009) (Fig. 3.8). Moreover, farm animals may be returned to the food chain for human consumption provided they were not treated with compounds that might endanger the human consumer.
 - Studies using large model animal species extend knowledge about farm animals that may be relevant for farm animal health and welfare (Reynolds et al., 2009).
- Large animal models are less well established than rodent models. This entails some disadvantages, in particular caused by gaps in our knowledge, such as lack of comprehensive ethograms. A number of the listed disadvantages are expected to dissipate with increasing experience and use of these models:
- Less “historical” data are available for large farm animal model species than for “classical” model species [e.g., rodents (efficacy, safety, toxicology), dogs (safety)], although relevant information is increasingly available for pigs (in particular Göttingen minipigs) in risk assessment (teratology, toxicology) research (Swindle et al., 2012).
 - Lack of external funding opportunities for research and technological development (Chiba et al., 1994; Golden et al., 2012; Ireland et al., 2008; Reynolds, 2009; Roberts et al., 2009).
 - Lack of validated models, that is, models that have survived a validation process (van der Staay, 2006; van der Staay et al., 2009).
 - Lack of validated tests (e.g., for pigs reviewed by Gielsing et al., 2011a; Murphy et al., 2014).
 - Lack of test equipment; only very few commercial suppliers offer test equipment for large animals; testing equipment very often is custom made (see also Chapter 39). Special demands regarding the testing equipment, for example, pig-proof apparatus (e.g., barn builders have expert knowledge for constructing pig-proof equipment).
 - The current education of scientists and technicians is “rodent” centered (Libby, 2015). Using large model animal species increases the need for trained technicians.
 - Large model animal species require special housing conditions, such as large stables and pens. Nearly all farm animal species are social (Estevez et al., 2007), requiring group housing.
 - Associated with group housing in cages/pens is the issue of “experimental unit” (Fig. 3.6 and 3.7).
 - Studies using large-animal models may be more expensive than studies using rodent models (e.g., purchase of the animals, housing facilities, testing equipment).

- Many large animal model species grow fast (e.g., the daily weight gain of pigs in the grower-finisher period: approximately 800–1000 g, Pardo et al., 2013; Vautier et al., 2013, and the brain volume of pigs doubles between 2 and 24 weeks of age, Conrad et al., 2012). Moreover, the high body mass in adulthood, for example, in pigs (Lind et al., 2007) may thwart their handling, drug administration (voluntary oral administration may be preferred, Turner et al., 2011), and behavioral testing.
- Compared with rodent research, large amounts of investigational drugs are needed when testing efficacy, safety, and toxicology in large animal models. In neuropharmacological research, it is worthwhile to investigate whether this may eventually be solved by intracerebroventricular (ICV) administration of centrally acting drugs. Such an approach may also prevent development of peripheral side effects. A disadvantage of ICV injections is the need to supply the animal with a cannula (Yao et al., 2014), which will need surgical interventions (e.g., stereotaxic placement), and which, in fast-growing species, eventually may grow out if implanted before they are full grown.
- One of the largest risk factors for many human diseases is aging (Harman, 1991; Niccoli and Partridge, 2012). Unfortunately, the life expectancy and longevity of large animal model species has not yet been documented well, but appears to be much higher than that of rodent species, making aging research with large animal models less feasible (Mitchell et al., 2015) (Fig. 3.14). Avian species, such as chickens are estimated to have a life expectancy considerably exceeding that of equally sized mammals (Holmes et al., 2001a; Wasser and Sherman, 2010).

Summarizing, existing models need to be modified to better serve their goals and new models need to be developed. They must undergo rigorous evaluation to ascertain their reliability and validity (Belzung and Lemoine, 2011; van der Staay et al., 2009). They must also measure up to high standards of preclinical evaluation to improve and ensure their translational value. “There is no single, perfect animal model that can completely predict the outcome of clinical trials. The challenge is to collect relevant and sufficient information from as many models as are required to make an informed decision regarding the potential benefits and risks to patients.” (Cibelli et al., 2013, p. 274). We are not advocating the use of large animal models *instead* of rodent models. We advocate use of the animal model that best suits the aim of a study and that best informs the researcher.

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