CHAPTER

39

Pigs as Model Species to Investigate Effects of Early Life Events on Later Behavioral and Neurological Functions

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OUTLINE

1 INTRODUCTION

Animal models are used to study causes of disease, search for and test potential treatments, and to study fundamental processes. They are often used to study human disease, but animal models can also be used in veterinary or animal sciences, in which an animal can serve as a model for another species, or indeed for its own species. The use of large animal models is discussed in depth in Chapter 3 of this volume.

Animal Models for the Study of Human Disease.

The importance of early (or even prenatal) life on later functioning in an organism has long been recognized. In humans, early life experience is often seen as influencing predisposition to develop a vast array of physical and mental diseases (Roseboom et al., 2006), including diabetes (Beyerlein et al., 2016), cardiovascular diseases (Barker and Martyn, 1992; Elford et al., 1992), schizophrenia (Matheson et al., 2013), and cognitive deficits (Rooij et al., 2010). Clearly, human studies are based on correlations, not on studies in which early life is manipulated experimentally (which would be unethical). This is an area of research where animal models can play an important role in aiding understanding of underlying mechanisms of effects of conditions during early life, and potential treatment of diseases and disorders resulting from maladaptive early life experiences.

It is increasingly recognized that pigs bear strong resemblance to humans perinatally and during early life, with respect to brain development, physiology, diet, and gastrointestinal function (Gieling et al., 2011a,b,c). This is in strong contrast to rodents, which constitute the most common animal model species at present, but which are vastly different from young humans in terms of development around birth (Gieling et al., 2011c). This implies that young pigs could be useful as models for young humans, and indeed, there are piglet animal models in use for neonatal processes including traumatic injury, neonatal nutrition, and effects of low birth weight (Gieling et al., 2011c).

In farming, management practices in early life can affect the ability of an animal to adapt to its circumstances later in life, and thus its welfare state (Ohl and van der Staay, 2012). Pigs are kept on farms, in some cases for years, as is the case for breeding sows and boars. Fattening pigs are kept until slaughter weight, usually at around 8–12 months of age. This is well past puberty, and in that sense these animals are young adults. Examining effects of management practices during early life on pig development, can give us information about the welfare of these animals and potential compromises to their welfare. In this case, the experimental pig can serve as a model for its own species on farms.

A number of tests have been developed to study the behavior and neurological state of pigs. In this chapter, these tests will be discussed, particularly with respect to their suitability for testing young animals. The potential implications of testing for pig welfare, and particularly the practical aspects herein, are reflected upon in the final section of this chapter.

2 OPERANT TASKS FOR TESTING COGNITIVE FUNCTIONING

In operant tasks, a subject is required to provide a response to elicit a specific outcome. Operant tasks can be very simple, as widely used in feeding stations in group housed pigs; in this type of housing, pigs learn to stand in front of a feeder, which reads a chip in its ear and then provides the pig's daily ration of food. Complex tasks are also possible, which can measure various forms of memory, behavioral flexibility, or internal state of an animal. Several operant tasks which have been developed and used in pigs are described further in the chapter.

2.1 The Holeboard Task

Spatial learning and memory tasks can be highly useful and suitable for testing pigs at both young and older ages. In these types of tasks, animals must learn to search for rewards within either an alley, with fixed starting positions and a single correct route to find all rewards, or within a "free choice" maze (Crannell, 1942; Lachman and Brown, 1957; van der Staay and Bouger, 2005). In the latter, animals are allowed to explore arenas in which rewards can be found in various places. Animals are free to determine in which order they search for rewards, and whether to return to a previously visited location or not. The most efficient strategy is to only visit locations containing rewards, and to visit the rewarded locations only once. The fact that pigs are mobile essentially from birth facilitates the use of spatial learning tasks in piglets, though it is important that the animals have the maturity to be able to learn the task at the age tested. For the full Pig Holeboard, this appears (in our hands) to be from about 6 weeks of age (Antonides et al., 2015b and informal observations).

In spatial memory tasks, an animal must remember where it has already been in order to avoid unrewarded revisits. This list of locations already visited is temporarily held in working memory (Olton and Samuelson, 1976). The information held in working memory is relevant only within a specific trial, as it reflects where an animal has been and already either consumed the rewards, or realized that a location is not baited. Reference memory (Olton and Samuelson, 1976) holds trial-independent information about, for example, the locations where the food reward can be found. One advantage of free choice mazes is that working memory and reference memory can be assessed simultaneously within the same test.

In the pig holeboard test, modeled after the rodent holeboard task, a fixed number of locations within a larger field is baited, for instance 4 locations of a possible 16 (so 1 in 4). For rodents, this test apparatus usually consists of literally a board with holes, some of which contain bait, and some of which do not. For pigs, various constructions suited to the pigs' size and strength have been used, including buckets affixed to the floor (Arts et al., 2009; Bolhuis et al., 2013; Clouard et al., 2016; Haagensen et al., 2013). In our lab, we use a setup in which plastic dog dishes are affixed to a slatted floor, covered by plastic balls. These plastic balls can be nosed

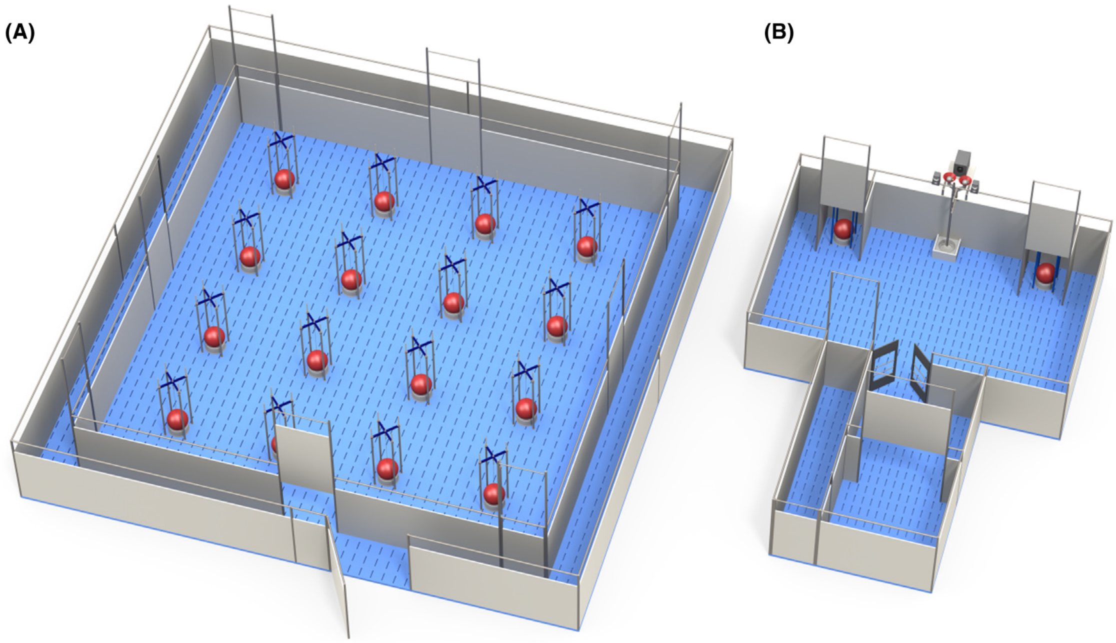

FIGURE 39.1 Holeboard apparatus to assess spatial discrimination learning in pigs (A), and an apparatus for assessing judgment bias and decision-making behavior in pigs (B). (A) In the holeboard task, rewards are hidden underneath hard plastic balls that the animal can raise using its snout. (B) In the gambling task and decision-making apparatus for pigs, the experimental animal enters the testing arena via an antechamber. Each goal-box contains a large hard-plastic ball which can be raised as operant response. Reward (usually M&M's) can be found in a central food trough. The accessibility of the bait in the trough is controlled by the experimenter operating a lid that covers the food trough. *Source: Modified from Antonides et al. (2015a), according to Open Access terms and used with permission from Murphy, E., Kraak, L., van den Broek, J, Nordquist, R.E., van der Staay, F.J., 2015. Decision-making under risk and ambiguity in low-birth-weight pigs. Anim. Cogn. 18(2), 561–572.*

aside to uncover the dishes, which may contain rewards. In our setup, scoring is automated using magnets and sensors installed in the balls and food dishes (Figs. 39.1 and 39.2), which are connected to a computer via an interface. We had specialized software compiled to register time stamps of visits to each bowl, and to calculate various measures, including, among others, working memory, reference memory, latency time to first visits, and order of visits.

The pig holeboard has been shown to produce highly reproducible results when comparing between studies. Independent experiments have used the pig holeboard to compare holeboard performance of low birth weight and normal birth weight animals (Antonides et al., 2015a; Gieling et al., 2012b), animals from large or small litters (Fijn et al., 2016), effects of pharmacological intervention with the M1R blocker biperidin (Gieling et al., 2013), effects of enriched housing (Bolhuis et al., 2013; Grimberg-Henrici et al., 2016), effects of overnight social isolation (van der Staay et al., 2016), and effects of iron deficiency during early life (Antonides et al., 2015b) show highly similar performance between control groups, indicating replicability of the test between studies. The test also shows sensitivity for deficits in performance (Antonides et al., 2015b; Gieling et al., 2013), as well as enhanced performance (Antonides et al., 2015a; Grimberg-Henrici et al., 2016), although the latter will reach ceiling-level

FIGURE 39.2 Details of the "holes" used in both the pig holeboard setup and the active choice setup used in judgement bias and decision making. Plastic balls are fitted with magnets and hung from a stainless steel wire in between four stainless steel rods. The balls lay in plastic dishes which contain sensors that can detect movement of the magnet. When the animal displaces the ball, either to gain access to a reward or as an operant response, the sensor detects movement of the magnet, and sends a pulse through the interface to a computer running registration software. *Source: Modified from Antonides et al. (2015a), according to Open Access terms.*

performance relatively quickly as normal animals reach near-perfect performance, particularly on working memory.

Behavioral tests can give valuable information, provided that the tests used are valid and reliable. Validity can be defined as the degree to which a test measures what it is supposed to measure. Reliability on the other hand is defined as the repeatability of scores, obtained from unchanged patients (humans or animals), under diverse scoring conditions (Mokkink et al., 2009). Four different types of reliability are defined: tests-retest, internal consistency, interrater and intrarater reliability. In simpler terms, a test that is valid, gives "right" results while one that is reliable, gives "good" results (Martin and Bateson, 2007). Behavioral tests for pigs are not frequently tested for reliability, which is a major hindrance to the development of the pig as a model species for cognition testing.

We have tested intrarater and interrater reliability in the pig holeboard test, calculating intrarater reliability by comparing data obtained from live video scoring against data obtained from scoring the video recordings of the same trials. We also tested interrater reliability by comparing the results of working and reference memory of the computer program used to register hole visits, the results of working and reference memory as visually scored by the observer. Results from this study showed high interrater reliability when comparing manual scoring to scoring by the specialized software, which points to high reliability of the test setup. The study also showed, however, that for manual observation, experience may play an important role in interrater reliability; interrater reliability was low, possibly due to a learning curve in the observer (Meléndez Suárez, 2014). See Box 39.1 for a detailed description of the methods and results of this reliability study.

BOX 39.1

METHODS AND RESULTS OF A VALIDATION STUDY OF THE PIG HOLEBOARD TASK

Originally reported in Meléndez Suárez (2014).

Procedure: Twenty average weight piglets [(Terra × Finnish landrace) × Duroc], 10 males, and 10 females, from the farm at Utrecht University were selected at 3 weeks of age from 10 different litters. Males and females were housed separately in enriched pens with straw and a ball. The holeboard training started when the piglets were 41 days old. The animals were habituated to the experimenters for a period of 2 weeks, during which they were fed M&M's to get them used to the bait. Each pig was assigned randomly to one of the four configurations, with different locations of the baited bowls. Males and females were divided into two subgroups, with a total of four groups consisting of five pigs each. The four groups were tested randomly using the ABBA and BAAB testing orders on alternate days, where letter A represented the female group and letter B the males. The pigs within the subgroups were tested in a random order as they presented themselves to the door. Each pig was tested twice during a day, one trial right after the other (massed trials).

Intrarater Reliability: On day 18 of training, a veterinarian, with no previous experience at scoring animal behavior, live scored the visits to the bowls for 20 pigs, two trials each. Live scoring was done using a monitor that was connected to a camera located above the Holeboard. Trials were recorded for a second evaluation. The visits to the bowls were scored using a computer with JWatcher Version 1.0 installed. One week after the live scoring, the same observer scored the video recording, in order to compare both files and obtain the intrarater reliability. JWatcher Version 1.0 was used to calculate the percent of agreement and the kappa coefficient.

Interrater Reliability: Based on the results obtained from the intrarater reliability the working and reference memory of the 40 trials were calculated using the formulas:

$$\text{WM}: \frac{\textit{Number of rewarded visits}}{\textit{Number of visits and revisits to the rewarded set of holes}}$$

$$\text{RM}: \frac{\textit{Number of visits and revisits to the rewarded set of holes}}{\textit{Number of visits and revisits to all holes}}$$

The results obtained for working and reference memory from the computer were compared to those obtained by the observer.

Statistical Analysis: For the intrarater reliability kappa statistics were used to obtain the percentage of agreement and the kappa coefficient.

For the interrater reliability a Wilcoxon *t*-test and a Pearson correlation test were used to see the association between the results from the computer program against the observer, for reference and working memory of the two scoring procedures (automatic vs. observer). All statistical analyses were carried out using a SPSS for Windows (version 16.0) computer program (SPSS Inc., IL, USA).

Results: The kappa coefficient values obtained from the 40 trials are shown in Table 39.1. The highest kappa coefficient score has a value of 1 and represents that the two scoring procedures that are compared have the same sequence of key codes. In this table, we can see that there are several scores with a value of 1, but there are also many low values. The average kappa coefficient for the 40 trials is 0.8.

Table 39.2 contains Pearson correlations from the two data sets and the *P*-values of the correlations. The data sets are highly correlated.

TABLE 39.1 Intrarater Reliability Results (Kappa Coefficient)

Pig	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
RT1	0.73	0.84	1	1	0.49	1	1	1	1	0.25	1	0.32	1	1	1	0.47	1	0.64	0.75	0.75
RT2	0.08	1	1	1	0.26	0.68	1	1	1	1	1	1	1	1	1	1	1	1	1	0.18

The animals were identified with letters from A to T. The reliability of trail 1 and trail 2 were calculated (RT).

TABLE 39.2 Pearson's Correlation for Working Memory (WM) and Reference Memory (RM) of Trial 1 and 2

	Pearson correlation	Pearson correlation (*P*-value)
WM T1	0.995[a]	<0.0001[b]
T2	0.928[a]	<0.0001[b]
RM T1	0.964[a]	<0.0001[b]
T2	0.971[a]	<0.0001[b]

[a] *Correlation is significant at the 0.01 level.*
[b] *P-value is significant <0.05.*

2.2 Judgement Bias

Judgement bias tests (JBTs) measure behavioral responses to ambiguous stimuli after animals have been trained to discriminate between a stimulus (or set of stimuli) predicting a positive consequence (S^+; reward) and another stimulus (or set of stimuli) predicting a negative consequence (S^-; punishment or lower-value reward). The "quality" of the ambiguous stimulus lies somewhere between S^+ and S^- (Fig. 39.3). Go/Go and Go/No-go tasks form the two different classes of JBTs in which Go/No-go entails the suppression of the response at S^-, whereas Go/Go entails that the animal responds to both stimuli types with an active response (Murphy et al., 2013a). JBTs provide a cognitive measure of optimism and/or pessimism since a negative emotional state is expected to cause a negative (pessimistic) judgement of an ambiguous stimulus, whereas a positive emotional state is expected to cause a positive (optimistic) judgement of the same ambiguous stimulus (Boleij et al., 2012; Roelofs et al., 2016).

The pig is one of the species in which JBTs became a frequently used tool to assess emotional states (Brajon et al., 2015; Roelofs et al., in press; Roelofs et al., 2016). Fig. 39.4 shows the JBT used, for example, by Murphy et al. (2013b) for pigs. Since it is known that early life experiences (e.g., stress) can have consequences for later emotional functioning (Pechtel and Pizzagalli, 2011), JBTs might be a useful tool to investigate, especially, the effects of early life experiences. Murphy et al. (2015) succeeded in showing that effects of low birth weight in piglets can be detected in a JBT. Table 39.3 summarizes points to consider when testing pigs in a JBT.

2.3 The Pig Gambling Task—A Variant of the Iowa Gambling Task

Strategies in animal decision-making may relate to differences in early life experiences as suggested by Potenza (2009) for environmental factors in general, by Andrews et al. (2015) for rodents and birds, or by Murphy et al. (2015) for birth weight in pigs. Negative (early life) experiences can induce negative mood states later in life (Mendl et al., 2010), which can consequently affect decision-making when outcomes are uncertain (Mendl et al., 2009). Studying decision-making strategies might thus allow drawing conclusions on emotional states. Decisions have to be made when conditions are uncertain (Kacelnik and Bateson, 1997) and might involve a risk (with a known probability of each outcome) or ambiguity (with an unknown outcome) (Bechara, 2005; Krain et al., 2006).

A common test to study decision making is the Iowa Gambling Task (IGT; Bechara et al., 1994). Animals have

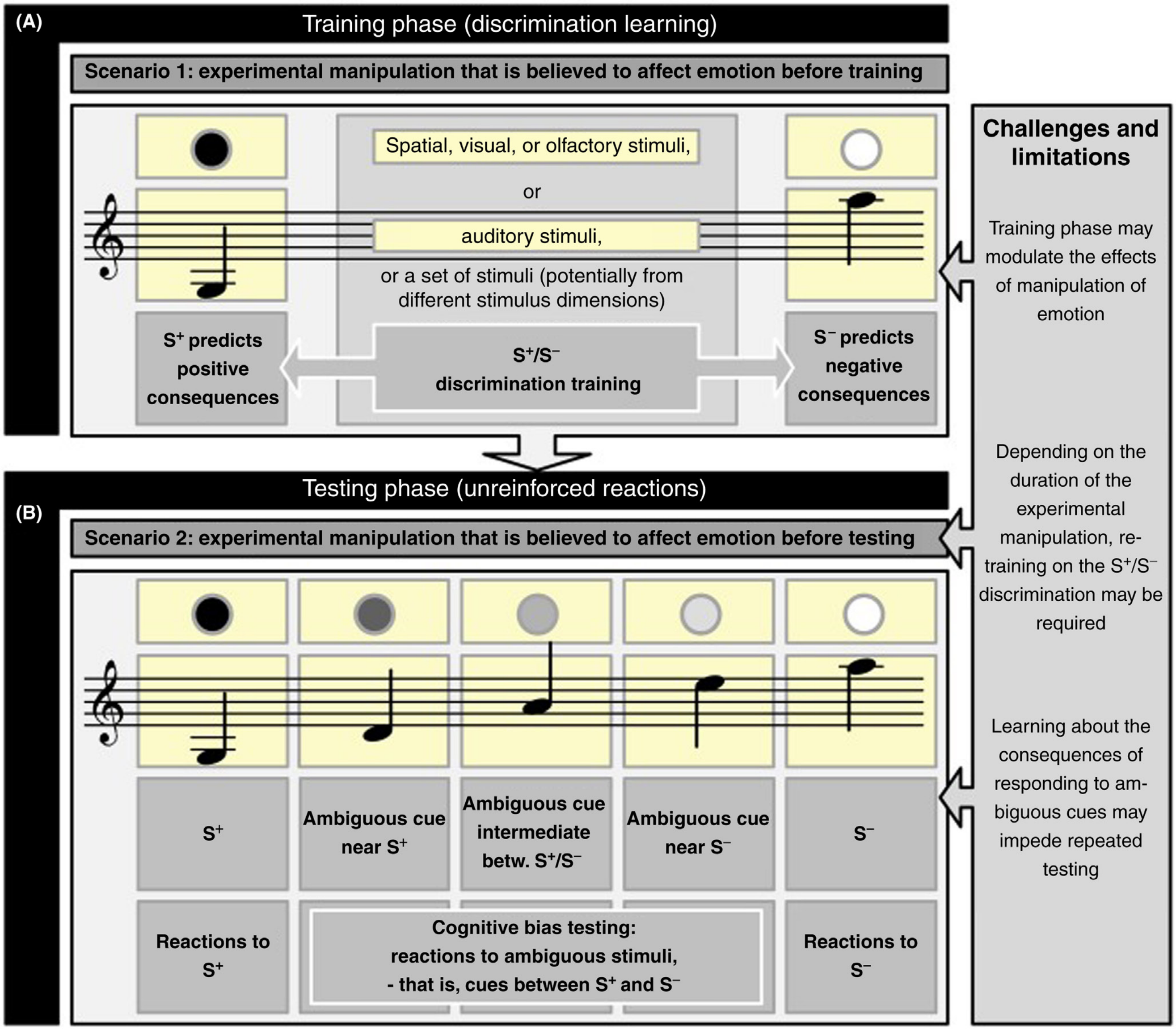

FIGURE 39.3 **Schematic representation of judgment bias training and testing using visual, olfactory, spatial, or auditory cues, or a combination of cues from different stimulus dimensions.** The experimental manipulation that is believed to affect emotion precedes the training phase (A; Scenario 1) or the testing phase (B; Scenario 2). Refreshment of the discrimination acquired during the training phase may be necessary, if the experimental manipulation preceding phase (B) lasts for a longer time period. An example of scenario 1 is studying the effects of growing up in different housing systems, whereas scenario 2 may be applied in a study assessing the effects of shorter lasting experimental manipulations, such as confinement, on emotion. Phase (B) may be repeated multiple times (Douglas et al., 2012) to test the effects of different experimental manipulations in the same animal. Specific challenges and limitations may be connected to the different phases. See Fig. 39.3 for an example of the specific contingencies connected with responding to S^+, S^-, and ambiguous cues. *Source: Reprinted from Roelofs, S., Boleij, H., Nordquist, R.E., van der Staay, F.J., 2016. Making decisions under ambiguity: judgment bias tasks for assessing emotional state in animals. Front. Behav. Neurosci. 10, 119, according to Open Access terms.*

to choose between two options: a "safe" or "risky" one. Risk is manipulated by varying the amount of reward, the probability of reward occurrence, or time delay until rewards are delivered (Murphy et al., 2015). Risk-prone individuals have been found to prefer risky options, while risk-averse individuals prefer safe options (Mazur, 1988). Anxiety led to increased risk-prone behavior in humans, as well as in rodents (Miu et al., 2008; de Visser et al., 2011). Positive mood on the other hand, led to earlier choices for safe options (de Vries et al., 2008).

Murphy et al. (2015) developed the Pig Gambling Task (PGT; see Figs. 39.1 and 39.5), inspired by the IGT. This two-choice probabilistic decision-making task allows studying decision-making under risk, in which the safe, or advantageous option yields greater overall gain. In the setup used by Murphy and coworkers an

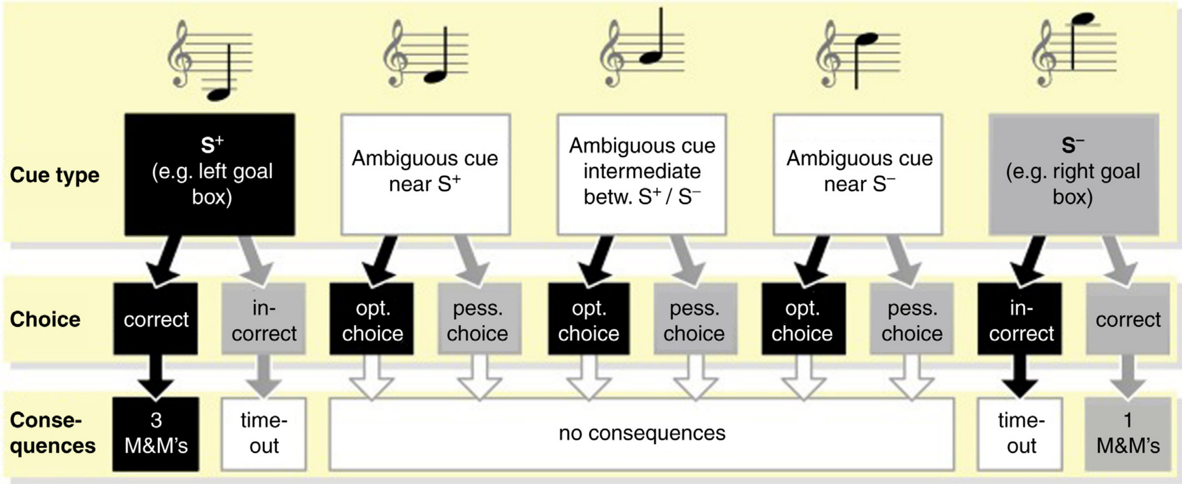

FIGURE 39.4 **Example of the exact contingencies connected with responding to the different cues presented during the testing phase (Fig. 39.1B) in a judgment bias tasks (JBT; programmed consequences of choices as used in Murphy et al. (2013b).** *Source: Reprinted from Roelofs, S., Boleij, H., Nordquist, R.E., van der Staay, F.J., 2016. Making decisions under ambiguity: judgment bias tasks for assessing emotional state in animals. Front. Behav. Neurosci. 10, 119, according to Open Access terms.*

TABLE 39.3 Points to Take Into Consideration When Testing Pigs in a Judgement Bias Task (JBT)

Effects of training/testing on emotional state	Procedural pitfalls
• Training within JBTs might be seen as cognitive enrichment, *improving the welfare* of the study subjects and affecting its emotional state (Carlstead and Sheperdson, 2000; Guldimann et al., 2015; Pomerantz and Terkel, 2009; Puppe et al., 2007; Zebunke et al., 2011).	• Pigs need *extensive habituation* before training and testing of individual animals can take place (Murphy et al., 2013b, 2015). • Initially ambiguous stimuli might *lose their ambiguity* within repeated testing (Roelofs et al., 2016). • Animals might *fail in reaching a required criterion* during training (Brajon et al., 2015).

FIGURE 39.5 **When tested for decision-making strategies, for example, in the PGT, pigs can decide on an advantageous or disadvantageous side.** Rewards used here were chocolate M&M's (Mars Nederland b.v., Veghel, The Netherlands). To illustrate, 8 rewarded trials per series of 10 trials (each yielding 2 M&M's as reward) (advantageous choice) are presented against 3 rewarded trials per series of 10 trials (each yielding 4 M&M's as reward) (disadvantageous choice). The number of rewards used might be varied. Advantageous choices yield, in the long run the largest number of rewards (here 16). *Source: Reprinted from van der Staay, F.J., Schoonderwoerd, A.J., Stadhouders, B., Nordquist, R.E., 2015. Overnight social isolation in pigs decreases salivary cortisol but does not impair spatial learning and memory or performance in a decision-making task. Front. Vet. Sci. 2, 81, according to Open Access terms.*

advantageous option was characterized by small but frequent rewards, whereas a disadvantageous option was characterized by large but infrequent rewards. The advantageous option thus led to greater overall gain in the long run (see also Fig. 39.5). Table 39.4 summarizes some of the considerations when testing pigs in the PGT

2.4 Discrimination Learning

Discrimination learning can be used as a part of training for more difficult tasks, including the judgement bias tasks and Iowa gambling task described earlier in the chapter. It can, however, also be used as a task in and of itself, to determine the ability of animals to discriminate between two stimuli and the capacity of animals to learn and perform tasks based on discrimination in different modalities.

TABLE 39.4 Points to Take Into Consideration When Testing Pigs in a Pig Gambling Task (PGT)

Effects or training/ testing on emotional state	Procedural pitfalls
• Training within JBTs might be seen as cognitive enrichment, *improving the welfare* of the study subjects and affecting its emotional state (Carlstead and Sheperdson, 2000; Guldimann et al., 2015; Pomerantz and Terkel, 2009; Puppe et al., 2007; Zebunke et al., 2011)	• Pigs need extensive habituation before training and testing of individual animals can take place (Murphy et al., 2013b, 2015). • Means by which risks are manipulated can influence preferences (Kacelnik and Bateson, 1997). • It is unknown whether pigs can assess maximum number of rewards or minimum number of punishment. They might decide on the option that provides reward in the majority of trials (van der Staay et al., 2016) . • It is unknown how many trials are needed to train pigs to consistently make advantageous choices (van der Staay et al., 2016).

Visual discrimination is frequently used in discrimination learning in various species. This can entail the use of lights, including discrimination between light color, intensity, or frequency of flashing lights. Visual stimuli can also include the use of pictures or patterns. Pigs have visual acuity which is inferior to humans, sheep, and cattle (Entsu et al., 1992; Tanaka et al., 1995; Zonderland et al., 2008) but which should, in theory, be quite sufficient for learning visual discriminations. In practice, however, discrimination based on visual stimuli in pigs has proven quite difficult, requiring lengthy training to show operant responses to distinct 2D shapes (Gieling et al., 2012a; Graf, 1976; Haagensen et al., 2013). Discrimination of conspecifics based on photographs, which has been demonstrated in domestic sheep (Ferreira et al., 2004) and cows (Coulon et al., 2009) did not seem to be possible in pigs (Gieling et al., 2012a).

Discrimination tasks based on auditory stimuli have been more successful, with pigs showing distinct operant responses to auditory stimuli of different frequencies (Murphy et al., 2013a). Other modalities, such as odor cues or tactile cues, have yet to be tested in pigs. Given their strong olfactory and tactile abilities (the snout is particularly sensitive), this may be an interesting avenue to explore to improve discrimination learning.

2.5 Practical Aspects of Training in Operant Tasks

Operant tasks, such as the pig holeboard, judgement bias, or gambling task, are work-intensive in terms of extensive time needed to habituate the animals to being alone in the setup, and because the animals need to be moved from the home pen to a test setup each time testing occurs. The latter can be partly offset by providing a waiting area where animals can stay for up to several hours between trials (thus with at the very least access to water). Training, while relatively fast in terms of learning tasks, still takes several weeks of daily training. On the other hand, the pig holeboard provides a number of measures of spatial memory and motivation within a single test, which can save time relative to conducting multiple sequential behavioral tests.

Training of very young animals in an operant conditioning task needs to be carried out keeping their cognitive, emotional, and physical abilities in mind. One manuscript reporting an attempt to train very young piglets (starting at 17 days of age) in a visual discrimination task reports that attempts at visual discrimination were suspended, as the first response of inserting the snout into a hole to be rewarded with milk was already quite difficult for unimpaired piglets (Andersen et al., 2016). In our hands, we found that piglets before the age of 4–6 weeks had difficulty habituating to being alone in the holeboard setup, and had difficulty learning the operant response of lifting a ball to gain a reward. This may reflect too high demands of the emotional and cognitive maturity of the animal before these ages to successfully perform an operant response.

For any task in pigs that involves intensive training, there will be a number of practical considerations having to do with the size of the animals. One of these is habituation of the animals to allow movement of the animals from a home pen to a test setup, and testing with as little interference from stress as possible. Even juvenile pigs are quite strong, and within a few weeks after birth it is very difficult to fixate or restrain piglets. If the animals can be fixated, this certainly will lead to stress in the piglets, with all of the welfare implications and potential for confounding the experimental result that this entails. To avoid this, extensive habituation to human contact, handling, and the test setup are highly desired before embarking on cognitive training. For holeboard testing, this entails daily habituation for 2–4 weeks (Fijn et al., 2016; Gieling et al., 2014a). The time involved in habituation of animals will, of necessity, increase the age at which animals can be tested. This may be a hindrance to the use of the holeboard test for testing very young animals. For testing of very young (preweaning) animals, one might consider the use of test setups that can be used either in the presence of or very near the sow and/or siblings. The 8-arm radial maze and the T-maze have been successfully used in very young animals (Dilger and Johnson, 2010; Elmore et al., 2012; Naim et al., 2010). If space allows these apparatus could be placed in the same room as the sow and littermates, allowing auditory and olfactory contact, which may reduce habituation time needed.

For any operant task, the type of reinforcer used will need consideration. Given that the animals need to be repeatedly trained and that the size of the animals requires that they voluntarily enter a test setup, it is advisable to work with a reward rather than an aversive stimulus. We have successfully used M&M's chocolate candies, which are easy to dose and highly palatable to pigs. For very young animals, M&M's have proven to be difficult to chew. In that case, we used small marshmallows. Other groups have used chocolate-covered raisins (Arts et al., 2009; Bolhuis et al., 2013), fruit, and for preweaning animals, milk replacers (Dilger and Johnson, 2010; Elmore et al., 2012) or applesauce (Bertholle et al., 2016; Meijer et al., 2014a).

Automation is also an important consideration in measuring any animal behavior. There are a few obvious advantages to at least some level of automation. First, automatic detection of responses can lead to much more detailed and fine-grained data, particularly regarding time (latency times, total time on task, etc.). This can be nearly impossible to score by hand for fast or repeated behaviors, or when more than one behavior is being scored, but can be relatively easily built into software. Second, automation can vastly reduce the effects of the experimenter, allowing multiple experimenters to work on a single test and produce similar results. As the experimenter will always have contact with the animals, even if just to move them from the home pen to the experimental setup, there will always be some effects of the experimenter. Different experimenters will be more or less gentle or patient with the animals, and some combinations of personalities of animals and experimenters may provide for either positive or negative interactions, that may differ for a single experimenter with individual animals. Moreover, automatic registration of behavior will reduce the observer bias (Bello et al., 2014).

There are some challenges to automation. For pigs specifically, experimental setups need to be built extremely robustly. Automation involves sensors and wires, which certainly can be built to withstand pigs, but this is (1) specialized work that is frequently not included in standard setups built for automation of behavioral measures, (2) can become quite costly, and (3) needs to be extremely well thought through if flexibility in the setup is required, that is, for disassembling and reassembling a setup if a space is to be used for multiple purposes. Stable builders who are experienced in building pig dwellings can be instrumental in building robust, automated setups.

An important aspect for implementing automation, is that one must know exactly what one wishes to measure in order to operationalize the measures in sensors and software. This can be an issue in pigs, where there is much less data on behavioral responses in tasks or to stimuli than, for instance, the vast amount of data available for rodents. It is highly advisable when introducing a new task, to first observe the animals closely in the task to determine which behaviors and which parameters should be scored. Once the automated setup has been built, the data should be validated (usually by comparing with hand-scored data, see Box 39.1 on validation of the pig holeboard). Raw data should be carefully examined after each experiment, to ensure that no malfunctions are taking place (i.e., dirty or old sensors, loose wires, software bugs, and so on) that can influence results. Some behaviors or parameters will not be easy or possible to detect automatically,as discussed further in the section gait scoring, for example, determining which foot produced a certain footfall. Advances in automation techniques, particularly in sensor development, make automation of more and more parameters possible.

3 NONOPERANT BEHAVIORAL TESTS

Behavior can also be tested in tasks which do not require extensive training, as operant tasks generally do. This may provide an advantage when one wishes to test very young animals; clearly one cannot test a 2-week-old piglet in a task that requires 4 weeks of training. Operant tasks can often give detailed information on cognitive functioning, but for specific questions on cognition or neurological function, nonoperant behavioral tests may provide more useful data. A number of tasks have been developed and used in pigs.

3.1 Measuring Motivation

Motivation is the process within the brain controlling which behaviors and physiological changes occur and when (Fraser et al., 1990). It is a reflection of the inner state of an animal. The inputs for this process may arise both from internal and external sources. For example, the decision to start foraging for food may be based on both internal cues, such as blood glucose level or gut distension and external cues, such as the presence of predators. Changes in motivational state can give rise to changes in appetitive behavior (i.e., searching for stimuli) or consummatory behavior.

Measuring the motivation of an animal to perform a certain behavior may provide information on the relative importance of that behavior for the animal. Captive animals, such as farm animals, may be restricted from performing some behavior. For example, nest-building in prepartum sows is impossible in many of the barren farrowing pens that are used in modern pig farming. Knowledge on the strength of motivation to perform behavior may help to understand the welfare implications of husbandry systems that restrict that behavior.

The motivation of pigs can be assessed in several ways. Perhaps the simplest method is to assess how often or how long an animal performs a behavior. For example, pigs that are food-restricted spend more time performing foraging behavior than nonrestricted animals (Day et al., 1995). Runways or obstacle courses have been used to assess the effect of feed composition on feeding motivation (Souza da Silva et al., 2012) and the difference between low-birthweight and normal-birthweight pigs on feeding motivation (van Eck et al., 2016). In the latter experiment, novel objects were added as obstacles to the runway in order to increase the difficulty of the task.

In operant conditioning, the animal is taught to perform a certain task in order to obtain access to a resource that allows them to perform a particular behavior. The “cost” of the resource can be manipulated by increasing the difficulty of the task (e.g., by increasing the amount of weight an animal needs to displace) or by increasing the number of times an animal needs to perform the task in order to get rewarded (e.g., increasing the number of times a lever needs to be pressed). Operant conditioning tasks have been used in pigs to assess motivation for, for example, food (Lawrence and Illius, 1989; Souza da Silva et al., 2012; van Eck et al., 2016), illumination (Baldwin and Start, 1985), straw (Pedersen et al., 2002), and heat (Baldwin and Ingram, 1967) and to get away from ammonia or draught (Baldwin and Ingram, 1967; Jones et al., 1998). Operant responses in a food-motivation task using nosewheel turning showed large interindividual variation (Souza da Silva et al., 2012), therefore within-subject designs should be considered when designing experiments using this task. Another parameter that may influence the performance in this test is social context: demand curves for food differ between pigs that are tested alone or together with another pig (Pedersen et al., 2002).

Other tasks may be used to assess motivation as well. The spatial holeboard task (see earlier for more information) includes parameters, such as trial duration and intervisit-interval (reflecting the speed of searching for bait) that can be used as measures for food motivation (Antonides et al., 2015b).

3.2 Mirror Use

The ability to use a mirror to guide movement or decision making has long been considered an indicator of complex cognitive ability. Instrumental use of mirrors (as opposed to the use of mirrors to show self-awareness) has been indicated in some types of primates (Anderson and Gallup, 2011; Heschl and Burkart, 2006; Menzel et al., 1985), dolphins, birds (Medina et al., 2011; Pepperberg et al., 1995), and elephants (Povinelli, 1989).

A paper by Broom et al. (2009) described a study in which pigs appeared to use a mirror to navigate an obstacle to gain access to food, indicating use of high cognitive functioning to solve a spatial problem. This would be an important finding, influencing our perception of pigs and their cognitive abilities. A later study failed to replicate the finding by Broom and coworkers (Gieling et al., 2014b). This indicates that mirror use is not universal in pigs, and may be at the periphery of their cognitive ability.

3.3 Open Field Test

The open field test, originally developed as a test for emotionality in rodents, has been used in pigs since the 1960s (Beilharz and Cox, 1967). It is generally performed in an unfamiliar square, rectangular, or circular arena. The pig is placed in the arena for a certain amount of time, usually between 4 and 10 min, and the behavior of the pig is recorded. Several categories of behaviors can be scored, such as locomotion and exploration (Andersen et al., 2000; Beattie et al., 1995; Donald et al., 2011; Fijn et al., 2016; Fraser, 1974; Gieling et al., 2014a; Meijer et al., 2015; van der Staay et al., 2009), location within the open field (Andersen et al., 2000; Donald et al., 2011; Fijn et al., 2016), vocalizations (Beattie et al., 1995; Donald et al., 2011; Fijn et al., 2016; Fraser, 1974; Gieling et al., 2014a), elimination (Andersen et al., 2000; Fijn et al., 2016; Fraser, 1974; Gieling et al., 2014a), and escape attempts (Fijn et al., 2016; Meijer et al., 2015).

The open field test is often used as a test for anxiety, exploration, and locomotion. Since it is known from several animal studies, as well as from human studies that stressful experiences in early life can modulate adult behavior (Taylor, 2010), the open field test may be useful to study these early life effects on anxiety later in life. Moreover, in a recent study in pigs, open field test locomotor activity was used as part of behavioral profiling in order to explain differences in learning abilities (Brajon et al., 2016) and it may therefore be a helpful tool when designing experiments.

Although the open field test is often used as a test for anxiety, there are some considerations that need to be taken into account. In general, the open field test may cause anxiety to animals due to social isolation and due to fear of novel environments and open spaces. Social isolation has been linked to indicators for stress by several studies (Kanitz et al., 2009; Poletto et al., 2006; Ruis et al., 2001). However, unlike rodents which show a tendency to remain close to the walls in unfamiliar surroundings, no evidence for this "wall-hugging behavior" in pigs has been found. Treatment with anxiolytics, such as diazepam (Andersen et al., 2000) and azaperone (Donald et al., 2011; Prut and Belzung, 2003), did not result in an increased time spent in the center of the open field. Location within the open field may therefore not be an ethologically valid indicator of anxiety in pigs (Fijn et al., 2016; Murphy et al., 2014).

When designing experiments using open field tests, the behavioral responses that are scored and their interpretation should be carefully considered (Forkman et al., 2007; Murphy et al., 2014). Scoring of the behavioral parameters can either be performed directly while observing the pigs, or from video recordings, or using video-tracking devices (van der Staay et al., 2009). The advantage of using video recordings is that the arena can be divided into several areas without physically having to place lines on the floor of the pen, which may influence the locomotor behavior of the pig. In a study looking at several behavioral parameters scored by two observers and both directly or from video, interater reliability was ≥0.73 and intrarater reliability was ≥0.78 (Fijn et al., 2016). Open field behavior is generally repeatable over time (Fraser, 1974; Jensen et al., 1995), although repeated exposure within a short period of time will decrease the novelty of the open field and may influence activity and vocalizations (Donald et al., 2011).

3.4 Clinical Examination of Central Nervous System Function

The clinical examination of the central nervous system in pigs has been extensively described in the setting of veterinary medicine, and is still the cornerstone in diagnosing neurologic disease in pigs (Radostits et al., 2007). In research settings, the clinical examination can also provide valuable insight into the functioning of the central nervous system. Some neurological conditions, presenting with one or several well-described symptoms, may be assessed by clinical examination alone. In other cases, the clinical examination may help to narrow down the possible localizations of the problem and may therefore help to limit the necessary ancillary techniques (such as imaging), saving both time and resources and limiting invasive and stressful procedures for the animal. The development of the central nervous system in young animals may be followed by using functional tests (Fox, 1964a,b; Muir, 2000), and may therefore be useful to study the effect of early life influences on development and functioning of the central nervous system later in life.

The clinical examination can be performed by any veterinarian, and with some training biomedical researchers, lab technicians, or students will be able to perform the clinical examination reliably as well. No specialized equipment is needed, and it is therefore an inexpensive technique. An additional advantage is that it is minimally invasive, and therefore may have minimal impact on the welfare of the experimental animals.

There are, however, also some drawbacks to using the results from the clinical examination as outcome parameters in experiments. Adult pigs are large and may be aggressive. This may limit the possibilities to perform some parts of the clinical examination, for example, tests that require the animal to lie down. Another drawback is that the clinical examination only provides functional information but no information on the underlying structural characteristics of the dysfunction.

3.5 Components of the Clinical Neurologic Examination

The components that are described further are for a large part derived from veterinary diagnostic protocols (Hajer et al., 2000). Not every component may be needed for each experiment, and some experiments may benefit from modified or additional tests. Table 39.5 provides an overview of common components of the neurologic examination, how to perform the test, and the expected response from the animal. It is important to perform the tests in an order that causes the least stress to the animal. Tests that require little or no fixation should be performed before more invasive tests and tests that require fixation.

3.6 Proprioception

Proprioception, the awareness of deep pressure and the position and movement of limbs, is mediated through receptors in muscles, tendons, and joints. They relay information to the spinal cord and brain via large Aα and Aβ myelinated fibers. Proprioceptive information is used to adapt body position and gait, and defects in the proprioceptive system may lead to ataxia. The lack of position sense in a limb may be demonstrated by placing the limb in an abnormal position. Unimpaired animals will correct this abnormal position directly. Commonly used tests are dorsal placement of the feet (Fig. 39.6A, Table 39.5) and crossing of limbs (Fig. 39.6B, Table 39.5).

3.7 Cranial Nerve Function

Many cranial nerve deficits can be identified by careful inspection of the head and neck. An abnormal position of the eye may be indicative of lesions to either the oculomotor nerve (divergent strabismus, often accompanied by a drooping eyelid and dilated pupil), trochlear nerve (rotated eyeball), or abducens nerve (converging strabismus). Abnormalities in the motor component of the trigeminal nerve may lead to a drooping lower jaw, facial nerve paralysis may present as a drooping ear, lip, and nose and an inability to close the eye. Atrophy of the neck muscles may be caused by accessory nerve dysfunction, and a paralysed tongue hanging from the mouth may be due to hypoglossus nerve dysfunction.

Several tests specific for certain cranial nerves are possible to perform in pigs (Fig. 39.6C, Table 39.5), although they require a relaxed animal. They may be difficult to perform in very young animals, either because the response is difficult to see (pupillary light reflex) or because animals only develop the adult-like response over time (menace response) (Enzerink, 1998).

3.8 Motor Function

Muscle tone and muscle strength as indicators of motor function may be assessed by observing the gait of the animal. Passive movement of the extremities, when the size of the animal permits, may give a more accurate impression of muscle tone.

3.9 Spinal Reflexes

A spinal reflex requires an intact reflex arc (muscle receptors, sensory axons within a peripheral nerve and dorsal root, lower motor neuron and its axon, muscle). No central input is required for a spinal reflex. However, sensory information may also be relayed to the brain and may result in additional behaviors. An example is the withdrawal reflex (Fig. 39.6D), in which stimulation

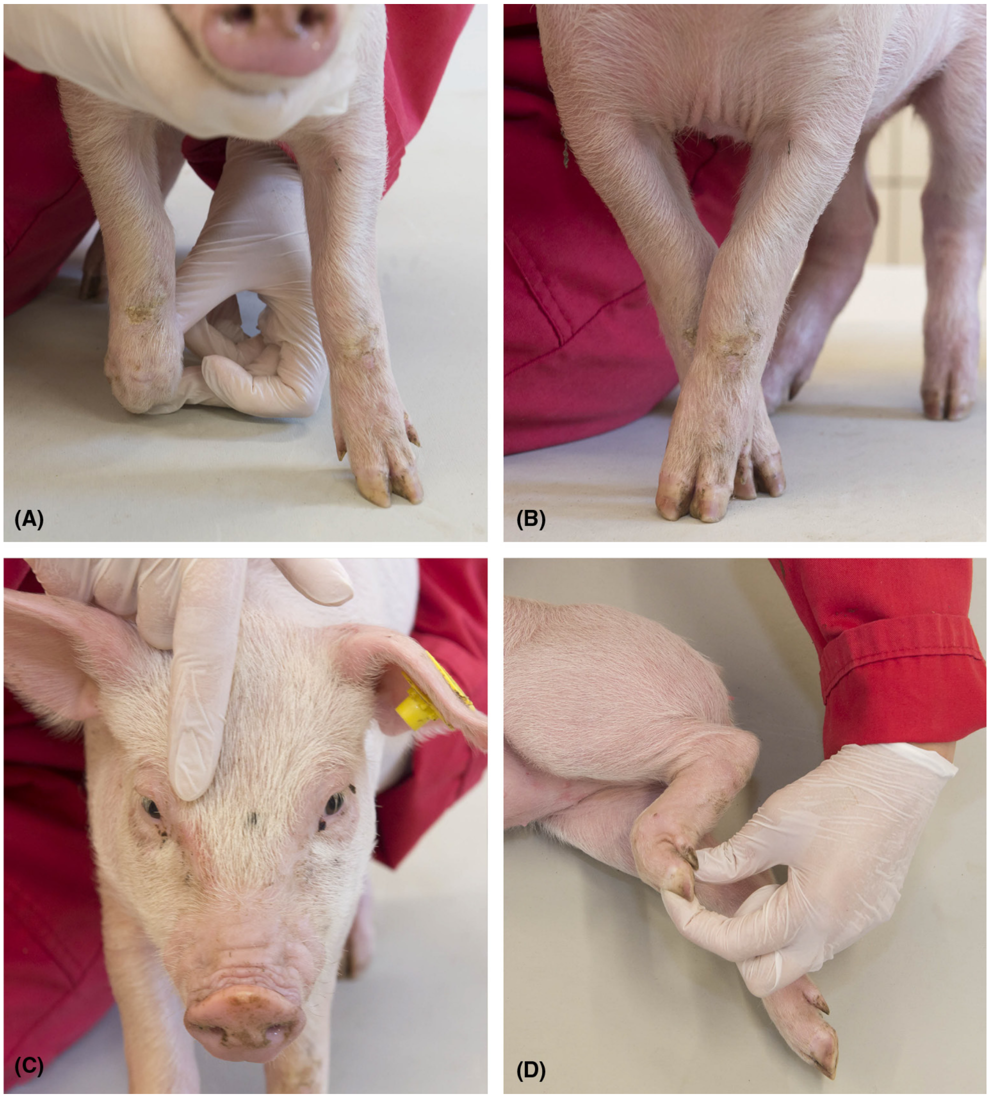

FIGURE 39.6 **Neurologic tests in pigs.** (A) Dorsal placement of foot, (B) crossing of limb, (C) Palpebral blink reflex, (D) withdrawal reflex.

TABLE 39.5 Common Components of the Clinical Examination of the Nervous System

Test	Action	Expected response
Dorsal placement of feet (Fig. 39.6A)	Placing dorsal side of foot in ground surface	Animal corrects abnormal position
Crossing of limbs (Fig. 39.6B)	Crossing the legs of the animal and let the animal put weight on it	Animal corrects abnormal position
Menace response	Moving an object fast in the direction of the eyes	Animal closes eyes
Pupillary light reflex	Shining into the eye with a flashlight	Pupil constricts
Palpebral blink reflex (Fig. 39.6C)	Tactile stimulation of the skin between the eyes	Animal blinks
Patellar reflex	Tapping the patella tendon with a reflex hammer	Flexion of the knee
Withdrawal reflex (Fig. 39.6D)	Pinching the skin between the claws	Flexion of the limb

This overview describes how the test is performed and the expected response from the animal.

of the skin between the claws results in flexing of the limb. Usually light stimulation of the skin is sufficient for the reflex to occur, but when stronger stimuli are needed, pain may be relayed to the brain and may result in guarding behavior or aggression.

In normal animals, central modulation of some reflexes is possible. In some conditions, such as complete transections of the spinal cord, this central modulation is not possible anymore, resulting in so-called "pathological responses." An example is the crossed extensor reflex. When performing the withdrawal reflex, the limb opposite the one being examined is extended. Another example is the occurrence of a massive response or repeated responses after performing the patellar reflex.

3.10 Gait Analysis

The ability to move from one place to another is an important ability for many species, including pigs. Locomotion is achieved through gait, "a complex and strictly coordinated, rhythmic and automatic movement of the limbs and the entire body of the animal, which results in the production of progressive movements" (Back and Clayton, 2013a). In order to produce gait, the integrated efforts of nervous tissue, muscles, bones, joints, tendons, and specialized skin are required. Many parts of the nervous system are involved in healthy gait.

3.11 Why Measure Gait in Neurological Research in Pigs?

Since the nervous system is so heavily involved in locomotion, problems in this system may produce a variety of gait disorders. Therefore, the analysis of gait may yield important information on the functioning of the nervous system. Several aspects of gait may be affected.

Strength may be affected when lower motor neuron function is impaired, resulting in weakness, paresis, or paralysis. Conditions affecting lower motor neurons may be limited to one muscle group, one limb, or may affect several or all limbs (e.g., in polyneuropathic conditions). Muscle tone is usually decreased, and weight bearing or propulsion may be affected, resulting in gait deficits.

Automated rhythmic movement is produced in the so-called spinal pattern generators. Although the presence of spinal pattern generators for limb movement has not been conclusively demonstrated in pigs, it has been shown in several other species, such as cats (Duysens and Van de Crommert, 1998). Due to the presence of these spinal pattern generators, even animals with complete transections of the spinal cord are able to produce rhythmic stepping movements. However, adaptation to the environment is only possible with additional input.

Coordination and adaptation are achieved through the integration of information from the visual, proprioceptive, and vestibular system. Integration and coordination take place in the brainstem, cerebellum, frontal cortex, and spinal cord (Nielsen, 2003; Sahyoun et al., 2004). Lesions in these systems may lead to ataxia, inability to correct for variations in the walking surface, deviations to one side, and hypermetric gait.

3.12 What Aspects of Gait are Important?

In the previous section some gait abnormalities resulting from neurologic impairment have been described. In order to determine the presence and severity of neurologic impairment, several aspects of gait may therefore be assessed.

Kinetic parameters assess the forces that affect motion. Examples of kinetic parameters are the amount of weight that is put on a limb, or the propulsive force that is used to produce forward motion. Kinetic parameters are therefore mainly indicative of the strength of a limb. Additionally, large step-to-step variations in the forces on a limb may be indicative of ataxia. There are of course other factors that may influence the forces involved in locomotion, such as mechanic impairments or pain resulting in voluntary modification of weight bearing.

Temporospatial parameters describe gait in terms of time and space. The timing of footfalls relative to each other, the distance that is bridged in one step or the deviations in the center of gravity are examples of temporospatial parameters. Rhythmicity may be assessed by looking at the consistency of timing parameters (Vrinten and Hamers, 2003). Ataxia may result in large variability in both temporal and spatial parameters and adaptations to neurological impairments, such as decreased balance that may result in wide-based gait (Givon et al., 2009; Gordon-Evans et al., 2009; Ishihara et al., 2009; Stolze et al., 2001).

3.13 How Can we Measure These Aspects of Gait?

There are several methods to measure the aspects of gait that we mentioned earlier in pigs. The most important ones, along with their advantages and drawbacks are described in short further in the chapter (Table 39.6).

3.14 Visual Assessment

Traditionally, visual assessment of gait is the technique that is used most often in research using pigs. Protocols specifically aimed at gait analysis in pigs do exist (Grégoire et al., 2013; Main et al., 2000; Mustonen et al., 2011; Van Steenbergen, 1989), but usually these protocols are geared toward recognizing orthopedic problems rather than neurologic dysfunction. Visual gait

TABLE 39.6 Advantages and Disadvantages of the Use of Pressure Mat Analysis of Pig Gait

Advantages	Drawbacks
Measurements can be performed fast and are minimally invasive	Expensive compared to visual scoring protocols
Several aspects of gait (both kinetic and temporospatial) are collected in one run	Assigning footfalls to the correct claw manually is time-consuming
Objective and repeatable, in contrast to visual scoring protocols	
Several footfalls can be recorded in one run, in contrast to force plate analysis	

assessment does not require costly or elaborate setups and is therefore fast and cheap. There are, however, several drawbacks to visual assessment of gait. It has only moderate interobserver agreement (D'Eath, 2012; Keegan, 2007; Main et al., 2000; Petersen et al., 2004), although the use of experienced observers increases the repeatability of results (Main et al., 2000), and it is inherently subjective (Arkell et al., 2006). Furthermore, since pigs are prey animals they tend to hide signs of disease, complicating the detection of abnormalities (Weary et al., 2008).

3.15 Kinematics

Kinematic analysis is performed by tracking body segments, either by following markers that are placed on anatomical landmarks, or by "markerless" tracking using dedicated software. The temporal and spatial characteristics of gait, along with angles between body segments can be analyzed. Kinematics in pigs have mainly been used to quantify gait abnormalities associated with orthopedic problems (Conte et al., 2014; Stavrakakis et al., 2015a; Thorup et al., 2007). Kinematic setups are usually elaborate and expensive, although efforts to develop simpler and more cost-efficient methods for use in pigs are underway (Stavrakakis et al., 2015b). Another drawback is the possibility of skin movement artifacts. Markers are placed on the skin over bony anatomical landmarks, but during movement skin slides over bones and markers may be displaced so they do not correctly represent the landmarks anymore.

3.16 Force Plates

Force plates are platforms that translate the magnitude and direction of forces exerted onto them (ground reaction forces) into electrical signals using either piezoelectrical transducers or strain gauges. They have been used since the late 1960s and are still considered the "gold standard" for kinetic gait analysis. A large body of knowledge on the use of force plates in quadrupeds exists, and they have been used, for example, in experiments on the effect of flooring on gait of healthy pigs (Thorup et al., 2008; Von Wachenfelt et al., 2009a,b, 2010) and to assess static weight distribution in standing sows (Karriker et al., 2013; Pluym et al., 2013; Sun et al., 2011). Force plates are able to measure forces in three dimensions and may therefore not only be suitable to assess weight distribution, but also the strength of propulsion which may in turn be an indication for overall strength.

An important drawback of force plates is that they cannot distinguish between feet that are placed simultaneously on the plate. Measurements in which two feet are on the plate simultaneously have to be discarded. This means that the optimal size of the plate is different for animals of different sizes, and that many trials may be needed in order to collect sufficient valid measurements. This may be a drawback in studies that follow growing animals over a longer period of time, for example, in studies investigating the effect of influences early in life on motor function in older animals. Also, there may be large variations between measurements, especially since velocity has a large influence on the ground reaction forces. In species, such as dogs and horses, which can be easily led by a collar or halter, this problem may be solved by setting strict limits for velocity and guiding the animal over the force plate at the required pace. In pigs, this is much more difficult since they resist to being led by a collar. A solution might be to use either an instrumented treadmill or several force plates in a row (Back and Clayton, 2013b), however, this setup has not been used in pigs to this date.

3.17 Pressure Mats

Pressure mats (also called pressure plates or pressure-sensing walkways) contain a dense array of sensors, each of them individually measuring the magnitude and the duration of the force exerted on them. When measurements of all sensors under one claw are combined, a pressure profile for each limb, but also for specific regions, such as the inner or outer claw can be constructed. Unlike force plates, pressure mats only measure vertical forces under the claw.

There are multiple pressure mat systems available, some of which are large enough to capture several footfalls in one measurement. In contrast to force plates, pressure mats allow to distinguish between individual footfalls. This opens up the possibility to eliminate influencing between-run factors, such as velocity, and to collect additional temporospatial parameters, such as step length, stance time, and base of support. These temporospatial parameters may be particularly interesting in the assessment of neurological causes of gait deficits.

In pigs, pressure mats have been used to assess pressure distribution under the claws of healthy sows

(Carvalho et al., 2009), to quantify experimentally induced lameness (Karriker et al., 2013), to assess the effect of pain medication on lameness (Meijer et al., 2015; Pairis-Garcia et al., 2015), to follow the development of normal gait in growing piglets (Meijer et al., 2014a), and to identify lameness due to several orthopedic conditions (Bertholle et al., 2016; Meijer et al., 2014b).

3.18 Pressure Mat Analysis in Pigs

Since pressure mats are able to collect both kinetic and temporospatial information and are fairly straightforward to use in pigs, their potential as a research tool to detect and quantify neurological gait abnormalities will be discussed in more detail.

When a pig steps on the pressure mat, several pressure sensors will be triggered. The frequency with which the sensors measure the force exerted on them can be set manually and usually varies between 63 and 126 Hz. On each timepoint, a certain force will be recorded under the sensor. All forces of the sensors belonging to one footfall are grouped, creating an outline of each footfall with corresponding force-time curves (Fig. 39.7). From these force-time curves several parameters can be derived (Figs 39.7 and 39.8), the most common of which are:

Peak vertical force: the highest peak in the time-force curve.

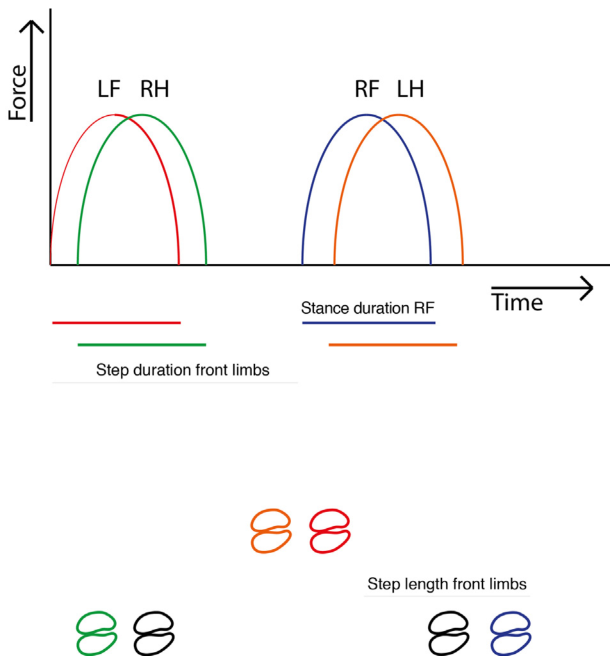

FIGURE 39.7 Schematic representation of footfall outlines with associated force-time curves. Outlines and their associated force-time curves have the same color. The temporal parameters Step Duration and Stance Duration in association with the force-time curves are shown in the upper part of the figure. The spatial parameter Step Length in association with the footfall pattern is shown in the lower part of the figure. *LF*, Left front; *LH*, left hind; *RF*, right front; *RH*, right hind.

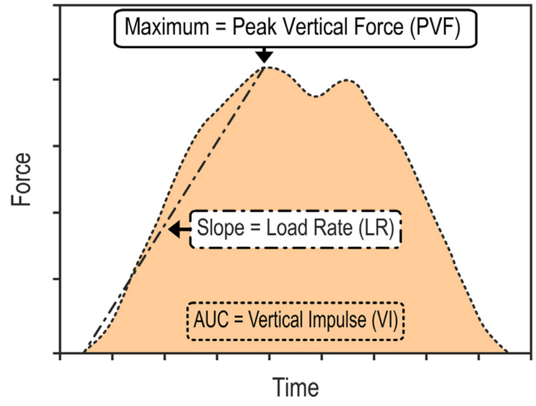

FIGURE 39.8 Force-time curve of one footfall with associated kinetic parameters. Peak vertical force *(PVF)*: The highest peak in the force-time curve. Load rate *(LR)*: The slope of the imaginary straight line from the start of the stance phase to the peak of the force-time curve. Vertical impulse *(VI)*: The area under the force-time curve.

Load rate: The slope of the imaginary straight line from the start of the stance phase to the peak of the time-force curve.

Vertical impulse: The area under the time-force curve.

Peak vertical pressure: The highest peak in the time-pressure curve, in which pressure is defined as the force at a timepoint divided by the contact area at that timepoint.

Stance duration: The time the limb is in contact with the ground surface.

Step length: Travelled distance between left and right limb.

Step duration: Time needed for one Step Length to be completed.

Stance percentage: Ratio between the time a limb is in contact with the ground and the duration of the swing phase of that limb.

Additionally, pressures can be visualized by color-coding pressure under each sensor, together creating an outline of each claw. Additional spatial parameters, such as Step Length can be derived from these footprints.

Pressure mat parameters may be influenced by several variables. Velocity has an important effect on most pressure mat parameters. In a study in growing pigs aged 5–15 weeks, velocity influenced Peak Vertical Force, Load Rate, and Peak Vertical Pressure, but not Vertical Impulse (Meijer et al., 2014a). Although no information is available on the influence of velocity on temporospatial parameters in pigs, it is known from horses (McLaughin et al., 1996; Khumsap et al., 2002) that velocity does in fact have a substantial influence on these parameters. Most quadruped species, including pigs, carry about 60% of their weight on their front limbs and the remaining 40% on the hindlimbs (Fernihough et al., 2004; Meijer et al., 2014a; Oosterlinck et al., 2011; Weishaupt, 2008).

It is therefore difficult to directly compare front- and hindlimb-kinetic parameters to each other. Depending on the experimental setup, there may be additional influences on pressure mat parameters, for example, measurement session and variations in body composition between animals or over time (Meijer et al., 2014a; Mölsä et al., 2010; Nordquist et al., 2011).

In order to deal with these influences, asymmetry indices (ASIs) may be used. Symmetry is considered as an important feature of normal locomotion. When one or more limbs of an animal are affected by neurological conditions, the symmetry of the gait may be affected. In kinetic, as well as kinematic gait analysis, this asymmetry may be quantified by comparing parameters of healthy and affected limbs to each other using ASIs. ASIs can be calculated in several ways (Budsberg et al., 1993), but they are most useful when limbs from an animal within one run are compared to each other. This minimizes the effect of interrun variations that may be caused by, for example, velocity differences. In pigs from 5 to 15 weeks old, time did not influence ASIs, which makes them particularly suitable for experiments that follow animals over time (Meijer et al., 2014a).

Perfect symmetry theoretically indicates that an animal is sound. The assumption that a sound animal moves perfectly symmetrically, however, has to be used with caution. In humans, symmetry indices of up to 7.6 (with 0 indicating perfect symmetry and 100 indicating complete lack of weight-bearing on one limb) for PVF were found in clinically healthy subjects (Herzog et al., 1989). Limb dominance and functional differentiation between limbs has been proposed as a reason for the frequently observed gait asymmetry in healthy humans (Sadeghi et al., 2000). Limb dominance has been shown in dogs (Colborne et al., 2011, 2008) and horses (Oosterlinck et al., 2011) and may also be present in pigs. In neurological conditions that affect all four limbs, ASIs may not be useful and other outcomes, such as coefficients of variation, may be more suitable (Hausdorff et al., 1998).

3.19 Obtaining Valid Runs Efficiently

As discussed previously, in order to obtain meaningful results, pigs need to ambulate in a straight line, at a constant velocity, and looking straight ahead. Data collection can be greatly facilitated by using a suitable setup, by habituating the piglets to the test apparatus and by training the animals to perform the desired behavior.

In order to facilitate that the pigs walk in a straight line over the pressure mat, borders on each side of the pressure mat should be placed to guide the pigs over the mat. Older, heavy pigs have a tendency to lean into walls when walking, influencing pressure distribution between limbs. This can be prevented by making the sides of the runway slightly inclined away from the pig. Furthermore, there should be enough space before and after the actual mat for the pigs to accelerate and decelerate, so that they cross the pressure mat at a constant velocity. The pressure mat should be level with the rest of the runway, and the pigs should not be able to see the borders of the mat. This can easily be achieved by placing a rubber mat over the entire runway including the pressure mat, but the mat should be recalibrated after the rubber mat has been placed. Manufacturers are usually able to provide advice on optimal properties for the rubber mat. Since pigs are curious animals and will chew on anything they find interesting, distractions in the runway (such as cables, screws, changes in color of the floor or borders), as well as in the environment should be removed. An example of an experimental setup is shown in Fig. 39.9. The effect of sudden noises can be minimalized by having a radio playing during the measurements, as long as the pigs are used to the sound of the radio.

Gradually habituating pigs to the test apparatus, social isolation, and the presence of humans increases the efficiency of data collection and decreases the amount of stress an animal experiences. Pigs are social animals and isolation of an animal is known to cause stress (Herskin and Jensen, 2000; Kanitz et al., 2009), which in turn may negatively affect cognitive functioning and thus make training of the animals more difficult (Mendl, 1999; Schwabe and Wolf, 2010). Fear of the experimenter may cause hesitation while crossing the runway and will disrupt the normal gait pattern.

Pigs are easily trainable and they can be taught to cross the runway at a predetermined gait (walk, trot, or canter). We have good experiences with operant conditioning using a secondary reinforcer (clicker training) and with food rewards, such as M&M chocolates (Mars, Incorporated), as the primary reinforcer. Although training is time-consuming, it greatly facilitates data collection. This is particularly important when animals are used that have painful or debilitating conditions, as prolonged testing that may be required in untrained animals to obtain reliable runs may seriously impair their welfare. Also, experimental setups where animals are followed over time and measurements are performed more often may benefit from properly trained animals.

3.20 Conclusions

Gait analysis in pigs may be interesting as an outcome measure for neurological impairment. Several techniques are available. Pressure mat analysis is a promising tool that allows collection of several gait characteristics and multiple footfalls in one run. Some advantages and drawbacks of the technique are summed up in Table 39.6. A suitable setup of the testing apparatus and training of pigs greatly facilitates data collection and may improve the quality of the recorded data.

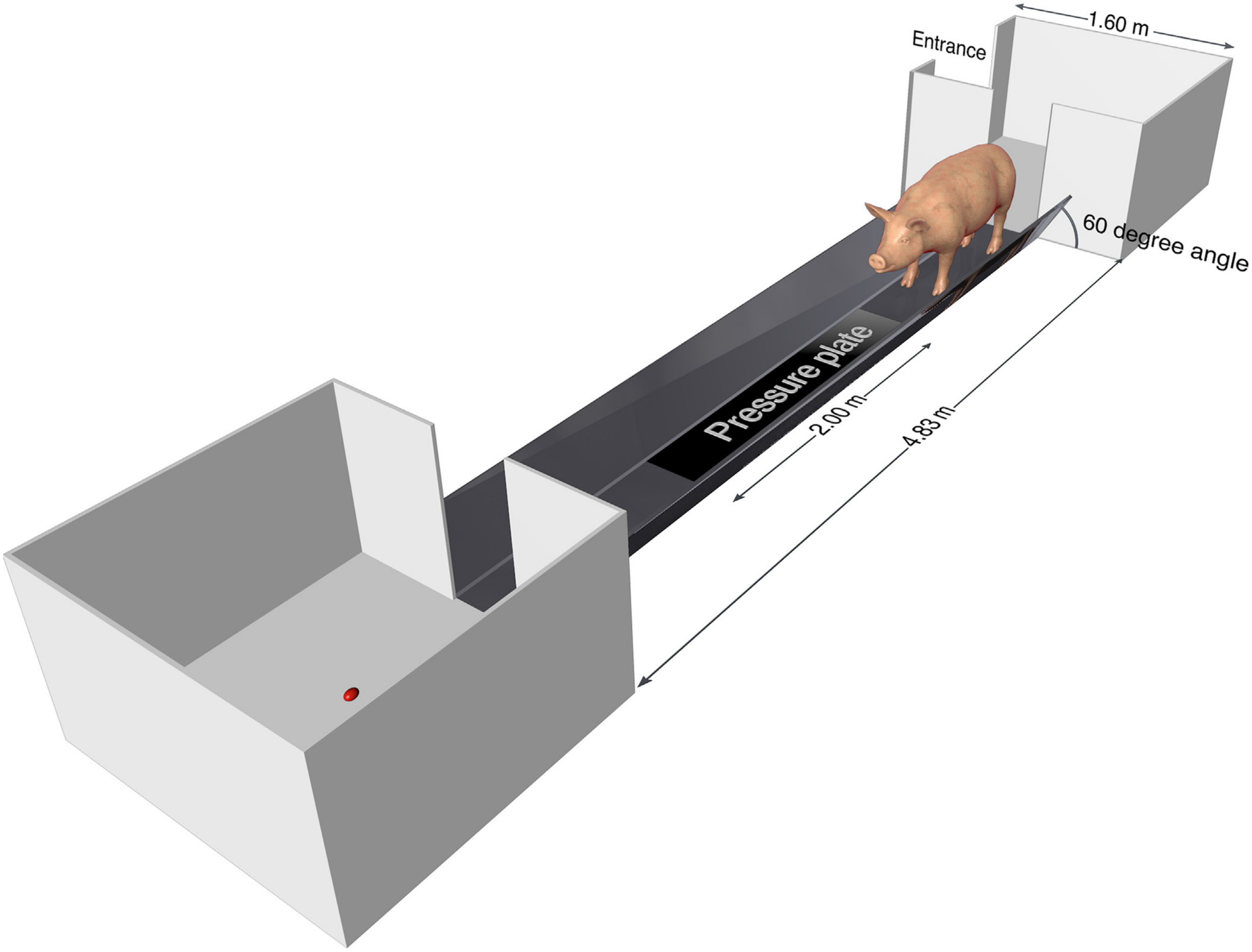

FIGURE 39.9 **Example of an experimental setup for collecting pressure mat data in pigs up to 60 kg of weight.** Walls of the runway are inclined to prevent pigs from leaning into them. Holding pens are situated at both ends of the runway. The pressure mat is built in level with the runway and the entire runway is then covered with a rubber mat (not shown).

4 WELFARE ASPECTS IN THE USE OF PIGS AS MODEL SPECIES

Minimizing unnecessary suffering and increasing the welfare of animals in general is imperative from a legal and ethical point of view. Regardless of the species of interest it is widely accepted that better welfare is inherent to better science. Poole (1997) was one of the first to state that "physiological and behavioral responses to avoidable suffering can act as experimental confounds…."

However, before elaborating on welfare aspects regarding the pig as model animal species we have to clarify some general questions.

4.1 Animal Welfare

The concept of animal welfare is poorly defined and, especially regarding farm animals like the pig, often based on the principle of the "five freedoms", which demand freedom from: (1) thirst, hunger, malnutrition; (2) suffering; (3) pain, injury, disease; (4) the freedom to express normal behavior; and (5) freedom from fear and distress (Newman, 1994) in order to safeguard welfare. The five freedoms, however, do not translate immediately into "measures" for animal welfare (Barnard, 2007; Cuthill, 2007; Ng, 1995; Terranova and Laviola, 2004; Weed and Raber, 2005) and have been criticized, for example, by Korte et al. (2007). Welfare of animals in their view is not at stake as long as the animal has the ability to meet environmental challenges. Other concepts of animal welfare, such as the concept of evolutionary salient welfare (Barnard and Hurst, 1996) are based on the idea that welfare is not at stake as long as the animal is able to fulfill its adaptive needs and is able to make its own decision. Ohl and van der Staay (2012) suggest a dynamic concept of animal welfare, taking the animal's ability to cope with environmental challenges, the relevance of positive emotions, and the importance of so-called "negative" emotions into consideration. They state that "An individual is in a positive welfare state when it has the freedom adequately to react to hunger, thirst or incorrect food; thermal and physical discomfort; injuries or diseases; fear and chronic stress, and thus, the freedom to display normal behavioral patterns that allow the animal to adapt to the demands of the prevailing environmental circumstances and enable it to reach a state that it perceives as positive." (Ohl and van der Staay, 2012). This appears to represent one of the most appropriate concepts.

Irrespective of which welfare concept is favored, welfare criteria based on scientific evidence are needed, for example, in order to estimate animal suffering. However, identification and validation of welfare-related measures, the prerequisite to improve animal welfare, remains a major challenge and is subject to several studies (Boissy et al., 2007; Duncan, 2005; Yeates and Main, 2008).

4.1.1 Assessment of Welfare

The question remains how to assess welfare of animals. The animals' perception of its inner, thus emotional state is central to present concepts of "animal welfare" (Fraser and Duncan, 1998; Nordenfelt, 2011; Ohl and Putman, 2014; Ohl and van der Staay, 2012; Taylor and Mills, 2007; Webster, 2011). This perception can be inferred indirectly using behavioral and physiological measures. Those measures form the (biological) basis of animal welfare, since they allow to define target values (for welfare states), based on the demands (needs) and behavior of the animal [note that the fact, that those target values are influenced by moral concepts will not be discussed here. For an elaboration see, Ohl and van der Staay (2012)].

4.1.2 Pig Welfare

Chapter 3 elaborates on the relevance of large animal models and large animal model species, such as pigs, for research related to productivity, animal health, and for translational biomedical research. One should, however, not forget another important role of pigs: they are well suited to serve as models in pig-welfare related studies (Renggaman et al., 2015), since using the same species as model offers the highest amount of the external validity (generalizability) (van der Staay et al., 2009).

It is inherent to experimental work that welfare of the study subjects might be impaired. Thus, welfare needs to be taken into consideration at any stage of a study and any attempt must be made to avoid unnecessary impairment of welfare. Since the conceptual and assessment related assertions above are species-transcending, they are applicable to pigs as well. Concerns regarding the welfare of pigs have been raised. However, attention is mainly paid to pigs in (industrial) farming. Many of these concerns are also applicable to pigs used in research. However, the latter are frequently exposed to additional situations or procedures potentially affecting their welfare even more.

Better welfare of pigs and better science with pigs "go hand in hand" (Hawkins et al., 2014). Next to investigating factors that might lead to an impairment of welfare it is of outmost importance to gain more knowledge regarding indicators of positive pig welfare states.

Especially young animals are prone to the effects of environmental factors and handling (Hargreaves and Hutson, 1990) but experiences, like exposure to testing and training have potential consequences for emotional responses and thus the welfare of adult animals as well (Boissy et al., 2007). It is important to realize that stimulation and experiences have a high potential to modulate emotional responses.

4.2 Assessment and Improvement of Pig Welfare

4.2.1 Health of Pigs

As stated earlier, good health is essential for animal welfare. Clinical evaluation needs to be the first step when attempting to assess welfare of pigs. Clinical signs of injury or disease provide a first indication that welfare might be affected. Body condition scoring tools [MAFF (Ministry of Agriculture, Fisheries and Food (1998)], cardiovascular responses, and stress-related hormone measurements might especially be helpful in gaining such first indications of affected welfare. It is obvious that impaired health is inherent to certain experimental manipulations performed on pigs, however, unintended health impairments need to be detected and solved to forestall welfare impairments.

4.2.2 Needs of Pigs—General Remarks

In order to allow pigs to fulfil their needs and/or to provide them with the necessary resources, we need to gain knowledge about their needs. Broom and Johnson (1993) defined needs as "a requirement, which is a consequence of the biology of the animal, to obtain a particular resource or respond to a particular environment or bodily stimulus." Needs furthermore (very likely) vary with factors, such as age, sex, and genetic background and previous experiences. However, the needs of recently created genetically manipulated pigs (Søndergaard and Herskin, 2012) are almost unknown. Comprehensive knowledge about the actual needs of pigs is urgently needed.

The animal's perception of its emotional (welfare) state might be derived by the use of behavioral and physiologicalmeasures. This chapter describes a series of behavioral tests, allowing to gain information on pigs emotional perception and thus, being also useful for gaining information about the effects of early life events on later emotional functioning.

We suggest that the assessment of welfare, regardless for which purpose an animal is used, should focus on the question whether an animal has the freedom and the capacity to adapt to environmental challenges, including those intrinsically tied to the experimental procedures applied in biomedical experiments.

4.2.3 Behavior in General

A prerequisite for conclusions on the adaptive capacity of an animal is knowledge of the normal/natural behavioral repertoire of the animal species in question (ethograms). Detailed information about the normal behavioral repertoire and the behavioral needs of farm animals is urgently needed. As early as 1978, Kilgour (1978) pointed to the exigency to obtain this basic information: "The ethogram of farm animals should have a high priority for current animal science goals and the farming system must be designed to fit the animal" (Kilgour, 1978, p. 1481).

For pigs, comprehensive ethograms are missing (although at least some ethograms for specific behavioral domains are available). To fall back on ethograms for the wild ancestor, the wild boar (*Sus scrofa*), is only an option under the assumption that domestication and selection on performance characteristics did not alter behavior. However, one needs to realize that the behavior of domesticated pigs is rooted in the wild pig (Baxter et al., 2011; D'Eath and Turner, 2009). Thus, certain behaviors seen in wild pigs might be important for the welfare of domesticated pigs as well.

4.2.4 Aggressive Behavior

Since 2013 sows on farms in the European Union must be housed in groups due to European legislation (DIRECTIVE 2008/120/EC) with the intention to improve welfare. Pigs are indeed socially living animals (Estevez et al., 2007) and individual housing of pigs is thus, generally speaking, a potential risk for welfare (see also Chapter 3). However, as discussed, for example, by Mendl et al. (1992) and Nicholson et al. (1993), aggression, as seen in group housed pigs, might result in an impairment of welfare since stress and injuries are very common consequences. Thus, several studies focus on measures of aggression [like, e.g., skin injuries (Meyer-Hamme et al., 2016)] in order to judge the effects on welfare. However, one has to realize that aggression is adaptive in nature and necessary for resource competition, offspring protection, defense and hierarchy-establishment (Koolhaas and Bohus, 1992). Thus, aggression might form a risk to welfare when its adaptive value is exceeded and consequences like stress and injuries (in receivers of aggression) are excessively present.

Several attempts have been made to reduce aggression among pigs to a minimum. Fredriksen et al. (2008) and Rydhmer et al. (2013), for example, recommend to socialize pigs early and to keep them in (sibling) groups. According to Day et al. (2002) the provision of rooting material (to distract the animals from penmates) successfully reduces aggression.

Dedicated mixing pens are recommended by Verdon et al. (2015). The authors, however, point out that the effects of housing in such mixing pens, on aggression (and stress), have not been investigated yet. Importantly, they point out that a secure development of a hierarchy may lead to less aggression when animals are mixed later in life with unfamiliar females and that such a secure development of a hierarchy might be realized by socialization of juvenile gilts (i.e., young female pig not yet mated, or not yet farrowed) to sows. In general, mixing sows that have been housed together earlier might help to reduce aggression as well (Verdon et al., 2015). The same authors furthermore mention hunger as a possible reason for aggressive behavior and recommend full-body length feeding stalls to reduce aggression (and stress). It is important to note that hunger as a transient state does not negatively affect welfare, because it represents a motivational state that triggers foraging and eating (Phillips et al., 2016). Thus, hunger is essential for the animal to protect its own welfare.

4.2.5 Affiliative Behavior

Pigs have been found to maintain preferential relationships (friendships) with other pigs (Stolba and Wood-Gush, 1984). Affiliative behavior may thus be essential for pig welfare. It is, however, important to identify valid measures of affiliation. Boissy et al. (2007) mention the risk of using proximity between individuals as a measure of affiliation. Proximity might simply be a result of limited space. Allogrooming belongs to the class of affiliative interactions and is believed to be part of pigs natural behavioral repertoire (Meynhardt, 1990), inducing relaxation in groomed pigs (Hansen and Von Borell, 1998). Nevertheless, pigs might experience disturbance due to being (excessively) groomed. Also here, more research is needed to verify the relevance for welfare.

4.2.6 Stereotypic Behavior

Stereotypic behavior has been investigated in pigs (Meunier-Salaün et al., 2001). However, whether the display of behavior reflects an impairment of welfare might be questioned, since, for example, Koolhaas et al. (1999) suggested that stereotypic behavior rather reflects a coping style.

4.2.7 Nest-Building Behavior

Damm et al. (2003) and Thodberg et al. (1999) found nest-building behavior to be essential for sows (at least approximately 1½ day before farrowing). Thus the provision of nesting material is essential for those sows (see also Baxter et al., 2011).

4.2.8 Anticipatory Behavior

Positive anticipation has been suggested to be useful in assessing positive emotional states (welfare) in animals (Boissy et al., 2007). Pigs indeed show increased locomotor activity and frequent behavioral transitions when anticipating food rewards (Dudink et al., 2006). The usefulness of anticipatory behavior in an attempt to draw conclusions on welfare must, however, be judged with care. If pigs do not show such reactions they might simply be fully satisfied or anhedonic (Boissy et al., 2007) or might not be able to learn to anticipate the delivery of feed at fixed time points of a day.

4.2.9 Emotions in General

The emotions discussed in the following can be inherent to certain experimental procedures, as discussed earlier in this chapter, and can thus affect the further life of the animal in question. Especially young pigs experiencing these emotions (unintended; excessively and thus adaptability-exceeding) might perform aberrant in experiments performed later in life (their welfare might have been affected, leading to long-term adverse consequences).

4.2.10 Fear and Anxiety

Fear and anxiety, investigated commonly in pigs (Forkman et al., 2007; Donald et al., 2011), are not negative by nature but trigger important adaptive responses (Ohl et al., 2008), and are not per se indicators of negatively affected welfare. Factors causing undesired, inappropriate, or prolonged anxiety (pathological anxiety; Ohl et al., 2008) need to be identified in order to remove or at least reduce them.

4.2.11 Pain

Like the emotions mentioned earlier, pain is an adaptive response, important for welfare (Ohl and van der Staay, 2012) and survival since it elicits protective vegetative and motor reactions, resulting in learned avoidance. The assessment of pain in animals is still a major challenge. There are some attempts to measurepain in pigs by investigating behavior (Meijer et al., 2015; Sutherland et al., 2012) and/or physiology [clinical and neuroendocrine approaches; Lonardi et al. (2015)]. However, pain is an individual sensation and thus, difficult to measure and compare among animals (Sneddon and Gentle, 2000).

Behavioral changes in general have been suggested to allow for the assessment of overall pain and discomfort by Hay et al. (2003), Moya et al. (2008), and Sutherland et al. (2012). Meijer et al. (2015) suggested locomotion asymmetry and spontaneous locomotor activity as potential indicators of pain associated with lameness in pigs. Classical physiological parameters used, probably indicative of pain, are clinical indicators, such as respiratory rate, blood pressure, rectal temperature, and heart rate in restrained awake piglets (White et al., 1995). A promising tool in assessing pain in pigs are changes in eye temperature due to sympatic and parasympathic systems variations (Lonardi et al., 2015).

All mentioned parameters potentially indicative for pain in pigs need, however, further validation. It is essential to take additional potentially confounding factors into consideration. Prunier et al. (2005), for example, made the important annotation that handling may interfere with physiological and behavioral responses of pigs.

Until valid measures of pain in pigs are available, one should, in particular, rely on the assumption that everything that causes pain in humans potentially causes pain in animals as well (Barnard and Hurst, 1996).

4.2.12 Stress

Stress is not negative by nature but an adaptive response. As outlined earlier, animals need to be able to adapt in order to safeguard welfare. In case the animal's adaptive capacity is reduced, the animal will experience chronic stress when adaptation to the environment is needed (Weiss, 1971) and welfare might be at stake (Korte et al., 2005; McEwen, 1998).

Unfortunately, valid measures for detecting when adaptive stress turns into distress (Holden, 2000) do not yet exist. Lack of control has been suggested to result in chronic stress (Heldt et al., 2002) and controllability has been suggested as being essential for animal welfare (Boissy et al., 2007). Thus, a controllable, or predictable environment is of high importance.

Fredriksen et al. (2008) suggested to socialize pigs early and to keep them in groups in order to reduce stress (Rydhmer et al., 2013). Furthermore, environmental enrichment might be helpful in preventing stress. Next to the fact that enrichment always needs to be decided on, taking the animals' biological needs into consideration, van der Staay et al. (see Chapter 3) made the important annotation that pigs need to be given the opportunity to learn to use enrichment. They furthermore elaborated on stressful situations for pigs on intensive farms. The majority of the discussed situations are applicable to pigs used as model animals in research: changes in housing systems, equipment, types of feed, introduction into groups of unfamiliar pigs, and the role of the human handlers. Thus, pigs in research need to be subjected to housing and experimental conditions with care, keeping possible consequences for welfare in mind.

4.2.13 Housing/Management Procedures

Welfare of pigs might be affected by several housing-related factors, such as space allowance, nutrition, group size, static and dynamic groups, mixing pens (Verdon et al., 2015).

Animals are highly motivated to perform behaviors that lead to the experience of positive emotional states. Housing or management procedures that allow the animals to perform those behaviors promote positive emotional states/welfare.

Pigs are highly motivated to play (Zupan et al., 2016). The degree of expression of this behavior has been considered to be indicative of good welfare (Lawrence and Appleby, 1996; Muller-Schwarze et al., 1982; Newberry et al., 1988; Siviy and Panksepp, 1985; Spinka et al., 2001). Furthermore, play seems to be essential for the development of social and cognitive abilities of many animal species (Fagen et al., 1981; Smith, 1982; Spinka et al., 2001) and of high importance especially during the neonatal period (Spinka et al., 2001). As summarized nicely by Martin et al. (2015) pigs show the whole spectrum of play behavior—including object-, social-, and locomotor play—and point out that play in pigs appears to be sex and age dependent and might form a training for the future.

Play behavior (in pigs) might thus be used as an indicator of welfare and should be stimulated whenever possible. The, probably, most important factor in promoting play is the environment since it can either stimulate or restrict (Martin et al., 2015).

If we intend to stimulate a certain behavior, such as play, we need to familiarize ourselves with the characteristics of the different categories of play (which holds true for all ethological readout parameters). The literature provides several ethograms for play in pigs (Martin et al., 2015; Zupan et al., 2016). As soon as we know what we are looking for we can test play-stimulating procedures/conditions.

Giving pigs the opportunity to play during neonatal development is essential, especially when exposed to behavioral testing later in life. Martin et al. (2015) observed that piglets kept in enriched, thus play-stimulating, environments develop greater sociocognitive abilities.

4.2.14 Space Allowance

Space is essential to pigs since they need to be able to move, assess resources, and to interact with each other and to explore (Verdon et al., 2015).

This factor becomes highly important within farrowing crates in which the execution of the behaviors listed earlier is severely restricted (Chidgey et al., 2016).

4.2.15 Enrichment

Enrichment is believed to improve animal welfare (van der Staay et al., 2009) allows, as stated (Ferguson, 2014) for pigs, the animal to fulfill its needs and to adapt to its environment. Based on these considerations, chains and bite sticks (Scott et al., 2006) and/or rooting material (Studnitz et al., 2007) are frequently given to pigs.

In order to decide on certain forms of enrichment, it is essential to take the animal's biological (behavioral) needs into consideration. The enrichment should be useful for the animal, as inappropriate enrichment can even lead to fear, anxiety, or stress. Pigs may lose interest in biologically nonrelevant enrichment quickly (Newberry, 1995; Van de Perre et al., 2011).

As pointed out by van der Staay et al. (Chapter 3) (cognitive) enrichment might be provided unintended: when testing animals, prior training is often required. This training and testing itself might act as (cognitively) enriching. Thus, while "unintended" enrichment might improve welfare on one side, at the same time it might mask the effects of experimental manipulations (Grimberg-Henrici et al., 2016; Westlund, 2014) In an attempt to make use of the (at least potentially) welfare increasing nature of such cognitive enrichment, the potentially experimental result-confounding characteristics need to be identified first.

4.2.16 Lighting Conditions

Operant preference tests in pigs revealed that they have a preference for lower light intensities (Roelofs et al., in press). This finding might be implemented in housing of pigs in general but also in experimental setups. Carrying out experiments under low light intensities does very likely correspond to the animal's needs and might thus improve welfare during testing.

4.2.17 Handling

Early life experiences, such as handling are quite influential as described earlier in this chapter and pigs have been found to be less fearful later in life when (positive) handling started early in life (Hemsworth et al., 1989).

4.2.18 Testing

Welfare might be at stake at different stages in a pig's life. This is especially important to realize when pigs are intended to be used as animal model species. Early welfare-impairing life experiences have a great potential to affect experimental results (e.g., during cognitive testing). But, when pigs are exposed to experimental studies their welfare might be at stake as well. Thus, within the test- and model evaluation process, the possible consequences of testing on the animals need to be investigated. Otherwise, the validity of the gained test results might be at stake (van der Staay et al., 2009).

Van der Staay and coworkers (see Chapter 3) raised another welfare-related concern within experimental animal work, applicable of course to pigs as well: widely accepted and implemented actions are taken in order to reduce the number of animals being used. This entails sometimes excessive reuse of animals. What does that mean for the welfare of those animals? It is of course desirable to reduce the number of experimental animals (while still guaranteeing the validity and thus quality of research). However, we urgently need to investigate the possible welfare consequences of reusing pigs in subsequent, additional tests or additional studies.

4.2.19 The 3Rs

Russell et al. (1959) introduced the principle of the 3Rs (refinement, reduction, replacement) to the research community. It is inherent to "replacement" that welfare is not at stake since living animals are not used anymore. "Reduction" might entail welfare consequences if animals are excessively reused. "Refinement" appears to be the most important "R" when it comes to animal welfare since it represents the attempt to reduce negative effects on the animal's welfare by refining husbandry and experimental procedures (Nuffield Council on Bioethics, 2005). Adherence to the principles of the 3Rs is legally required in many countries. Research on the potentially welfare-improving qualities of certain refinement procedures can benefit pigs being used as model species but at the same time pigs used on farms. This becomes even clearer when one realizes that research with pigs is for the largest part carried out on farms and that thus experimental and farm animals are for a major part exposed to the same (e.g., housing and handling) procedures.

4.2.20 Humane Endpoints

A humane endpoint represents the very moment in an experiment at which pain and/or distress, experienced by the animal itself is alleviated or ended by means of discontinuing a procedure or humane killing of the animal in question (see e.g., "Humane endpoints in laboratory animal experimentation"; https://www.humane-endpoints.info/en).

There is ample literature dealing with humane endpoints in general, but almost no literature on humane endpoints for pigs exists. However, Ellegaard et al. (2010) elaborated on possible humane endpoints for the minipig based on the consideration that pain, distress, and discomfort are fundamental for the development and implementation of humane endpoints. The authors list the following signs as a potential basis for determining humane endpoints:

- decrease in bodyweight,
- locomotor or obvious signs of discomfort,
- not wagging the tail or the tail is held between the legs (look for tail bites),
- the animal crouches in a corner (and does not react to people entering),
- ears and head are hanging,
- lacks normal curiosity, and
- change in the normal vocalization pattern upon touch.

—*Ellegaard et al. (2010, p. 174)*

Next to the potential indicators of pain, stress, and discomfort described in this chapter these signs might be useful in determining humane endpoints for pigs.

5 CONCLUSIONS

Recent years have seen an increase in the use of pigs, particularly young pigs, in experimental animal models. While it seems clear that pigs have strong potential as models for young humans, and that examining effects of early life management practices on later life will give us insight into farm pig welfare, the utility of pig-based models can only be tested and validated with the use of reliable and valid tests. The reliability and validity of the tests described in the current chapter have to some extent been determined, but concerted efforts from groups involved in pig research should be undertaken to further develop tools for testing effects of early life events on behavior and welfare in pigs.

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