Technical Guide

Handling, Dosing and Training of Göttingen Minipigs

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GÖTTINGEN MINIPIGS

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Introduction

For any type of work with animals, a profound understanding of the species involved is paramount. Conducting studies with Göttingen Minipigs in a research environment is no exception. Proper handling and training of the Minipig will greatly contribute to the success of the study. In lack of scientific data, it is assumed that the basic ethology of the Göttingen Minipig is no different from other porcine strains, either wild or in captivity. Practical experience backs up this assumption, but relevant research in this field is needed, especially concerning the extent to which selective breeding may have modified the needs and behaviour of the Göttingen Minipig (Ellegaard 2010)[1].

People with experience working with dogs can still apply their basic animal interaction skills but should be aware that dealing with a Minipig requires a different approach altogether. As dogs have been bred for human companionship for thousands of years, understanding their behaviour almost comes naturally to us. Pigs have been domesticated for many years as well, but only as a source of food, despite the fact that pigs can be excellent companions. Our attitude towards them, and vice versa, is therefore slightly different. Another important difference in respect to the dog is that pigs are not predators, but prey. This means that we are dealing with a wary and shy animal. It takes some effort to gain its trust, but once established Göttingen Minipigs can be accustomed and trained to actively participate in a study. This creates a situation where humans work with the Minipig rather than against it, thus drastically reducing the stress factor for everyone involved.

Biology – facts about the pig

This chapter provides a general explanation of some of the important aspects of the biology of the domesticated (*sus domestica*) and wild pig (*sus scrofa*) and the Minipig which are relevant in this context. Basic behaviour and senses have not changed much through domestication; minor variations from strain to strain have not been accounted for.
In this booklet, growth, genetics and reproduction refer strictly to the Göttingen Minipig.

Ethology of the pig

Pigs are social animals. In the wild they live in family groups usually comprising related females and their offspring. Young males typically band together once they are sexually mature and form their own groups. Mature boars leave these bachelor groups to lead solitary lives once they become sexually active.

Even in a domesticated setting there is evidence of pig bonding and grouping, forming a dominance order. Pig groups develop stable hierarchies of a simple linear type that are maintained through the avoidance and submissive behaviour of lower ranking individuals (Curtis 2001)[2]. The largest animals are not necessarily the dominant individuals (Ewbank 1971)[3] but Francis (1996)[4] suggests that mixing pigs by weight heterogeneously will reduce hierarchical conflict and decrease the intensity of fighting by clear weight differentiation. If new pigs are introduced into a group there will be fierce fighting, but the level of aggression drops dramatically after about one hour (Symoens 1969)[5]. Odour masking by applying pheromones and/or artificial compounds to all pigs when mixing then has little, if any effect in limiting aggression and increasing hierarchical stability (Gonyou, 1997)[6], (Friend et al. 1983)[7]. Unless they are used for breeding, boars can be kept in groups. In a production context, the boars are grouped as early as possible, usually at weaning (4–5 weeks of age) and kept together until they are shipped to the recipient unit. Aggression among boars is rarely a problem in such a group, even if they are temporarily parted, but they will mount each other. Pigs do not engage in allogrooming and there are no reports of strong individual affiliation. On the other hand, pigs in a group have a tendency to coordinate and synchronize behaviour in space and time. In spite of their tendency to synchronize, pigs differ a lot individually in their behaviour. Most probably the personalities of pigs consist of several dimensions, similarly to what has been learned about humans, other primates or dogs (Spinka 2009)[8].
Humans are important components in the social environment of domesticated pigs.
Feral pigs have home ranges which vary in size. Scientists do not agree on the specific area required by pigs, but it appears that pigs will occupy whatever space is provided for them, since exploratory behaviour is almost as important as foraging. If food availability is not an issue, then the space will be utilised for exploration and for avoiding dominant individuals.

Behaviour

- When the ears are held back against the neck, the pig is probably afraid.
- An erect tail signals danger and when clamped down signals submission.
- A wagging tail represents contentment.
- A stiff legged, arched back posture is most likely threatening.
- There are ranges of vocalisation that are relatively easy to recognise. A warning call sounds similar to a dog's bark and if a pig 'barks' in fear, the rest of the group will immediately repeat the sound and either run or freeze and listen intently. Pigs make grunts of greeting and have squeals which indicate submission. Sows and piglets have an interesting 'vocabulary' all of their own.
- Rooting, grazing and browsing are feeding behaviour in pigs. If given the opportunity, a pig will spend a large amount of its time rooting, which is a need specific to pigs. Pigs are also strongly motivated to explore environments: rooting, chewing and checking scent are examples of exploratory behaviour and, as with rooting; pigs will spend a lot of time on these activities.

- Another behaviour worth mentioning is wallowing. Pigs need to wallow to keep their body temperature down in warm weather. They prefer wallowing in mud although a bath in water is also acceptable.
- Pigs have specific resting sites, which are often made into nests using grass, and the whole group will sleep in this nest. Pigs huddle, irrespective of the amount of space provided in domestic settings for resting nests, and huddling is innate behaviour. If materials are provided, pigs will spend time in nest-building activities before resting.
- Sexual behaviour includes males using their snouts to root the side of female pigs, resting the head on the back of others and mounting. This behaviour is exhibited by pigs as young as 5 weeks old. Immature males 'ride' each other; presumably this behaviour has an element of practice but is principally related to status. Boars are indiscriminate and will attempt to mount sows regardless of whether they are in oestrus. They will also mount lower ranking males. Thus, mounting behaviour will not disappear from a group of pigs with age.
- Abnormal behaviour tends to appear when the environment is inappropriate. In domestic settings, stereotypical behaviour such as bar biting (where pigs close their mouths over the bars of their housing and run their heads repeatedly from side to side) are believed to have developed as a result of frustrated foraging behaviour. Where pigs are overcrowded, they may initiate tail-biting and belly-nosing (persistent rooting the snout into the belly of another pig in the pen, prolonged and potentially injurious, and differs from sexual 'nosing' due to persistence). Tail-biting is also believed to be associated with impoverished environments and may result from frustrated exploratory behaviour.

Biology

Vision

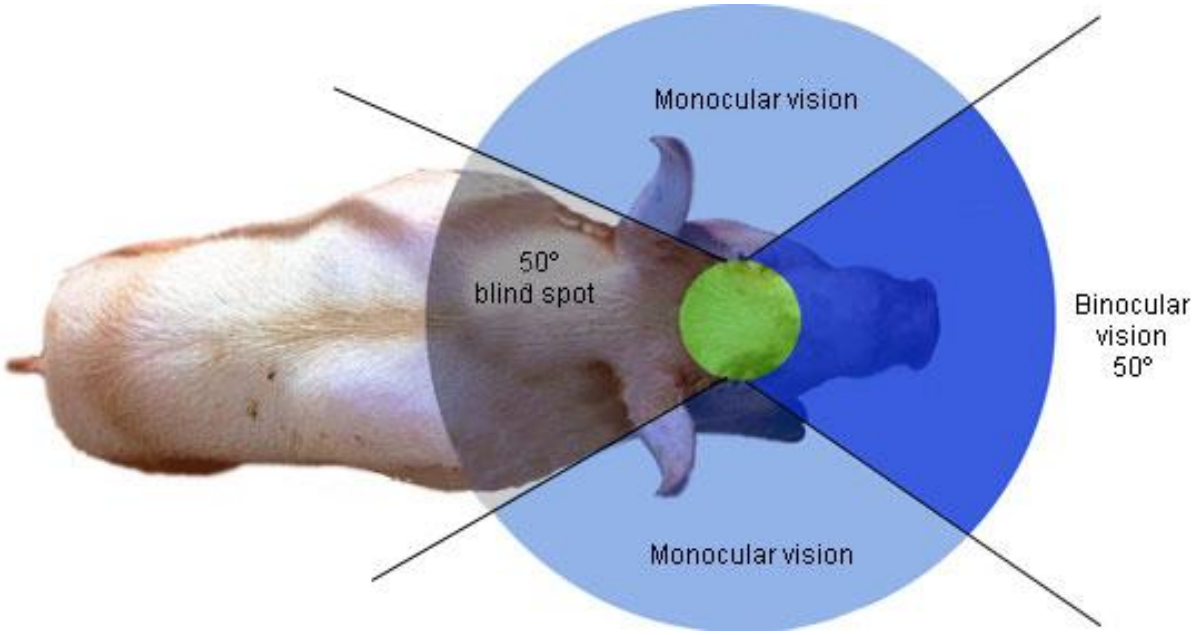
Little is known about the visual capacity of the pig. The studies that have addressed the ability to distinguish size and shape found large individual variation. E.T. Gieling et al (2011)[9] concluded that contrasts are difficult to distinguish, as are smaller symbols at close range.

As for pig colour vision, it is still a source of debate. Most probably pigs can discriminate blue from other colours by hue and exhibit red–green colour blindness but do not perceive contrasts well. Tanida (1991)[10] Neitza and Jacobsa (1989)[11] examined pigs' eyes with electroretinogram (ERG) flicker photometry. They revealed an average maximum sensitivity (λ max) at 439 nm and 556 nm (blue to green), thus a retinal basis for dichromatic colour vision.

Tanida also found that visual as well as auditory cues are more important than olfactory cues in distinguishing between people. (Tanida 1998)[12]. On the other hand, Kittawornrat (2010)[13] concluded that pigs have some ability to discriminate among colours but not much information is available on the impact of colour on pig behaviour.

Pigs generally prefer areas with light rather than dark spaces (Tanida 1996)[14] even though in nature they are most active at dusk and dawn. That might have something to with the relative safety from predators during this period. This preference for bright areas should be keep in mind when moving pigs. Have the place where you want the pigs moved to well illuminated but do not shine the light directly into the eyes of the animals (Grandin 1982)[15].

Their field of vision is typical for a prey animal. The visions panoramic range is 310° (Prince 1977)[16] but out of that binocular vision accounts only for a relatively small angle of 35-50°. Binocular vision is sacrificed for the benefit of a large field of monocular vision to remain alert to and detect any danger.

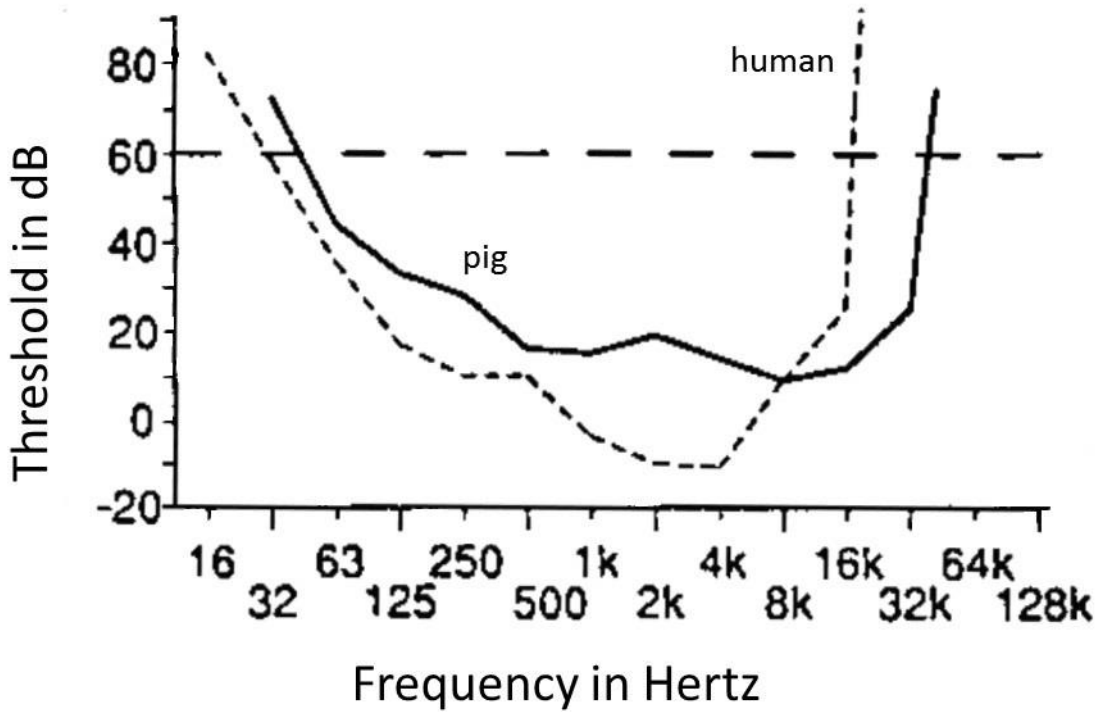


From: https://www.pig333.com/what_the_experts_say/pig-vision-and-management-handling_981/

Hearing

The pig's hearing range is from 42 Hz to 40.5 kHz with the highest sensitivity at 8 kHz. It exceeds the human hearing range in the ultrasound range (Heffner 1990)[17]. In regard to sound localisation, pigs are more accurate than many carnivores with a threshold of 4.6°, not far behind humans (1.2°) (Heffner 1992)[18].
Sounds that cannot be heard by the human ear can affect pig behaviour. Practical experience shows that pigs quickly accustom themselves to a wide variety of sounds, even at high levels, but react to sudden noises or changes. However, sudden as well as constant loud noise is aversive to pigs and should therefore be avoided. Noise levels in buildings where pigs are housed should be kept below 85 dB (Broucek 2014)[19]. It is not quite clear if playing music has a beneficial effect on behaviour or welfare of pigs. This area seems to be notably under-investigated.

Pigs have a rich repertoire of vocal signals, there might be up to 20 different signals. We do not yet fully understand their vocalization, the pig's vocal ethogram is not fully mapped, but some are easily recognizable. A warning call sounds similar to a dog's bark and if a pig 'barks' in fear, the rest of the group will immediately repeat the sound and either run or freeze and listen intently. Pigs make grunts of greeting and have squeals which indicate submission. The grunt is one of the most common sounds, given in response to familiar sounds or while looking for food (rooting). A short grunt is given when the pig is excited, while a long grunt is a contact call and normally associated with pleasurable stimuli. When pigs are aroused, or anticipated they may squeal, and they may scream when hurt. Squealing occurs as well when they are unhappy with a situation. Sows and piglets have a distinctive 'vocabulary' all of their own.



From: Heffner H.E. & Heffner R.S (1992)[18]

Olfaction

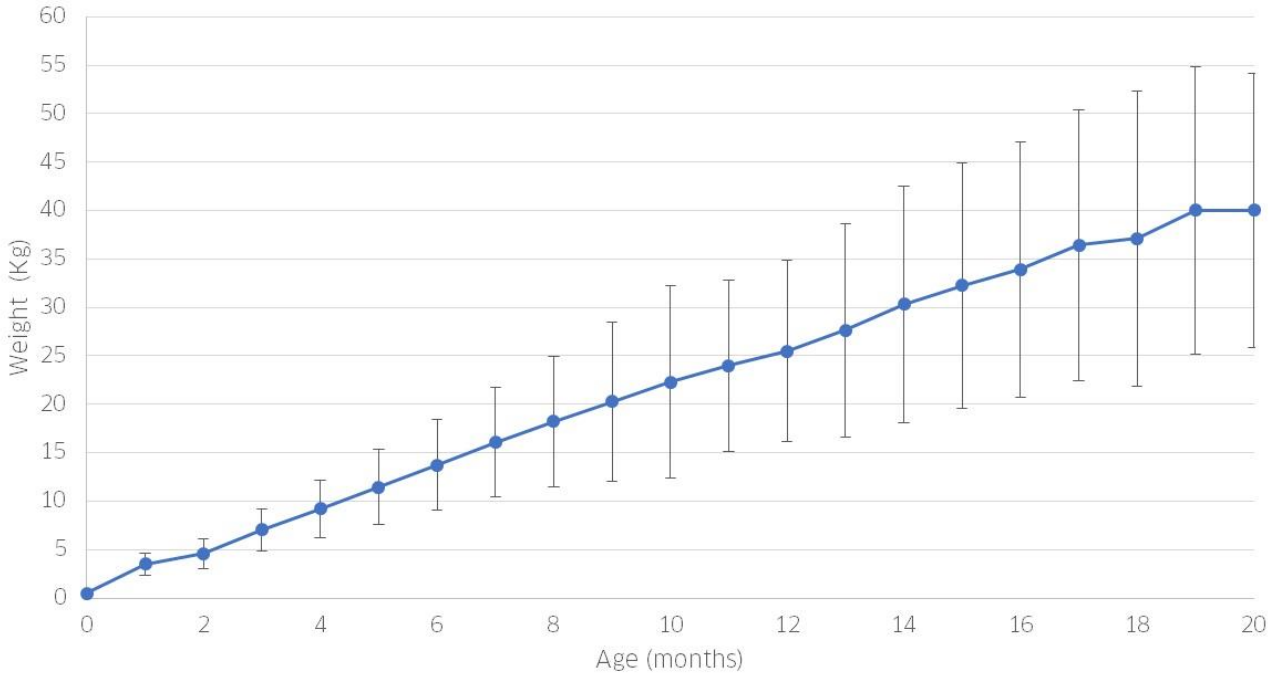
Olfaction is the principal sense in pig communication although there are visual and auditory signals of importance as well. Pigs have a very acute sense of smell that is developed early in the life. It is important for survival and pigs use a wide range of olfactory cues in their natural behaviour. They use this type of information as the predominant basis for individual recognition and it plays a special role in reproductive behaviour. In an operant testing situation, sows have been able to distinguish between otherwise identical cards they have previously touched or not touched. (Hafez 1962)[20]. A study by L. V. Søndergaard provided evidence that the Göttingen Minipig has olfactory discrimination, which makes it possible to obtain reliable measurements of olfactory sensitivity to detect early behavioural changes in future porcine models of Alzheimer's disease[21].

Cognition

Cognition refers to the conscious mental processes – e.g. feeling, thinking, learning, remembering, communicating, imagining and solving problems – which are presumed to occur in a pig. Having more thorough knowledge of pig cognition would broaden our basis for better management of these animals by employing ways and means that are more supportive of health, performance, and overall well-being. The cognitive abilities of pigs have not yet been thoroughly characterised. It is known that pigs communicate vocally as well as by olfaction. Also, they can be easily trained to perform operant tasks to gain rewards. I. G. Cerbulis[22] found that pigs were indeed capable of complex learning in tasks requiring discriminative responses to different gestural and verbal symbols regarding a variety of objects and actions. Consideration must be given to the visual capacity of the pig. The pig's ability to make use of symbols in these ways reflects the cognitive ability to abstractly represent stored information, which is essential for understanding concepts and using language. Hence, there is reason to expect that pigs are able to use language.

Growth of the Göttingen Minipig

The average weight at birth is 450g, and the Minipig will have added between 2 and 3kg by the time of weaning (4–5 weeks). In the first year, Göttingen Minipigs gain roughly 2 kg a month and after this first year they weigh between 20–25kg. They end up weighing about 35 - 40kg at maturity, i.e. when they are 2 years old.



Reproduction and sexual maturity of the Göttingen Minipig

Males are sexually mature at the age of 2-4 months[23], whereas females come into oestrus for the first time at the age of 4-6 months and subsequently have an oestrous cycle of 21 days, unless they are pregnant. Recent studies (Peter et al., 2012[24] and Peter et al., 2016)[25] show that there is a great variation in the onset of sexual maturity in females and it can be as late as 7 months. The signs of approaching oestrus are seen in form of reddening and swelling of the vulva, increased general activity, nosing and mounting other sows. The signs of oestrus can be more, or less noticeable, depending on age or if any sexually mature boars are in the vicinity. However, the lack of obvious signs does not necessarily mean the animal is not in oestrus or has not reached sexual maturity. The Full oestrus lasts between 30 and 72 hours. Gestation is 115 days.

Sexual behaviour, females

Proceptive behaviour starts about 2 days before the so-called standing oestrus, when the sow will adapt a standing reflex, a rigid stance with some lordosis when courted by the boar or when pressure is applied to the rump. This proceptive behaviour can include seeking out a boar to initiate courtship. In controlled settings, where females are kept away from males, this behaviour is often redirected towards other pen mates and will include nosing their flanks and mounting them. If allowed to mate the female will stand still and let the boar mount when she is ready to conceive.

Sexual behaviour, males

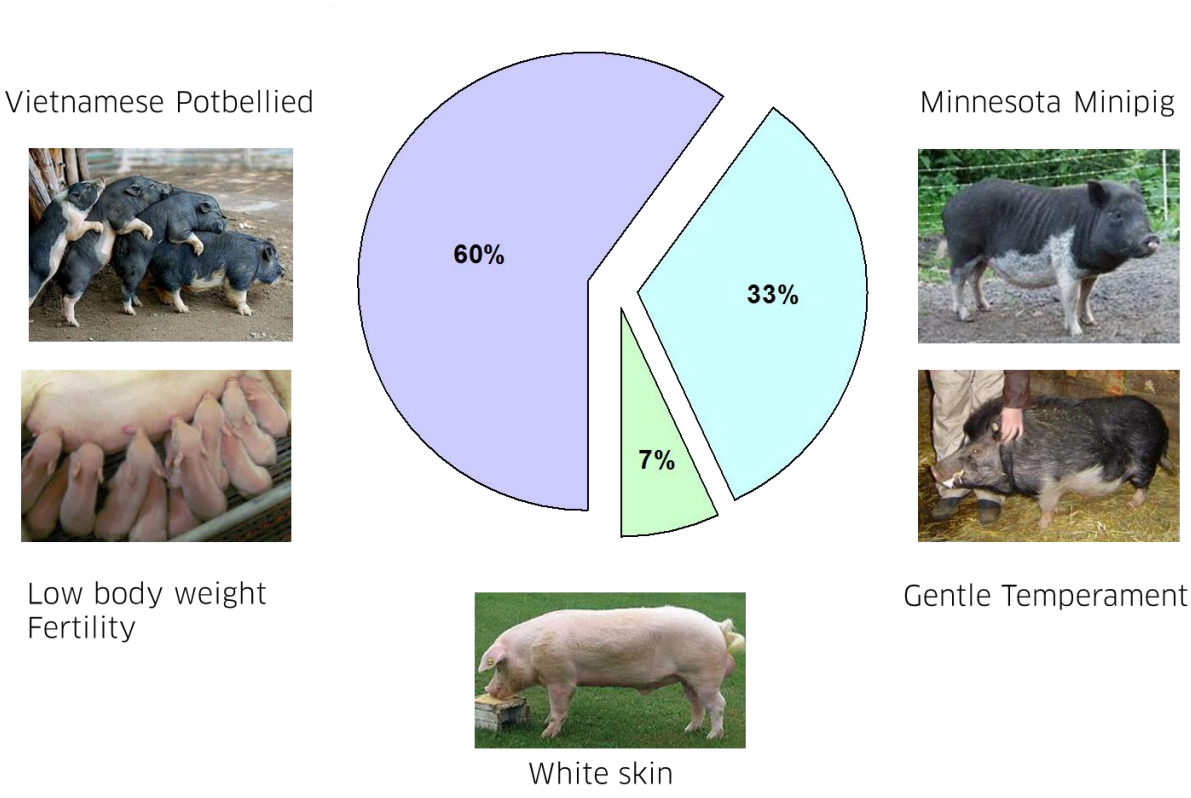
Boars are less active in seeking oestrous females and show relatively poor discrimination when given a choice between oestrous or anoestrous individuals. If kept in groups they will mount each other to perform

sexual activity. When allowed to mate, the courtship however plays an important role in inducing the standing reflex. During courtship boars approach females, utter characteristic short grunts and champ their jaws while salivating until it froths. Other courting behaviour includes stretching the forelegs to emit a scent from glands located there, sniffing the sows head and genital region, nosing and nudging the flanks, until he mounts.

Genetics

The Göttingen Minipig breed was established at the University of Göttingen from 1960 to 1970 by crossing two strains of Vietnamese Potbelly pigs with Minnesota Minipigs and adding the German Landrace for un-pigmented skin. Currently there are four breeding populations in the world: in Germany, the US, Japan and Denmark respectively. The genetic management of these four production sites is provided by the University of Göttingen for the following purposes:

- to maintain genetic integrity by avoiding inbreeding (as far as possible) and genetic drift
- to pursue desired breeding objects (smaller size, gentler temperament)
- to balance the adverse effects of inbreeding
- to maintain the genetic uniformity of the subpopulations (Simianer et.al. 2010)[26]



Breakdown of the different pig breeds in the Göttingen Minipig.

Animal Training

This section explains the basic principles of animal training and the part this can play when working with the Göttingen Minipig. It is important to note that whether we are aware of it or not, we train animals in our care simply by interacting with them. Because of this fact, it is important to understand these principles and use them to our advantage. Practical examples are given for various situations, followed by a brief guide on how to implement a formal training programme and train with the help of a clicker.

Background

The capacity to learn is a fact of life in almost any organism with at least a rudimentary nervous system and takes place through classical and operant conditioning. Classical conditioning takes place in simple animals, and continues to occur in humans, because any animal can benefit from making anticipatory reactions. All animals need to prepare for the future. Creatures like a sea slug follow the simplest rule: "if it happened before, it may happen again." After A has been paired with B a few times, A is treated as if A predicts B. Because it is so simple and basic, classical conditioning does not require conscious thinking or language. It can influence people or primitive organisms without a conscious thought process. (Dewey 2007)[27].
If there is a potential to learn, then things can be taught and trained as well. This process will happen repeatedly throughout their lives and has no starting or ending point per se. If we hold or take part in a training session, then this is only a defined part of the entire life-long learning process. On the other hand, we are often learning or training without being aware of it.

Animals have been trained by humans for centuries, although humans have never really understood the underlying mechanisms. Developments in behavioural psychology produced some understanding in the 20th century. Scientists like Pavlov, Watson and Skinner used animal models to understand and describe behaviour and learning. Later, this information was used to train animals effectively and responsibly – with the voluntary participation of the animal.
Pigs are particularly swift at grasping the training 'game'. This guide deals in general terms, but there can be significant individual differences and personalities in the animals we work with, and these will be clearly expressed when training.
Conditioning pigs to work with the clicker is the most effective. They are an intelligent species and, like all animals, they learn according to a set of scientifically proven principles, collectively known as Associative Learning, including the principles of Classical and Operant conditioning.

Learning is achieved through trial and error: pigs do what works for them!

Although training has become widely popular in working with animals as pets, in zoos and as performing animals, it is still not a standard procedure for working with laboratory animals. The benefits, however, are manifold: improved welfare, reduction of stress for human and animal, enrichment and exercise, all leading to better results for the study in which the animals are involved. Apart from this, the workload is often reduced (no lifting, less personnel) for the benefit of better working conditions and saved man-hours.

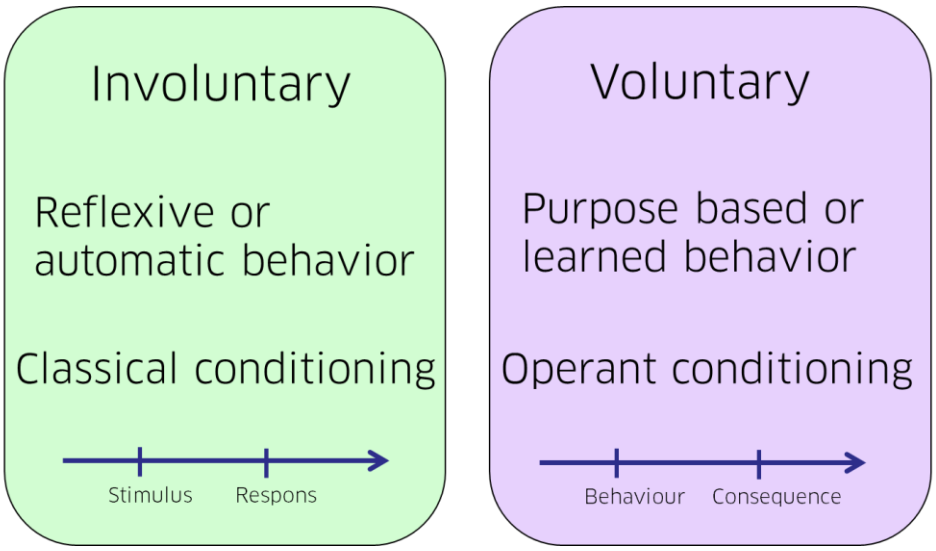
An initial investment of time and effort is necessary to get started on a training project, but companies that have implemented a training programme will agree that the dividends are enormous. Training skills acquired by trainers can of course be used with other animals in care, as the fundamental principle of animal training is the same for all species.

Areas where training has been successfully applied with Minipigs:

- socialising and general habituation to equipment and procedures
- husbandry tasks, such as weighing, trimming of hoofs and moving
- dermal applications
- subcutaneous dosing
- nasal dosing

Behaviour and learning

Two terms are important here: classical conditioning and operant conditioning. Extensive explanation and descriptions of these terms are available in any book about psychology or dog training (e.g. Dewey 2007[27] and Prior 1984[28]). An abridged version of them is provided in the following:



In real life, classical conditioning and operant conditioning are not always clear-cut and definitely not as separate from each other as they seem on paper. When we train or teach, we commonly refer to this as operant conditioning as well. Often classical conditioning is initially used to condition the animals to our tools, such as a clicker which is turned into a conditioned stimulus. Then, building on natural or reflexive behaviour, we teach the animal a purpose-based behaviour that will be consciously executed to bring about a desired consequence.

Under operant conditioning, behaviour has consequences. There are four possibilities.

Action - Reaction	Reinforcement behaviour increases	Punishment behaviour decreases
Positive Something added	Positive Reinforcement Something added increases behaviour	Positive Punishment Something added decreases behaviour
Negative Something removed	Negative Reinforcement Something removed increases behaviour	Negative Punishment Something removed decreases behaviour

There can be a great deal of misunderstanding surrounding these terms, mainly because of the words "positive" and "negative". In this context, their value is strictly defined in a mathematical sense: something is added or removed. In other words, the terms "positive" and "negative" have nothing to do with "good" or "bad".

By definition, reinforcement increases behaviour and punishment decreases behaviour, regardless of whether this is intended by the trainer. I.e. if the trainer intends to reinforce a behaviour by scratching the animal behind the ear and the behaviour decreases as a consequence then it is in fact positive punishment, rather than positive reinforcement.

Even though these four terms can be confusing, it is important to understand them for training purposes.

Examples:

Behaviour	Consequence	Result	Term
I go to work	I get paid	I will go to work again	Positive reinforcement
Pig steps on scale	Pig gets food reward	Pig will do it again	Positive reinforcement
I touch something hot	I get burned	I will not do it again	Positive punishment*
Cattle touches electric fence	Cattle gets an electric shock	Cattle will not go near the fence again	Positive punishment*
I get into my car and do not buckle my seatbelt	Alarm sounds	I am annoyed by this alarm	Positive punishment* followed by Negative reinforcement
I buckle my seatbelt	Alarm stops	I am relieved	

**Important notes regarding punishment*

When using positive punishment in a training session, it is very important that the consequence is ***immediate*** *and* ***unmistakably*** *linked to the behaviour. This is the case in the first two punishment examples. If not, we produce animals that are confused and frightened.*
It is equally important that there is an easy alternative to the behaviour being punished, either avoidance of the punished behaviour or doing something else.
The last example illustrates that the link between behaviour and punishment must be understood: If I do not know why the alarm is sounding, I will not be able to stop it. As positive punishment is linked to negative reinforcement in this case, avoidance is not being trained, as it is in the other two examples. To avoid being punished requires behaviour that is negatively reinforced.
For novice and less experienced trainers it is advised to train by positive reinforcement exclusively. Mistakes, which inevitably are made by the trainer, will not harm the animal with this approach, unlike when using punishment; they only slow down the progress.

Minipig training with positive punishment – negative reinforcement

Positive punishment includes all corporal punishment; this is very powerful and should only be used by experienced trainers, as adverse and counterproductive results can occur. In conjunction with negative, reinforcement, however, it provides a fantastic tool for training. There is a correlation between positive punishment and negative reinforcement as indicated by the arrow in the table above. In order to execute negative reinforcement (to remove something to increase behaviour) this must be added before, through

positive punishment. This method can be used in daily interaction with the Minipig as well as for specific training sessions.

Example 1: I pick up the Minipig and hold it in my arms. The Minipig starts to struggle, so I increase my grip and hold it a bit tighter (positive punishment).
The pig stops struggling and relaxes; I loosen my grip immediately and give it some space (negative reinforcement).
I repeat this and eventually the Minipig has learned not to struggle. After this I can reinforce calm behaviour with a treat (positive reinforcement) if I wish.

Example 2: Sling training: I put the pig carefully in the sling; the pig tries to get out. It is gently but unmistakably pushed into the sling (positive punishment).
As soon as the animal relaxes, I remove my hands and with that the pressure (negative reinforcement).
It is important to note here that only one person is dealing with the pig and that the signals are precise and correctly timed so they can be understood with the right connection.

Minipig training with positive reinforcement

Positive reinforcement training is basically to reward desired behaviour and includes treats (something added). For practical reasons this is often food but could be all sorts of things that would reinforce the behaviour.

Food treats are easy to come by, easy to administer and very effective. For Minipigs, ordinary lab diets can be used, but the animal should not be fed just before training. By setting aside the amount of lab diet used for training from the daily ration, the total food intake can be controlled, if this is an issue. Otherwise pieces of apple are fantastic and, as pigs have a sweet tooth. Raisins and chocolate also work but beware of the possible weight gain from using the latter.

Here is the most important message regarding treats:

Every treat given must serve a purpose, i.e. reward behaviour or establish trust– do not just feed the animal (with special food) and hope it will love you for it!

Training the animal

In formal training using positive reinforcement, the use of a conditioned reinforcer like words, a whistle or a clicker is a great help. The sound of a whistle or clicker has no meaning to the animal by itself but is neutral. Only once it has been conditioned by pairing it with a treat, it becomes a significant signal that makes it possible to communicate with the animal. The clicker has become so popular that positive reinforcement training is often called “clicker training”.

Using the clicker has the following advantages:

1. It is a fast, accurate precision tool.
2. It enables the trainer to accurately reward the pig for behaviour performed a short distance away, or to single out a specific behavioural trait amid a behavioural chain.
3. It eliminates the problem of the pig having to single out information from other sources, e.g. lots of words or handling.
4. It is not voice-dependant and can be used for training by any staff member.
5. It is unemotional.

These five points combine to make the clicker a very powerful communication tool.

The word **communication** should be emphasised here, as all the trainer really wants is to communicate with the animal: by doing this, you get that – and the pig will happily oblige the trainer if the reward makes it worthwhile.

Clicker training is a "hands free" method of training, as the power and clarity of communication through the clicker/reward represents the most efficient and effective use of a limited training session compared to other training methods.

Being hands free, clicker training also has the advantage of being a powerful tool in relationship-building with animals that have little previous experience of handling or being trained and consequently minimises the stress associated with handling in other training methods.

Training the trainer

It is important to note, however, that results of animal training are inextricably linked to and reliant on the mechanical skills and technique of the trainer. Once trainers have mastered the clicker technique and understand and apply the principles of associative learning, it is possible to teach pigs anything of which they are physically and mentally capable.

Trainers need to be skilled and knowledgeable in the following areas:

1. Observation of the pig and behaviour.
2. Introducing and using the clicker.
3. Use of the clicker to mark a specific behaviour.
4. How to initiate new behaviour
    - utilising spontaneous behaviour
    - luring
    - targeting
    - shaping
    - using a combination of the above
5. How to lengthen and strengthen specific behaviour.
6. How to add cues and signals.
7. Putting behaviour under cue control.
8. Generalising responses and combating distraction.

It may sound complex and difficult, but do not be discouraged: it is not. Of course, it takes a lot of effort to become a master trainer, but any person with a keen interest can learn the technique and get results within a few hours. In the list above, the first 5 points are most important in a research (short-term) situation.

When using positive reinforcement or positive punishment/negative reinforcement, it is important to keep track of the progress you are making. If you do not make progress in two consecutive sessions, the training procedure should be questioned. It is possible that you are using the technique ineffectively, that the animal is distracted/tempted by things in the surroundings or that you are trying to train incompatible behaviour. Many possible causes may need to be investigated.

Brief instructions for targeting

Targeting involves teaching the pig to touch part of you, something you are holding or some other object. Touching will be done using the snout. Targeting is a very good start, as it is simple, and results are achieved quickly. The trainer and animal will gain confidence in the system and can move on from this base to train behaviour.

Stage 1:

With a naive pig, you always start out with socialising: the pig should not be scared in the training situation.

If the pig is comfortable in the situation and will eat a food reward thrown, then you can start conditioning with the clicker. This is done by clicking and throwing a food reward towards the pig, a fraction of a second later. Do this for two minutes and repeat after a two-minute break. Most Minipigs will have understood the principle of the clicker after the sequence has been repeated sequence three or four times. Now that the clicker has been conditioned, you can start to work with it.

Stage 2:

Teach the pig to "TARGET" your hand or a hand-held target. A hand-held target offers more possibilities, especially if the pig is shy. Offer your target to the pig by placing it close to its snout. Click and treat (C/T) as its snout contacts your fingers/target. Repeat several times until the sight of your target produces an enthusiastic bump from its snout on it. Make the pig look for your target by offering it in different positions in relation to its head.

Stage 3:

Change position to encourage the pig to move short distances to reach your target and to move in different directions and on different levels.

Stage 4:

If your pig is confidently looking for your hand, moving towards it and bumping it strongly with the snout as soon as you present it to the pig, you may be ready to add a cue for this behaviour. Try the following: use your cue ("touch" is a suitable choice) and then present your target to the pig. C/T as it touches the target. Keep offering your target in a variety of places and at different levels.

Stage 5:

Encourage your pig to follow your target by giving the cue and presenting your hand but as the pig advances towards it, move your hand a few inches so that your pig has to "chase" it to keep up. Gradually make the movement into a larger sweep, encouraging your pig to follow by C/T when it catches up to and touches your target. Always make sure that the moment comes when the pig gets the opportunity to touch the target. Keep the "following" distances short to begin with and expand the distance covered. One aim of this behaviour is to move pigs to the weighing scales, or any other place you want it to go.

There is no need to use cues, but for long-term training situations it can be useful.

Terminology

Reinforcement	Anything that occurs with or follows behaviour that tends to increase the likelihood that the behaviour will recur.
Primary Reinforcer	Any stimulus whose reinforcing effect is immediate and not a function of previous experience (e.g., food, water, warmth).
Secondary Reinforcer	Any stimulus whose reinforcing effect is a function of its association with a primary reinforcer (clicker).
Punishment	Anything that occurs with or follows behaviour that tends to decrease the frequency of recurrence.
Positive	Something is added.
Negative	Something is removed.
Extinction	Method of eliminating behaviour by not reinforcing it any longer.
Bridge	Any stimulus that tells the animals that it just did something correct and reinforcement is on the way (clicker).
Continuous Reinforcement	A schedule of reinforcement where the desired response is reinforced every time it occurs. Trainers typically use a continuous reinforcement schedule when the animal is in the process of learning a new behaviour.
Discriminative Stimulus (cue)	A conditioned stimulus which, when presented, results in the occurrence of a specific response.
Target (noun)	Any object that identifies a location for an animal (e.g. a trainer teaches a rhino to touch its lip to the end of a stick). When this technique is used to shape behaviour, it is called “targeting.”
Shaping	Using selective reinforcement (approximations) to modify a general response into a specific response.

Handling

Introduction

Animals continually observe and gather information from their surroundings, then process those stimuli and respond to it. This process can be described as learning. A similar definition could be that learning is a change in behaviour that results from previous experience.

Regardless of whether we are aware of it or not, as animal caretakers/handlers/technicians, we influence what the animals in our care learn. In other words, anyone who works with animals is teaching or training them at the same time. Sometimes we are aware of what we are teaching or training; we make conscious efforts to "train" animals to exhibit a variety of behaviours for husbandry or other purposes. Sometimes we influence (train) animals' behaviour inadvertently through our actions, our husbandry routines or through other stimuli present in the captive environment. In effect, animal-care staff is always training, and they need to be aware of this fact. Training is all about associations. The key to an optimal captive environment is to facilitate animals' opportunities to make associations that enhance their well-being.

The above is fundamental to all handling and handling in this context is every interaction with the animal. Personnel need to be familiar with pig behaviour and the greatest attribute of the handler is probably patience. Overreaction will greatly contribute towards the animal being frightened.

Anyone who works with animals – whether they are cleaners, caretakers, technicians or scientists – exert equal influence on how animals react and deal with certain situations. Everyone needs to be aware of this because everyone contributes to the successful outcome of a study.

As a general rule, imagine your Minipig is like your baby; it might be struggling with the same fears and uncertainties in a novel situation. Help it along by creating a comforting atmosphere, reassuring it and gently guiding its behaviour in the right direction. Pigs do not like stress and appreciate it if they are shown a way around it.

Acclimation, socialising and interaction

There is always an acclimation period, the length of which depends on company policy or type of study. Acclimation should not be regarded as idle time. In fact, during acclimation, we can already set the switches for easy handling and a successful study. It pays to make good use of the acclimation period (Tsutsumi 2001)[29]. Leaving animal alone in this time is not acclimation. Pigs are motivated to have visual and physical contacts with humans and refusal to have physical interaction and/or eye contact is aversive for most pigs. (Terlouw 2005) [30].

After delivery, let the pigs settle in for a day, give them peace and time to sniff around and become familiar with their new environment.

The next day you can start to socialise with the animals: feeding time provides a good opportunity, as this is a situation where they naturally get a reward. Remember that we are dealing with an animal of prey, so most likely it will be shy and wary, maybe even a little frightened.

It is important to understand “flight zone”. Pigs like most animals of the Artiodactyl order have a flight zone. This could be considered personal space and if a person enters this zone the animal will escape or try to do so. An animal's flight zone will vary depending on how calm or how tame it is. The flight zone gets bigger when an animal becomes excited. The flight zone is also bigger when you approach "head on". Your first efforts should be concentrated on reducing this flight zone or with other words gain its trust. Move slowly when you are around pigs and do not make abrupt movements, as this will frighten them. Squat down to seem less imposing and talk to the pig in a low, pleasant and encouraging voice. Throw a food pellet or treat to encourage it to come closer. Pigs are curious and inquisitive as well, so offer a hand to sniff as well, but let the pig come to you rather than the opposite. It takes a while to gain enough trust so that you can touch the animal. At first, Minipigs do not like to be patted, but once they trust you, they love to be scratched quite roughly with fingernails behind the ear or on the back. A gentle belly rub will be appreciated as well and tender fondling of the cheek shows your trust towards the Minipig. But be careful, because a frightened or frustrated animal might try to bite you in the latter situation. Keep in mind that they are individuals and not all of them might like or enjoy the same thing.

Here, too, you will discover that, in addition to these general traits, behaviour will vary greatly from one individual to another – as in all species.

If you have a formal training programme for Minipigs, start as soon as the Minipigs are not afraid anymore and eat the treats they are offered. This might not occur at the same time for all animals in the group.

Even if you do not have a training schedule, it is advisable to desensitise the Minipigs to the upcoming procedures: If a pig must be carried, then accustom it to being picked up and held on the arm; or if you need to take a blood sample, then train it for being placed on the V-trough, and so on.

Remember the principles of animal training: reward desired behaviour only and reward for a purpose. If the animal vocalises when you pick it up, do not put it down immediately, otherwise you reward squealing and teach the Minipig to vocalise to be put back down. The appropriate action is to use a gentle voice to calm the animal (like a baby) and give a food reward as soon as the noise stops. You can translate this to all other situations.

Whenever you are dealing with Minipigs, you should create a pleasant, undisturbed atmosphere: make it easy for everyone, set up the situation to succeed and take account of the hearing and visual capacities of the Minipig. Handle the animal with confidence and certainty but be gentle and caring at the same time. The Minipig needs assurance and clear guidance, and in this sense staff training is just as important as Minipig training.

If you have many Minipigs in your care, it would be advisable to keep records of the progress with socialisation in the acclimation phase. This will allow you to concentrate your resources and spend more time with the animals that are a bit behind, not as relaxed and friendly. The following table is a suggestion to record socialization and other welfare activities during acclimation. You can adjust it to your needs.

Socializing Minipigs during Acclimation **Week No.:**

Study No.: Room No: Study start:

Pig No.	Touching	Picking up	Behaviour		In sling							Walking/Exploring							Remarks
			on Arm	In Sling	M	T	W	T	F	S	S	M	T	W	T	F	S	S	

Give marks 1 to 3 every time the animals are in the respective situation

Touching:

1: actively looking for contact and likes to be touched and scratched
2: seeking contact, accepts some touching
3: shy, moves away when trying to touch, not seeking contact

Picking up:

1: calm no vocalization or struggling
2: short vocalization or some struggling
3: strong vocalization or struggling

Behaviour:

1: calm and relaxed during the whole procedure
2: some struggling, but relaxing after a while
3: fighting all the time and trying to get out of the situation.

Restraint

All restriction of movement can be considered as restraint. For Minipigs, less is often more and if a base of trust has been established during acclimation the job will be much easier.

Minipigs can be gently cornered for brief and minimally aversive treatment, or they can be trained to walk up a ramp into a box to give the user a more comfortable working position.

Dermal application, injections (i.m., s.c.), nasal dosing, catheter handling and similar would be considered minimally aversive treatments in this context.

Restraining a Minipig on the arm

Depending on their size, Minipigs can easily be picked up and carried on the arm. Grab onto a hind leg, put your other hand under the thorax and lift the pig with this grip. Hold it close to your body, forelegs over your arm and hold it loosely if the pig is happy. Try to avoid unnecessary shifting as this will unsettle the Minipig.


Picking up a Minipig supporting the thorax and holding it on loosely on the arm.


Minipig in a box for dermal application. Feed at the same time to distract and reward.
The same system can be used for other minimally aversive treatment like subcutaneous injection, attending to catheters, etc.

Restraining a Minipig in a sling

For various applications, restraining the Minipig in a sling is appropriate and quite well tolerated by the animal. Some training may be necessary to get it used to the device. Put the Minipig gently in the sling, hind legs first. You might want the assistance of another person to help guiding (without pulling) the legs through the openings. Otherwise, only one person should perform the task to control the positive punishment/negative reinforcement sequences.

Suggested applications for Minipig in a sling are: blood sampling, access to auricular or tarsal vein, attending to catheters ECG, NIBP, ophthalmology and vaginal dosing – in short, procedures that take slightly longer than would be comfortable for carrying the pig on the arm (size dependent). Even single dose oral gavage has been performed in the sling. Trimming hoofs as a husbandry task can also be done while the pig is in a sling.

When restraining the Minipig in a sling for blood sampling put the head at the short end of the sling and gently lift it up to stretch the neck. If you experience some resistance in lifting the head do not use force but take a good grip and firmly guide it higher and higher in steps, relaxing in-between. Alternatively, the fabric of the sling can be modified so that the head rests in the sling but is raised so the skin of the sample area is stretched. Access to the sample area is through a hole in the fabric from below. This hole should be covered by a flap and Velcro© at the time of placing the Minipig in the sling to avoid getting its head down that way.

Placing a Minipig in the sling. Take your time and do it gently, especially the first time.

For blood sampling the Minipig can be restrained in a short sling where the head is lifted and supported by an assistant.

Restraining in the V-trough for blood sampling

Restraining the pig in a V-trough for blood sampling from the jugular vein/vena cava requires some training for personnel and Minipig. Take your time if it is the first time for the animal, do it preferably during acclimation without taking a sample.

The Minipig is picked up as described under “Restraining a Minipig on the arm”, the tail tucked under and lowered into a seating position on the device. Secure the front legs and support the neck well and then roll it into dorsal recumbency. During this process the Minipigs might try to turn around in which case you will need a firm grip. If it is a larger Minipig an assistant can gently spread the hind legs to make turning around more difficult. Most likely the Minipig has its head raised upwards at this stage; gently extend the snout with one hand and lay the head down as the Minipig relaxes. With larger animals, an assistant may take control of the head with both hands. Covering the eyes has a calming effect at times. Up to three people may be required to hold the Minipig, depending on its size (forelegs, hind legs and head). Do not use excessive force and secure the hind legs in their natural position without pushing and pulling. If the Minipig is relaxed you only need to hold it loosely, but be ready to tighten up when it starts to move.

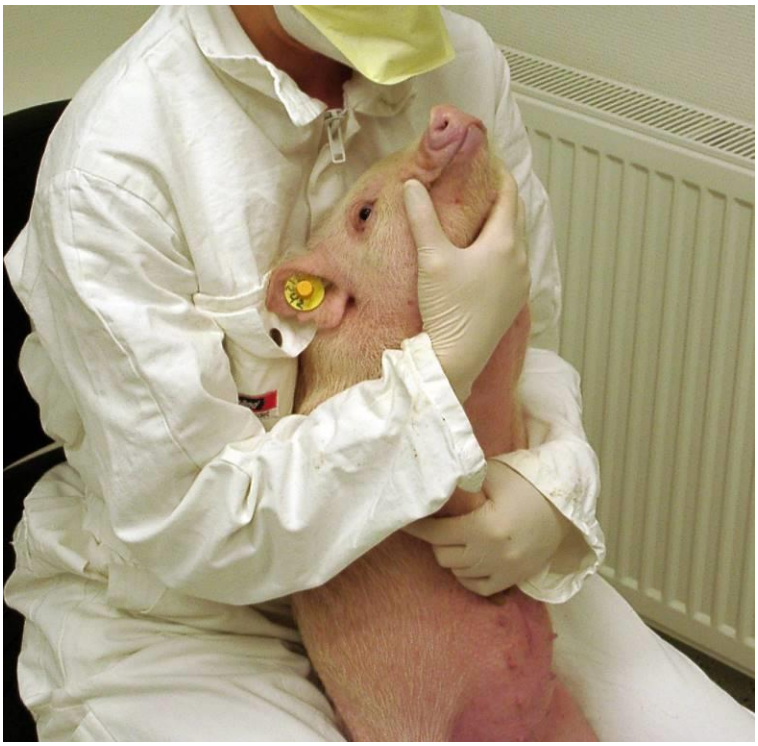
After sampling the Minipig is rolled in the reverse order onto the arm of the person controlling the forelegs.

Sequence of restraining a Minipig in a V-Bench.

Restraining by siting technician for oral dosing or blood sampling

Oral dosing is a stressful and unpleasant procedure for the Minipig. Training is not very beneficial here and should be restricted to the restraining procedure; a level of acceptance can be achieved. This procedure is also the one that requires the most restraining force. Find a good stable place to sit with the pig. A specially designed chair is helpful, especially for larger Minipigs. (Ellegaard Newsletter 47) Hold the pig close to your body and restrict its movements, particularly the head. Try to hold the spine straight and the head only in a slight angle. Perform the gavage quick and swiftly to reduce the time the animal is put under stress.

When restraining for blood sampling, stretch the neck and make sure the manubrium and jugular fossa, your landmarks for placing the needle correctly, are exposed.



Restraining a Minipig for oral dosing on a chair or bench.

Procedures

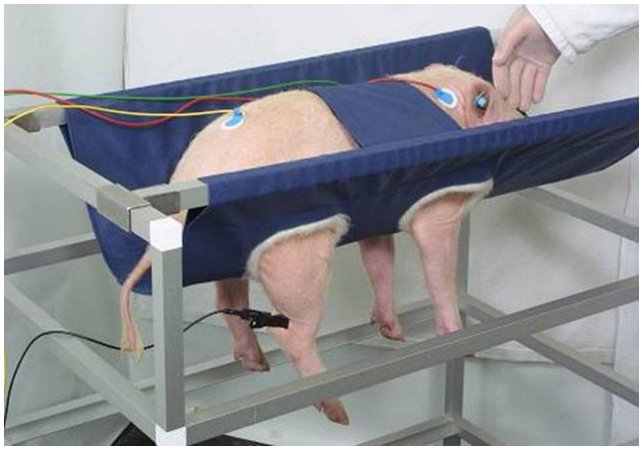
Weighing

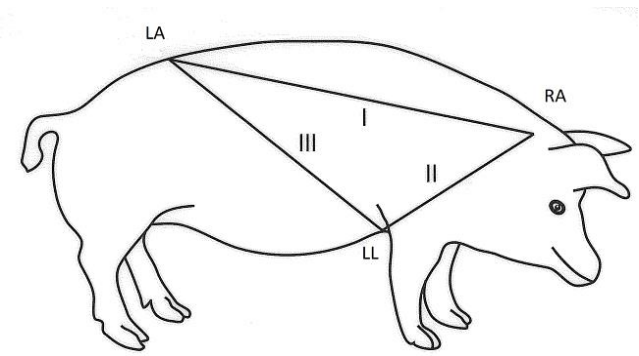
Smaller animals can be picked up and put in a box on a scale; the box will help to keep the Minipig calm and restrict it movements. Larger pigs can walk by themselves into a specially designed scale that can open on both ends: walk in – walk out. A Minipig can also be held on the arm by a technician that steps on a scale – the technician's weight will then be deducted.

ECG

The heart of the Minipig and other hoofed animals is oriented differently than in other common lab animal species, such as dogs. The projection of the Minipig heart is between the 2nd and 5th intercostal space, whereas the projection of the dog heart is between the 3rd and 6th intercostal space; the apex of the Minipig heart is projected toward the cartilage of the sixth rib. The QRS vector in swine and other hoofed animals is negative as it is directed from the sternum toward the spine. Because of these differences, standard limb leads are not ideal for recording ECG measurements in swine. Interpretation of the QRS complex is enhanced by using the axial lead system as the wave is significantly amplified, and the axial lead method may provide more accurate results (Nahas 2002)[31].

To record ECG from conscious Minipigs, first place them in a sling. Clip the hair at each location before placing the tabs for the electrodes. ECG electrode tabs developed for humans work well with Minipigs. Traditionally an alligator clip is used as the ground with minimal discomfort observed in the Minipig. However, if discomfort is observed, using cuffs or ECG lead pads avoids pinching.



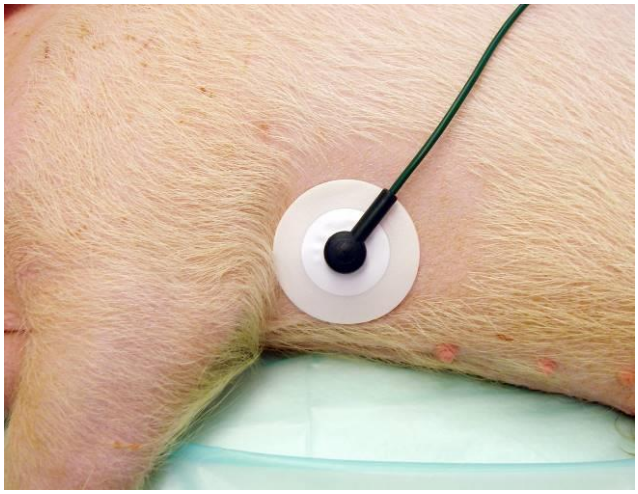


We recommend positioning the electrodes according to Nehb-Spoerri. The axial leads will give less variability and higher amplitudes than standard limb leads in Minipigs.

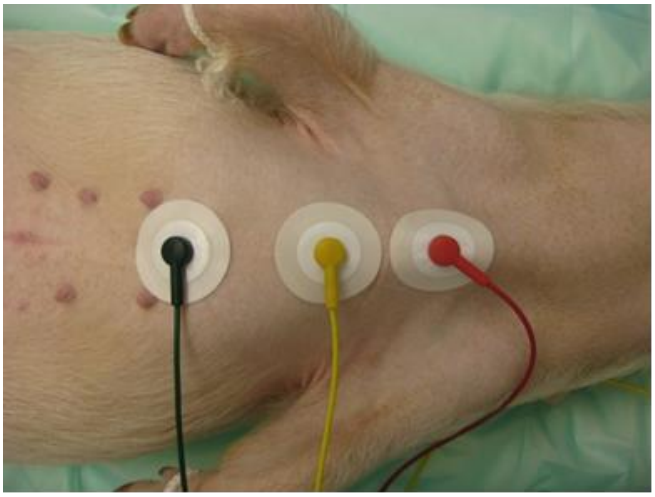
LA (yellow):	Proximal of sacrum	I or Dorsal
RA (red):	Right mastoid process	II or Axial
LL (green):	Apex of heart, intercostal space of 5-6 rib, close to sternum	III or Inferior
RL (black):	right leg (ground)	

For monitoring ECG during surgery an oesophagus probe could be used or place electrodes like the following if Nehb-Spoerrri is not feasible:

RA (red): manubrium sterni
LA (yellow): 1/3 from RA
LL (green): xiphoid sternum
RL (black): right leg (ground)



Placement of LL electrodes Nehb-Sporri.



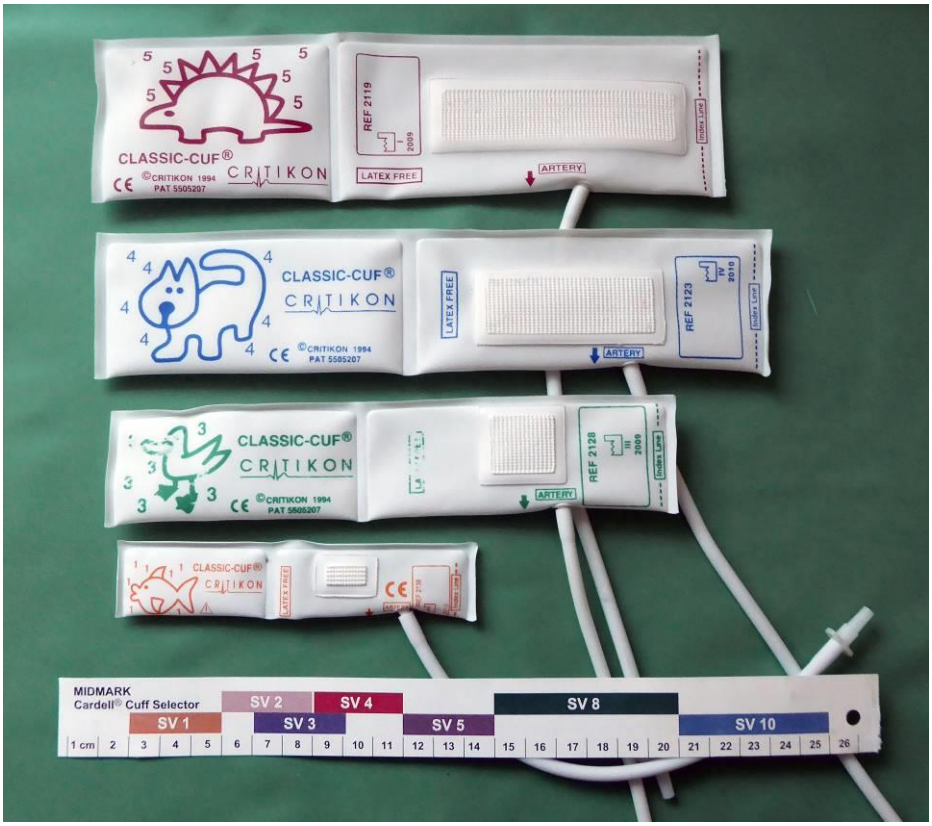
Electrodes placement for surgery .

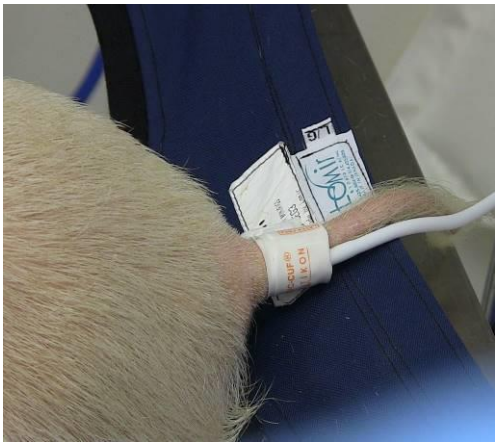
There are two different colour-coding and labelling schemes used for identifying electrode connections. One is recommended by the International Electrotechnical Commission (IEC), while the other is recommended by the American Heart Association (AHA).

	AHA		IEC	
Location	Inscription	Colour	Inscription	Colour
Right arm	RA	White	R	Red
Left arm	LA	Black	L	Yellow
Right leg	RL	Green	N	Black
Left leg	LL	Red	F	Green
Chest	V	Brown	C	White

NIBP

Measurement of non-invasive blood pressure can be done by placing a cuff around the limbs or tail of the Minipig. Choosing the right cuff is important for accuracy. The width of the cuff should be 40% of the circumference. A cuff that is too small will give falsely high readings and a cuff that is too large gives falsely low readings but the first case causes proportionally the bigger mistake. The cuff should be snug, however not so tight that it impedes venous return between BP measurements. Some cuff manufacturers provide a measure tape to help selecting the right size cuff.
The cuff should ideally be placed in the height of the hearth so on a conscious Minipig in the sling the best site would be the tail, but it can be difficult finding a cuff small enough for the younger animals. Placing a cuff on the legs can be tricky because of the conical shape of the limbs.

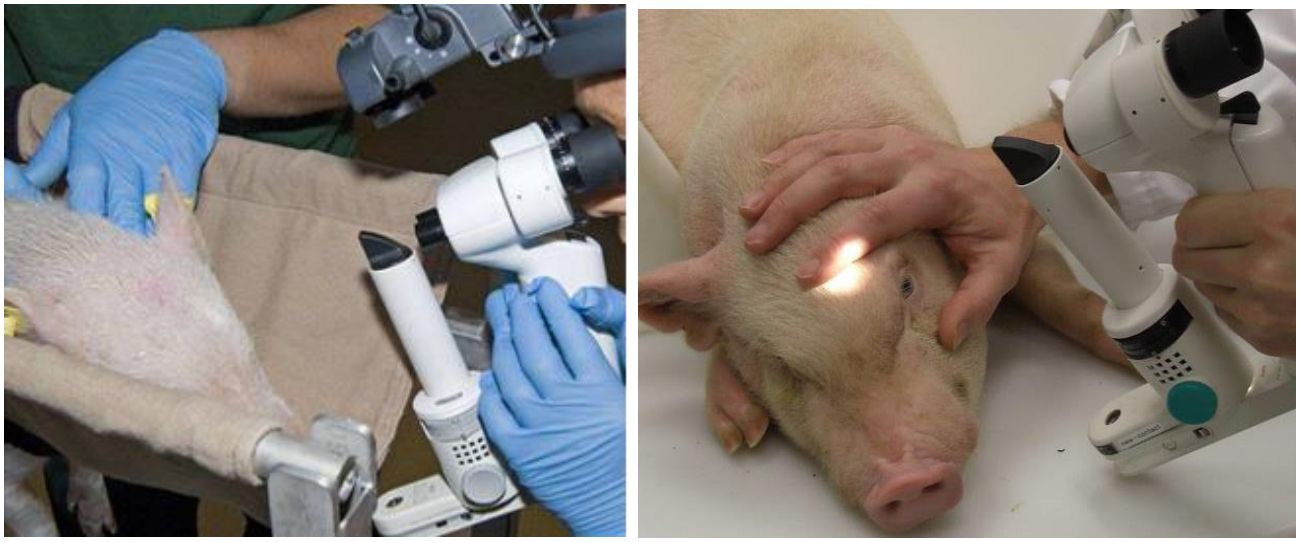




Placement of cuff for NIBP.

Ophthalmology

Small Minipigs can be held on the arm and carried into a dark room where the examination can be carried out. Larger animals can be put in the sling for this procedure or they can be gently cornered, possibly offering some treats to distract them.



Performing the procedure on a conscious and an anesthetized Minipig.

Dosing

As a general rule, it is a good idea to arrange the study, whenever possible, that the animals are fed right after dosing to reward the pigs for enduring the dosing process.
Needle size and dosing volumes in this chapter are indicative only. Considerations for choosing needle size include viscosity, volume and anatomical site. It is recommended to use the smallest size that delivers the injection appropriately. Volumes are based on collection of published data and in-house guidelines of various places that are made public in an article from Diehl K.H. et al. (2001)32. Animal welfare may be affected by the amount of compound injected and the number and frequency of the administration. Every application however should be evaluated by its own merits and always consult with your facility's SOP and veterinarian.

Orally administrated drugs or compounds can often be given in the Minipigs food or hidden in treats. They may also accept liquid, flavoured medication directly from a syringe.
It may not work to give less palatable drugs or compounds with diet and there are numerous study related reasons why Minipigs cannot be dosed with the compound in the diet.

Oral dosing by gavage

Oral dosing can be a challenging procedure and proper restraint is imperative (see restraining). It is typically very stressful for the Minipig. Handlers should be well trained to restrain the Minipig appropriately and perform the procedure quick and smooth to minimize stress (D.B.Morton et al.[33]).

Material: *Mouth gag:* Several types of bite bars and fork shaped devices in different sizes available – and deciding which one to use is a matter of taste and preference. Use the one that works most successful for you.
Catheter: lengths from 400 to 600mm and diameter from 12 to 18Fr. depending on the size of the pig. There are a variety of brands available, i.e. suction tubes from Vygon or urinary catheter from Rüsch. They are made of different materials and therefore a different stiffness. Try a few and pick the one you like best.

Length of insertion:

In order to be sure the end of the tube is placed in the ventricle, the insertion length is the distance from the last rib to the snout of the pig in question.

Procedure: Restraining the Minipig requires one or two people depending on size and temperament. Insert the gag gently in the mouth; make sure the whole of the pin is slightly off centre. If using a fork, use it to open the mouth. It is difficult to elicit the swallowing reflex in a Minipig, so having a bit of water in a moist tube can help when inserting it. Insert, dose and rinse swiftly and without much delay. Reward with a very tasty treat if protocol allows.

Dose: 10ml/kg is good practice. 15ml/kg is the maximum dose.

Oral dosing-capsules

Material: *Mouth gag:* as above.

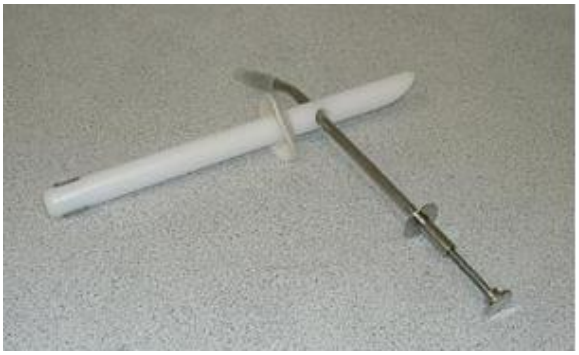
Dosing gun: two types are available. One of them is reusable, made of stainless steel and can be ordered through Ellegaard, and the other one is a commercially available device made of plastic.

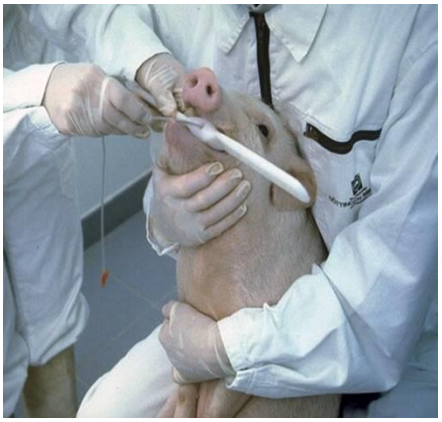
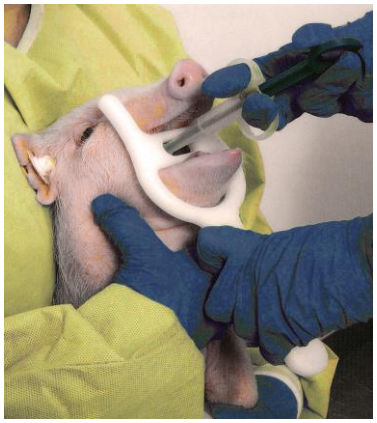
Procedure: Restrain the pig and use the gag as above. Try to place the capsule as far back in the mouth as possible. Here, too, getting the pig to swallow can be tricky. Water can be added in the plastic dosing gun or can be dosed right after the capsule. Stroking along the neck also helps.

Dose: Maximum of two capsules sized 000.



Different types of mouth gags - Instruments for capsule dosing, below.

Gavage. *Capsule dosing.*

Dermal dosing

Dermal dosing is one area where pigs can easily be trained to cooperate and this greatly reduces stress levels (for humans and Minipigs), implements good animal welfare and reduces working hours and costs.

Material:

A box with a ramp for the pig to walk on is a good help. Otherwise a table with a guardrail could be an advantage.
For a semi-occlusive bandage, gauze tape and net-bandages are used. Netting can be affixed to a dog collar with jeans buttons.
For occlusive bandages, the use of a plastic covering like Saran™ wrap, dental dam or Tegaderm™ can be used and held in place with Stockinette®, Tubifast® or Vetwrap®.
Another material that has very good adhesive properties on pig skin is Polsterplast® and it can be used for various tasks.

Procedure:

Train the pig to walk up into the box or place the box on a table. It helps to feed the animal at the same time as the dosing to reward and distract it. Apply the compound to the marked and shaved area. Bandage the pig and let it walk back to its pen.

Dosage area:

A maximum of 10% of the body surface can be used. Use the following formula to calculate the body surface: [34]

$$BS = \frac{70 \times BW0.75}{1000}$$

BS = body surface (m2)
BW = body weight (kg)

The calculation of the body surface is based on metabolic body weight (BW0.75). Pigs have a specific heat production of 70kcal/BW0.75 and produce 1000kcal of heat per square metre. Combining this results in the mentioned equation which returns the body surface.

In long-term studies, the area might have to be recalculated due to the growth of the animal.

Shaving and marking the area.

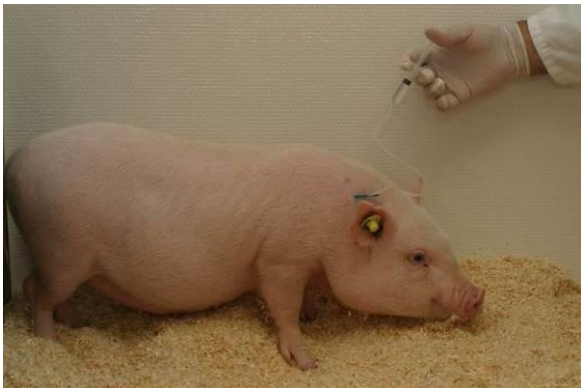
On the table, eating (above), semi-occlusive bandage (below) and collar (below right).

IM injection

Intramuscular injections are preferably given in the neck muscle just behind the ear. This site is generally more acceptable than elsewhere and has the advantage of being accessible on freely moving pigs when using an extension or butterfly cannula.

Material:	Syringe and needle 19–23ga, 20–30mm, according to the size of pig. Note that there could be a thick layer of fatty tissue in the neck. Extension tube or butterfly cannula.
Dose:	0.25ml/kg is good practice; 0.5ml/kg is the maximum.
Sites:	Cervical muscle behind the ear. Note that there could be a thick layer of fatty tissue as you move away from the area usually covered by the ear. The muscles in the hind leg may also be used but cause more resentment to the injection than the neck region.



Subcutaneous injection

Subcutaneous injection in Minipigs is slightly different from other laboratory animals, as Minipig skin is firmly attached to underlying tissue, as in humans. This reduces the maximum dose that can be injected. Two sites are commonly used for subcutaneous injection: the lateral side of the neck caudal to the ear and in the groin where the loosest skin can be found.
There is often a layer of fatty tissue just below the skin, and subcutaneous injections are often administered into subcutaneous fat.

Material:	Butterfly cannula, 21–25ga.
	Syringe and hypodermic needle.
	Infusion set.
Dose:	1ml/kg is good practice; 2ml should be the maximum per site with taut skin. Where the skin is loose up to 5-10ml may be administered.
Sites:	Neck, flank or inguinal area.



Intradermal injection

Intradermal injections can be performed by jet injector on conscious Minipigs, using hypodermic needles the Minipig should preferably be sedated (Ploemen I. et al.)[35].

Material: Syringe and needle 23 - 25ga, 15–20mm.
Jet injector.
Sedatives.

Dose: 0.2ml per site is the maximum.

Sites: Flank, behind the ear.

ID injection by injector and needle.

Intraperitoneal injection

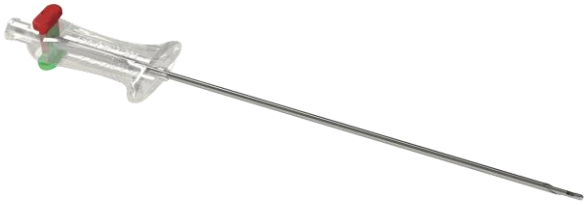
Intraperitoneal injections are not very common in Minipigs, however they can be successfully accomplished on anesthetized animals. There is a high risk of penetrating intestines as the abdominal cavity is tightly packed. The use of a veress needle is recommended. If it is not available a large bore needle can be used to penetrate the skin when it is pulled up. Proper placement of the needle tip is tested by injecting saline first and immediately withdraw it again. If liquid is clear or withdraw is impossible the position is most likely fine. If the liquid is cloudy or faecal matters can be seen intestines might have been penetrated.

Material: Veress needle, or
10 ml Syringe and needle 17- 19ga, 25-40mm.
Saline.
Sedatives.

Dose: According to study.

Sites: Abdomen.


Anatomy of the abdomen of a Minipig.




Veress needle.

Sequence of IP injection; saline contaminated with facies.

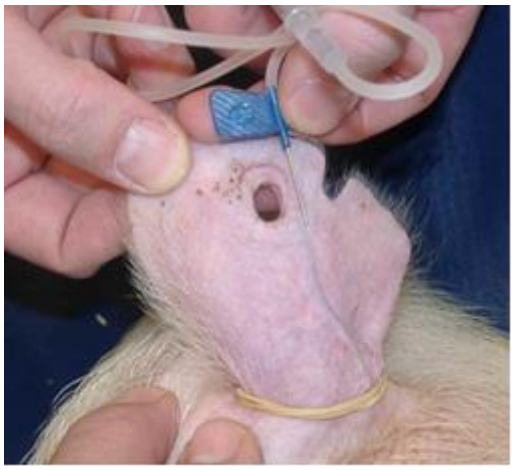
IV injection

Few superficial veins in a Minipig are easily accessible. The ear vein is the most commonly used site. The saphenous/tarsal vein is an alternative, but the skin on older animals can be very tough to penetrate at this site. It might be necessary to sedate the animal or use topical anaesthetic agents, such as Lidocaine or EMLA, for this procedure. The use of an over-the-needle catheter can be useful for multiple dosing and can be left in place for up to three days. The use of a mandrin to prevent coagulation is an option otherwise the use of a lock solution is appropriate. (Zeltner A. [36]).
Good stasis will facilitate the procedure, especially concerning the ear vein, and for that a rubber band tied with an artery clamp is helpful.
Consideration should be given to the properties of the compound and vehicle (in terms of pH, osmolality, viscosity, protein content corrosiveness, etc.) in deciding whether a peripheral vein is the right route of dosing or whether catheterisation of a large vessel is more appropriate.

Material: Butterfly cannula, 22–24ga, for auricularis, 21–23g for saphenous vein.
Over-the needle catheter, 22–24ga for auricularis, 20–22g for saphenous vein.
Corresponding mandrins.

Dose: 2.5ml/kg bolus, 5ml/kg slow (infusion pump).
Continuous IV infusion: 24ml/kg Rate: 1ml/kg/h.

Sites: Ear vein (Tarsal Vein).



Vascular access via ear vein.

Vascular access v.saphena.

Blood sampling and vascular access

Blood-sampling sites and restraining method depend on several factors such as the volume of blood required, the sampling frequency, size of the Minipig or study related considerations. The following will outline the possibilities and it is up to the user to decide what works best it the given situation and will allow collecting a sample effectively while maintaining the comfort of the animal.

In any case the Minipig needs to be restrained properly (see Restraining) and staff needs to be well trained.

Jugular vein/vena cava:

This is the preferred vessel for venepuncture as large volumes can be withdrawn from this site. For multiple sampling, it is advisable not to access the vessel more than 8 times in 24 hours. If more samples are required, catheterisation should be considered. Procedures for implantation of indwelling catheters and VAP are well documented[36] and not part of this paper.
A mature, healthy Minipig at an adequate nutritional level has an average circulating blood volume of 65ml/kg. The potential withdrawal in % of circulating blood and reconstitution times can be deduced from the table below (Adapted from Diehl K. et al. 2001)[32].

Single sampling (e.g. toxicity study)		Multiple sampling (e.g. pharmacokinetic study)	
% of circulatory blood volume removed	Approximate recovery period	% of circulatory blood volume removed in 24 h	Approximate recovery period
7.5%	1 week	7.5%	1 week
10%	2 weeks	10–15%	2 weeks
15%	4 weeks	20%	3 weeks

Anatomy of the vessels in the neck of a Minpig.

Sampling with restraining in the V-trough

Material:

V-trough on high adjustable table or similar.
Padding.
Alcohol for disinfection.
Vacutainer™ system or similar or Syringe.
20–21ga x 1½inch needles fitting the above devices. The length of the needle is depending on the size of the Minipig; for animals, less than 8kg, a 1inch needle can be used, very large animals may require needles above 1½inch.

Procedure:

Roll the Minipig gently into the V-trough as described in section "Restraining in the V-trough for blood sampling". Palpate the sternum, manubrium and the first rib. Disinfect the skin and insert the needle approximately 5-10mm cranially to the manubrium sterni and approximately 15 mm laterally in an angle of 45-60^0 into the jugular fossa, aiming at the dorsal end of the opposite shoulder blade. Apply or activate the vacuum as soon as the skin has been penetrated and advance until the vessel is reached and you have a flash back. If you are not successful in the first approach, withdraw the needle in the same direction, but do not remove completely. Change the angle slightly and advance again. The depth of insertion will vary based on age, size and individual anatomy and it might be necessary to bury the entire needle to access the vein.
After removing the needle, apply good pressure for a minute or more on the site to reduce the risk of hematoma.
As it is a blind stick there is also a risk of hitting the artery. If you suspect you have been withdrawing arterial blood (colour), apply extra-long pressure at the end of the procedure.

Blood collection with the Minipig in dorsal recumbency, restrained in a V-trough.

Sampling with restraining in the sling

Material:

Sling frame, preferably high adjustable.
Sling to fit the size of Minipig, short at the Head end.
Alcohol for disinfection.
Vacutainer™ system or similar or Syringe.
20–21ga x 1½inch needles fitting the above devices. The length of the needle is depending on the size of the Minipig; for animals less than 8kg, a 1inch needle can be used, very large animals may require needles above 1½inch.

Procedure:

Place the Minipig gently into the sling with the head at the short end as described in section "Restraining in the V-trough for blood sampling". Raise the sling to a comfortable working height. The head is supported and lifted so that the ventral side of the neck is nicely stretched.
Palpate the sternum and locate the jugular fossa.
Disinfect the skin and insert the needle approximately 20 - 15mm cranially to the manubrium sterni and approximately 15 -20 mm laterally in an angle close to 80^0 into the jugular fossa, aiming at the dorsal end of the opposite femur. Apply or activate the vacuum as soon as the skin has been penetrated and advance until the vessel is reached and you have a flash back.
If you are not successful in the first approach, withdraw the needle in the same direction, but do not remove completely. Change the angle slightly and advance again. The depth of insertion will vary based on age, size and individual anatomy and it might be necessary to bury the entire needle to access the vein.
After removing the needle, apply good pressure for a minute or more on the site to reduce the risk of hematoma.
As it is a blind stick there is also a risk of hitting the artery. If you suspect you have been withdrawing arterial blood (colour), apply extra-long pressure at the end of the procedure.

Taking blood samples with the Minipig restrained in a sling.

Sampling with restraining by siting technician

Material:

Bench, chair or oral dosing chair.
Alcohol for disinfection.
Vacutainer™ system or similar or Syringe.
20–21g x 1½inch needles fitting the above devices. The length of the needle is depending on the size of the Minipig; for animals less than 8kg, a 1inch needle can be used, very large animals may require needles above 1½inch.

Procedure:

Pick up the Minipig and sit with it in a similar fashion as for oral gavage. Hold the head straight, so that the ventral side of the neck is nicely stretched.
Palpate the sternum and locate the jugular fossa.
Disinfect the skin and insert the needle approximately 15-20mm cranially to the manubrium sterni and approximately 15mm laterally in an angel between 70-80^0 in to the jugular fossa, aiming at the dorsal-caudal point of the opposite shoulder blade.
Apply or activate the vacuum as soon as the skin has been penetrated and advance until the vessel is reached and you have a flash back. If you are not successful in the first approach, withdraw the needle in the same direction, but do not remove completely. Change the angle slightly and advance again. The depth of insertion will vary based on age, size and individual anatomy and it might be necessary to bury the entire needle to access the vein.
After removing the needle, apply good pressure for a minute or more on the site to reduce the risk of hematoma.
As it is a blind stick there is also a risk of hitting the artery. If you suspect you have been withdrawing arterial blood (colour), apply extra-long pressure at the end of the procedure.

Taking blood samples with Minipig restrained by sitting technician.

Ear vein

It is generally not possible to collect blood by applying vacuum at this site. The vein is too thin and will collapse. However, up to 3–400µl of blood can be obtained by puncturing the vein and collecting it in a capillary. Due to the nature of this procedure, its frequency should be low.

Material: 21ga hypodermic needle.
Capillary, possibly with vial.

Procedure: The Minipig is preferably placed in the sling and a topical anaesthetic can be applied to the ear. Disinfect the skin and apply stasis, a standard rubber band is helpful. If the pig is conscious, apply a good grip on the ear, as the pig will shake it at the time of penetration. Puncture the vein with a quick smooth motion and withdraw tool immediately after. A blood drop will form on the ear. Collect it in a capillary.

Collecting blood from ear vein.

Saphenous vein:

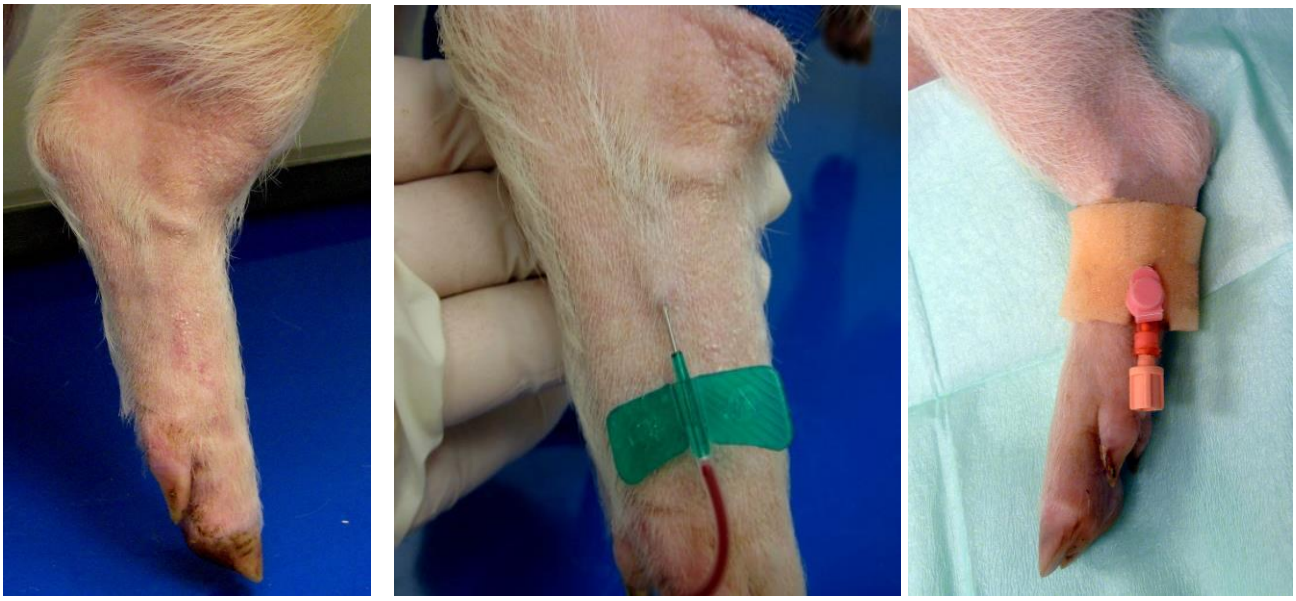
This vein is sometimes also referred to as lateral plantar vein and is often clearly visible. The accessing technique is rather unusual, as the skin is very tough in this area and the dewclaw can interfere with the angle of penetration. With a bit of training, however, it is possible to several millilitres of blood by applying gentle vacuum to the syringe. It is possible to catheterise this vessel.

Material: Butterfly needle, 21–23ga, or
Over-the-needle catheter, 18–21ga.
Syringe.

Procedure: Accustom the animal to be in the sling and having its leg touched and manipulated. Shave and disinfect the leg and apply stasis with your thumb above the ankle. If using a butterfly, aim at the vessel and push it through the skin with a quick smooth movement, as this is the painful part. You can numb the area with ice prior to disinfection, if you like. If the vein is not penetrated immediately, then move the needle forward and change the angle slightly until you are successful. Sometimes a gentle massage of the ham improves the blood flow.

Apply prolonged and firm pressure after sampling to avoid haematomas.

If you are using an over-the-needle catheter, it is advisable to make a small nick with a scalpel in the skin first; otherwise the catheter might break due to the strength of the skin. This is especially the case with older animals. The device can be left in place for up to 24h to facilitate multiple blood sampling.



Saphenous vein on shaved leg and with cannula in place.

Other vascular access

All the vessels above, as well as others, can be catheterised, short or long term, surgically implanted (with VAP or exteriorised) or with Seldinger technique, all depending on the purpose. As this subject is not part of this guide, please inquire about courses or refer to the corresponding information material.[36]

Courses

Ellegaard Göttingen Minipigs A/S regularly holds Handling and Dosing courses at the facility in Dalmose, Denmark. These courses are held at the best convenience for the parties involved and scheduled by mutual agreement. All the above techniques can be trained in practice. The maximum number of participants is four, so there is plenty of hands-on training. The course has a standard programme, but the contents can be adjusted to your particular needs.
As another option, a tutor could visit your facility and provide training on site. The programme would have to be adjusted according to your licensing terms, the availability of pigs and materials and your requirements.
To achieve basic knowledge and practice in the training of Minipigs, Ellegaard provides rudimentary one-day courses to get you started.

We hope that this educational package regarding handling, dosing and training of the Göttingen Minipig will be useful when you work with Minipigs.

More copies of this educational package are available.

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References

1. Ellegaard L., Cunningham A., Edwards S., Grand N., Nevalainen T., Prescott M. & Schuurman T. *Welfare of the Minipig with special reference to use in regulatory toxicology studies*. The Rethink report, Journal of Pharmacological and Toxicological Methods 62 (2010), 167-183.

2. Curtis S.E., Edwards S.A., Gonyou H.W. *Ethology and Psychology, Biology of the Domestic Pig*. Pond W.G, Mersmann H.J Cornell, University Press.

3. Ewbank, R., and G. B. Meese. 1971. *Aggressive behavior in groups of domesticated pigs on removal and return of individuals.* Anim. Prod. 13:685–693.

4. Francis, D.A., Christison, G.I. and Cymbaluk, N.F. (1996) *Uniform or heterogeneous weight groups as factors in mixing weanling pigs.* Can. J. Anim. Sci. 76, 171-176.

5. Symoens, J. and Van Den Brande, M. 1969. *Prevention and cure of aggressiveness in pigs using the sedative azaperone.* Vet. Rec. 85:64-67.

6. Gonyou, H.W. (1997) *Can odours be used to reduce aggression in pigs?* 1997 Annual Research Report, Prairie Swine Centre, Saskatoon.

7. Friend, T. H., D. A. Knabe, and T. D. Tanksley, Jr. 1983. *Behavior and performance of pigs grouped by three different methods at weaning.* J. Anim. Sci. 57:1406-1411.

8. Špinka M. *The ethology of domestic animals, Behaviour of pigs, p* 177 second edition CAB international 2009.

9. Gieling E.T., Nordquist R.E. & van der Staay F. J. *Assessing learning and memory in pigs.* Anim Cogn (2011) 14, 151-173.

10. Tanida, H., Senda, K., Suzuki, S., Tanaka, T., Yoshimoto, T. *Color discrimination in weanling pigs. Anim. Sci. Technol.* 1991;62:1029–1034 (Jpn.).

11. Jay Neitza, Gerald H. Jacobsa. *Spectral sensitivity of cones in an ungulate. Visual Neuroscience.* Volume 2,Issue 02, February 1989, pp 97-100.

12. Tanida H, Nagano Y. *The ability of miniature pigs to discriminate between a stranger and their familiar handler.* Appl Anim Behav Sci. 1998;56:149–159.

13. Kittawornrat A, Zimmerman JJ. *Toward a better understanding of pig behavior and pig welfare.* Anim Health Res Rev. 2010 Jun;12(1):25-32.

14. H. Tanida, A. Miura, T.Tanaka, T. Yoshimoto. *Behavioral responses of piglets to darkness and shadows.* Appl Anim Behav Sci 1996;49:173–183.

15. T. Grandin. *Pig behavior studies applied to slaughter-plant design.* Applied Animal Ethology. Volume 9, Issue 2, December 1982, Pages 141-151.

16. Prince, J.H. (1977) *The eye and vision.* In: Swenson, M.J. (ed.) Dukes' Physiology of Domestic Animals. Cornell University Press, pp 696-712.

17. Heffner H.E. & Heffner R.S. *Hearing in domestic pigs.* Hearing Research, 48 (1990) 231-240.

18. Heffner H.E. & Heffner R.S. Auditory Perception. *Farm animals and the environment.* Clive Philips and David Piggins (Eds) 1992, C.A.B. International, Wallingford UK.

19. Broucek J. *Effect of noise on performance, stress and behaviour of animals.* Slovak J.Anim.Sci., 47 2014 (2): 111-123.

20. Hafez E.S.E., J.P. Signoret. *The Behaviour of Swine. The behaviour of Domestic Animals.* Hafez E.S.E (Ed.). Williams & Wilkins, Baltimore, 1962.

21. Søndergaard L.V. et al. *Determination of Odour Detection Threshold in the Göttingen Minipig.* Chem. Senses 35 2010, 727-734.

22. Cerbulis, I. G. 1994. *Cognitive abilities of the domestic pig (Sus scrofa).* PhD Diss. The Ohio State Univ., Columbus.

23. Navratil N, Taberner E, Jasmin B, Salerno M, Grambo B, Althouse G.: *Spermatogenesis in the Göttingen Minipig: Assessing the Onset of Puberty.* Poster: American College of Toxicology 35th Annual Meeting, Orlando, Florida. November 9-12, 2014.

24. Peter B., Lorentsen H., van Duijnhoven E.A.J., van Rozendaal A.W.M., Emmen H.H. (2012) *Determination of onset of sexual maturity in female Göttingen Minipigs.* Society of Toxicology(SOT) 52nd Annual Meeting.

25. Peter B., Eveline P. C. T. De Rijk, Adrian Zeltner, and Henricus H. Emmen (2016) *Sexual Maturation in the Female Göttingen Minipig.* Toxicologic Pathology 1-4.

26. Simianer H., Köhn F. *Genetic Management of the Göttingen Minipig.* The Rethink report, Journal of Pharmacological and Toxicological Methods 62 (2010), 221-226.

27. Dewey Russell A. 2007 *Psychology: An Introduction.* Published online: http://www.intropsych.com Chapter 05: Conditioning.

28. Prior, Karen. *Don't Shoot the Dog.* 1984 Ringpress Books.

29. Tsutsumi H, Morikawa N, Niki R, Tanigawa M., *Acclimatization and response of minipigs toward humans.* Lab Anim. 2001 Jul;35(3):236-42.

30. Terlouw E.M.C. and Porcher J., *Repeated handling of pigs during rearing. II. Effect of reactivity to humans on aggression during mixing and on meat quality.* J. Anim. Sci. 2005;83:1664–1672.

31. Nahas K, Baneux, P, Detweiler D., *Electrocardiographic Monitoring in the Göttingen Minipig.* Comp Med 52; 258-264 (2002).

32. Diehl K. et al. *A good practice guide to administration of substances and removal of blood, including routes and volumes.* J.Appl.Toxicol. 21 (2001), 15-23.

33. D. B. Morton, M. Jennings, A. Buckwell, R. Ewbank, C. Godfrey, B. Holgate, I. Inglis,R. James, C. Page, I. Sharman, R. Verschoyle, L. Westall, and A. B. Wilson, *Refining procedures for the administration of substances.* Lab Anim January 1, 2001 35: 1-41.

34. Bollen P.J.A., Hansen A.K., Olsen Alstrup A.K. *The Laboratory Swine.* Second edition,p 87, CRC Press 2010.

35. I. HJ Ploemen, H. JHB Hirschberg, H. K., A. Zeltner, S. van Kuijk, D. PK Lankveld, M.Royals, G. FA Kersten, JP. Amorij, *Minipigs as an Animal Model for Dermal Vaccine Delivery.* Comp Med. 2014 Feb; 64(1): 50–54.

36. A. Zeltner. *Guide for the Implantation of Catheters in the Göttingen Minipig.* Published on www.minipigs.dk

Recommended reading

- Jeremy N. Marchant-Forde. *The welfare of Pigs.* Springer 2009, ISBN 978-1-4020-8908-4.

- Cox M.L. *Training Göttingen Minipigs.* Ellegaard newsletter 33, 2010, www.minipigs.dk

- Gonyou H.W. *How animal handling influences animal behaviour.* Prairieswine, Annual report 1995 pp 72-74.

- Grandin T., *Minimizing Stress in Pig Handling in the Research Lab.* Lab Animal Vol 15;3 1986.

- Grandin T., *Animals in Translation.* 2005 Bloomsbury Publishing, London.

- Harvinder S.G., Prausniz M.R., *Does needle size Matter?* J Diabetes Sci Technol Vol 1 Issue 5, 2007.