

Case study

EPAA's Refinement Prize 2021

Novo Nordisk submission

Case study describing the Refinement approach developed

What are the gains?

The training of the pigs is always focused on the individual experiment, to meet the exact needs. The goal of the training is that the animals voluntarily participate in central parts of the experiment. This approach has many advantages. First of all, the welfare of the animals is increased, as they are being stimulated; secondly, stress is reduced, which is considered an important refinement for the laboratory animals. Further, reduced stress is beneficial for the scientific study, as stress hormones can act as a scientific confounder. Reduced stress levels and calmer animals in the experimental situation is also desirable, to avoid mistakes, have more precise measurements (e.g. precise dosing) and to decrease the variability between each experiment. The time used on the experimental procedures is also reduced, as the animal and the animal caretaker cooperate.

To train the animals and to handle well-trained animals, adds to the job satisfaction for the animal caretakers. They build a relation – or bond - to the animals in the first phase of the training and build further to this during the training sessions. For people who care for and care about animals, as animal caretakers do, this positive interaction is a rewarding part of the daily work and strengthens the 'culture of care'. When the experimental set-up is calm, this also further reduces stress for the personnel involved in the experiment, yet another benefit.

The physical work environment is also positively influenced by this. For the procedures with the animals, the animal caretakers can sit down next to the animals and not bend over it. There is less risk of undesirable twists in the body, due to the animals standing still during experimental procedures. When transporting the animals from A to B, this is usually also done by positive enforcement and following a target stick, and this results in fewer cases of heavy lifting.

How is this method applied?

The training is divided into three phases: Socialising, specific training and maintenance. Socialising starts on the day the animals arrives and continues the following days. It consists of being with the animals, petting them, making them used to be touched, talking to them etc. This can be done in the acclimatisation period where the animals are also getting used to their new habitat (habituation) and a few minutes per animal per day makes a huge difference in the behaviour of the animals. They become more relaxed when there are humans inside their pens and have a reduced tendency to try escape from the situation.

The specific training is easily implemented in the socialisation, as the animals find it rewarding to learn new things.

We use 'clicker-training', where the animals initially (See Fig 1) learn to associate the 'clicker' with a reward ("bridging") and from there the click is used as a positive reinforcer to precisely signal to the animals when it is performing the desired behaviour.

When the animal is performing the desired behaviour voluntarily (e.g. standing still) it receives a click and a reward.



Figure 1 The picture shows the initial and basic step of the clicker training. The end of the stick has the (green) target which the minipig learns to touch with the snout and to follow. The clicker which gives the auditory signal to the minipig is attached to the other end of the stick, easily accessible for the trainer. The reward (yogurt or similar, easy to 'dose' immediately) is in the bottle. careful timing of observed desired behaviour, the click and the reward is essential. Note the short distance between the minipig and the trainer.

You can also guide it gently to the desired behaviour; however, this method is less effectful.

The specific training is targeted to the specific experiment and a training manual is made. Here, close communication with the people planning the experiment, is essential. Often, in pharmacology experiments, the experimental set-up changes during the planning phase, as the training planned may needs adjustments. The specific training most often centres around a target stick or a suction cup (also used as a target). When the animal seeks towards the target, it receives a reward. Once this part of the training is learned, the training may be expanded; for instance, the animal can learn to "freeze to target", making for example an injection (See Fig 2) or a blood sample a very easy procedure.

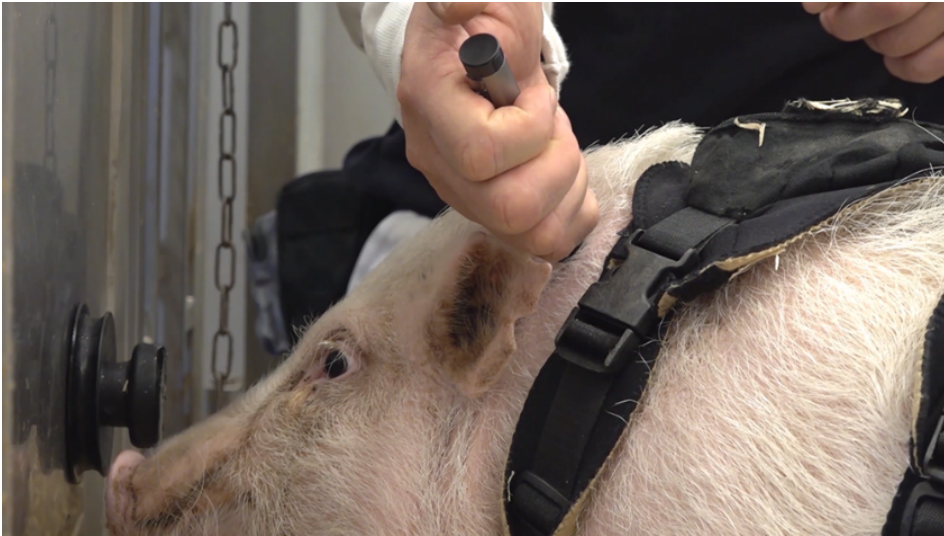


Figure 2 The picture shows a 'freezing' minipig. The minipig has been trained to fix the snout on the black suction cup placed on the wall in front of it and it will voluntarily stand absolutely still for 10 – 15 seconds. The requirement for a motionless animal is necessary for some subcutaneous injections, where needle must stay in for about 10 seconds to preventing backflow of the drug.

Another example from our facility was an experiment where exhalation air from pigs was a desired endpoint. Anaesthesia would be a confounder for the experiment, so a set-up where the animals were awake and relaxed, would be preferable. For this the specific training resulted in the animals froze on a suction cup within their own pen, then exhaled quickly in a small plastic bag (see Fig 3); all on voluntarily basis and with rewards for all steps in the process.



Figure 3 The picture shows the training situation for sampling of the exhalation air from the minipig. The animal is trained to place the snout in the plastic-cone where the bag for collection of the exhaled air is collected. As the pigs tended to hold their breath, the final step was to give the reward when it exhaled. Alternatively, the animals in the study would need to be fixated or restrained manually or mechanically.

This training will also be implementable in e.g. inhalation studies of regulatory testing or similar where the current set-up is to physically fixate or restrain the animals.

In the actual experimental setting, the environment around the animals is often different than during the training sessions. To accommodate this, 'distraction' is another important element of the training, meaning that the animals are getting used to all sorts of distractions during training. For instance, training is performed in different places, with different noises around, with different people, which could be differently dressed etc.

Maintenance is performed when the animal has learned the task and consists of repetition, repetition and repetition. If the animal finds the exercise hard, always decrease the difficulty, so that the experiment is set up for success. This way, motivation for the animal stays high.

How have the employees in scope added specific value to this set-up?

Stine and Mie have taken on a huge responsibility for implementing this training method for all animals and for teaching their colleagues about the theory and the method and for including all their colleagues in their considerations and plans for the training. When they have trained their colleagues, they have empowered their colleagues to further develop the specific training, this way ensured involvement and engagement from all animal caretakers in the unit.

They have shown a high dedication, and with high energy brought up issues related to the training at various meetings, keeping the focus of this important refinement for the entire team. Through dialogue with management, they have ensured that training is prioritised in the daily work, even when days are busy. They have shown an active example when training the animals, been supportive and they have been available for sparring from their colleagues.

Their continued focus has resulted in training being an integrated part of the work in our facility, always discussed for each specific study and important just as other parts of animal caretaking.

Potential for wider impact beyond immediate area

The method can easily be applied by other facilities housing laboratory animals. We have been inspired by and learned from modern zoos where the method is frequently used. Mie Johansson and Stine Larsen have with creativity and an innovative mindset transferred the method from one sector to the use of laboratory animals in pharmaceutical research.

In principle, the method is applicable for all laboratory animal species. Training of the animals only takes few minutes per animal per day to make a difference so it can also be applied for studies of shorter duration too (e.g. acute toxicology studies), which thereby is a possible refinement for many laboratory animals all over the world.

The training is relatively simple and easily learned and animal caretakers have from their education a solid background in understanding this method and further developing this and to use each other for sparring.

The time required to train the animals is often perceived as a barrier to introduce and implement this method. However, - it is our firm belief that this 'investment' in time is multiple returned: without training you easily spend more actual time 'struggling' with a non-cooperative pig or minipig and in non-fiscal terms you get the benefit of improved animal welfare, which lead to more robust scientific results (which ultimately benefits the patients), and you get employees which are motivated with a caring attitude and also have a high degree of job satisfaction.