CATHETER COURSE

We offer one-day courses in vascular access for injections and blood samples.
At this practical hands-on course you will try out different methods of catheterization and you will have time to repeat the methods and thereby increase your skills.
The specific course contents will be tailored to your exact needs and wishes and the course will be arranged to match your schedule.
Examples of techniques are central venous catheters - either percutaneous (Seldinger Method) or surgically implanted with a subcutaneous port (VAP).
Furthermore, we will introduce you to the various types of materials and kits used for vascular access and discuss their pros and cons.
After the course, we offer all the supervision you need to succeed with catheterization of minipigs.
The course instructors are our Laboratory Technician and DVM.
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NEW BEDDING MATERIAL

We have tried different kinds of bedding material for minipigs, and we recently decided to use straw instead of the bedding material used previously.
We use straw for bedding material in our barriers and in the transport boxes used for transporting minipigs to our customers.
The new bedding material is wheat straw from our own farmland near the minipig barrier facilities. The wheat straw is cultivated without the use of hazardous plant growth regulators.
The wheat straw is cleared of dust at Box Straw, chopped, wrapped in a double layer of plastic bags and treated with gamma irradiation. After this procedure the straw enters the barrier units through the airlocks.
By using our own straw for bedding material, we are assured that the quality is exactly as we want it and we have also seen that the minipigs like it.
You are welcome to contact us if you have any questions or if you would like to buy some of the bedding material for your next consignment.

Measuring spatial in (mini)pigs using

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Introduction

In recent decades, a broad range of cognitive tests has been used to investigate learning and memory in pigs (for recent reviews see: Gieling et al., 2011; Kornum and Knudsen, 2011). Unfortunately, most of these tests have not been standardised, and studies attempting to replicate published findings have been performed only sporadically. We are developing and validating a spatial hole-board task for pigs that allows for a simultaneous assessment of spatial working memory (short-term memory) and reference memory (long-term memory) in pigs.
The holeboard task has been deemed suitable for testing cognition in many different species. Holeboard-type tasks are sensitive to a broad range of experimental manipulations (summarised in van der Staay et al., 2012). We expect this task to be suited for safety pharmacology and toxicological risk assessment studies and for pharmacological evaluations of putative cognition-enhancing compounds.
Holeboard-type tasks comprise of an arena in which a matrix of holes (e.g. 4 x 4 holes) can contain a food treat. Usually only a subset of these, for example 4 of the 16 holes, contains a treat. For optimal foraging, animals must learn to visit the locations with a treat only, and to avoid revisiting locations from which they collected the treat during a trial (Olton, 1987). The most efficient behaviour is to visit each location with a treat only once. To achieve this, the animal must apply a win–shift strategy and remember the places already visited to avoid revisits.
If food can only be found in a subset of potential places, two memory components can be distinguished: spatial working memory and spatial reference memory. The list of places visited is held in the working memory (WM). By contrast with WM, which holds information that is only relevant within a specific trial, reference memory (RM) holds trial-independent information, such as where food is hidden.
WM is considered a form of short-term memory, and RM a form of long-term memory (Bimonte-Nelson et al., 2003; Dudchenko, 2004; Mizuno et al., 2002). It should be stressed that WM and RM are *operationally* defined in spatial orientation tasks: WM is regarded as memory for trial-unique stimuli and events, whereas RM stores information which remains unchanged between trials (Prior et al., 1997). WM and RM performance is reflected by an animal's choosing behaviour, i.e. by its visits and revisits of locations with and without a treat. WM and RM are considered operational definitions of presumably psychologically distinct mnemonic processes with different neural substrates.
Spatial WM reflects the ability of an animal to avoid revisiting holes with a treat during a trial. WM errors are revisits to holes with a treat, i.e. visits to holes which contained food, but had already been visited in the ongoing trial. Often, instead of counting WM errors, WM is defined as the number of rewarded visits divided by the number of visits to the set of holes with a treat.

discrimination performance the cognitive holeboard task

Spatial RM holds information about the performance of the hole-board task, e.g. about the localisation of food and about the actions necessary to get the treat, such as dipping into a hole with food, or lifting up a ball covering a hole (Gieling et al., 2012). RM stores the general rules of a task. This information retains its relevance across many trials of an experiment and is thus trial-independent, but may be learning-task specific.

RM errors are defined as the number of visits and revisits to holes that never contain a treat. Instead of counting RM errors, RM is often defined as the number of visits to the set of holes with a treat divided by the number of visits to all holes.

Ratio measures of WM and RM are less biased if a pig does not find all food rewards (in which case WM and RM performance,

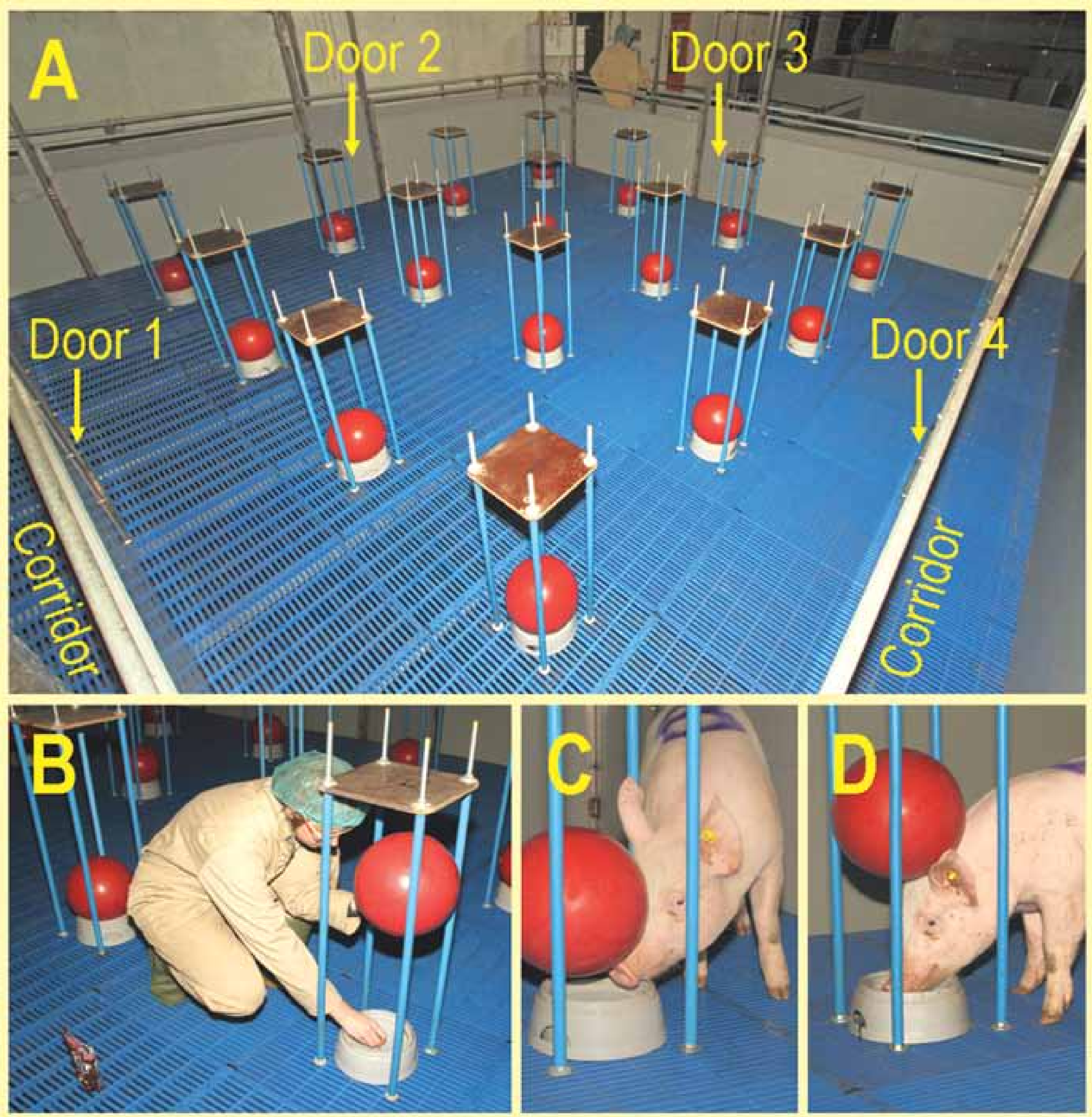

Figure 1. A fully automated holeboard device for measuring spatial WM and RM in (mini)pigs. (A) Overview of the testing arena with a matrix of 4 x 4 holes; (B) Experimenter puts M&M chocolates in one of the baited holes. (C, D) Pig lifts up the ball covering a hole to gain access to the hidden M&M chocolates. For the position of the four doors, see Fig.2C,E. (photographs: Annemarie Baars)..

expressed as the number of errors, may be overestimated). We routinely use the ratio measure.

Achievement of the holeboard task by conventional pigs and Göttingen Minipigs

We performed a series of experiments for developing and validating a holeboard task which is suited to assess the effects of experimental manipulations on spatial WM and RM in pigs.

Animals: Four groups of pigs were tested. Group 1 consisted of 20 conventional pigs from a study by Arts et al. (2009) which were approximately 8 weeks old when holeboard learning started. Group 2 consisted of 18 conventional pigs from a study by Gieling et al. (2012) which were approximately 9 weeks old when they were trained in the holeboard task. Group 3 comprised 8 conventional pigs and group 4 comprised 8 Göttingen minipigs from an unpublished study by Gieling, in which these two lines of pigs were compared directly. The pigs in groups 3 and 4 were approximately 13 weeks old when trained to use the holeboard.

Device: For testing group 1, a manually operated holeboard (8 m x 7.6 m) was used, in which buckets served as holes. The animals always entered the holeboard through the same door (see Fig. 2B; for further procedural details see: Arts et al., 2009).
A semi-automatic holeboard (5.4 m X 5.4 m) – adapted to testing pigs with a body weight of up to approximately 120 kg – was used for group 2. The device was fully automated when testing groups 3 and 4. The device, manufactured by Ossendrijver BV (Achterveld, The Netherlands), is a cognitive pig holeboard (Figure 2A) consisting of a square arena with a 4 x 4 matrix of food bowls. The blue synthetic floor is slatted and the grey synthetic walls (height: 80 cm) have a steel bar across the top. The arena can be entered through any one of the guillotine doors positioned in each of the four side walls, operated from the outside. The entry door for each animal was determined individually by permutations of the numbers 1–4 (with a maximum of twice the same door in a row). By voluntarily walking down a small corridor that surrounded the arena, the animals found the open door and entered the holeboard. The device (arena and corridor) was elevated above the floor to facilitate cleaning. The entrance could be accessed by a little ramp covered with a rubber mat.

Habituation: Habituating piglets to the test device and testing procedure is of great importance. Pigs naturally live in groups and are not used to being alone in an unfamiliar environment. After getting them used to the experimenters (by means of movements, sounds and stroking), they were brought to the waiting area located next to the holeboard device. As a group they explored this area and the holeboard. As soon as they showed signs of relaxation, the group size was gradually decreased. Finally, the animals entered the holeboard device alone in at least 4 successive habituation sessions. During habituation, all bowls were always rewarded with M&Ms to increase search behaviour and prevent the development of place (bowl) preferences. The total habituation period lasted approximately one hour a day for 1–2 weeks.
To prevent the animals from locating rewards on the basis of smell, the food rewards (fresh M&M milk chocolates, replaced daily) were placed under the false bottom of the food bowls (Fig. 1B). To prevent the animals from locating the rewards visually, each bowl was covered with a synthetic red ball (JollyBall Dog Toy, diameter: 24 cm, weight: 400 g). The animal had to lift this ball with its snout to gain access to the reward (Fig. 1C,D). The design of the device was such that the ball fell back into place and covered the bowl, once the animal retracted its head.
Each ball contained a magnet that operated a sensor under each of the food bowls. Lifting up a ball was automatically registered as a visit. All data were gathered through an interface (LabJack) and stored on a Personal Computer (OS: Windows XP) using the

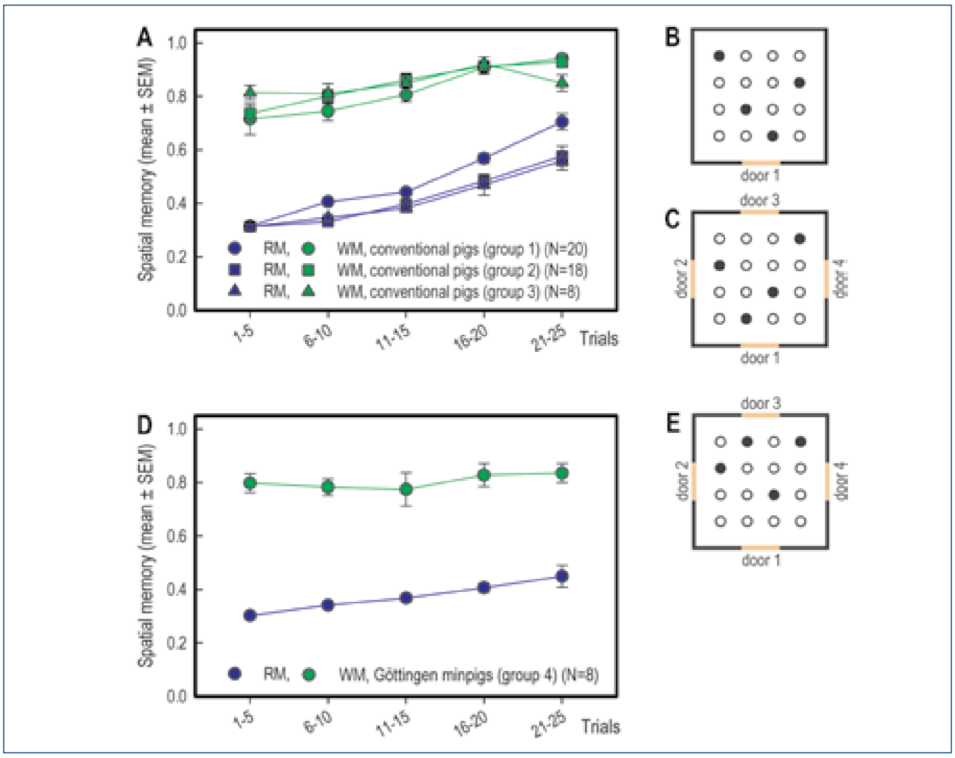

Figure 2. Group 1: (Arts et al., 2009), Group 2: (Gieling et al., 2012), Groups 3 and 4: comparison of conventional pigs and Göttingen minipigs (submitted publication).
Note that for comparison purposes, 5 block averages of 5 trials each are depicted. Panels B and C show typical configurations of holes with a treat as used for testing groups 1 (with one entry to the holeboard) and 2 (with 4 entries to the holeboard). Panel E shows a typical configuration of holes with a treat as used for testing groups 3 and 4.

custom-made program ‘Experiment control for the University of Utrecht’ (Blinq Systems, Delft, The Netherlands). This program controlled the experiment (e.g. chose randomly per trial through which door a pig was allowed to enter the holeboard arena) and collected the data (start and end of trial, holes visited, and the time of each visit). The program also calculated WM and RM per trial. A camera was mounted above the holeboard, which recorded every trial to allow controlling the registrations by the automatic system and as backup in case of system failure.

Statistical analyses: The early learning curves for WM and RM (first 25 trials) of the four groups of (mini)pigs, measured in three different experiments, are depicted in Fig. 2. For comparison purposes, the first 25 training trials were considered.

The mean of five trials each for WM and RM was calculated and subjected to an analysis of variance (ANOVA) with the factor “Groups” and the repeated measures factor “Blocks of trials”. Note that these comparisons were performed across data from three different experiments. In addition, we subjected the data of the third experiment in which conventional pigs and minipigs were directly compared to an ANOVA with the factor Groups [conventional pigs, group 3, vs Göttingen minipigs (group 4)] and the repeated measures factor Blocks of trials.

Results

All pigs were able to learn the task.

Working memory: The average WM performance of the four groups of pigs was similar (Groups: $F_{3,50}$=0.78, NS). The four groups of pigs slightly improved their WM performance in the course of training (Blocks of trials: $F_{4,200}$=13.28, p<0.0001). The rate of improvement, however, appeared to be different between groups [Groups (1,2,3, and 4) by Block of trials interaction: $F_{12,200}$=1.81, p=0.0491). When comparing the conventional pigs (group 3) with the Göttingen minipigs (group 4) which had been trained in parallel in the same experiment in a separate analysis, the following picture emerged: the average WM performance of the Göttingen minipigs and of the conventional pigs was similar [Groups (3 vs. 4): $F_{1,14}$=1.97, p=0.1817]. Both groups performed at a very high level of WM from the beginning of training, and they did not improve their WM performance across the first five blocks of training [Blocks of trials: $F_{4,56}$=1.84, p=0.1347; Groups (3 vs. 4) by Block of trials interaction: $F_{4,56}$=0.59, p=0.6713]. Note that, after 79 additional training trials, WM performance in these two groups of pigs did increase (the results of the full study will be reported elsewhere).

Reference memory: The average RM performance of the four groups of pigs was different (Groups: $F_{3,50}$=9.65, p<0.0001). The four groups of pigs improved their RM performance in the course of training (Blocks of trials: $F_{4,200}$=106.43, p<0.0001), differently between groups (Groups by Block of trials interaction: $F_{12,200}$=4.48, p<0.0001). It appears that the pigs of group 1 (which were trained in a version of the holeboard with one entry door) improved their RM performance faster than the other groups of pigs. The version with one entry is considered less complex than the version in which four doors were used.

Direct comparison of the RM performance between conventional pigs and minipigs revealed a marginal difference in the average performance level of the two pig breeds (Groups: $F_{1,14}$=3.38, p=0.0875). Both breeds learned the task (Blocks of trials: $F_{4,56}$=28.89, p<0.0001), but the conventional pigs tended to improve their RM performance slightly faster than the Göttingen minipigs (Groups by Block of trials interaction: $F_{4,56}$=2.42, p= 0.0588).

Conclusions

We are validating the holeboard task for pigs as a cognitive task for assessing WM and RM performance in pigs. Pigs are willing to perform the task without the need to apply food deprivation. The normal food ration is distributed, but 1/3 is given in the morning and the remainder in the afternoon. Compared with published learning curves in rodents, pigs learned the task faster, and they reached a nearly error-free performance level after extended training.

Göttingen minipigs appeared to learn the task slightly slower than conventional pigs, as assessed in a direct comparison (group 3 vs group 4), but they reached the same high asymptotic performance level by the end of the study (data not shown; paper submitted for publication). Commenting on a study by Manton (2010) who trained minipigs in a holeboard task for a total of 18 trials, Downes (2012) concluded that “the results of testing for learning and memory in minipigs were equivocal and ultimately disappointing”. The minimum prerequisite for testing whether putative cognition impairers or neurotoxic compounds affect learning in minipigs, however, is that the compounds are tested in a stage where the animals show clear learning under drug-free conditions. Our data demonstrate that minipigs are fully capable of learning the holeboard task, which contradicts Downes’ (2012) pessimistic view.

This task is well suited for testing the spatial memory performance of Göttingen minipigs. We experienced that it is difficult to train conventional pigs which had reached a body weight of over 100 kg, in terms of both handling and motivation. These problems did not occur when testing Göttingen minipigs.

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