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Plasma cortisol and noradrenalin concentrations in pigs: automated sampling of freely moving pigs housed in the PigTurn® versus manually sampled and restrained pigs

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Abstract

Minimising the effects of restraint and human interaction on the endocrine physiology of animals is essential for collection of accurate physiological measurements. Our objective was to compare stress-induced cortisol (CORT) and noradrenalin (NorA) responses in automated vs manual blood sampling in pigs. A total of 16 pigs (30 kg) were assigned to either: (i) automated blood sampling via an indwelling catheter using a novel-penning system called PigTurn® which detects the pig's rotational movement and responds by counter-rotating, allowing free movement while preventing catheter twisting; (ii) automated sampling while exposed to visual and auditory responses of manually sampled pigs; or (iii) manual sampling by jugular venipuncture while pigs were restrained in dorsal recumbency. During sampling of (i), personnel were not permitted in the room; samplings of (ii) and (iii) were performed simultaneously in the same room. Blood samples were collected every 20 min for 120 min and measured for CORT (ng ml^{-1}) using mass spectrometry and NorA (pg ml^{-1}) using High Performance Liquid Chromatography (HPLC). Effects of treatment and time were computed with mixed models adjusted by Tukey post hoc. *CORT and NorA concentrations were lowest in group (i) followed by group (ii), which were not different. However, CORT and NorA levels in manually sampled animals (iii) were highest compared to automated methods (i) and (ii). Plasma concentrations across time were not different for CORT, but NorA concentration at time 0 min was higher than at 120 min. The presence of visual and auditory stimuli evoked by manual sampled animals did not affect non-handled pigs' responses. Restraint and manual sampling of pigs can be extremely stressful while the automated blood sampling of freely moving pigs, housed in the PigTurn® was significantly less stressful for the animals.*

Keywords: *animal welfare, blood sampling, cortisol, noradrenalin, pigs, restraint*

Introduction

The development of drugs for medical purposes is a long and extremely costly process and invariably uses animal experimentation. The current estimated cost for bringing a new drug safely to market is about US$1.8 billion (Paul *et al* 2010), with less than 1 in 10,000 investigated compounds making it all the way from being an initial compound of interest to a medicine in clinical use (GAO 2006). The majority of compounds fail during the first two stages of development, namely the drug discovery and preclinical stages (which includes animal testing), with perhaps only 250 compounds beginning the clinical trial stage. All research involving the use of animals in the preclinical stage should be guided by the three chief principles of humane technique, as described by Russell and Burch (1959), namely those of Replacement, Reduction and Refinement — commonly referred to as the 'Three Rs'. From an animal welfare standpoint, the greater the use of replacement methodologies and/or the refinement of data collection in animal studies that decreases data variation, the greater will be the reduction of animal use. From an economic standpoint, if refinement results in better science that leads to earlier prediction of potential clinical failure, it will reduce the amount of wasted financial investment in further drug development.

The vast majority of animal species used in animal experiments continues to be rodents. In the UK, which has the most comprehensive animal-reporting statistics, just over 80% of

all animals used in experimental procedures are rodents, with 90% of this total being mice (*Mus musculus*) (Home Office 2011). The use of rodents as models for humans and their applicability continues to be debated (Olson *et al* 2000) and there is concern voiced within the pharmaceutical industry that better animal models should be developed (Fenwick & Fraser 2005). One species that has potential to be an improved animal model is the domestic pig *(Sus scrofa*; Lunney 2007) and its use in biomedical research is attracting increasing attention (Schook *et al* 2005). As a large-animal model, the pig offers potential advantages because of its supposed physiological and anatomical similarities to humans, though there are also many differences. The pig may also have a disputed increased ethical 'acceptability' as an experimental animal when compared with dogs or non-human primates (Webster *et al* 2010), although this question has yet to be asked of the public.

The domestic pig is also an important, globally widespread farm animal species and a great deal of research has been conducted into assessing the welfare of pigs kept under various conditions. The assessment of animal welfare invariably requires an amalgam of measures including, but not limited to, behaviour, health, productivity, immunology and physiology (Broom 1991). Whereas some measures, such as behaviour and heart rate, may be relatively easy and non-invasive to collect, others may require handling and restraint of the animal, and perhaps sampling a tissue, blood or body fluid for further analysis. Although often the information gained by taking samples may be useful, there is also the risk that the sampling methodology itself affects the very measures for which the samples are being taken (Cook *et al* 2000). There has been a move to collect samples from pigs non-invasively, such as by the use of heart-rate telemetry (Marchant *et al* 1995) or sampling saliva (Parrott *et al* 1989), urine (Pol *et al* 2002) and faeces (Carlsson *et al* 2007) instead of blood. However, sometimes the physiologically active compounds of interest, such as catecholamines, can only be measured in blood and that necessitates taking blood samples from the animal. Consequently, minimising the effects of restraint and human interaction on the endocrine physiology of animals is essential for collection of accurate physiological measurements. Furthermore, much of the pig welfare research is carried out in complex environments, with multiple stressors acting upon the subject animals meaning that a pig's responses to discreet elements of stress cannot be discerned or quantified.

Against this background of both pharmaceutical development and welfare assessment, we have developed a novel housing system which allows the automatic and serial collection of blood from a free-moving pig — the PigTurn® — with potential applications across both (and other) disciplines. However, a major element of the housing system must be that the system itself and the method of sampling should not induce stress. The objectives of this study were to firstly compare the effects of automatic and manual blood-sampling on plasma cortisol (CORT) and noradrenalin (NorA) concentrations and, secondly, to determine if manual blood sampling of conspecifics induced cortisol or noradrenalin responses in pigs being sampled automatically in the same room.

Materials and methods

Study animals and housing

The subjects consisted of a total of 16 male, crossbred (Yorkshire × Landrace), commercial pigs (30.1 [± 1.4] kg) from Purdue University's Animal Sciences Research and Education Center. Two replicates of eight pigs each arrived at the Veterinary Animal Holding Facility and were placed in pairs into four raised, perforated metal-floored, holding pens (2.4 × 1.2 m; length × width), with *ad libitum* access to appropriate feed (Laboratory Mini-Pig Grower Diet 5081, PMI International LLC, St Louis, MO, USA) containing 14.0% crude protein and 2.41 kcal g^{-1} digestible energy, and to water. Partitions between pens were made of stainless steel mesh so that pigs had visual, olfactory, auditory and limited tactile communication with pigs in neighbouring pens. After one week of acclimatisation, one pig from each pen was randomly assigned to one of two housing systems: i) the PigTurn® housing system (n = 8); and ii) remaining in the home pen (n = 8).

The PigTurn® (BASi, West Lafayette, IN, USA) consisted of a 1.2-m diameter animal enclosure in an octagonal shape giving 1.12 m^2 floor area (see Figure 1). The floor was plastic-coated perforated steel and the side walls were solid, clear plastic with aluminum supports. A stationary waste basin under the floor captured and directed urine to an external collection point. A feeding and watering area were built into a wall panel to allow easy access and a functional watering system without external connections. The access door to the animal enclosure consisted of two sidewall panels that were hung on heavy-duty hinges. When the access door was locked in the open position, it provided a secondary function by forming an enclosed area to restrict animal movement for manipulation or dosing. The upper panels on the walls were removable to allow easier access to the pigs. The animal enclosure was supported by a central-bearing assembly that allowed the whole enclosure to rotate in either direction. A drive system consisting of an electric motor with gear reduction, a belt drive, and an adjustable speed motor controller allowed external control of clockwise and counterclockwise rotation of the enclosure. The feedback control of the drive system consisted of a tether connecting the pig via a harness (see Figure 2) to an optical sensor array suspended above the enclosure. The sensor array converted the pig movement outside an allowed 270° arc into a signal that triggered the drive system to rotate the enclosure in the opposite direction of the pig movement. This allowed the pig to remain relatively constant in relation to the room while allowing movement in the pen. Once the pig stopped moving, the drive system was triggered to stop rotating the enclosure. Acceleration and deceleration of enclosure rotation was controlled to occur in a gradual manner. This system also enabled automatic recording of time, direction, and duration of animal movement. The harness was an adjustable H-design with additional strapping to control movement or slippage while on the pig. Two PigTurns® were housed in each of two adjoining rooms.

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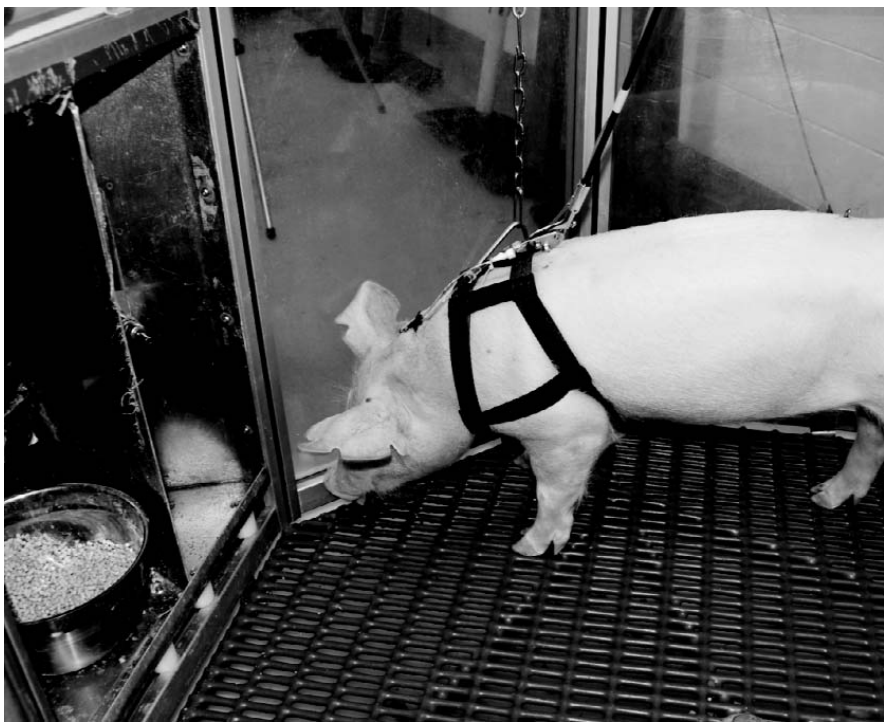
Figure 1

The PigTurn® housing system showing the octagonal pen, the tether attached to the optical sensor arm and the cabinet unit containing the blood sampling system and the motor for rotating the pen.

Figure 2

A pig in the PigTurn® housing system showing the harness with tether attached. Also visible is the plastic-coated, perforated wire floor, and the panel containing the feed and water systems.



In both housing systems, pigs had *ad libitum* access to appropriate feed and water, which was checked on a daily basis. Additionally, all pens were cleaned on a daily basis each morning.

Surgical procedures

All surgical procedures were approved by Purdue University Animal Care and Use Committee (PACUC Protocol # 05-057). Pigs remaining in the home pen did not undergo surgery, whereas pigs being placed into the PigTurn® housing underwent catheterisation surgery. Two pigs underwent surgery on each of two consecutive days. For catheterisation, feed was withdrawn 12 h before planned surgery and anaesthesia was induced by IM injection of 2.2 mg kg^{-1} of 50 mg ml^{-1} each tiletamine, zolazepam (combined as Telazol, Fort Dodge Animal Health, Fort Dodge, IA, USA), ketamine (Ketaset, Fort Dodge Animal Health) and xylazine (Sedazine, Fort Dodge Animal Health). Anaesthesia was maintained by cone delivery of 1 to 4% of isoflurane with oxygen. Effective anaesthesia was tested by ensuring that palpebral reflexes were diminished, interdigital pinch yielded no response, and there was absence of movement to physical stimuli. Instruments were packed and autoclaved. All disposable surgical supplies such as suture material, drapes, and catheters were sterile and not reused. Pigs were clipped and scrubbed as surgical preparation, then draped. Capped and masked surgeons and assistants were scrubbed, gowned and gloved to ensure sterile technique.

For the surgery, pigs were placed in dorsal recumbency with forelimbs pulled caudally and an incision was made over the jugular fossa. The external jugular vein was isolated and two loose ligatures of 2-0 non-absorbable suture (Ethilon, Ethicon Inc, Somerville, NJ, USA) were placed. The incision was packed with sponges soaked in sterile isotonic saline and the pig was rolled to lateral recumbency. The dorsal cervical exit site was re-scrubbed and draped. An approximate 3-cm incision was made and bluntly dissected 3 to 4 cm subcutaneously towards the ventral cervical incision. A trocar was passed from the dorsal site to the ventral cervical incision as an assistant protected the vessels. The stylet of the trocar was withdrawn and the catheter was fed through. The trocar was withdrawn as the surgeon set the 7-french, double-lumen, central venous catheter (Arrow International, Reading, PA, USA) into the dorsal incision. A mattress suture of 2-0 absorbable material (Monocryl, Ethicon Inc, Somerville, NJ, USA) in the subcutaneous tissue secured the catheter and the same suture was used to close the dorsal incision in a continuous pattern. The pig was then brought back to dorsal recumbency and jugular catheter installed by making a small nick in the vessel and utilising a vessel pick to feed the catheter towards the heart. Placement was verified by the ease of pulling blood into the catheter using a sterile syringe pre-filled with isotonic saline. When the surgeon was satisfied with placement, the ligatures on either side of entry of the catheter into the jugular were tied and the incisions closed. The H-harness was fitted to the pig and the catheter end was attached to the harness using a zip-tie. The catheter was blocked with a heparin lock and the pig was given an analgesic injection of flunixin meglumine (Banamine-Merck Animal Health, Summit, NJ, USA) at 2.2 mg kg^{-1} IM and an antibiotic injection of Cefazolin at 50 mg kg^{-1} IM.

The pig was then transported to the inactivated PigTurn® for recovery after voluntary movement was evident. Towels were used under and over the animal to maintain body temperature and continuation of recovery was in a darkened room with monitoring every 15 to 30 min until the animal was standing steadily. After standing, lighting was increased. All surgeries took approximately 45–60 min and were carried out between 0830 and 1130h. Twenty-four hours post surgery, the pig's harness was hooked up to the tether and the pen rotation system was activated. The catheter was attached to a 2-m catheter extension fixed to the automatic sampling system (Culex-L, BASi, West Lafayette, IN, USA), which was programmed to tend the catheter at 6 min intervals — ie to push 1 ml of heparinised saline into the pig to maintain catheter patency.

Experimental procedures

All experimental procedures were approved by Purdue University Animal Care and Use Committee (PACUC Protocol # 05-057). Seventy-two hours after surgery and 48 h after activation of the PigTurn® functionality, the pigs in the PigTurns® underwent automatic blood sampling. The computer controlling the Culex-L sampling system was programmed to collect 1 ml blood samples every 20 min over a period of 2 h, beginning at 0900h. After the programme was established at 0830h, the experimenter pressed the start button and exited the room, so that the 0900h (0 min) sample would be undisturbed. All samples were then collected without human presence by the Culex-L system. During sampling, the blood was drawn up through the catheter and the extension into a reservoir and the 1 ml blood sample was then pushed from the bottom of the reservoir into sealed EDTA vials contained within a chilled carousel, ensuring that the sample contained whole blood. Once the sample was collected, the remaining blood in the reservoir was pushed back into the pig, together with heparinised saline to replace the 1.0 ml blood volume that had been withdrawn and ensure catheter patency. The total volume of the implanted catheter and catheter extension was 6.6 ml. After 2 h, the collected samples were removed and centrifuged 13,000 × g for 10 min at 4°C for separation of plasma. Plasma was stored at –80ºC until analysis.

On the day after automatic sampling from the undisturbed PigTurn® pigs, blood samples were taken from the corresponding holding-pen pigs using manual restraint. For this part of the study, two holding-pen pigs were placed together into a wheeled cart and transported to the PigTurn® room. The Culex-L system was again set to collect 1 ml blood samples every 20 min over 2 h, and as the system began to take the first sample from the PigTurn® pigs, each of the holding-pen pigs were picked up simultaneously, placed into dorsal recumbency in a V-trough and a manual 1-ml blood sample was taken by jugular venipuncture into an EDTA vacutainer, which

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was immediately stored on ice. After the manual sample, these pigs were placed back into the cart, but then picked up and sampled again every 20 min over the next 2 h at the same time as the Culex-L was sampling the PigTurn® pigs. After the final samples were collected, the holding-pen pigs were transported back to their home pens and all blood samples were centrifuged at 13,000 × g for 10 min at 4°C, plasma separated and stored at –80ºC until analysis.

Thus, blood was collected under three conditions: i) automatic sampling under undisturbed conditions (n = 8); ii) manual sampling by jugular venipuncture (n = 8); and iii) automatic sampling under disturbed conditions, with experimenters present and pigs undergoing manual sampling in the same room (n = 8). There were no concerns about any possible carry-over effects from undisturbed automatic sampling to disturbed automatic sampling of the same pigs, as our treatments were applied in the perceived order of 'less stressful' to 'more stressful'. Also, the total blood withdrawal of 7 ml per day was well within the accepted guidelines of 0.05% of bodyweight for daily blood withdrawal for our 30 kg pigs (15 ml).

Assays

Cortisol

Pig plasma samples were extracted with methyl tertiary butyl ether. The organic layer was blown down to dryness under nitrogen and reconstituted with 0.1% (v/v) formic acid in water:methanol (50:50 v/v). Analysis was performed by High Performance Liquid Chromatography (HPLC) using a Zorbax XDB C8 column (Agilent Technologies Inc, Santa Clara, CA, USA) and a Tandem mass spectrometer (AB Sciex, Framingham, MA, USA) for detection. Detection was by MS-MS monitoring negative ions produced in the TurboIon Spray source of the Sciex API 365 (AB Sciex, Framingham, MA, USA). The intra-assay CV was 5.0% and the inter-assay CV was 5.6%.

Noradrenalin

Noradrenalin was extracted from proxy matrix and K_2EDTA pig plasma treated with sodium metabisulfite using acid-washed anodic aluminum oxide (AAO) in a 96-well protein precipitation filter plate. Before the extraction, ethylnoradrenalin hydrochloride was added as an internal standard. The analytes were eluted with the 0.1 M perchloric acid solution and the eluate was injected into an HPLC system with electrochemical detection using a SymmetryShield™ RP18 analytical column (Waters Corp, Milford, MA, USA) with BASi MP-2 Catecholamine mobile phase (BASi, West Lafayette, IN, USA). The intra-assay CV was 2.7% and the inter-assay CV was 4.3%.

Statistical analysis

For cortisol concentrations, a total of 14 out of 156 samples were either uncollected or had undetectable concentrations. Complete data sets for all time points within a 2-h collection period were therefore obtained for six undisturbed automatically sampled pigs, six manually sampled pigs and four disturbed automatically sampled pigs. For noradrenalin concentrations, a total of 19 out of 156 samples were either uncollected or had undetectable concentrations. Complete data sets for all time points within a 2-h collection period were therefore obtained for five undisturbed automatically sampled pigs, six manually sampled pigs and four disturbed automatically sampled pigs. Both data sets were log-transformed. The Mixed Procedure of SAS with repeated measures mixed models was applied to the cortisol and noradrenalin results and time was applied as a repeated measure. The analysis model included the terms of fixed effects for treatment, time and their interaction. Pig nested within treatment was included as a random effect. For all analyses, main effects and the interaction were computed depending on the significance of the higher order interaction and *P*-values were adjusted according to a Tukey *post hoc* test. All means, their respective SEM and *P*-values are presented in figures or are described in the text. Mean differences of $P < 0.05$ were considered statistically different. Data are presented below as the means (± SEM) of the seven samples per treatment.

Results

Cortisol

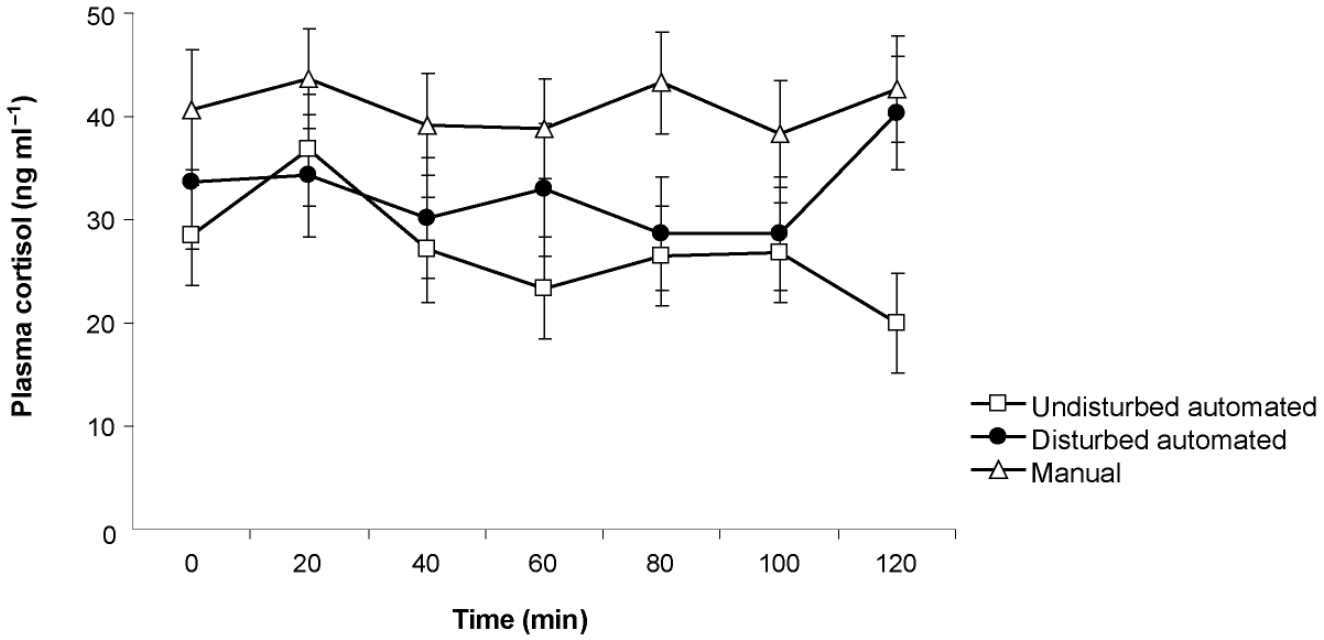
The main effect of treatment was highly significant ($P < 0.01$). Plasma cortisol concentration from undisturbed automated blood collection pigs (27.0 [± 2.1] ng ml^{-1}) was not different from that of disturbed automated collection pigs (32.7 [± 2.7] ng ml^{-1}; $P = 0.559$). However, both the undisturbed automated treatment concentration ($P < 0.001$) and the disturbed automated ($P < 0.05$) treatment concentration were different from the cortisol concentration of manually sampled pigs (41.0 [± 2.3] ng ml^{-1}). The main effect of time (differences between sampling time points) was not different ($P = 0.426$). The interaction of treatment by time was not significant (see Figure 3; $P = 0.286$), and there were no significant differences between any of time points.

Noradrenalin

The main effect of treatment was highly significant ($P < 0.001$). Plasma noradrenalin concentration from undisturbed automated blood collection pigs (260.9 [± 47.4] pg ml^{-1}) was not different from the noradrenalin concentration from disturbed automated collection pigs (268.5 [± 54.8] pg ml^{-1}; $P = 0.985$). However, both the undisturbed automated treatment concentration ($P < 0.001$) and the disturbed automated ($P < 0.001$) treatment concentration were different from the noradrenalin concentration from manually sampled pigs (747.0 [± 61.5] pg ml^{-1}).

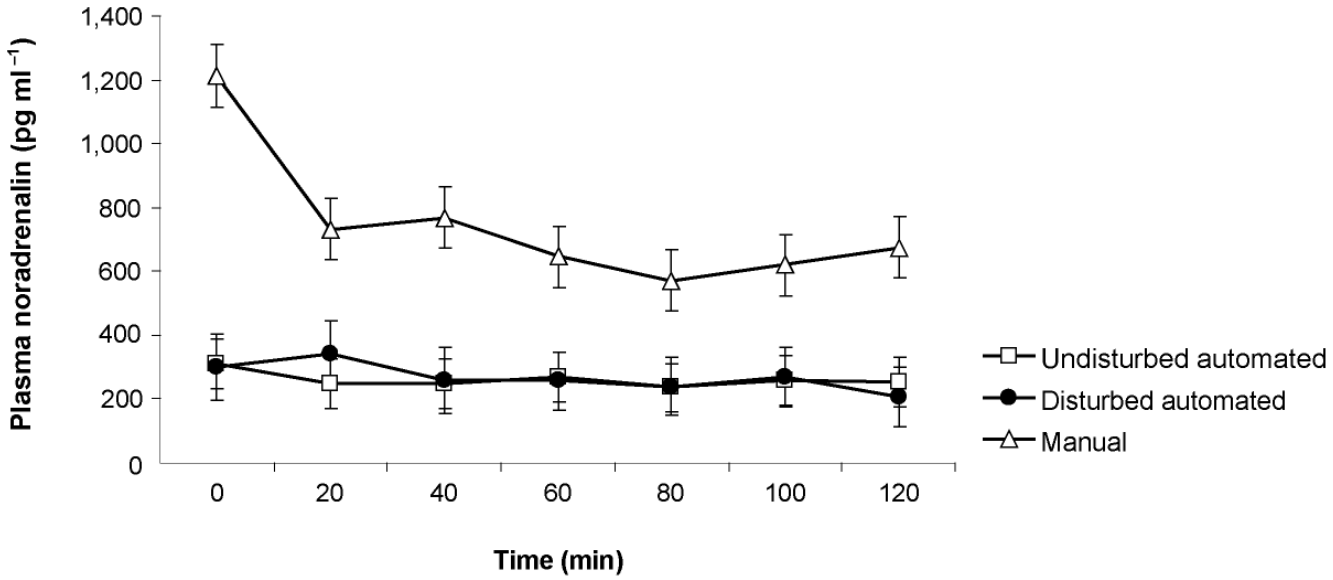
The main effect of time (differences between sampling time points) was different ($P < 0.004$) with the concentration at the first sample point (0 min) being significantly higher than noradrenalin concentrations at 80, 100 and 120 min, due to the influence of the manually sampled treatment. The interaction of treatment by time was not significant (see Figure 4; $P = 0.498$).

Figure 3



Mean (± SEM) plasma cortisol concentrations (ng ml^{-1}) of pigs sampled under three conditions; undisturbed automatic sampling, manual sampling and disturbed automatic sampling. Overall treatment differences — manual sampling was greater than undisturbed automated sampling ($P < 0.001$) and disturbed automated sampling ($P < 0.05$).

Figure 4



Mean (± SEM) plasma noradrenalin concentrations (pg ml^{-1}) of pigs sampled under three conditions; undisturbed automatic sampling, manual sampling and disturbed automatic sampling. Overall treatment differences — manual sampling was greater than undisturbed automated sampling ($P < 0.001$) and disturbed automated sampling ($P < 0.001$).

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Discussion

The collection of blood for scientific purposes is generally acknowledged to be stressful (Morton *et al* 1993). Depending on the methodology, collecting a manual blood sample will involve exposing the animal to multiple stressors simultaneously. For single-point samples, animals are usually exposed to short-term restraint, short-term human presence and handling and venipuncture, which may elicit some pain. For serial blood samples, depending on the time scale, animals will be exposed to longer term or repeated short-term restraint and longer term or repeated short-term human presence and handling. Blood may be collected via a previously implanted jugular catheter or by repeated venipuncture. Within manual sampling, it is known that nose-snaring and jugular venipuncture will cause a rapid increase in plasma cortisol concentrations in swine, peaking within 15 min of the first sample being taken (Merlot *et al* 2011). Other species show similar plasma cortisol trends but perhaps different time-courses of response. Dairy cattle sampled every 15 min show a peak in plasma cortisol concentrations 60 min after the first sample (Hopster *et al* 1999). Silver foxes (*Vulpes vulpes*) sampled by jugular puncture every 30 min also showed an increase in plasma cortisol, but the time-course of the response was dependent on the novelty of the procedure (Moe & Bakken 1996). Foxes that had been blood sampled regularly showed a peak cortisol concentration 30 min after the first sample, followed by a decline, whereas foxes being sampled for the first time showed a continuous rise in plasma cortisol up to 120 min after the first sample (Moe & Bakken 1996). Our manually sampled pigs showed no clear peak in plasma cortisol, but rather a sustained elevation and no habituation. This is most likely due to the timing of the first sample relative to removal from the home pen and transport to the experimental room. Thus, our 0 min sample did not represent a true baseline. For noradrenalin, there are no comparative data available. Whereas the plasma cortisol concentration may take as little as 2 min to increase in response to restraint and manual sampling (Stilwell *et al* 2008), catecholamine release is known to be virtually instantaneous and thus any manual sampling method will show only the catecholamine response to the acute stress of sampling rather than a measure of a longer term stressor. The higher concentration of our 0 min noradrenalin sample in the manually sampled pigs likely represents the combined effects of removal from the home pen, transport to the experimental room, restraint and sampling. The subsequent flat lining of the noradrenalin concentrations in this treatment reinforces the lack of habituation to this type of restraint and sampling, as seen with the cortisol response.

Instead of repeatedly collecting samples by venipuncture, many studies will employ catheterisation or cannulation techniques to enable serial samples to be collected without repeated needle sticks. The efficacy of catheterisation in decreasing stress hormone responses may be further influenced by how familiar the animals are with repeated sampling. Knol *et al* (1992) compared venipuncture with catheter sampling in experimental dogs and found no difference in the plasma cortisol response, but the dogs were trained experimental dogs that were routinely subject to repeated sampling. Vachon and Moreau (2001) compared jugular puncture in anaesthetised rats (*Rattus* spp) with blood withdrawal from catheterised rats and found that in both cases plasma corticosterone increased and peaked at 30 min after the first sample, but, thereafter, catheterised rats showed a rapid decrease in plasma corticosterone concentrations so that the 1- and 2-h samples were significantly lower than the same samples in the venipunctured rats (Vachon & Moreau 2001).

The results show that blood samples taken automatically using the PigTurn® and Culex-L combination, in the absence of the experimenter and without any restraint or disturbance, contained lower plasma concentrations of both cortisol and noradrenalin compared with blood samples taken using jugular venipuncture from a restrained and inverted pig, in the presence of an experimenter. No directly comparable experiments investigating different sampling methodologies have been reported in pigs, however Säkkinen *et al* (2004) carried out a study on reindeer (*Rangifer tarandus*) comparing manual restraint and sampling through an in-dwelling catheter with automatic sampling using a back-pack mounted sampling system also connected to an in-dwelling catheter. They found that plasma cortisol was five to six times higher and plasma noradrenalin was two times higher in blood sampled manually compared with concentrations in blood sampled automatically (Säkkinen *et al* 2004). In our study, cortisol was about two times higher and noradrenalin about three times higher in our manual samples than our automatic samples. Our manually sampled pigs were moved from the home pen, kept in pairs but given repeated, short-term, high-intensity restraint with repeated jugular venipuncture, with blood sampled every 20 min over 2 h. In the Säkkinen *et al* (2004) study, the manually sampled reindeer were separated individually from the herd and given repeated, short-term, cattle-crush-type restraint with first time non-surgical catheterisation followed by repeated blood draws every hour over 24 h. The differences in magnitude of responses may be attributed to species and methodological differences, but both studies show agreement in automatic sampling being less stressful than manual sampling.

Automatic sampling is not a new idea (Farrell *et al* 1970) but given the impact of sampling methodology on blood constituents, it has received relatively little attention. Within the laboratory animal community, automated blood sampling systems have been increasingly utilised since the mid-1990s (Holmberg & Pelletier 2009). Ideally, automated sampling is integrated into a system that allows the animal to have freedom of movement yet protects catheter patency. This can be achieved either by use of a liquid swivel — for example, the AccuSampler® (DiLab AB, Lund, Sweden), or by employing a caging system that counter-rotates against the animal's movements — for example the Raturn® (BASi, West Lafayette, IN, USA), suitable for rodents. For farm animal species, most automatic systems

have been developed to be animal mounted and carried in 'backpacks', which contain the necessary mechanics to sample and store the blood until removed by the experimenter — for example the IceSampler® (Ice Robotics Ltd, Edinburgh, UK). The backpack-mounted samplers have a finite sampling capability due to storage capacity and the animal has to be restrained and the equipment reloaded for studies requiring greater than 14 (Goddard *et al* 1998) or 16 samples (Fønss & Munksgaard 2008). The PigTurn® is based on the Raturn® technology and attached to a Culex-L sampler which can hold up to 24 samples in a refrigerated carousel and which can be accessed and replenished without disturbing the pig. The system has previously been demonstrated to have little effect on behavioural time budgets in pigs (Poletto *et al* 2006).

Our results also showed that human presence and indeed manual sampling of pigs in the same room did not affect either plasma cortisol or plasma noradrenalin concentrations in the pigs that were being sampled simultaneously within the PigTurns®. It has been postulated that pigs can convey alarm or distress by a combination of auditory (Marchant *et al* 2001) and olfactory (Vieuille-Thomas & Signoret 1992) output. However, the pigs in the PigTurn® did not appear to show any hormonal response to their conspecifics undergoing restraint and sampling in the same room. Similar results have been seen among pigs witnessing slaughter of conspecifics (Anil *et al* 1997) or being exposed to conspecific distress calls (Dujpan *et al* 2011).

Animal welfare implications

Automatic sampling of pigs within the specially designed penning system enabled us to collect blood samples containing baseline levels of plasma cortisol and plasma adrenalin compared with samples collected using manual restraint and jugular venipuncture, thus demonstrating an advantage of the system for collecting serial samples. The successful design of the integrated system allows the collection of refined physiological data from conscious, free-moving pigs within a laboratory setting which has potential implications in reducing the number of animals needed to obtain significant results thereby improving overall animal welfare. It also has the potential application to investigate the effects of discreet imposed stressors on pigs that may be related to pigs in commercial settings, thereby improving our understanding of the effects of stress on swine in commercial production.

Conclusion

Restraint and manual sampling of pigs can be extremely stressful while the automated blood sampling of freely moving pigs, housed in the PigTurn® was significantly less stressful for the animals. The presence of olfactory and auditory stimuli evoked by manually sampled animals did not affect non-handled pigs' plasma cortisol or noradrenalin concentrations.

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