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Validation of a digital video tracking system for recording pig locomotor behaviour

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Abstract

We are introducing a system for automatically tracking pig locomotor behaviour. Transposing methods for the video-based tracking of rodent behaviour engenders several problems. We have therefore improved existing methods, based on image-subtraction, to offer increased flexibility and accuracy in tracking large-sized animals in situations with a constantly changing background. The improved tracking algorithms introduce a reference frame, which does not include the animal and is automatically updated, and implementation of an automatic threshold detection algorithm. This makes the system more robust to the tracking environment, which could even be of the same colour as the animal, and allows the tracking environment to change during recording.

We validated the system by estimating the repeatability, accuracy, and basic noise level, and tested the system in different levels of animal activity evoked by administration of apomorphine (APO) to minipigs in an open field test.

Seven pigs each received the vehicle and three doses of APO (0.05, 0.1, and 0.3 mg/kg i.m.), and the locomotor behaviour of each session was recorded for 60-min. The calculated coefficient of repeatability was 0.6%, indicating high repeatability and the basic noise level of the tracking system was estimated to be 2%. Administration of the two lowest doses of APO was accompanied by increased locomotor activity of the pigs.

Thus, this digital video-based tracking system for automatically tracking the spontaneous locomotor behaviour of pigs is highly reliable and accurate, and was able to detect well-known effects of APO in pig locomotor activity.

Keywords: Apomorphine; Pig; Swine; Behaviour; Activity patterns; Locomotion; Video tracking; Image analysis

1. Introduction

Behaviour is an important parameter in several neuroscience disciplines. It is central to studies of animal welfare, neurobehavioural genetics, and brain function, and the number of behavioural studies is vast. Assessment of locomotor behaviour is especially crucial in neuro and psychopharmacology.

Pigs are most often studied in relation to animal welfare, but have recently been regarded with increased interest in neuroscience (Mikkelsen et al., 1999; Parrott et al., 2000; Danielsen et al., 2000; Cumming et al., 2001; Moustgaard et al., 2002; Arnfred et al., 2003a,b, 2004). In spite of the many ethological studies that have been made of this species, the use of automated tracking is uncommon. Pig behaviour is usually recorded manually, or semi-automatically with the aid of, for example, an event recorder. Since manual recording suffers from inherent problems of fatigue, drift, subjectivity, and other error sources (Martin and Bateson, 1993), automated recording is more reliable and accurate. Several automated systems for laboratory use have been developed for quantifying rodent locomotor behaviour, based on photo beams (Clarke et al., 1985; Sanberg et al., 1987; Gapenne et al., 1990; Robles, 1990; Minematsu et al., 1991; Young et al., 1993), infrared (Kramer, 1998) or ultrasonic (Vatine et

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al., 1998) motion sensors, sensors detecting of mechanical vibration (Van de Weerd et al., 2001), mechanical devices (joy stick connected to neck collar) (Brodkin and Nash, 1995) and video-based tracking systems (Torello et al., 1983; Vorhees et al., 1992; Schwarting et al., 1993; Hoy et al., 1996; Spink et al., 2001; Marchand et al., 2003).

Pig locomotion is commonly recorded manually; in terms of the number of zones entered. However, this method is fairly rough, as it is based on approximation and not on exact values. Automated methods for recording locomotor behaviour in rodents based on photo beams likewise suffer from having poor resolution in time and space. Tracking an animal is more precise, and delivers a high resolution. The principles of video-tracking analysis are often based on image-subtraction algorithms (Hoy et al., 1996; Wu et al., 2000; Noldus et al., 2001), grayscale threshold (Torello et al., 1983; Vorhees et al., 1992; Schwarting et al., 1993; Hoy et al., 1996; Noldus et al., 2001), statistical models (Tillett et al., 1997; Hu and Xin, 2000), or colour (Noldus et al., 2001). The use of an image-subtraction algorithm is advantageous when testing takes place against a varied background, but often some degree of contrast between the moving object and the tracking environment is required. Colour tracking is based on the identification of a coloured mark, unique in terms of by saturation and hue, placed on the animal (Noldus et al., 2001), and this method can also overcome problems with varying backgrounds. This commercially available system, however, it too expensive for some research budgets.

In the present study, we chose to use a tracking algorithm based on image-subtraction techniques. With high contrast between the object and background, a simple threshold brightness value can be estimated that separates the animal from its background. However, this is not possible if the contrast is low, i.e. if the colour of the animal is close to that of the testing environment. However, if a reference frame containing only the background, without the animal, is available, it is possible to use the threshold technique with a slight modification.

We have developed inexpensive software (freeware) for quantifying pig locomotor activity. The software is based on a combination of image-subtraction and automatic threshold-detection methods for tracking moving pigs. To validate this video-based tracking tool one must assess the accuracy and repeatability of the system. Additionally we assessed the ability of the software to detect different levels of animal activity by using the compound apomorphine (APO) to evoke quantitative changes in locomotion (hyperlocomotion).

2. Materials and methods

2.1. Hardware

The system for recording of pig locomotor activity consisted of a monochrome video camera (TOPICA TP-606D/3, Videosystemer A/S, Hedehusene, Denmark) connected to a digital video recorder (VSD-1000, Videosystemer A/S, Hedehusene, Denmark). The camera monitored the entire arena through an 8 mm wide-angle CCTV lens (with auto iris). The video was recorded and compressed to the MPEG2 format and stored on a 120 GB hard disk with a ∼96 h recording capacity. After completing the behavioural recordings, the hard disk was removed from the video recorder and the recorded behaviour was analysed offline using a PC (2.0 GHz Intel® Pentium 4 processor, 256 MB RAM, WinFast GeForce4 64 MB). The raw video stream was decompressed and adjusted to compensate for lens distortion, which resulted in video frames with a resolution of 384 pixels × 288 pixels. These video frames served as the input to the analysis algorithm. The video spatial resolution, or pixel size, was less than 2 cm, which can be determined by a simple calibration. As compensation for lens distortion is not entirely perfect, there is a small deviation on the pixel size, the effect of which will be discussed in a following section. The system is currently configured to work offline, where tracking can be performed at normal speed or faster; the speed only limited by computer specification (in the present study ∼4 times normal speed).

2.2. Software

The video sequences were analysed using software containing a collection of purpose-written definitions and algorithms, which will be described in detail in the following.

2.2.1. Lens distortion compensation

To be able to cover the entire arena with one camera, a wide-angle camera lens was used. This, however, has the effect of applying a fish-eye like distortion to the image. General methods for geometric image transformation are used for correcting this distortion, and are described in detail elsewhere (Carstensen, 2001). Here we used the following extension of the general polynomial transform, which results in an approximate correction for the distortion:

$$\tilde{x} = \frac{\alpha_0 + \alpha_1 x + \alpha_2 y + \alpha_3 x^2 + \alpha_4 y^2 + \alpha_5 xy}{\gamma_3 x^2 + \gamma_4 y^2 + \gamma_5 xy}$$
$$\tilde{y} = \frac{\beta_0 + \beta_1 x + \beta_2 y + \beta_3 x^2 + \beta_4 y^2 + \beta_5 xy}{\gamma_3 x^2 + \gamma_4 y^2 + \gamma_5 xy} \quad (1)$$

where $(\tilde{x}, \tilde{y})$ is the pixel location in camera frame and (x, y) the pixel location in the corrected frame. The 15 model parameters $\alpha_{0,...,5}$, $\beta_{0,...,5}$ and $\gamma_{3,...,5}$, must be determined prior to the processing.

To process a frame using Eq. (1), each pixel in the target frame (x, y) is taken from the corresponding location in the recorded frame $(\tilde{x}, \tilde{y})$. For speed optimisation, simple nearest-neighbor interpolation was used.

The model parameters can be determined as the result of an optimisation problem. If a number of corresponding (x, y) and $(\tilde{x}, \tilde{y})$ points are known (called ground control points) for a sample frame, the model parameters can be determined by minimising $\sum_i (\tilde{x}_i - x_i)^2 + (\tilde{y}_i - y_i)^2$ with respect to the parameters, where i is the index of the known point. In our experiments, the model parameters were determined using eight ground control points: The four corners of the arena and the center of each wall. The true positions (x_i, y_i), of these was determined by measuring the arena, and the recorded positions $(\tilde{x}_i, \tilde{y}_i)$, was located by examining the raw video frames.

2.2.2. Tracking

The basis of the tracking algorithm is the image-subtraction of a reference video frame from subsequent video frames containing a moving object. We will here consider tracking in a monochrome digital video recording, i.e. with each pixel value representing a brightness level ranging from black (0) to white (255) with 8-bit precision.

We denote the frame being analysed **I** and the background reference **B**, with **I** and **B** being matrices whose elements are pixel values. Then an image for analysis, **A**, can be computed by subtracting **I** and **B**:

$$\mathbf{A} = |\mathbf{I} - \mathbf{B}| \tag{2}$$

With this new image, **A**, the threshold technique can be used (see example in Fig. 1).

The location of the animal in **A** can be found as the weighed average of the co-ordinates of all bright pixels, where the weight is the pixel value and bright is defined as the value being above a certain threshold. The weighed average is computed as follows:

$$x = \frac{\sum_{i=1}^{M}\sum_{j=1}^{N} j \times p(i, j)}{\sum_{i=1}^{M}\sum_{j=1}^{N} p(i, j)} \quad y = \frac{\sum_{i=1}^{M}\sum_{j=1}^{N} i \times p(i, j)}{\sum_{i=1}^{M}\sum_{j=1}^{N} p(i, j)} \tag{3}$$

where (x, y) is the estimated animal location. M and N here represent the height and width of the video frame in pixels, and $p(i, j)$ is defined as

$$p(i, j) = \begin{cases} a_{i,j} & \text{for } a_{i,j} \geq \tau \\ 0 & \text{for } a_{i,j} < \tau \end{cases} \tag{4}$$

where $a_{i,j}$ denotes an element in **A**.

It should be emphasised that this is not a weighed average of bright pixels in the video frame, but rather a weighed average of differences from the background where the difference is greater than τ. This is closely related to computing the "centre of mass" of the pixel values with a difference from the background greater than the threshold. The weighing procedure ensures that pixels with a value close to that of the background colour assume less significance in the position computation, because there is a greater risk that these are in fact only noise.

The threshold, τ, must be selected either manually or automatically. Our software determines τ automatically for each frame by examining the histogram of **A**. The total number of pixels in the frame is MN, and we have used only the $\lfloor MN/f \rfloor$ brightest pixels to determine the position. Given this, τ should be set to the value of the darkest of the $\lfloor MN/f \rfloor$ brightest pixels. In other words, if we sorted all MN pixels by brightness (in descending order), we would set τ to the value of number $\lfloor MN/f \rfloor$ in the ordered list. Setting $f = 100$ would mean that we used 1% of the pixels.

The optimal value of f depends on the digital video resolution, the contrast between the animal and the background, and the noise (electronic and MPEG2 artefact) in the video recording. A large value of f increases the separation of the animal from the background, which can be necessary if the colours are nearly identical. On the other hand, reducing f means that more pixel values are used in the average, and averaging more values is known to reduce noise. Therefore, the choice of f is a balance between good separation from the background and the noise in the estimated position. In general, a range of 50–400 is suitable, but since the method is not sensitive to choice of f, finding the optimal value is not imperative; in the present study we selected $f = 100$ for animal tracking.

2.2.3. Updating the background reference frame

Changes in the background during the recording period affect **A** and, therefore, also the calculated position of the animal (Eq. (3)). Consequently, the background reference,

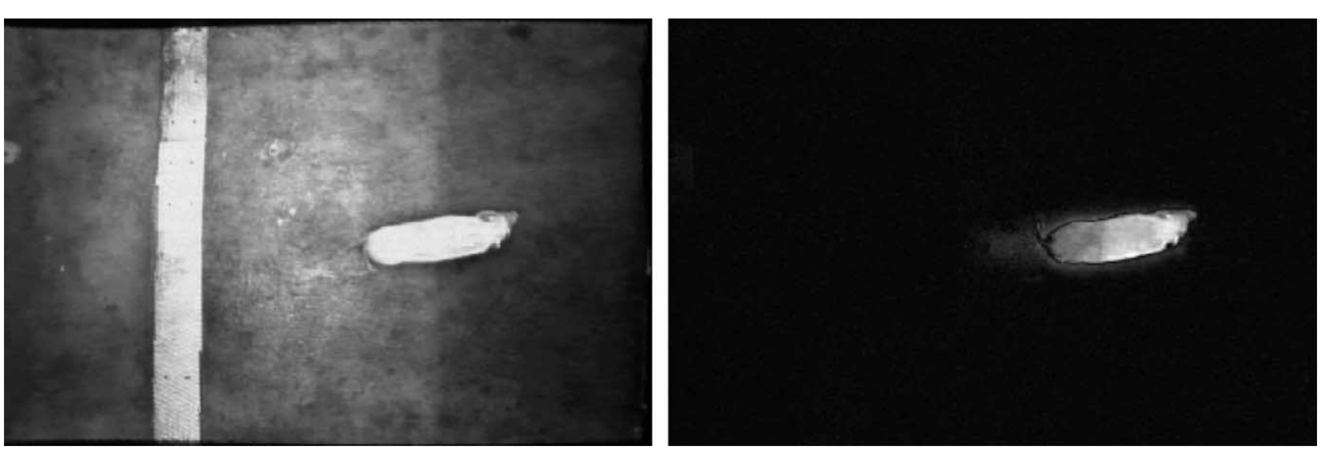

Fig. 1. An example of a video frame **I** (left), and the corresponding image for analysis **A** (right). All bright pixels in **A** are clearly part of the animal. See text for details.

B, must be updated to include these changes. In the software we have implemented an automatic update of **B** every 25 frames. When **B** is updated, it is imperative that the animal is not accidentally included, and that a single faulty frame, because of a hardware glitch, does not ruin the reference. This is avoided by computing the updated **B** from the current **B**, the current **I**, and four previous frames:

Let $\mathbf{B}_t$ and $\mathbf{I}_t$ denote the current reference frame and video frame, and $\mathbf{B}_{t+1}$ the updated reference. First, an estimate of the background frame, $\mathbf{E}_t$, is created from only $\mathbf{B}_t$ and $\mathbf{I}_t$. Most of $\mathbf{E}_t$ is copied from $\mathbf{I}_t$, except for a square around the animal which is taken from $\mathbf{B}_t$. In Matlab notation this could be written as follows:

$$\begin{aligned}\mathbf{E}_t &= \mathbf{I}_t; \mathbf{E}_t(y-r:y+r, x-r:x+r)\\ &= \mathbf{B}_t(y-r:y+r, x-r:x+r), \end{aligned} \tag{5}$$

where r is a parameter determining the square size. In our case the video frames are 384 pixels × 288 pixels, and r was set to 64, which is sufficiently large to cover the animal. This gives *one* estimate of the background image. Then $\mathbf{B}_{t+1}$ is computed as the median of $\mathbf{E}_t$ and the corresponding matrix from four previous updates, $\mathbf{E}_{t-1}$, $\mathbf{E}_{t-2}$, $\mathbf{E}_{t-3}$, and $\mathbf{E}_{t-4}$. The median is here computed element wise, meaning that with a frame rate of 25/s, information from the last 5 s of recording is used to update the background reference. This algorithm for updating **B** assumes that the background variation is slow, so that not all of the background changes all of a sudden. This is not an unrealistic assumption and posed no problems in the experiments.

2.2.4. Position noise reduction

Even if the animal is not moving, the (x, y) position estimate computed may vary a few pixels because of noise in the video recording and the changing background.

To reduce the effect of this "noise" on the position, the x- and y-values output from the tracking to the statistical analysis is median filtered. This is achieved by computing each output value as the median of the positions in the last n frames. For both this position noise reduction and the background reference computation, the median was chosen because of its ability to suppress noise and ignore outliers in the data.

For the position median filtering, n was chosen equal to 4. This value is sufficient to exclude single outliers in the data, but small enough to avoid the "staircase" shape inherent in running median smoothing. As described in the next section, the method used for computing the distance travelled has an additional build-in smoothing effect. We should note, however, that if there is a need to compute velocity or acceleration from the tracking results, more advanced smoothing methods are necessary (Hen et al., 2004).

2.2.5. Estimation of travelled distance and specifications of user-defined zones

On the basis of the calculated position estimates for the animal, the software can estimate the travelled distance, D, using the formula

$$D = \frac{1}{4}\sum_{m=1}^{4}\sum_{i=1}^{T/4-1} \sqrt{(x_{4(i-1)+m} + x_{4i+m})^2 + (y_{4(i-1)+m} + y_{4i+m})^2}, \tag{6}$$

where T is the total number of frames and (x_i, y_i) is the position in frame i after median filtering. The distance is computed in steps of four frames to avoid the effect of noise and very small movements. There are obviously four ways of choosing every fourth frame, namely choosing either frame 1, 5, 9,..., frame 2, 6, 10,... or the next two sets. To obtain the best distance estimate, we averaged these four sets, hence the outer summation.

Furthermore, the software has an in-built capacity to estimate the time spent in user-defined zones. An unlimited number of rectangular zones can be defined. In the present study, we applied a central zone dividing the arena in two equal-sized, 3.1 m^2 zones, thereby providing information regarding both central and peripheral activity.

2.3. Experimental animals

Seven female Göttingen minipigs (Göttingen minipigs™, Dalmose, Denmark) aged 18 months and weighing 15–25 kg were used in this study. A year prior to entry into the study, the animals had been trained in a series of cognitive and behavioural tasks for a period of 6 months. During this time, each pig was treated with D-amphetamine 5 times. The final exposure to amphetamine was at least 6 months prior to the study. The minipigs were housed in three pens with bedding of wood shavings and straw, illuminated by natural daylight, and an ambient temperature of between 18 and 20 °C. Animals were fed according to the breeder's recommendations with a commercial pelleted diet for minipigs (Altromin, Brogaarden, Denmark). Water was provided ad libitum. Housing and all experimental procedures were performed in accordance with the Danish Animal Experimentation Act (based on the Council of Europe Convention ETS 123) under a license granted by the Ministry of Justice.

2.4. Drugs

Animal testing took place every third day, starting with administration of the vehicle (2 ml 0.9% NaCl) and followed by three administrations of apomorphine hydrochloride (0.2%) in increasing doses of 0.05, 0.1 and 0.3 mg/kg. All injections were given i.m.

The dose regime was chosen on the basis of a prior study of the effect of APO in pigs, which found increased locomotion, though with differing intensity, at the chosen doses (Terlouw et al., 1992).

The study design was chosen on the basis of pilot studies showing that the APO-elicited behaviour had a pronounced carry-over effect, even with a test interval of one week. Since this study is not a pharmacological study per se, but a methodological study, a more uniform carry-over effect was desirable.

2.5. Procedures

The animals were familiar with the testing arena (2.00 m × 3.15 m) from prior behavioural testing carried out seven months before this study. Thus, habituation to the arena consisted of only three 30-min sessions in the week immediately before testing began. Testing took place between 10:00 and 15:00 h. After briefly isolating the minipig in a small capture pen, where it received the injection, it was immediately led to the arena and left there for an hour undisturbed. The arena floor was washed with tap water and swept between sessions. The behaviour was video recorded for the entire 60 min post injection.

2.6. Repeatability

It is crucial that testing equipment have low variability of accuracy between repeated measures. The precision term "repeatability" refers, according to the International Organization for Standardization (International Standard ISO 5725, 1986), to an estimate of the variability of the results between tests performed under conditions that are as constant as possible, the tests being performed over a short period of time, in one laboratory by one operator using the same equipment. "Repeatability" is, therefore, a measure of closeness of agreement between mutually independent test results. Our calculation of the repeatability of the tracking process was adapted from suggestions by Bland and Altman (1986). Since the best approach to examine repeatability is to take repeated measurements of a series of subjects (Bland and Altman, 1986), in a control session we tracked each of the seven animals twice for a 10-min period, i.e. the very same 10-min sequence was tracked twice for each animal separately. The starting point for tracking was randomised within the 1-h recording of each of the animals' behaviour. Differences in distance (in metres) between the two trackings were calculated for each of the animals, and a coefficient of repeatability was determined as the standard deviation of these differences divided by the average response (mean distance for all the animals). The coefficient of repeatability is low if the variability between the repeated measurements is low.

2.7. Estimation of basic noise level and accuracy

The basic noise level arising from electronic and analytic variation was estimated by tracking a stationary object—a box of approximately same size (30 cm × 47 cm) and colour (light beige) as a minipig. The box was placed in the open field and "tracked" for 10 min, and the travelled distance computed from this recording can be interpreted as an estimate of the accuracy of the method for distance calculations.

The accuracy of the tracking process with regard to moving objects was estimated using a turntable. A 15 mm white spot was placed 14 cm from the centre on a black disc (34 cm in diameter), and using a mean speed of 0.02 m/s, the distance estimated by the tracking software was compared to the actual distance moved by the spot. The calculations were performed on a recording of the spot moving exactly two rounds and the degree of deviation between the two measures was expressed in percent of the true distance. Due to differences in lighting and background conditions, as compared to the arena, the appropriate value for determining the threshold, f, was found to be 500 in this tracking.

The effect of tail wagging on the estimation of travelled distance was investigated by post hoc analysis. A 10 s sequence in which the pigs were standing still, and only wagging their tails, was located for each of the pigs, as was a 10 s sequence in which the same pigs were standing still without any body movement. These sequences were compared with respect to estimated travelled distance, thus quantifying the effect of tail wagging on this measure.

2.8. Statistical analysis

Analysis of the effect of APO on distance travelled was performed using repeated-measures ANOVA in the SAS statistical package (version 8.2, SAS Institute Inc., 1999–2001), treatment and time blocks being used as repeated factors. Where significant effects were found, relevant post hoc comparisons were made according to the differences between least-square means (t-test), and the p-values Tukey-adjusted.

Due to the inherent dependency of the two zone measures, a one-sided t-test was applied to the differences in the amounts of time spent in the two zones (zone 2–zone 1) for comparison of zone-specific activity. For the individual zone measures (i.e. peripheral or central zone), a repeated-measures ANOVA was applied to investigate the significant effects of drug treatment in the time interval, according to the outline given above.

The effect of tail wagging on the estimated distance travelled was tested for statistical significance using a paired t-test (two-tailed, level of significance 0.05).

3. Results

3.1. Repeatability, and estimation of basic noise level and accuracy

In the repeated-measurements study, the average distance travelled of the seven pigs in the control session was 29.6 m (S.D. 9.6 m), while the average difference between the two trackings was 0.1 m (S.D. 0.02 m). A requirement for using the method for calculating repeatability, as mentioned in Section 2.6, is that individual differences between the two trackings should not be related to the individual mean distances. This was investigated and found not to be the case

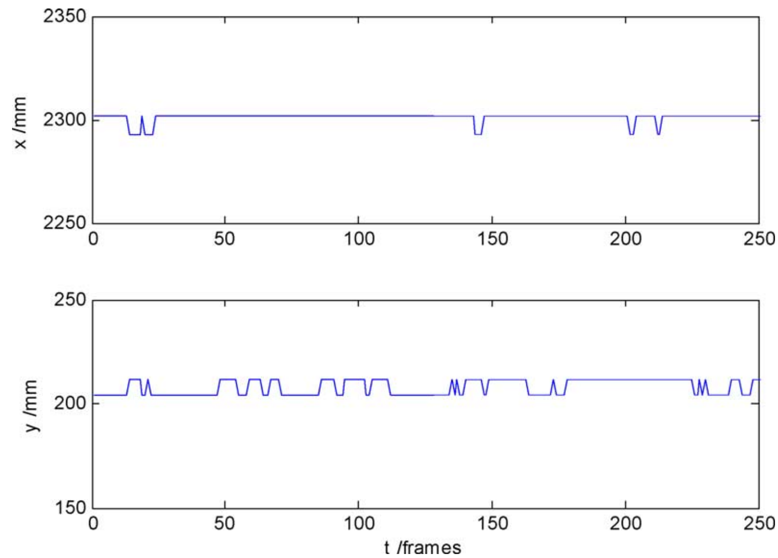

Fig. 2. Illustration of the 1-pixel limitation of variation of the recorded position when recording a stationary object: *x* and *y* position resulting from tracking a 10 s period where the animal was not moving.

(data not shown). Thus, the coefficient of repeatability for these trackings was 0.6%.

Estimation of the basic noise level by recording of a stationary object (10 min) showed that the variation in the recorded position was limited to 1 pixel (which also was found to be the case for pigs standing still, as shown in an example in Fig. 2) and the estimated distance travelled was 2 m. The impact of this error depends on the total distance travelled by the pig in the recorded period. In our study the average distance travelled in a 10-min time block ranged from 42 m (S.D. 17 m) (mean of vehicle session) to 157 m (S.D. 46 m) (mean of 0.05 mg/kg APO session) (Fig. 2) corresponding to an expected error in the estimated distance of about 1–5%.

Tracking a moving spot on a turntable showed that the path of the spot, as registered by the tracking system, agreed very well with the actual path (Fig. 3). The actual moved distance of the spot was calculated to be 176 cm, while the tracking software estimated this distance to be 192 cm. This corresponds to a general 9% overestimation by the tracking system of the distance travelled. Investigations into the source of this bias found that it was primarily due to deviant pixel size. The tracking system identified the spot as being 15.2 cm from the centre of the turntable. This corresponds to a distance travelled of 191 cm, very close to the actual estimated travelled distance as given by the tracking algorithm (192 cm). Thus, the main component of the bias of the track-

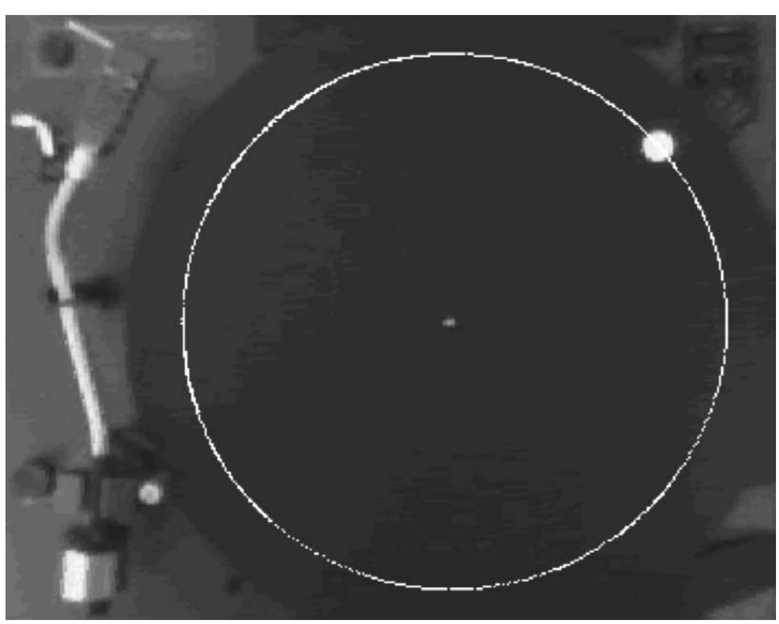

Fig. 3. The estimated path (white line) of a moving spot on a turntable. Please see text for details.

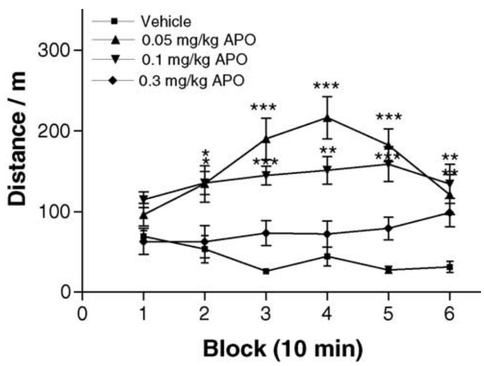

Fig. 4. The effect of APO on the distance travelled by pigs in an open field. Values represent mean ± S.E.M. for six time-blocks of 10 min each. Effects of treatment within a time-period are indicated. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

ing software in estimating the distance travelled is due to lens distortion, resulting in a deviant pixel size.

To investigate the effect of tail wagging, it was possible to locate 10 s sequences in which the pigs were standing still with their tails wagging for 5 of the 7 pigs. The mean estimated distance travelled for these samples was 0.17 m (S.D. 0.09 m), while for corresponding sequences where the pigs were standing still without any other body movement it was 0.08 m (S.D. 0.05 m). Although the mean estimated distance travelled for sequences with tail wagging was twice as high as it was in sequences with no body movements, the paired *t*-test comparing these two sequence types indicated that had a non-significant effect of tail wagging ($t = 2.26$, $p = \text{NS}$).

3.2. Effect of apomorphine

Treatment with APO increased the amount of locomotor activity. There was a significant dose–time-block interaction [$F(15, 132) = 2.73$, $p = 0.001$] on the distance travelled, such that animals when receiving the two lowest doses of APO travelled significantly farther than when receiving the vehicle in all time blocks except the first (Fig. 4).

In terms of zone-specific activity, the animals spent equal amounts of time in the two defined zones in the control situation ($p = 0.48$) and when receiving the two highest doses of APO ($p_{(0.1\text{ mg/kg})} = 0.06$; $p_{(0.3\text{ mg/kg})} = 0.33$; this did not, however, seem to be the case when they received the lowest dose (0.05 mg/kg) of APO ($p < 0.003$). However, this was not a significant finding when analysing peripheral activity in a repeated-measures ANOVA for effects of drug treatment and time block. Here, a main effect of treatment was present [$F(3, 152) = 3.93$, $p = 0.01$], but post hoc analysis revealed no differences in time spent in the peripheral zone between the pigs administered the vehicle and those administered any of the APO doses when these were adjusted for multiple comparisons. There was no main effect of time-block or interaction between time block and dose on the time spent in the peripheral zone. An example of the results of tracking a representative animal receiving the vehicle and APO (0.05 mg/kg) is presented in Fig. 5.

4. Discussion

We have presented a system for automatically tracking the spontaneous locomotor behaviour of minipigs in an open field. The basic tracking technique has previously been applied in a few systems for automatically tracking animal movement, and is based on subtracting a previously recorded image of the background that either does (Hoy et al., 1996, 1997) or does not (Spink et al., 2001) contain the animal. This procedure dramatically enhances the contrast between the animal and the background, and thus improves the accuracy of tracking of the animal. This technique is also able to compensate for a heterogeneous background.

We further improved this method, the better to suit the special needs connected with tracking of pig movements. First, we used a reference frame without the animal that is automatically updated; second, we implemented an automatic threshold-detection algorithm.

Preliminary studies showed that a former tracking method based on subtracting the previous frame (Hoy et al., 1996, 1997) was overly sensitive to the vigorous tail wagging that minipigs perform as part of their natural behaviour. This substantial movement bias was problematic for two reasons: first, since the amount of tail wagging varies between individuals, the bias is not systematic; and second, as tail wagging is affected by drug intervention, drug effects on the spontaneous

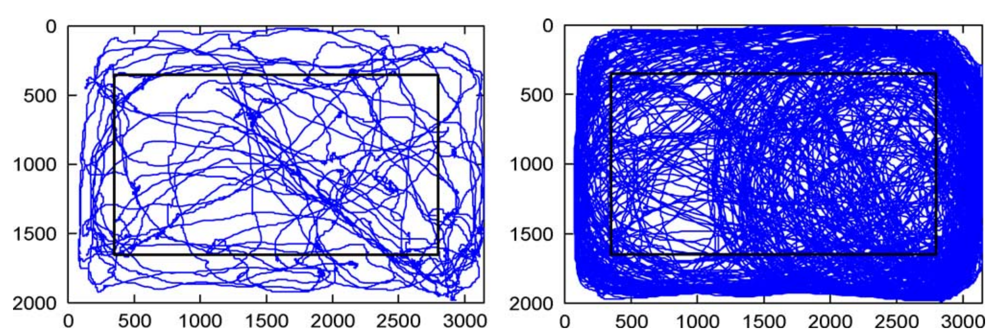

Fig. 5. Graphic presentation of the recorded path of a representative animal receiving either vehicle (left) or APO (0.05 mg/kg) (right). The test arena is divided in a central and a peripheral zone. Axis units are mm.

locomotor activity cannot be recorded reliably. The present algorithm applies a reference frame that does not contain the animal; therefore, the result of the subtraction is not only the part(s) of the animal that is (are) moving, but the entire animal. This is important since it minimizes the error arising from tail wagging, and furthermore increases the number of pixels used in determining the position of the animal. This is particularly relevant in calculating the position of large-sized objects, such as pigs, since the use of a large number of pixels in the calculation minimises the frequency of recognition errors on the part of the tracking system. A recognition error can cause substantial overestimation of the distance travelled, since the distance from the animal to the erroneously estimated position and back again is added to the total distance travelled. The extent of recognition errors in the present study was limited to one pixel (Fig. 2). Furthermore, post hoc analysis indicated that tail wagging was of no statistical significance, illustrating how the effect of this phenomenon on the calculation of the distance travelled had been reduced.

The technique of using a reference frame that does not contain the animal is also used in a commercial tracking system (Spink et al., 2001); we have, however, made an important refinement to this tracking algorithm by extending it to handle a background that itself varies during recording, by implementing automatic updating of the reference frame.

This was important for several reasons: first, open-field testing is commonly performed on a concrete floor—as was the case in our study. However, the water used to clean the arena between tests darkens the concrete, which dries off gradually during the 1-h recording period, resulting in a constantly changing background. Alternatively, if testing is conducted in an arena containing shavings, the hoof prints in the shavings also constantly change the background. Second, a similar effect is obtained with the animal's deposition of urine or faeces on a dry or semi-dry concrete floor.

Automatically updating of reference frame has also been used in model-based tracking (Hu and Xin, 2000), but this method was not adequate for our purpose since still objects would gradually become part of the reference frame over time.

The second improvement we made to the tracking method was to modify the threshold technique. In principle, this system can be applied to all possible tracking environments including to a background (e.g. shavings) of the same colour as the animal, by adjusting the parameter, f, that determines the number of pixels included in calculating the position. As well, our software determines the threshold, τ, automatically, while this is a manual procedure in other tracking software packages (Hoy et al., 1996). The determination of τ is based on f. Our empirical findings indicate that this can be an advantage because the result can be very sensitive to the choice of threshold value. We observed that the sensitivity of the system with respect to f was less than it was to τ. Earlier methods were also very sensitive to the choice of τ, whereas with our software, for example, setting f to 100 or 150 will not make much difference. The method is adaptive, so that the same value of f value will be appropriate under different lightning conditions and with different backgrounds—the software has a feature, whereby pixels different from the background above the threshold can be displayed. This is used for empirically determining f by choosing a value whereby a minimum number of the background pixels are displayed, while a maximum of pixels of the animal are displayed.

The system has high temporal (25 frames/s) and spatial resolution (< 2 cm), which is important for more accurately measuring path length, since higher resolution can detect smaller meanderings of the path (Paulus and Geyer, 1993). This is especially important in states of hyperlocomotion accompanied by circular movements, as is the case for APO-treated animals. With low resolution the true path length is underestimated, since the system assumes the path to have been straight rather than curved. Hyperlocomotion increases this error, so the bias is thus greater in the drug situation. This renders the foundation for comparing the two states problematic, since the degrees of error accrued in the two states are unequal, high-resolution systems can decrease this bias.

We found that the tracking software overestimates the distance travelled by 9%. However, this is a bias stemming mainly from lens distortion, which renders it difficult to determine the pixel size accurately. It is not a result of difficulties in recognising the true path pattern, as is the case with the low-resolution systems. The impreciseness in the lens correction is partly due to the approximate nature of the correcting procedure, and partly due to the manual assignment of the eight ground control points, which may be a few pixels off the exact locations. Fig. 3 illustrates the precision in recognising of the path.

We also found that even when an object was not moving there was a small bias present in estimating distance travelled. This is the basic noise level, which stems from electronic as well as analytic variation. Recordings of pigs standing still, however, showed that the estimated distance travelled was twice as high as was found from the recordings of the stationary object (4.8 m versus 2 m per 10 min). Since the size of the animals and the stationary object (a box) was comparable, this most likely reflects the fact that the pigs, although standing still, indeed had minor body movements. It illustrates how tracking methods based on image-subtraction or grayscale thresholding detect motions of the entire body and not only forward locomotion. The smoothing algorithm we use in the present study nevertheless proved able to reduce the effect of tail wagging.

An important prerequisite of any method is a high degree of repeatability—we investigated this, and found a coefficient of repeatability of 0.6%, indicating that the tracking process has a very high degree of repeatability.

The system has an in-built ability to display tracking results for user-defined zones, and we divided the arena into a central and a peripheral zone. We found no evidence of a spatial preference for either of the zones in the control situation, indicating that healthy pigs explore a familiar arena without spatial preference. This is in contrast to rats, which display thigmotactic scanning in an open field, a behavioural asymmetry defined as locomotion along the walls, while maintaining contact between the wall and the vibrissae on the side facing it (Schwarting et al., 1993). Also, in pigs, the number of entries into the centre of a circular arena was not affected by administration of the anxiolytic drug diazepam (0.8 mg/kg) (Andersen et al., 2000b) as is the case in rats (Stefanski et al., 1992; Fernandez et al., 2004), factor analysis did not link open-field activity to anxiety or fear of novelty (Andersen et al., 2000a), further indicating that the behavioural motivations behind open-field activity in rats and pigs are not identical.

The user-defined zones can be applied with advantage to other experimental situations involving zones of interest, such as the spontaneous object-recognition task (Moustgaard et al., 2002) or the novel-object test (Beattie et al., 2000; Herskin and Jensen, 2000). In particular, this approach of placing an animal in an empty arena containing an unfamiliar object has been hypothesis generating in terms of defining the biology of the exploratory behaviour of rodents (Drai et al., 2001), and automated tracking would be advantageous in future studies along this line in pigs. Also, investigations of how other large animals react to a novel object (King et al., 2003; Van Reenen et al., 2004) could well benefit from the use of an automated tracking system.

Administration of APO significantly increased locomotor activity (Fig. 4), which agrees with the findings of others (Terlouw et al., 1992; Bolhuis et al., 2000), and the described system for tracking pig locomotor activity was able to detect different levels of APO-evoked activity. However, considering specific doses, the highest dose (0.3 mg/kg) was not found to increase the distance travelled in any of the time blocks in contrast to the findings of a prior report (Terlouw et al., 1992). Instead, when subject to the highest dose, pigs were often occupied in stereotypic exploration with persistent snout contact with a limited area of the floor for much of the recording period, decreasing the recorded travelled distance. This phenomenon is also in accordance with the findings of others (Terlouw et al., 1992; Bolhuis et al., 2000), where such behaviour had a large peak at the 0.3 mg/kg dose (Terlouw et al., 1992).

Even though the tracking system has proved reliable and accurate in recording pig locomotor behaviour, it has its limitations. One is the confinement to the horizontal plane, which poses no problem in recording of pig behaviour, since pigs rarely perform vertical activity. Another limitation is the necessity of lighting, a feature shared with most other video tracking systems. However, pigs are day active, so for most test situations this not problematic. We are currently investigating possibilities for tracking in darkness using infrared light.

The open-field test was chosen as the experimental setting, since this is a common way of studying the effect of dopaminergic drugs on locomotor behaviour. However, the test could also have been performed in a single-animal pen. Since such is often the case in toxicological or pharmacological studies, our tracking system could be applied as an additional tool in this kind of research.

We are also testing the use of the software for tracking multiple animals in a pen. We have already successfully tracked a group of animals in their home pens, distinguishing them by adding a unique identifying mark. The results of the group tracking will be presented in another paper.

In conclusion, we have presented a digital video-based tracking system for automatically tracking the spontaneous locomotor behaviour of pigs. This system is based on an improved tracking technique that makes tracking horizontal animal motor activity very accurate, even under sub-optimal recording conditions, such as those involving a changing background. Furthermore, the system is inexpensive, highly reliable, has high temporal and spatial resolution and was able to detect the well-known effects of APO in pig locomotor activity.

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