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Application of 3-D imaging sensor for tracking minipigs in the open field test

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HIGHLIGHTS

- EthoStudio and 3-D sensor were applied for automated tracking of minpig behavior.
- 3-D sensor can trace white, black and agouti minipigs in the open field test.
- Agouti minipigs explored arena more than white or black animals.
- 3-D imaging system can be used to study behavior of farm-bred animals.

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ABSTRACT

Background: The minipig is a promising model in neurobiology and psychopharmacology. However, automated tracking of minipig behavior is still unresolved problem.
New method: The study was carried out on white, agouti and black (or spotted) minipiglets ($n = 108$) bred in the Institute of Cytology and Genetics. New method of automated tracking of minipig behavior is based on Microsoft Kinect 3-D image sensor and the 3-D image reconstruction with EthoStudio software. The algorithms of distance run and time in the center evaluation were adapted for 3-D image data and new algorithm of vertical activity quantification was developed.
Results: The 3-D imaging system successfully detects white, black, spotted and agouti pigs in the open field test (OFT). No effect of sex or color on horizontal (distance run), vertical activities and time in the center was shown. Agouti pigs explored the arena more intensive than white or black animals, respectively. The OFT behavioral traits were compared with the fear reaction to experimenter. Time in the center of the OFT was positively correlated with fear reaction rank ($\rho = 0.21$, $p < 0.05$). Black pigs were significantly more fearful compared with white or agouti animals.
Comparison with existing method: The 3-D imaging system has three advantages over existing automated tracking systems: it avoids perspective distortion, distinguishes animals any color from any background and automatically evaluates vertical activity.
Conclusion: The 3-D imaging system can be successfully applied for automated measurement of minipig behavior in neurobiological and psychopharmacological experiments.

1. Introduction

The minipig is a promising model for study of the mechanism of neuronal pathologies and effects of psychotropic drugs (Mikkelsen et al., 1999; Parrott et al., 2000; Cumming et al., 2001; Moustgaard et al., 2002; Arnfred et al., 2003, 2004). Behavior of minipigs and other farm-bred animals is usually recorded manually or semi-automatically with an event recorder. Therefore, the development of more reliable and accurate automated tracking system for quantification of behavior of farm-bred animals is an actual and still unresolved problem (Lind et al., 2005).

At the present time, there are several commercially available digital video tracking systems for automated quantification of rodent behavior (Noldus et al., 2001; Spink et al., 2001; Kulikov

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et al., 2008). However, there are two unresolved technique problems associated with an application of these rodent-oriented systems to automated tracking behavior of big farm-bred animals. The first problem is the low contrast between animal and background. Indeed, accurate object recognition needs special arenas and lighting systems (Spink et al., 2001; Kulikov et al., 2008). Such arenas and lighting system hardly can be technically realized for big farm-bred animals such as the cow, sheep or pig. At the same time, it is difficult or even impossible to achieve necessary contrast for correct computer recognition of a farm-bred animal in its home pen. Some authors tray to compensate low contrast condition by the sophisticated software which can distinguish an animal from a complex background (Lind et al., 2005). Although this software can trace white pig on a dark background, it hardly can trace a dark animal on a dark background or a white animal on a white background due to low contrast. The second problem is the perspective distortion which results in marked errors during tracking a big animal on a large arena.

A principled solution of these problems is a reconstruction of 3-D image of the arena and the animal. Now the problem of 3-D reconstruction can be easily resolved by means of application of Microsoft Kinect 3-D imaging sensor including digital video camera combined with a pair of near-infrared sensor and projector that measures disparity between emitted and received infrared pattern. This disparity in each point is converted into distance value from the point to the sensor. However, no application of Microsoft Kinect for automated tracking has been published. Moreover, there is no means of saving 3-D image on hard disk.

The open field test (OFT) is the most used behavioral test for measurement of locomotion, exploration and anxiety-related traits in a mild stress conditions in laboratory rodents (Denenberg, 1969; Prut and Belzung, 2003; Tecott, 2003; Standford, 2007) and in farm-bred animals (Lind et al., 2005; Forkman et al., 2007; Pedernera-Romano et al., 2011; Donald et al., 2011). It consists of a large and brightly illuminated arena. An animal is placed on the arena and its behavior including horizontal and vertical activities are recorded by a rater (farm-bred animals) or automatically tracked with a digital camera connected to a computer (laboratory rodents). In laboratory rodents the horizontal (distance run) and vertical (number of rearing) activities are usually interpreted as locomotor and exploratory activities, respectively, while time in the center is considered to be negatively correlated with fear (Denenberg, 1969; Prut and Belzung, 2003). However, there is no evidence on association between time in the center and fear in farm animals (Forkman et al., 2007). At the same time, a fear reaction of a farm-bred animal can be successfully evaluated with the fear response test (FRT) (Lankin, 1997; 2014; Lankin and Bouissou, 2001).

The aim of the present study was to apply Kinect 3-D sensor to automated tracking minipigs in the OFT. It was also intended (1) to construct equipment and software for tracking 3-D image and saving 3-D image stream on a hard disk, (2) to evaluate effect of animal's sex and color on the behavior in the OFT and FRT; (3) to compare pigs behavior in the OFT with that in the FRT.

2. Materials and methods

2.1. Animals

Experiments were carried out on 108 minipiglets (males and females) selectively bred in the Institute of Cytology and Genetics SD RAS (Novosibirsk, Russia) (Tikhonov, 2000). All animals were 2–3 months of age and weighting 3–4 kg. At the moment of OFT the animals were kept in the home pens (237 cm × 195 cm × 90 cm) together with the mothers and the sibs. After OFT and before the FRT the animals were isolated from their mothers, separated by sex and kept in groups of five in pens of the same size. There were three color-genetic groups: white (II), black, black and white (E^dE^d E^pE^p) and agouti (EE AA) (Ollivier and Sillier, 1982). Since in spotted pigs the black spots covered more than 70% of animal's back, these spotted animals were classified as black-colored.

Fig. 1. The open field apparatus: 1 – arena (pen), 2 – halogen lamp, 3 – Kinect camera on the mount.

The animal were bred and kept in compliance with the European Communities Council Directive of December 18, 2008 (2008/120/EC). All experimental procedures were in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

2.2. The OFT apparatus

The OFT was carried out in a concrete rectangle arena (237 cm × 195 cm × 90 cm). The size of this arena was similar those usually used for the OFT in adult minipigs (Lind et al., 2005; Donald et al., 2011) or piglets of German Landrace (Zebunke et al., 2013). The center of the arena included the rectangle region (118.5 cm × 97.5 cm). The pen was brightly illuminated with two halogen lamps of 30 wt each attached to the opposite walls, 75 cm above the floor. The floor was covered with sawdust. Microsoft Kinect 3-D sensor used as a video capture device was mounted 290 cm above the floor and connected to a PC – compatible notebook using an USB cable via an USB port (Fig. 1).