

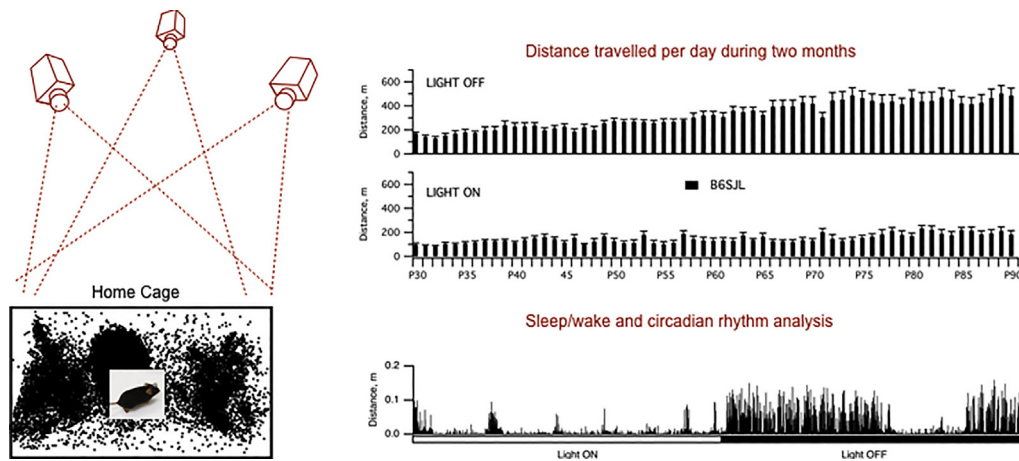
3-D motion capture for long-term tracking of spontaneous locomotor behaviors and circadian sleep/wake rhythms in mouse

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GRAPHICAL ABSTRACT



HIGHLIGHTS

- Simultaneous tracking of locomotor movement in tens of freely behaving animals in their home cages.
- Does not require separate monitoring devices for the individual animal.
- A cost effective system for making continuous and fully automated long-term recordings (up to 3 months).
- Capability of recording at all ambient light levels, including during darkness, with high spatial and temporal resolution.
- Actigraphy-based movement detection allows for the analysis of sleep patterns and circadian rhythms.

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ABSTRACT

Background: Locomotor activity provides an index of an animal's behavioral state. Here, we report a reliable and cost-effective method that allows long-term (days to months) simultaneous tracking of locomotion in mouse cohorts (here consisting of 24 animals).

New method: The technique is based on a motion capture system used mainly for human movement study. A reflective marker was placed on the head of each mouse using a surgical procedure and labeled animals were returned to their individual home cages. Camera-recorded data of marker displacement resulting from locomotor movements were then analyzed with custom built software. To avoid any data loss, data

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files were saved every hour and automatically concatenated. Long-term recordings (up to 3 months) with high spatial (<1 mm) and temporal (up to 100 Hz) resolution of animal movements were obtained.

Results: The system was validated by analyzing the spontaneous activity of mice from post-natal day 30–90. Daily motor activity increased up to 70 days in correspondence with maturational changes in locomotor performance. The recorded actigrams also permitted analysis of circadian and ultradian rhythms in cohort sleep/wake behavior.

Comparison with existing method(s): In contrast to traditional session-based experimental approaches, our technique allows locomotor activity to be recorded with minimal experimenter manipulation, thereby minimizing animal stress.

Conclusions: Our method enables the continuous long-term (up to several months) monitoring of tens of animals, generating manageable amounts of data at minimal costs without requiring individual dedicated devices. The actigraphic data collected allows circadian and ultradian analysis of sleep/wake behaviors to be performed.

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1. Introduction

Spontaneous locomotor activity is an instructive indication of animal (Pierce and Kalivas, 2001) or human (Sadeh, 2011) behavioral states. The exponential development of genetic models for psychiatric (Pawlak et al., 2008), human neurological and neuromuscular diseases (Trancikova et al., 2011), as well as for screening in pharmacological studies has led to the development of a wide range of appropriate behavioral analysis. A notable problem with such approaches, however, is that large differences can be observed between session-based experiments and home cage recorded activity due to experimenter intervention, anxiety arising from handling, circadian variations, etc. (Galani et al., 2001). To readily assess behavioral changes, such as those occurring during neurodegenerative processes, it is therefore important to be able to monitor activity for extended periods with a minimum of perturbing experimenter intervention.

For years, a wide variety of procedures has been developed to automatically track and quantify spontaneous locomotion. Other than the manual collection of behavioral data, automated systems based on photo-cell counters (Clarke et al., 1985) or mechanical devices (Brodkin et al., 2014; Ganea et al., 2007; Mollenauer et al., 1991; Rainer et al., 2011) have been extensively used, although such approaches require placing the animal in a dedicated experimental environment. Other systems based on implantable telemetric transmitters (Clement et al., 1989; Gegout-Pottie et al., 1999) or magnets (Baier et al., 2002; Storch et al., 2004) have been valuable in collecting both locomotor activity and physiological parameters, although these also require dedicated sophisticated devices that increase both the cost and experimental design complexity for monitoring each animal's activity. Besides these specialized devices, video-based tracking methods are currently commonly used (Spink et al., 2001) and with the continual development of novel algorithms, they have allowed the collection of increasing quantities of data (Brodkin et al., 2014; Giancardo et al., 2013; Gomez-Marin et al., 2012). However, these approaches also have several limitations, such as an inability for usage in a dark environment (which is critical for behavioral analysis of the nocturnal rodent) or to simultaneously track in a simple manner a large number of animals, as well as the requirement for a large amount of data treatment. Moreover, the reliability and accuracy of such procedures is highly dependent on the quality of the collected images.

The aim of the present study was to develop and validate a new method based on a 3D kinematic recording system. The main advantage of our experimental set-up is that it allows the simultaneous monitoring of movements in a large number of animals in their home cage or in any other open field area. Furthermore, we were able to automatically track the same animals for several

weeks (up to 3 months) with minimal experimenter intervention. Our movement monitoring system is continuous, fully automated and extremely precise (with a spatial precision of <1 mm and a sampling frequency of up to 100 Hz), and can function in both dark or lit environments. It can apply to a virtually unlimited number of animals and is cost efficient since it does not require devices dedicated to individual animals.

Results based on the use of this system have been published in two previous studies (Bellocchio et al., 2016; Du et al., 2016), although with minimal details on the methodology.

2. Material and methods

The mouse strain used in this study (B6SJL) was obtained from IFFA/Credo, (Lyon, France). Animals were kept in our laboratory animal housing facility under constant room temperature (22 °C) with a 12:12 light/dark cycle (lights on at 8 AM). Throughout the study, the mice had unlimited access to food chow (SAFE, Augy, France) and water in their separate home cages. Experiments were performed according to European Commission directives (86/609/EEC).

For animal housing, the original lids of standard polycarbonate cages (33 × 15 × 14 cm; Tecniplast, Limonest, France) were replaced by a wire netting of 1 × 1 cm mesh size to unmask the external environment. Food was placed directly into a bowl in the cage and the feeding bottle was attached on one of cage's sides. On a 2 × 2 m board fixed below the cage floor, a grid was marked so that when the cage was moved for weekly cleaning, it could be subsequently repositioned exactly at the same place (Fig. 1).

The markers used (Fig. 1A; B&L Engineering, Santa Ana, USA) were reflective spheres (diameter 7.9 mm, weight <0.5 g and fixed with a 3 mm metal nut) that reflected the infra-red light emitted by the cameras (see below). Each sphere was chronically implanted under isoflurane anaesthesia after weaning at PND 28. After a small skin incision, a 3 mm nylon screw was glued on the skull of the animal using dental cement. This allowed changing the marker if its surface became degraded with time. Animals were given analgesic and anti-inflammatory agents (carprofene, 10 mg/kg) during and after surgery. The marker was screwed on two days after the surgery. Alternatively, if only a short recording period (<5 days) was intended, the hemisphere markers were glued directly onto the head of the animal using cyanoacrylate adhesive.

2.1. Recording system and acquisition

Data were acquired using a Mac Mini computer (Apple, Cupertino, USA) with an Intel CPU 1.83 GHz/2 GB of RAM and a hard drive of 120GB. Marker positions were tracked using a 3-D motion

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