



METHOD ARTICLE

REVISED COMPASS: Continuous Open Mouse Phenotyping of Activity and Sleep Status [version 2; referees: 4 approved]

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Abstract

Background: Disruption of rhythms in activity and rest occur in many diseases, and provide an important indicator of healthy physiology and behaviour. However, outside the field of sleep and circadian rhythm research, these rhythmic processes are rarely measured due to the requirement for specialised resources and expertise. Until recently, the primary approach to measuring activity in laboratory rodents has been based on voluntary running wheel activity. By contrast, measuring sleep requires the use of electroencephalography (EEG), which involves invasive surgical procedures and time-consuming data analysis.






Methods: Here we describe a simple, non-invasive system to measure home cage activity in mice based upon passive infrared (PIR) motion sensors. Careful calibration of this system will allow users to simultaneously assess sleep status in mice. The use of open-source tools and simple sensors keeps the cost and the size of data-files down, in order to increase ease of use and uptake.


Results: In addition to providing accurate data on circadian activity parameters, here we show that extended immobility of >40 seconds provides a reliable indicator of sleep, correlating well with EEG-defined sleep (Pearson's $r > 0.95$, 4 mice).

Conclusions: Whilst any detailed analysis of sleep patterns in mice will require EEG, behaviourally-defined sleep provides a valuable non-invasive means of simultaneously phenotyping both circadian rhythms and sleep. Whilst previous approaches have relied upon analysis of video data, here we show that simple motion sensors provide a cheap and effective alternative, enabling real-time analysis and longitudinal studies extending over weeks or even months. The data files produced are small, enabling easy deposition and sharing. We have named this system COMPASS - Continuous Open Mouse Phenotyping of Activity and Sleep Status. This simple approach is of particular value in phenotyping screens as well as providing an ideal tool to assess activity and rest cycles for non-specialists.

Open Peer Review

Referee Status: 

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REVISED Amendments from Version 1

We have added further methodological details to the paper and have extended the discussion to highlight other promising work in this field. We have also added two supplementary figures, with further details relating to the comparison of PIR and EEG-derived measurements of sleep (Figure2_suppl) and relating to the variation in patterns of activity and sleep in wild-type mice (Figure5_suppl). Finally, we have added an additional electronic notebook to the online resources, highlighting one possible approach to the analysis of bouts of sleep from COMPASS data.

See referee reports

Introduction

24 hour rhythms of activity and rest occur in virtually all organisms. Remarkably, these circadian ('around a day') rhythms persist in the absence of external stimuli, demonstrating the presence of an internal biological clock. The timing of many biological processes, including locomotor activity and sleep, are regulated by the circadian system and disruption of these rhythms has been associated with a wide range of health consequences, including cognitive impairment, metabolic and cardiovascular disease and even cancer^{1,2}. In laboratory mice, patterns of activity and sleep provide valuable markers of health and disease. Animals display characteristic changes in physiology and behaviour associated with illness, including increased sleep, fever, weight loss and reduced social interaction³, yet changes in activity and sleep are rarely used as welfare indicators⁴. Furthermore, changes in circadian rhythms and sleep may precede symptoms in other disorders, as has been shown in neurodegenerative and neuropsychiatric disease⁵⁻⁷. As a result, many researchers working with laboratory mice are interested in studying longitudinal patterns of activity and sleep in disease models.

Measuring long-term locomotor activity and sleep in mice requires specialised resources and expertise, and may require surgical intervention. Cost and complexity have previously placed limits on the widespread use of such measures. Whilst many commercial systems are available, these are often targeted at those with specific research interests in circadian rhythms or sleep, and are not suitable for widespread implementation. When a potential behavioural phenotype is predicted in a transgenic model and the mice are available in another facility, the ability to examine activity and sleep patterns, non-invasively, within the home facility would be beneficial. Moving transgenic animals often requires specialist facilities for import and re-derivation of the model, a process often required to maintain health standards within facilities. Avoiding the need for such procedures would reduce the number of animals bred for research and curb increased economic costs of the research.

A wide range of methods are used to monitor locomotor activity, such as running-wheels, beam-break or video monitoring. Whilst home cage running wheels are widely used in circadian biology^{8,9}, it is recognised that exposure to a wheel may modify the behaviour of the animal¹⁰. Beam-break methods are ideal for short duration assessment of exploratory behaviour¹¹, but require specialised caging and are therefore not suitable for long-term monitoring.

Sleep is a complex physiological process resulting in coordinated changes in locomotion, body posture and responsiveness to stimuli¹². Whilst sleep provides an ideal welfare indicator, it is rarely used in laboratory rodents due to the requirement of electroencephalography (EEG) and electromyography (EMG) which involve surgical intervention. Whilst the detailed study of sleep stages require EEG/EMG, a number of non-invasive correlates including video-based monitoring and piezo-electric sensors have been described which enable high-throughput assessment of sleep status, enabling total sleep, sleep timing and sleep fragmentation to be measured¹³⁻¹⁵. Whilst video monitoring has been increasingly used¹³, such methods generate large amounts of data and are computationally intensive, making them unsuitable for large scale implementation or real-time analysis.

To address these issues, we established a minimal system for measuring activity in relation to the lighting environment. The use of microcontrollers (simple, single-chip computers) and in particular the Arduino family of open-source hardware has been discussed as a flexible solution to a number of scenarios where current lab equipment and technologies are either insufficient or prohibitively expensive^{16,17}. From the outset, the design of the system was kept as simple as possible, whilst maintaining integration of the sensors at the level of the microcontroller. Using pyroelectric or passive infrared sensors (PIRs) provide a cheap means of measuring activity which is easily incorporated in home cages and is easily scalable.

Methods

Activity monitoring system

The PIR used incorporated an integrated digital amplifier (model: Panasonic EW AMN32111) alongside a light-dependent resistor (LDR, Excelitas Tech - VT90N1). The use of PIR sensitive to slight movements and with an inbuilt amplifier will help to ensure consistency of the data generated. In this regard, the essential considerations for reproducibility will be the type of sensor used, the distance from sensor to mouse and the temporal resolution of the measurements. In the system built for this paper the sensors were incorporated into simple circuits (see [Supplementary material](#)). The sensors were read using the Arduino Uno (Rev3) board, featuring an ATmega328 microcontroller (<http://arduino.cc/en/Main/ArduinoBoardUno>). Printed circuit boards were designed using Fritzing (<http://fritzing.org/home/>, versions up to 0.9.3b), and manufactured through their fabrication service. All other components were from Farnell (<http://uk.farnell.com/>, Leeds, UK), or Mouser Electronics (<http://uk.mouser.com/>, London, UK). Software (sketches) for the system was written and tested using versions 1.0.1–1.0.5 and 1.5.6–1.6.8 of the Arduino integrated development environment (IDE, available at <http://arduino.cc/en/Main/Software>). This software is written in Java and is both open-source and cross-platform¹⁸. Data storage and visualization was carried out using Processing (also free and open-source and available for multiple platforms, see <http://processing.org/>). The programs detailed in the current paper work with versions up to 2.2.1.

Positioning of sensors

The cages used in the study were either Techniplast 2154F (Techniplast S.p.A., overall size 482 × 267 × 210 mm), with a modified top and externally-mounted holders for food and water, or an MBI

mouse cage (North Kent Plastic Cages, Overall size: 450 × 280 × 130mm), with wire top. An accurate measurement of activity and rest relies on the infrared radiation from the mouse being able to reach the sensor at all times. For this reason the small gaps under the food and water hoppers on the MB1 cages were blocked using Perspex blocks and no environmental enrichment was used that could fully obscure the animal (e.g. Perspex tubes). The nesting materials used in the study were either paper-based Sizzle-Nest, or cotton fibre Nestlets (Datesand Group, Manchester, UK), with a minimum of 10g in each cage (in excess of that required for successful thermoregulation by mice with similar materials¹⁹). Excessive nesting materials could potentially obscure the mouse from the sensor and nesting boxes will prevent the accurate assessment of activity and sleep. Cages require wire tops as most plastics show very low transmissivity for the wavelengths of energy detected by infrared sensors (approximately 5–10µm).

The timing and collection of data

In order to establish an efficient system, the collection of data was based around previous work showing that periods of immobility >40s in mice were an accurate indicator of sleep^{13,15}. Preliminary experiments with the PIRs showed that a polling time of 100msec was a good balance between capturing brief movements and over-estimating movement due to the dwell-time of the PIR (the time the sensor stays active after movement stops). With 10s bins this provides 100 separate 100msec measurements and therefore a simple % activation of the sensor can be calculated for each of the 10s epochs. A serial message containing all the activity data and a single measurement of environmental light from the LDR is then sent to the PC connected via USB, where it stored alongside a timestamp. Using ISO8601 UTC (Coordinated universal time) timestamps for the data as saved to file helps to minimise future errors caused by difference in time zones and daylight savings times.

Calculation of sleep status from activity

With data collected as a percentage activity in 10s bins, the calculation of 40s of immobility is as simple as a rolling sum over the last 4 bins. This can be achieved in many software programs, including the Microsoft Excel (using a function such as “=IF(SUM(A1:A4)=0,1,0)”). For the current study these calculations were carried out using the python [PyData stack](http://pydata.org/), (<http://pydata.org/>), including the Pandas library (version 0.18.0)²⁰ that will allow easy import of the .csv files produced by the system, resampling and rolling-sum calculations. Examples of the processing of data are provided in a series of interactive notebooks online ([Data and software availability](#)).

The use of animals

All work was carried out in accordance with Animal [Scientific Procedures] Act 1986, with procedures reviewed by the clinical medicine animal care and ethical review body (AWERB), and conducted under project licence PPL 30/2812 and personal licences I459D3D59 and IDB24291F. Young-adult male wild-type C57BL/6J mice (RRID:IMSR_JAX:000664), were obtained from Envigo (Alconbury UK), with all experiments carried out when the mice were between 12 and 24 weeks of age. Animals were

housed in specific pathogen free conditions, with the only reported positives on health screening over the entire time course of these studies being for *Helicobacter hepaticus* and *Entamoeba spp.* All animals were singly-housed, provided with food and water ad libitum and maintained on a 12h light:12h dark cycle (150–200 lux, measured at the cage floor), in light-tight environmental enclosures (in groups of 6 cages). Where constant light was used to establish circadian parameters, the same intensity of 150–200 lux was used. Comparison of EEG and PIR-derived sleep was carried out within individual mice, and no mice were excluded from any group in this work.

Comparison of EEG and PIR-estimated sleep

A telemetric transmitter (volume, 1.9cm³; total weight, 3.9g; TL11M2-F20-EET; DSI, St. Paul, MN, USA) connected to electrodes for continuous EEG and EMG recordings was implanted in 4 adult male C57BL/6J mice (15.8 ± 0.6 weeks old, mean ± S.E.M.) as described in (and adapted from Hasan *et al.*, 2014)²¹. Briefly, in mice under anesthesia (isoflurane induction 4.5%, maintenance 0.7–2.25%), 2 stainless-steel EEG electrodes (length of screw shaft, 2.4mm; outer diameter of screw thread, 1.19mm) were implanted epidurally over the right frontal and parietal cortices²² and connected to the telemetry transmitter via medical grade stainless-steel wires (surrounded by silicone tubing). The EEG electrodes and connections to the subcutaneous wiring were covered with dental cement (RelyX Arc; Kent Express, Kent, UK). Two EMG stainless-steel leads were inserted into the neck muscle ~5mm apart and sutured in place. The telemetry transmitter was placed into the abdominal cavity of the mouse. Perioperative analgesics were administered at the onset of surgery (buprenorphine, 0.1mg/kg; meloxicam, 5mg/kg) and the next day (meloxicam, 5mg/kg). Saline (0.9%, 500µl) was also administered by subcutaneous injection at the end of the surgery. After surgery, the animals were allowed to recover for more than 2 weeks and 24 h EEG data-collection began when the mice were 20.1 ± 0.4 (mean ± S.E.M.; n=4) weeks of age. The telemetric transmitters were activated 1-2 days before recordings, and EEG/EMG signals were then recorded continuously (Dataquest ART; DSI). The EEG and EMG signals were modulated with a one pole high-pass (-1.1dB at 1.0Hz; -3.8dB at 0.5Hz) and a two pole low-pass antialiasing (-1.6dB at 50Hz) analogue filters built in the transmitter. An additional (30th order low-pass FIR) digital filter was selected at 49Hz (2dB of attenuation). These signals were visually classified into 10s epochs of vigilance states according to standard criteria²².

Statistics and figures

Correlation coefficients (Pearson's r) were calculated and correlation plots and hierarchical clustering produced using the Seaborn package for Python (<http://stanford.edu/~mwaskom/software/seaborn/>, version 0.7.0). Actograms and Chi-squared periodograms in [Figure 1](#) were generated using the ActogramJ plugin (version 0.9-1.0, <http://actogramj.neurofly.de/>)²³ for the ImageJ program (version 1.5.1g, <http://imagej.nih.gov/ij/>), and based on 10min bins of mean activity to improve clarity. Other figures were generated using Matplotlib²⁴ (<http://matplotlib.org/>, 1.5.1) and final figures were arranged using Adobe Illustrator and Photoshop (CS5, Adobe Systems Inc.)

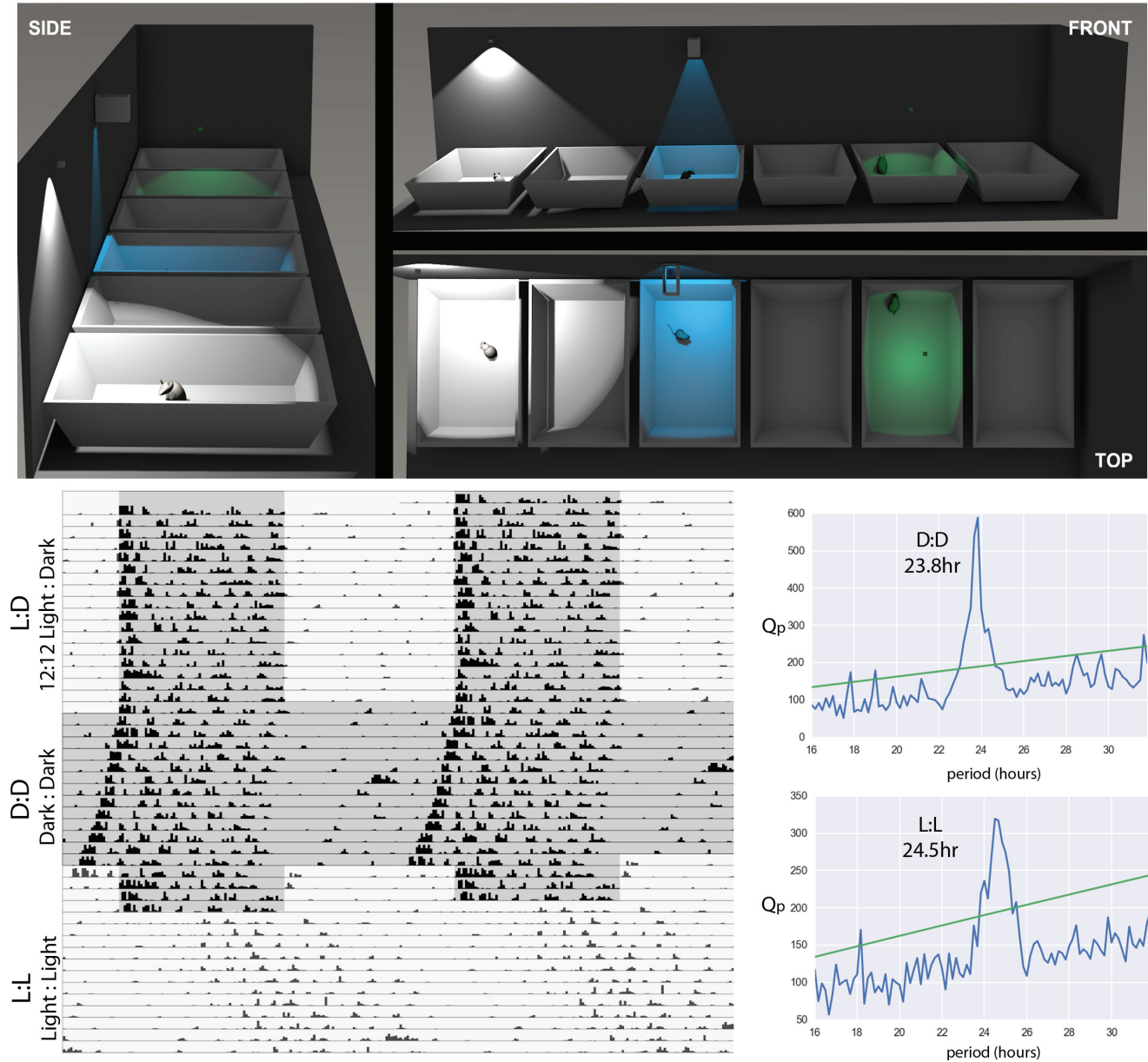


Figure 1. Considerations for a system for monitoring activity and rest. (Top Panel) Positioning of sensors and shielding to ensure accuracy of readings: Positioning of the sensors and any required shielding can be calculated using the angle of detection for any given sensor. In the case above, a sensor positioned 440mm above the cage floor at the back of the cage (WHITE) will also sense movement in neighbouring cages. This can be prevented by shielding the sensor (BLUE), or moving the sensor centrally, 220mm above the floor (GREEN). At different heights the sensor will be activated by different degrees of motion. Models created in the Blender 3D modelling program (version 2.68a, <http://www.blender.org/>) with each PIR represented as a lamp with the field of illumination the same as the field of detection for the PIR. Interspersed cages in the illustration are left empty to aid with clarity. Accurate positioning of the sensors will negate cross-talk and remove the need for empty cages.; (lower panel) Examples of actograms collected from a single C57BL/6J mouse under a 12h:12h light:dark-cycle, constant darkness and constant light, with Chi-squared periodograms showing the main rhythmic component of activity under these 2 constant conditions (sensor in equivalent position to blue light in top panel).

Results

PIRs with inbuilt amplification and a binary digital output, used alongside a light-dependent resistor (LDR) provide the simplest configuration that could be used for consistent measurement of activity. Whilst LDRs provide a good marker for gross changes in the light environment, this is no replacement for a correctly measured and calibrated light source, especially when considering circadian and visual processes²⁵. The experimental setups are shown visually in [Figure 1](#) and further details of the experimental setup can be found in the [Supplementary materials](#).

Circadian measures of activity can be established using PIRs. Studies initially ensured that no cross-talk between cages was occurring, before characterising the degree of motion that was required for activation of the PIR sensor. The sensitivity of the detectors is dependent on the lensing and the distance of the sensor from the cage floor. Using information about the angle of detection (in this case 91°) the field of detection can either be calculated, or the environment modelled using 3D-modelling software (Blender, version 2.68, <http://www.blender.org/>), see [Figure 1, upper panel](#)). This approach is useful when considering the environment in different animal facilities to show the options for positioning or shielding sensors, to prevent cross-talk. PIRs were then incorporated into the microcontroller system, where data could be analysed using existing tools²³. This demonstrated circadian entrainment, with elevated nocturnal activity and clear activity onsets, free-running activity in constant dark (DD) with a circadian period of <24h and period-lengthening in constant light (LL) with a circadian period >24h ([Figure 1, lower panel](#)). For comparison, these PIR sensors were also incorporated into an established system for studying circadian behaviour (ClockLab, Actimetrics, IL), providing comparable results.

To determine the activity threshold required to activate the PIR, video of mice in cages was recorded, while the output of the PIR was linked to a circuit including a near-infrared LED (emission peak 850nm). This LED was placed in the corner of the field of view of the camera, outside and below the top of the cage. As sensitivity is determined by how close the PIR was positioned relative to the cage floor, this was assessed under two PIR positions 440mm and 220mm (equivalent to BLUE and GREEN positions in [Figure 1, top](#)). At 440mm, activation every 1–2 steps was observed (gross locomotion), whereas at 220mm, activation of the PIR was also observed with small movements, such as head turns and rearing (see [Video](#)). This ability to separate small movements from immobility raised the possibility that this approach could be used to identify sleep in addition to locomotor activity^{13,15}.

Sleep scored by immobility under PIR correlates well with total EEG-scored sleep

Using a criteria of >40sec of immobility as used previously^{13,15}, a PIR at 220mm above a cage floor was found to provide a high correlation with sleep assessed by video-tracking. As such, PIR-determined sleep was directly compared against sleep measured using EEG/EMG in the same animals. C57BL/6 mice (n=4) were implanted with dual biopotential telemeters (DSI, St. Paul, MN, USA). Following recovery and entrainment to a 12:12 light:

dark-cycle, 24h of activity from PIRs at 220mm above the cage floor was compared to EEG/EMG scored sleep ([Figure 2](#)). A high degree of correlation was observed with the total amount of sleep reported by EEG/EMG (>0.95 in all mice, Pearson's r , [Figure 3, left](#)). A Bland-Altman comparison of methods²⁶ showed a good agreement of the total sleep in each 30min bin (PIR-EEG = +1.9min, -3.5 to +7.3min, 95% confidence intervals), with a slightly lower agreement in the 12h of light ([Figure 3, right](#)). This is likely to be due to reduced overall movement in the light leading to over-estimation of total sleep by the PIRs as has been reported for other immobility-based methods¹⁵.

Long-term monitoring of activity and immobility-scored sleep

Removing the need to collect and process video files allows the assessment of activity and sleep (as immobility over 40s) over longer periods of time. [Figure 4](#) shows an example of the length and detail of recording that is possible. A time series of activity data (and from immobility, sleep) over 1 month can be examined in more detail in sections of 1 week, or a single day.

Wild-type laboratory mice show repeatable patterns of activity from day to day, but these patterns differ between individuals

When studying any biological process, rhythmic variation in measurements is often important but either ignored or not studied. Attempts to study variation over time will usually involve collecting time series of data. These can either be longitudinal (multiple measurements within the same subject) or transverse (a time series constructed from measurements from many individuals). The data in [Figure 5](#) reveals the importance of longitudinal measurements where possible. Unsupervised hierarchical clustering of 1 week of data from 24 male wild-type mice shows that although many animals show repeating patterns of activity from day to day (high interday stability), these patterns of activity and sleep are often different to those mice in neighbouring cages. Clusters do not just consist of those mice in the same environmental chamber (4 chambers, with mice numbered 1–6, 7–12, 13–18 or 19–24 housed together, [Figure 5](#)). The clustering also indicates the measure of sleep from immobility does not result in the same clustering as activity, showing that sleep is not just the absence of activity and that cycles of activity and sleep are distinct (yet related) biological processes. Furthermore, differences in the positions of individual mice in the heatmaps of all immobility (>0s) vs sleep defined as extended immobility (>40s) show that, although the estimation of sleep is related to immobility, use of immobility (>0s) alone will overestimate sleep. Moreover, the relationship between these two measurements is not the same for all individuals ([File 5_supplementary](#)). Longitudinal measurement over multiple days also reveals additional ultradian rhythms in activity and sleep in some mice ([Figure 5, lower panels](#)).

Examples of movements of a mouse required to activate PIR sensors at different heights from the cage floor

1 Data File

<http://dx.doi.org/10.6084/m9.figshare.4072701>

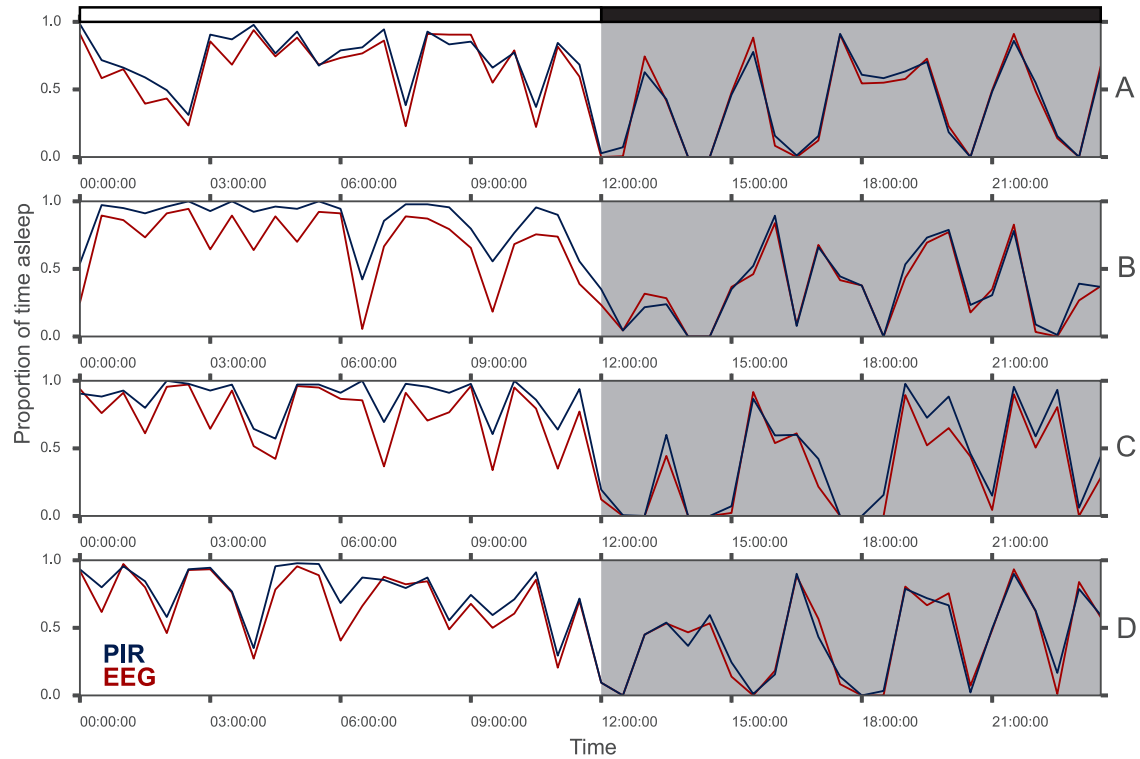


Figure 2. Sleep, as assessed by EEG recordings and by immobility. Comparison of periods of immobility (> 40s) measured by PIR to manually-scored sleep from EEG telemetry (as proportion of 30min bins), in 4 male C57BL/6J mice, entrained to a 12h:12h light:dark-cycle (labelled A to D).

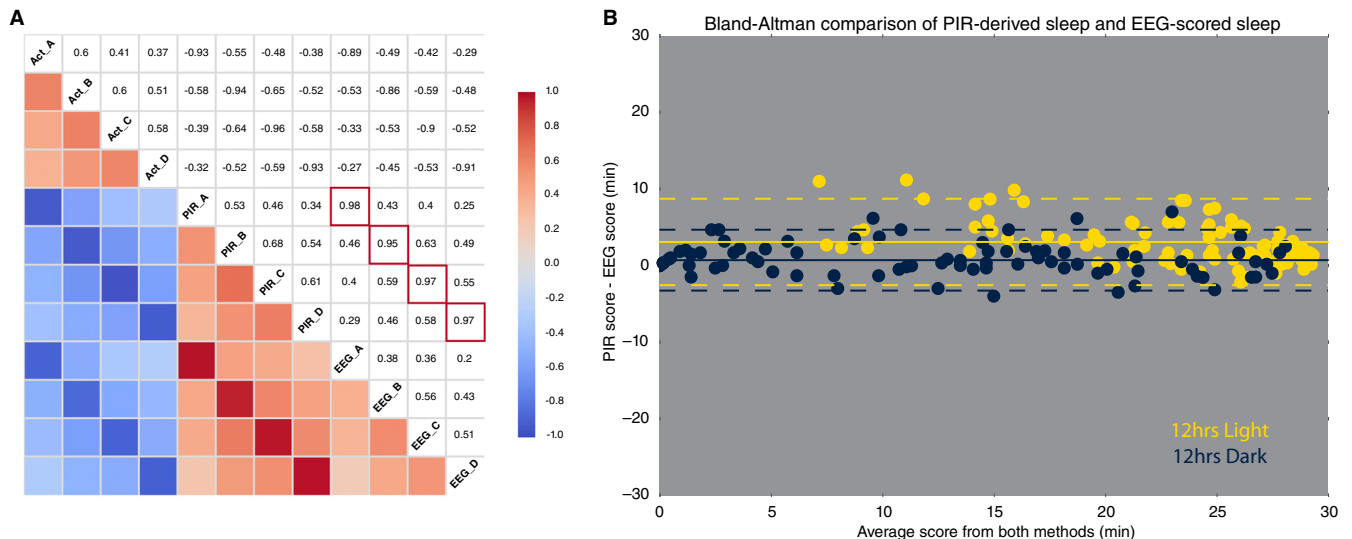


Figure 3. Immobility as a correlate of sleep, and compared to total sleep scored from EEG/EMG records. a) Correlation plot shows the relationships between the activities of 4 C57BL/6J mice, the sleep scored from periods of immobility between bouts of activity and the total sleep scored by EEG/EMG in the same mice. High correlation between PIR and EEG: Pearson correlation coefficients of 0.95-0.98 for sleep per 30min bin (scored by immobility >40sec by PIR and by manual scoring of total sleep by EEG traces, red boxes). b) Bland-Altman comparison of methods for scoring total amounts of sleep. Good agreement of the total sleep in each 30min bin is achieved in the 12hrs of dark (PIR-EEG = +0.7min, -3.3 to +4.7min 95% C.I.). As is apparent from the individual traces (see Figure 2) the agreement is lower in the 12hrs of light (PIR-EEG = +3.1min, -2.6 to +8.7min 95% C.I.). This is likely to the reduced overall movement in the light leading to over-estimation of total sleep by the PIRs. A closer look at the data from Figure 2, with 5min bins for sleep as measured by EEG and PIR, supports the suggestion that bouts of quiet wakefulness (with minimal or no associated movement) would be detected by EEG but may be missed in any measure of sleep based on extended immobility (see File 2_supplementary). Although these bouts of waking make minimal difference to overall measurement of time asleep, they can lead to longer bouts of sleep being reported.

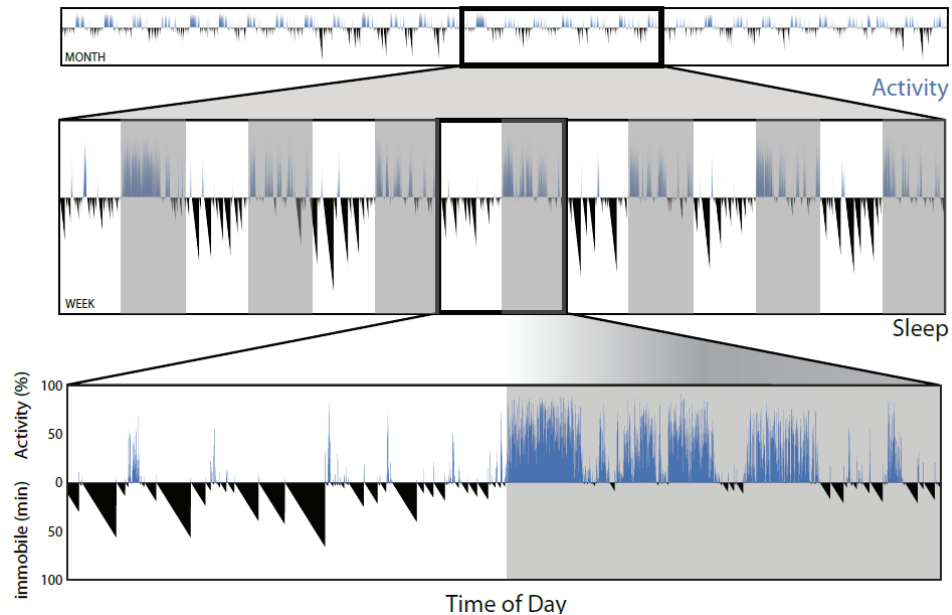


Figure 4. Minimal data processing allows for long-term longitudinal studies of patterns in both activity and sleep. Traces for activity (blue, upward deflection) and sleep (black, downward increasing deflection in minutes for immobility longer than 40 seconds) from a single male C57BL/6J mouse for 1 month. Lower panels show one week (middle), or a 24h period lower panel) from within this data at higher resolution.

Discussion

Here we have shown that passive infrared sensors (PIRs) coupled with an LDR and a readily available microprocessor provide a simple, affordable system for the combined measurement of activity, light and sleep over days, weeks, or even months (Figure 4). We have named this system COMPASS - Continuous Open Mouse Phenotyping of Activity and Sleep Status. COMPASS enables researchers working on transgenic mouse models in any discipline to easily use activity and sleep as biomarkers without the need for specialist resources or expertise. Longitudinal monitoring of activity and sleep will also help to assess the effect of any novel therapeutic interventions in pre-clinical models and act as potential objective welfare indicators. Early incarnations of the system have already proved valuable for studies of a number of mouse models of disease^{27,28}, with phenotypes described using video-based assessment of sleep and the COMPASS system appearing the same (both offering an important comparison to patterns of wheel-running behaviour)²⁹. Measures of immobility will potentially have difficulty detecting phenotypes such as narcolepsy, if the onset of sleep is sudden and sleep duration is short. However, as recent studies of orexin knockout mice with a non-invasive piezo-electric system to assess have suggested, the total daily amount of sleep between wild-type and transgenic animals was the same, suggesting that missed bouts of narcoleptic sleep (occurring with or without preceding inactivity), may lead to different total amounts of sleep being recorded³⁰. As always, users should always be mindful of the temporal resolution and assumptions in the measurements they employ.

There are a number of labs working to explore non-invasive methods of assessing sleep and both video-tracking and piezo-electric

systems have proved to be valuable in the study of sleep in mutant mouse models of disorders such as narcolepsy³⁰ and Huntington's disease³¹. Furthermore, modelling and machine learning using EEG/EMG data³², video-tracking³³, or piezo-electric data³⁴ are helping to improve the scoring of different stages of sleep, especially for Rapid Eye Movement (REM) sleep. There are also systems for monitoring the behaviour of multiple co-housed animals, including patterns of activity and rest³⁵. COMPASS prioritises simplicity and efficiency for assessing sleep, while additionally providing a measure of locomotor activity. Although this system is simple, this allows both the cost (from around £20 per cage) and size of the data (1-2Mb per cage per month) to be kept to a minimum. Affordability and minimal data requirements mean such a system is ideally suited for web-based data logging, enabling researchers to monitor their ongoing experimental animals remotely and the open nature should allow for integration of other environmental and physiological sensors.

It is important to note that PIRs will only measure total movement in a cage, necessitating single-housing to establish individual patterns of activity and sleep. Although this is the standard experimental approach used in circadian and sleep laboratories, group housing is preferable for long-term colony housing. However, COMPASS is also suitable for monitoring total cage activity in group housed mice, where altered behavioural patterns or health status in one animal may be identified via changes in whole cage activity. Further studies may be required to establish standards for group monitoring in this manner. Non-invasive, longitudinal measurement of both activity and sleep status would be transformative for many fields of research, as well as animal welfare. Recent studies have shown that, in models relevant to psychosis, depressive and anxious states

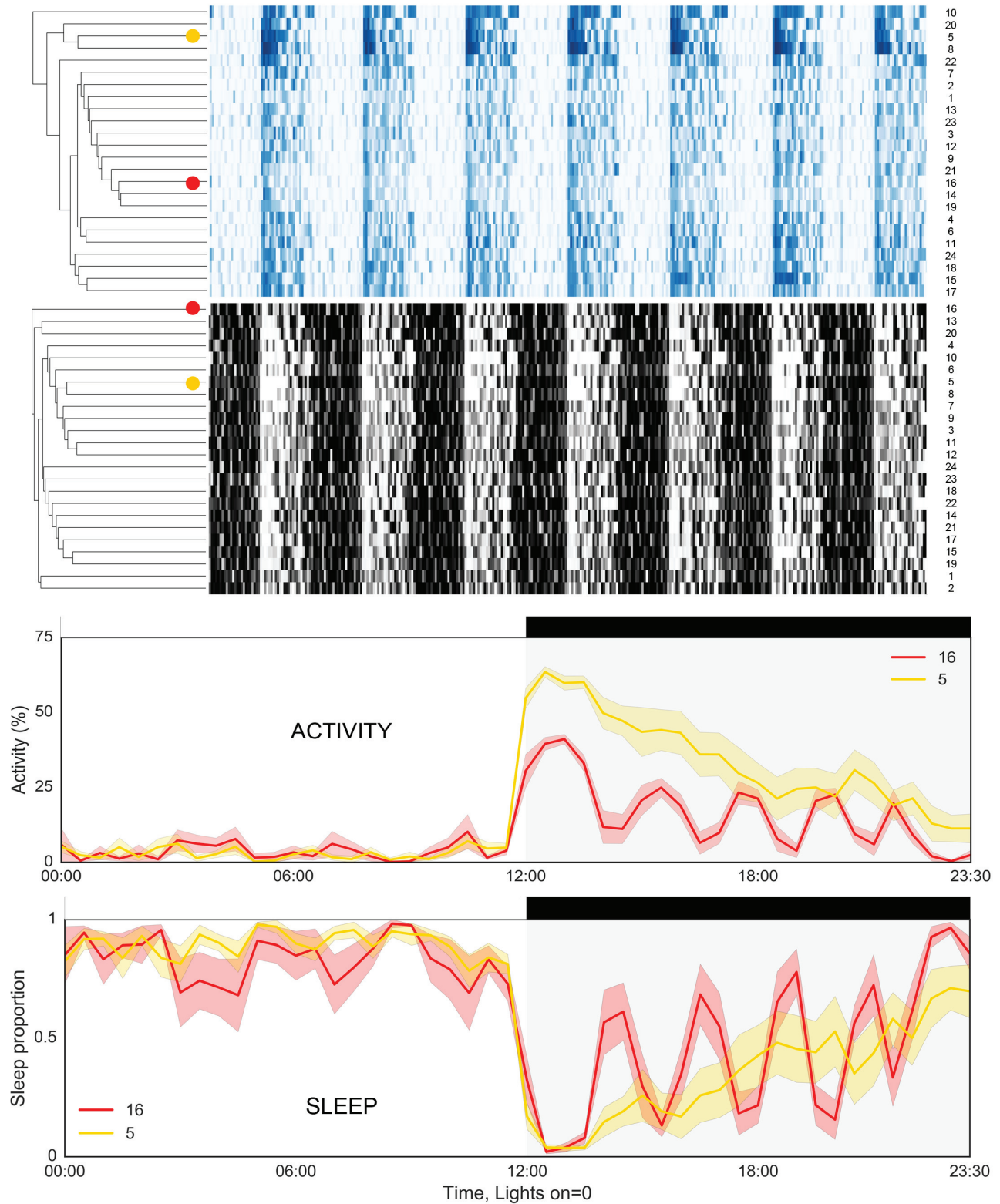


Figure 5. Variation in the daily patterns of activity and sleep between wild-type mice. Hierarchical clustering of activity (blue, top) and sleep (black, bottom), for 24 male wild-type C57BL/6J mice shows a range of different patterns. Lower graphs indicate that from day to day each mouse repeats a similar pattern over each 24h cycle (lower intra-day variation). However, variation in amplitude and distribution of activity and sleep can be much greater between mice. Example traces (taken from the marked positions on the heatmap above) show mean activity (for 7 days) for a single male mouse in 30min bins across the day, with shaded areas showing the standard error of the mean (S.E.M.).

are correlated to infradian variation in home cage locomotion³⁶. Furthermore, recent work shows that the success of haemopoietic stem cells transplants in repairing blood and bone in a recipient mouse are abrogated when the donor is sleep deprived³⁷. This suggests prior activity and sleep history are important variables in many if not all animal experiments, ones that are currently often ignored. This study details an important step towards the goal of widespread longitudinal measurements of activity and sleep in laboratory animals.

Data and software availability

The programs (sketches) for the Arduino microcontroller and data-collection (via Processing) are provided online ([current GitHub repository](#)), alongside a series of interactive Python notebooks (<http://jupyter.org/>). All PIR data for assessing activity and sleep in mice is also provided in this repository ([Figure 2–Figure 5](#)), as is the sleep scoring from the EEG data used for comparison in [Figure 2](#) and [Figure 3](#).

Zenodo: Dataset 1. The programs for hardware, Python notebooks, and files from Dataset 2, [10.5281/zenodo.345396](https://zenodo.org/record/345396)³⁸

Zenodo: Dataset 2. PIR data, and sleep as scored from EEG files (.csv), [10.5281/zenodo.160344](https://zenodo.org/record/160344)³⁹

Zenodo: Dataset 3. The raw EEG data, 4 files (EEG_A to D), in European data format (.edf), [10.5281/zenodo.160118](https://zenodo.org/record/160118)⁴⁰

Figshare: Examples of movements of a mouse required to activate PIR sensors at different heights from the cage floor. doi: [10.6084/m9.figshare.4072701](https://doi.org/10.6084/m9.figshare.4072701)⁴¹

Author contributions

LAB and SNP designed the study. LAB designed microcontroller/PIR systems, and developed the software. LAB and SH carried out collection and analysis of data. All authors contributed to writing the paper.

Competing interests

No competing interests were disclosed.

Grant information

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We would also like to thank Lindsay Benson for surgical advice and assistance and members of the NDCN (including Stephen Hicks and Iain Wilson) and UCL (Clare Stanford, Ashley Porter and Katie Pillidge) for helpful discussions. Finally the authors would like to thank the open-source communities connected to [Arduino](#), [Processing](#), [Python/PyData stack](#) and [Blender](#) for the tools used to illustrate the methods in this paper.

Supplementary material

Supplementary File 1. Additional details for construction of a 6-channel system.

[Click here to access the data.](#)

Supplementary File 2. Sleep, as assessed by EEG recordings and by immobility (5min averages).

Comparison of periods of immobility (> 40s) measured by PIR to manually-scored sleep from EEG telemetry (as proportion of 5min bins), in 4 male C57BL/6J mice, entrained to a 12h:12h light:dark-cycle (labelled A to D).

[Click here to access the data.](#)

Supplementary File 3. Fritzing circuit board design for sensors.

[Click here to access the data.](#)

Supplementary File 4. Fritzing circuit board design for microcontroller.

[Click here to access the data.](#)

Supplementary File 5. Variation in the daily patterns of inactivity and sleep compared.

Hierarchical clustering of inactivity (red, top) and sleep, as defined by ≥ 40 s in such immobility (black, bottom), for 24 male wild-type C57BL/6J mice. Differences in the positions of individual mice in the heatmaps show that, although the estimation of sleep is closely tied to overall immobility, the relationship between these two measurements is not the same for all individuals.

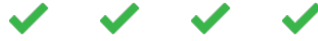
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Open Peer Review

Current Referee Status:



Version 2

Referee Report 03 May 2017

doi:[10.21956/wellcomeopenres.11937.r22134](https://doi.org/10.21956/wellcomeopenres.11937.r22134)



Christopher Colwell , Dawn Loh

Department of Psychiatry and Biobehavioral Sciences, University of California - Los Angeles, Los Angeles, CA, USA

I am satisfied with the response and feel that this work will make a solid contribution to this research area.

Competing Interests: No competing interests were disclosed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 09 December 2016

doi:[10.21956/wellcomeopenres.10663.r17584](https://doi.org/10.21956/wellcomeopenres.10663.r17584)



Hugh Piggins

Division of Diabetes, Endocrinology & Gastroenterology, University of Manchester, Manchester, UK

In this manuscript, Brown and colleagues report a new, low cost (COMPASS) system based on passive infrared detectors (PIR) to longitudinally assess sleep in mice. This is an important advance since conventional methodologies to measure sleep in rodents are invasive and require considerable finances and infrastructure. Further, such approaches result in the acquisition of large volumes of data whose analyses are complex and time consuming. The quality and quantity of sleep is widely recognized as important for general health across a wide range of organisms and method described in this manuscript provides an interesting and readily adaptable system to enable many labs to quantify sleep in mice. This approach quantifies immobility (40s epochs) and uses this as an index of sleep state. They validate COMPASS through careful and sophisticated comparison to sleep as measured through the conventional electroencephalogram (EEG) method. In general this is a very well-written and nicely illustrated manuscript, with the details and validation of COMPASS readily understandable.

1. In the analysis of this PIR-based method, mice with fully functional circadian and sleep regulation were used (the C57BL6 animal). Can the authors speculate on the effectiveness of this approach in animals such as orexin knockout mice in which overall sleep in 24h does not differ to that of

controls, but where there is an increase in cataplexy? Specifically, how will this method distinguish between immobility due to a motor disorder versus a bonafide sleep alteration?

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 11 Apr 2017

Laurence Brown, University of Oxford, UK

Dear Professor Piggins,

Thank you for the helpful review of our work. We have added to the discussion in order to stress the importance of careful interpretation of results for sleep based on immobility alone. It is interesting that sudden-onset bouts of sleep (cataplexy) might be missed or underestimated using ≥ 40 s of immobility as criteria, but that in groups with the same overall amounts of sleep, this would lead to a decrease in the reported sleep each day and therefore a difference when compared to wild-type litter-mates.

Our recent use of the COMPASS system alongside voluntary running wheel activity would suggest that the PIRs often give a more sensitive and unbiased measure of overall activity. Mice are often awake active, but not running in a wheel. However, we acknowledge that more data is needed before activity (and consequently, immobility) alone could be used to reliably separate motor deficits from changes in the parameters of circadian rhythmicity or sleep, especially as many of the mutant mice studied have complex phenotypes

Competing Interests: No competing interests were disclosed.

Referee Report 06 December 2016

doi:[10.21956/wellcomeopenres.10663.r17583](https://doi.org/10.21956/wellcomeopenres.10663.r17583)



Christopher Colwell , Dawn Loh

Department of Psychiatry and Biobehavioral Sciences, University of California - Los Angeles, Los Angeles, CA, USA

There is a large interest in the role of sleep in our health and in the disease process. One of the major limitations in the study of sleep is the difficulty in measuring sleep using electroencephalography (EEG) with the required surgical procedures and time-consuming data analysis. The Peirson group has previously made good progress using a video based system to quantify sleep behavior. This system has been used by laboratories around the world. So we read with interest this study which describes simple, non-invasive system to measure home cage activity in mice based upon passive infrared (PIR) motion sensors. Careful calibration of this system will allow users to simultaneously assess sleep status in mice. The use of open-source tools and simple sensors keeps the cost and the size of data-files down. We looked over the plans and felt that we could implement this system. Broadly, this is a very promising approach to address an important issue i.e. measurement of sleep in rodents using a high-throughput system.

While we are generally supportive, we did have some problems with the present ms. First, as the authors well know, inactivity is not the same as sleep. Some more validation of their sleep measurements is important. For example, do hypnotics increase and stimulants alter PIR defined sleep? When the mice are sleeping can they demonstrate the increase in threshold to external stimuli? The authors undertook this type of validation in their prior study with video analysis and we feel that they should do the same type of validation with this new methodology.

In addition, we would like them to compare sleep fragmentation with the IR and compare that to values obtained with the EEG. Sleep fragmentation is an important parameter in disease models and should be detected with their methodology. Their prior video-based system was effective in measuring this parameter.

It was unclear if the mice needed to have an empty cage in between the measurement cages. We do not see why this would be the case but the illustration in Fig. 1 suggests the need for a separating cage. Please clarify.

We were uncertain as to the utility of the cluster analysis. We did like the clear demonstration that the intra-day patterns were stable between mice. But we felt that the cluster analysis would be more useful if it compared the inverse of activity in top panel with the IR-defined sleep below. This would help address the issue of whether IR-defined sleep is more than the absence of activity.

Finally, several labs have been exploring the use of behavior measures of sleep including IR measures as used in the present study. The authors should expand this part of their discuss to include others working in this space.

Competing Interests: No competing interests were disclosed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 11 Apr 2017

Laurence Brown, University of Oxford, UK

Dear Dr Loh and Professor Colwell

Thank you for your review and the important points that you raise. On the first point we agree that we should continually reassess of the use of immobility as a surrogate of EEG-defined sleep. However, we would consider the use of passive infrared sensors as a more efficient way of assessing immobility, rather than a new methodology to measure sleep. As you have discussed, the previous work from the group with video tracking showed that hypnotics increase and stimulants decrease immobility. It is definitely the case that data from the COMPASS system will allow efficient measurement of bouts of both activity and sleep, as well as measures of stability in these bouts. This is one area where long-term recordings will be especially useful. We are focusing current and future efforts on this aspect of analysis, but we wish to collect data from a number of known sleep and circadian mutant models first. One additional advantage of the .csv file format used is that it provides easier import of data in to existing programs for bout-analysis and measures of fragmentation with which groups might already be familiar. In order to help demonstrate how analysis of bouts could be approached we have included another notebook for

download with the revised paper (COMPASS_sleep_bouts.ipynb).

It is also possible to look at the PIR and EEG-defined sleep in **Figure 2** at a higher temporal resolution. Averaging sleep with each system over 5min bins further supports the suggestion that small waking movements in the light period would be the main source of error for the PIR-based estimate of sleep. Consequently, this would lead to longer uninterrupted bouts of sleep being recorded with PIRs. A note to this effect has been added to the revised manuscript (along with a supplementary version of **Figure 2**).

You are correct that, provided the sensors have been correctly tested, there is no need to leave empty cages between those in use. As one sensor is shown reading from a neighbouring cage, we left some empty in **Figure 1**. A note to this effect has been added to the legend of **Figure 1**.

We have added a comparison of the hierarchical clustering of the 24 wild-type mice from immobility (>0s) versus sleep defined as extended immobility (>40s) (now included in the revised paper as a supplement to **Figure 5**). This shows that sleep will be over-estimated based on immobility alone, and that the clustering of mice by their immobility (>0s) vs extended immobility (>40s) profiles differs, illustrating that sleep behaviour is not simply the inverse of locomotor activity.

Finally, we agree that by keeping the focus on the practicalities of this particular system, we neglected to describe the extent of other efforts towards a non-invasive estimate of sleep. We have expanded the discussion in an attempt to cover others working in this field, with regard to both improvements in methodology and uses of non-invasive measures of sleep in biomedical research.

Competing Interests: No competing interests were disclosed.

Referee Report 01 December 2016

doi:[10.21956/wellcomeopenres.10663.r17581](https://doi.org/10.21956/wellcomeopenres.10663.r17581)



Valter Tucci

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In the manuscript 'COMPASS: Continuous Open Mouse Phenotyping of Activity and Sleep Status', Brown *et al.* describe the development of a new method to approach the investigation of sleep in mice. In particular, by using passive infrared sensors the system recapitulates the main distribution of physiological sleep in animals. This is a very important study in the field of sleep, in which EEG time-consuming approaches are currently still the standard. The authors have provided a comprehensive evaluation of the PIR based system with EEG-based (10-sec epochs) sleep scoring, the manuscript is well written and the methods precise, as well as results and discussion. I have no major issues to raise but only two minor questions that perhaps would be useful to clarify.

- The authors report a high correlation between PIR at 220nm and sleep. If I understand correctly this was better than the PIR at 440nm. Was this a significant improvement?

- The main comparison reported in the paper is between PIR detected immobility and EEG. However, at Page 6 second column, the authors discuss of a high correlation with video-tracking. Was this paralleled with EEG or on different mice?

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 11 Apr 2017

Laurence Brown, University of Oxford, UK

Dear Professor Tucci,

Thank you for your time and effort reviewing the work. In answer to the points you raise we can confirm that the original positioning of the PIR sensor at 440mm was at the top of our environmental chambers, in which the cages sat. As is indicated by the video we published, the detection of small movements by the mice was poor at this height. Our findings in preliminary experiments looked at sleep assessed by the video-tracking protocol previously used in our lab and the PIRs. We found the correlation of video-tracking and PIRs at 440mm from the cage floor often fell below 0.75 (Pearson's r , over 24h), but with the sensors at 200-220mm from the cage floor, the correlation between methods was very high (Pearson's r , >0.98). These experiment were carried out on different mice to the EEG experiments. As both of these methods are based on immobility we felt it was best to focus on the comparison with EEG-scored sleep.

Competing Interests: No competing interests were disclosed.

Referee Report 22 November 2016

doi:[10.21956/wellcomeopenres.10663.r17579](https://doi.org/10.21956/wellcomeopenres.10663.r17579)



Dominic Wells

Department of Comparative Biomedical Sciences, Royal Veterinary College, London, UK

This is an excellent piece of work describing a simple, low-cost, non-invasive system to measure mouse activity in their home cage. The system is based on passive infrared (PIR) motion detectors. Using 40 seconds of immobility as an indicator of sleep, the authors demonstrate that PIR based estimates of sleep correlated very well with EEG recordings from implanted telemetry transmitters.

In general, the work is very well described, allowing other researchers to use this system in their animal facilities, but there are a few points that it would be useful to clarify:

1. Second paragraph of the introduction. The fourth sentence should be rewritten to make it clear that the import, re-derivation and breeding issues relate to access to specialised facilities.
2. In the methods it would be helpful if the authors could identify the cage type they used. By small gaps do they mean the spaces under the feed and water hoppers?

3. The authors note that no environmental enrichment can be used that might fully obscure the animal. Can the authors confirm that some nesting material was available but presumably was limited in quantity?
4. The authors used cages in light tight boxes. Have they tested their PIR system in more conventional animal caging?

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 11 Apr 2017

Laurence Brown, University of Oxford, UK

Dear Professor Wells

Thank you for your review. We hope the revised manuscript will address the important points you raised:

1. We agree this should be clarified. We have rewritten this paragraph in the revised manuscript.
2. This is correct. In some cages we have food and water hoppers mounted externally, so no further modification was needed, but where the water bottles and food are integrated into the wire of the cage lid, a Perspex block was used to ensure the mice were active and nested in view of the sensor. We have added the cages types used in the paper to the revised version.
3. We provided nesting materials for all of the mice in the study and in preliminary work. This was paper-based Sizzle-Nest, or cotton fibre Nestlets, with a minimum of 10g in each cage (in excess of that required for thermoregulation by mice with similar materials, Gaskill et al 2012, PLOS ONE, (<http://dx.doi.org/10.1371/journal.pone.0032799>)).
1. We are currently collecting data with collaborators in a range of cage sizes and types of racking. For some types of cage, particularly those with food and water hoppers recessed in the centre of the cage lid rather than at one end, it will be difficult to ensure complete coverage of the cage. Individually-ventilated cages (IVCs) would need modification for the integration of PIR sensors, as the polycarbonate material most are constructed with will block the 5-10µm infrared wavelengths of body heat.

Competing Interests: No competing interests were disclosed.