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Short communication

Automating mouse weighing in group homecages with Raspberry Pi micro-computers

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HIGHLIGHTS

- Cost-effective mouse automated homecage weighing using RFID and load cell.
- Open-source, cross platform modular code in python.
- Can be integrated in custom behavioural assessments, supports 10 group housed mice.

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ABSTRACT

Background: Operant training systems make use of water or food restriction and make it necessary to weigh animals to ensure compliance with experimental endpoints. In other applications periodic weighing is necessary to assess drug side-effects, or as an endpoint in feeding experiments. Periodic weighing while essential can disrupt animal circadian rhythms and social structure.
New method: Automatic weighing system within paired mouse homecages. Up to 10 mice freely move between two cages ($28 \times 18 \times 9$ cm) which were connected by a weighing chamber mounted on a load cell. Each mouse was identified using an RFID tag placed under the skin of the neck. A single-board computer (Raspberry Pi; RPi) controls the task, logging RFID tag, load cell weights, and time stamps from each RFID detection until the animal leaves the chamber. Collected data were statistically analyzed to estimate mouse weights. We anticipate integration with tasks where automated imaging or behaviour is assessed in homecages.
Results: Mice frequently move between the two cages, an average of 42+-16 times/day/mouse at which time we obtained weights. We report accurate determination of mouse weight and long term monitoring over 53 days.
Comparison with existing methods Although commercial systems are available for automatically weighing rodents, they only work with single animals, or are not open source nor cost effective for specific custom application.
Conclusions: This automated system permits automated weighing of mice ~40 times per day. The system employs inexpensive hardware and open-source Python code.

1. Introduction

Automated mouse home cage systems have been widely developed in the recent decade, facilitating the monitoring of mouse behaviours, physiology, brain activity, and body conditions for extended periods (Goulding et al., 2008; Ulman et al., 2008; Naoto Izumo 2015; Bolaños et al., 2017). These systems reduce the need for human interaction which may lead to elevated stress in animals (Balcombe et al., 2004). Measuring body weight (BW) is one of the important parameters in body condition scoring which is used to evaluate the animals' health, establish endpoints, side effects of drugs, and implementation of water/food restriction protocols. Water/food restriction is one way to motivate animals to cooperate in behavioural experiments. In this procedure, mouse weight needs to be carefully monitored (Tucci et al., 2006; Bekkevold

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et al., 2013; Chen et al., 2015). Moreover, the regulation of body weight by drugs, such as dexfenfluramine (Redux), which has been developed for obesity patients demands the frequent monitoring in animals (Bush et al., 2006). Based on the type of animal and experiments, frequency of weighing them can vary from several times per week to several times per day in small animals such as rodents. Non-invasive handling of mice can change plasma concentration of corticosterone, glucose, growth hormone, heart rate, blood pressure and behaviour (Balcombe et al., 2004). We have developed a system to automatically weigh and track group-housed mice. This system allows researchers to monitor fluctuation of body weight many times per day, without any interaction and facilitates accurate record keeping, protocol compliance, and integration with tasks where automated imaging (Scott et al., 2013; Murphy et al., 2016) or behaviour is assessed in homecages (Richardson 2015; Bains et al., 2016).

2. Methods

All procedures were approved by the University of British Columbia Animal Care Committee and conformed to the Canadian Council on Animal Care and Use guidelines and employed C57BL/6 mice. Two standard mouse housing cages of 28*18*12 cm capable of holding up to 10 mice were connected at 30*30 mm openings with a non-contacting floating chamber 100 mm long with a square cross-section of 30 mm per side. The chamber rested on a 100 g load cell (BONAD) which was connected to a breakout containing an AVIA Semiconductor HX711 load cell amplifier (Sparkfun) (see Section 3, Parts list). The 5 wires from the load cell (positive and negative signals: white and green, power: red, ground: black, and electromagnetic shield: yellow) were soldered to the corresponding labeled inputs on the breakout. The load cell amplifier included a built-in 24-bit digital to analog converter (DAC), which output data serially on a single pin (DAT), at a rate set by the signal input on the clock pin (SCK). DAT and SCK were connected to arbitrary GPIO pins on the Raspberry Pi. Vcc and GND on the breakout were connected to 5 V and ground on the Raspberry Pi, respectively.

Since the average weights of adult C57BL/6 female and male mice are 22 g and 28 g respectively (Reed et al., 2007) and the weight of the chamber was 20 g (which was set as a tare at the beginning of each trial), we used a load cell with the maximum capacity of 100 g and precision of 0.1 g. This level of precision was consistent with weight fluctuations in a day which present data showed can be more than 0.2 g. The load cell and HX711 amplifier combination was calibrated with 34 standard weights (0–12.98 g increments of 0.37 g). We applied standard weights from lowest to highest and collected outputs at 10 Hz for 5 s (50 samples for each weight). We averaged outputs for each weight and then calculated the standard deviation. The standard deviation of outputs was less than 0.002%. Results of a linear fit of the measured weight in grams to the 24-bit output of the load cell amplifier (slope = 0.01399e6, r = 0.99 and p = 0.0001) was used to convert the output of the load cell amplifier to weight in grams.

An RFID reader (ID-12LA RFID SparkFun) was mounted on a breakout board (SparkFun #13030) and attached to a post to suspend it above the chamber with a 3 mm gap that prevented the RFID reader from touching the chamber, thus biasing the weight. Pin 11 (Vcc) and pin 1 (GND) of the RFID reader were connected to 5 V and ground, respectively, of the same Raspberry Pi interfacing with the load cell amplifier. Pin 2 (Reset Bar) of the RFID reader was tied to 5 V, and pin 7 (Format Selector) was tied to ground. Pin 9 (Data 0) was connected to the Pi's serial input pin (RXD, GPIO 15), and pin 6 (TIR; Tag in Range) was connected to an arbitrary GPIO pin on the Pi. The RFID reader set the TIR signal high when a tag first entered the range of the RFID reader, and set it low only when the tag was no longer in range. The RFID reader sent the tag's data over the serial port only once when the TIR pin was first set high. While the TIR pin was high, the RFID reader would not read another tag, even if one came in range. Therefore, only one animal could be detected inside the chamber until that animal moved out of the RFID range.

RFID-tags (inert glass-encapsulated tags 125 kHz, Sparkfun) were injected into the nap of the neck (Bolaños et al., 2017) of five mice and the animals were placed in the dual cage setup. Water was provided in one cage, food was provided in the other and animals allowed to freely move between the two cages. Once an animal went inside the chamber and the RFID-tag was read by the RFID reader, the Raspberry Pi started recording data with a frequency of 10 Hz until the tag was out of range and the measured weight had dropped below an arbitrary threshold. The sequence of weights recorded was referred to as a single epoch. Data was output to files in 32 bit floating point format, with each epoch prefixed with the RFID tag number of the mouse (as a negative number to aid in epoch parsing), and the entry time in seconds from midnight.

An electronics schematic, plus the related Python code for controlling the HX711 and RFID reader with the Raspberry Pi can be found in the online **Supplementary 1** file. 3D view of system can be found in the online **Supplementary 2** file. 3D printer files-tube construction can be found in online **Supplementary 3** file. Python code to read the binary data files containing weights and identify the modal value of the weight distribution is included in the online **Supplementary 4** file. We also share a video about how to assemble electronic parts in YouTube: https://www.youtube.com/watch?v=FazQw4XZ-Ng&feature=youtu.be.

3. Parts list

The following parts were used: Raspberry Pi Model B2 from Newark #38Y6467, Chicago, Illinois, USA, http://canada.newark.com/raspberry-pi/raspberrypi-2-modb-1gb/sbc-raspberry-pi-2-model-b-1gb/dp/38Y6467; 32Gb SD card from Adafruit #1583, New York City, New York, USA, https://www.adafruit.com/product/1583; Pi Cobbler+ from Adafruit # 2028, New York City, New York, USA, https://www.adafruit.com/product/2028", RFID reader ID-12LA (125 kHz) from Sparkfun #11827 loc Boulder, Colorado, USA, https://www.sparkfun.com/products/11827; RFID reader breakout from Sparkfun #13030 located in Boulder, Colorado, USA, https://www.sparkfun.com/products/13030; RFID-tag glass capsule (125 kHz) from Sparkfun #08310, Boulder, Colorado, USA, https://www.sparkfun.com/products/8310; 100 g load cell from BONAD #CZL639 M Guangdong, China, https://www.alibaba.com/product-detail/Micro-Load-Cell-100g_702793819.html; load cell amplifier HX711 breakout from Sparkfun #13879; 7" touchscreen display Raspberry Pi from Element14 #49Y1712 located in Chicago, Illinois, USA, http://www.newark.com/raspberry-pi/raspberrypi-display/display-7-touch-screen-rpi-sbc/dp/49Y1712.

4. Results

5 female mice C57BL/6 mice that were 12 months of age and had transcranial brain windows (Silasi et al., 2016) and head-fixation bars (Murphy et al., 2016) were used for data acquisition. While the animals were equipped for optogenetics, they were tested without head-fixation to assess feasibility for future applications. The 5 mice were placed in the dual cage automatic weighing system continuously for 53 days. Although we have assessed 5 animals here, we have recently been approved for housing 10 mice in the double cage configuration. Preliminary work with 10 male mice indicates little issue with crowding or fighting (over standard caging) consistent with recent work in connected cages with up to 50 mice (Garner et al., 2015). Mice moved freely between the two cages and had con-

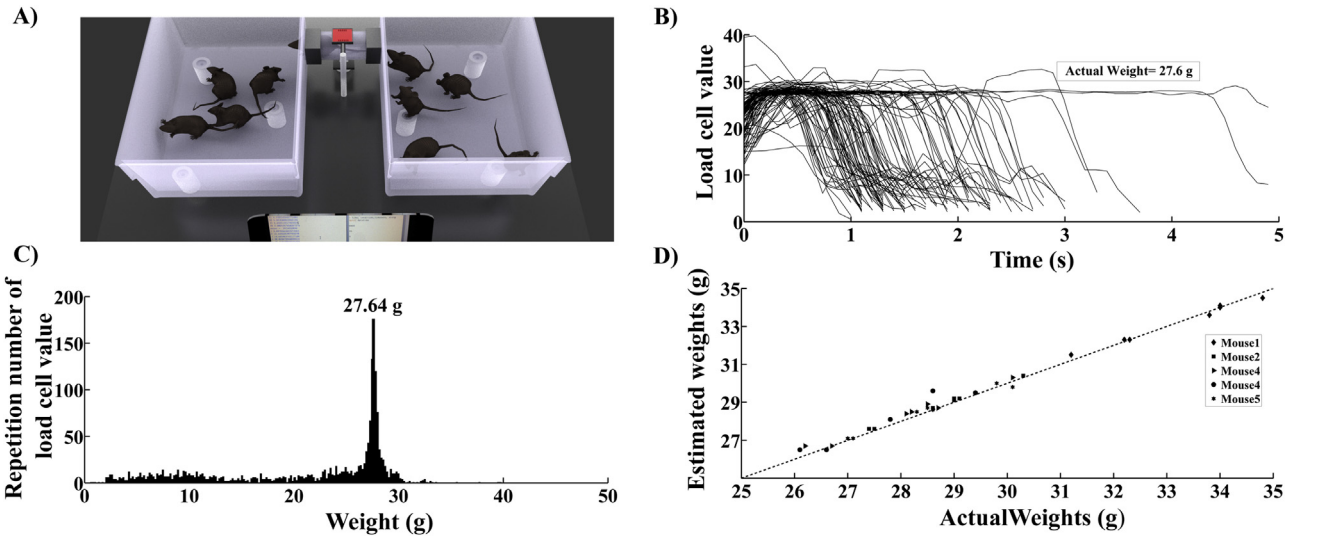

Fig. 1. Dual cage automatic weighing system for group housed mice. **A**) 3D view of automatic weighing system, designed with Blender software. Note the load cell is positioned between the two cages. A high resolution 3D rendering of the cage is available online in the **Supplementary 2**. **B**) A set of epochs for 12 h (contains 102 epochs) for one mouse showing the load cell weights as the mouse crosses the chamber. **C**) The estimating method based on extracting the most common value during 12 h of activity for one mouse. The set of epochs were merged together and binned by 0.2 g increment. The actual weight was 27.6 g which was equal to estimated weight in this case. **D**) Comparing actual weights with estimated weights for 5 mice. Mice were manually weighed 7 times with a digital scale ($r = 0.90$ and $p = 0.0001$).

tinuous access to food and water. During this period we recorded epochs, which corresponded to the time a mouse goes inside the chamber and load cell starts recording until the mouse goes out of RFID reading range.

The pattern of epochs for one mouse over 12 h is shown in Fig. 1B with 80% of epochs being less than 1.5 s in duration. These data indicate that mice do not rest in the tube for long periods, possibly blocking other mice from being measured, but make rapid traversals. At the onset of each epoch, values started below the actual weight of the mouse and steadily increased up to some maximum. Yet, the maximum value was not reliable as an estimate of weight since other animals may have simultaneously touched the chamber. In order to estimate weight, we considered a set of epochs taken together (for example, epochs within 6 h), binned the data with a bin size of 0.2 g, and found the most common weight, excluding out of range points based on conservative estimates of minimum and maximum weights (using MatLab). Fig. 1C shows one example of this estimation. (Python code to read the binary data files containing weights and identify the modal value of the weight distribution is included in the **Supplementary 4**) As expected, the distribution of values around the actual weight (27.6 g in this case) formed a clear peak. To assess the accuracy of this estimation method, we compared the estimated weights with actual mouse weights which we manually measured with a digital scale. Two hour sets of epochs before and after manual weighing were used to evaluate automated weight accuracy. Comparison of software estimated weights and actual ones (Fig. 1D) indicates that this method predicted the weights with an average error of 1.6%.

Results for five mice whose weights were monitored for 53 days is shown in Fig. 2. On average, mice were weighed 42 ± 16 times a day and there were no days when mice were unable to be weighed. There were significant inter-animal differences (ANOVA) between the number of tube entries; mouse 5 entered most frequently at 66 ± 27 times per day and mouse 4 least frequently at 27 ± 11 entries per day. Circadian cycles, albeit not extremely pronounced, were evident in weighing tube entries (Fig. 2A) and periodic patterns of weight fluctuation on the order of several days were also present in the data (Fig. 2B). Weights for some pairs of mice tended to fluctuate together, while the weights of other pairs were not correlated (Fig. 2C).

5. Discussion

Several systems have been previously developed to assess water and food intake in rats and mice and automatically weigh animals. Investigators measured the weight of water and food that an animal would take in each bout (Ulman et al., 2008) attaching load cells to the water and food hoppers outside of the cage. Another automated home cage was developed to monitor mouse weight (Naoto Izumo 2015), but only allowed a single mouse to be tracked. Moreover TSE systems (Phenomaster) has made an automated body weight measurement device which could be located inside a mouse/rat home cage. This device was a tube attached to a scale located outside of the cage. Once the animal stood inside the tube, weights were monitored, again without group statistics. The advantage of our system over the previous work (Naoto Izumo 2015) is that we could, by applying RFID readers and tags, track the weights of individual mice within a group rather than tracking a singly-housed mouse, or tracking an average weight of group housed mice. The system is modular in nature and can be the basis of more complex future tasks where homecage behaviour or brain activity is assessed.

Beyond monitoring weight for its own sake, the data collected by the system described here could be applied towards studies monitoring activity or behaviour over long periods, as evidenced by Fig. 2. For instance, the activity of aged mice is relatively higher during the light phase comparing to younger mice (Wimmer et al., 2013). The mice used in this experiment were more than 12 months old, yet still showed a circadian rhythm for tube entries. A longitudinal study might show a progressive decline in rhythmicity as reflected by weighing tube entries. Some aspects of social behaviours of group housed mice might also be teased out of the data from this system, as weights from some pairs of mice, but not others, tended to vary together. Relationships between tube entry times for pairs of mice have not yet been examined, but such temporal cross-correlations might also provide information on social interactions.

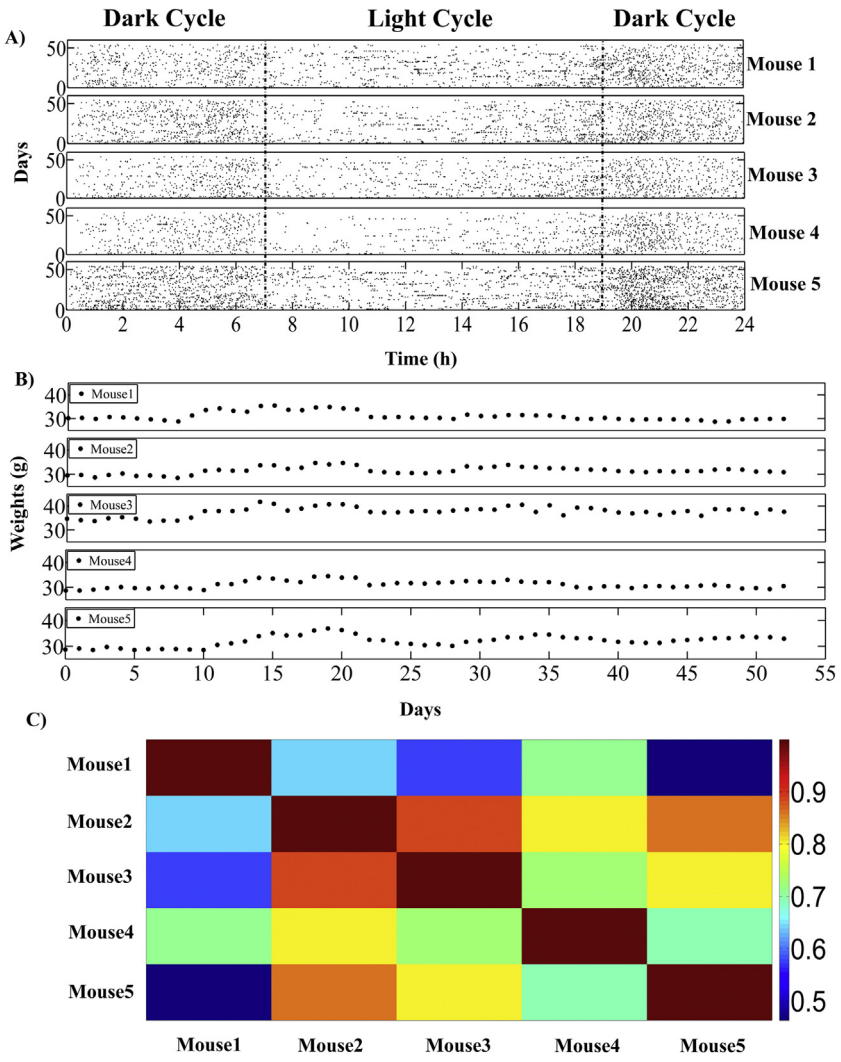

Fig. 2. Long term monitoring of weights in 5 group housed mice. **A**) Depicts the activity patterns of mice for 53 days during each day. Each tick mark indicates a weighing tube entry. **B**) Weight fluctuation over 53 days for 5 continuously tracked mice. C) Correlated changes in body weight between select mice over 53 days, for example the weights of mouse 2 and 3 or 2 and 5 were correlated while other pairs were not (color bar indicates r value).

6. Conclusion

We have developed an automated system to weigh mice several times per day for a long period of time without disruption by human interaction. We anticipate that the inexpensive hardware and open-source code that the system employs will promote uptake by the community.

Competing financial interests

The authors declare no competing financial interests.

Contributions

Omid Noorshams designed and assembled the automated weighing system and collected and analyzed data. Jamie Boyd developed the electronics and wrote the Python and C++ code. Timothy Murphy developed the 2 cage concept and assisted in the load cell design. Timothy Murphy and Omid Noorshams wrote the paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jneumeth.2017.05.002.

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