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Short communication

Cost effective raspberry pi-based radio frequency identification tagging of mice suitable for automated *in vivo* imaging

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HIGHLIGHTS

- Simple, cost-effective mouse identification using radio frequency identification.
- Open-source, cross platform modular code in Python
- Easily integrated into custom behavioural assessments.

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ABSTRACT

Background: Automation of animal experimentation improves consistency, reduces potential for error while decreasing animal stress and increasing well-being. Radio frequency identification (RFID) tagging can identify individual mice in group housing environments enabling animal-specific tracking of physiological parameters.
New method: We describe a simple protocol to radio frequency identification (RFID) tag and detect mice. RFID tags were injected sub-cutaneously after brief isoflurane anesthesia and do not require surgical steps such as suturing or incisions. We employ glass-encapsulated 125 kHz tags that can be read within 30.2 ± 2.4 mm of the antenna. A raspberry pi single board computer and tag reader enable automated logging and cross platform support is possible through Python.
Results: We provide sample software written in Python to provide a flexible and cost effective system for logging the weights of multiple mice in relation to pre-defined targets.
Comparison with existing methods: The sample software can serve as the basis of any behavioral or physiological task where users will need to identify and track specific animals. Recently, we have applied this system of tagging to automated mouse brain imaging within home-cages.
Conclusions: We provide a cost effective solution employing open source software to facilitate adoption in applications such as automated imaging or tracking individual animal weights during tasks where food or water restriction is employed as motivation for a specific behavior.

1. Introduction

Animal identification is a critical part of any basic or applied research, but correct animal identification is subject to human error. In research that uses rodents, ear clipping, toe clipping, or tail marks can be used to identify individual animals (Wang, 2005). Of these methods ear tagging is the least problematic from a welfare standpoint, but the marks can become ambiguous over time leading to errors in identification. Furthermore, in cases where there is pooling of cages, duplicate IDs may exist necessitating additional ear tagging that can be stressful for adult animals.

We propose a cost effective system that utilizes radio frequency identification to identify mice. A USB based RFID reader interfaces with the RFID tags that are implanted in the mice. The RFID reader is compatible with any USB enabled computer although the raspberry pi is arguably the most cost effective option. A previous method (Gruda et al., 2010), utilized light activated microtransponders; however a disadvantage of this method is that the animal must be immobilized by scruffing which is not possible as part of a self-directed behavioral assay as we have previously described (Murphy et al., 2016). There are commercially available animal RFID tags

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Table 1
Parts list for deploying RFID system.

Part	Supplier	Catalog #
RFID Glass Capsule (125 KHz)	Sparkfun	SEN-09416
RFID Reader ID-20LA (125 KHz)	Sparkfun	SEN-11828
Sparkfun RFID USB Reader	Sparkfun	SEN-09963
RFID Syringe 12 mm microchip	Alibaba	RFID-Syringe-embedded-12mm-microchip_1620554840.html

and readers such as the FriendChip and those from Kent Scientific, however these are not cost effective solutions and, due to their proprietary nature, cannot be as easily integrated into custom applications. The system we employ can be deployed on all major operating systems and can be readily incorporated into more complex behavioral assays. While other research-based animal RFID tagging systems have been described (Catarinucci et al., 2014; Howerton et al., 2012), our method is aimed at a cost effective solution employing open source software that will facilitate adoption by the community in applications such as automated imaging or tracking animal weights during tasks where food or water restriction is employed as motivation for a specific behavioral task.

2. Methods

2.1. Hardware Overview

The system uses inert glass-encapsulated tags (12.25 mm in length) (Fig. 1A) that are implanted subcutaneously at the nape of the mouse's neck. The unique tags are identified and logged using a USB RFID reader connected to a 3D printed stand (Fig. 1B). This system of identification has the advantage that duplicates can be eliminated and human errors are significantly reduced. An inexpensive $35 raspberry pi single board computer (Fig. 1C) can be used to interface with the reader, enabling data collection using the provided, flexible, Python open-source software.

2.2. Tag Implantation

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. A cage of five Thy1-GCaMP6 mice (C57BL/6J-Tg(Thy1-GCaMP6f)GP5.5Dkim/J, from The Jackson Laboratory) were used to demonstrate the procedure.

A sterile field was created by placement of a sterile surgical drape. Mice were briefly anesthetized with 2% isoflurane within an induction chamber. Unconscious mice were placed on the surgical drape and anesthesia was maintained with 1.5% isoflurane (Fig. 2B). A sterile needle and injector (Alibaba https://www.alibaba.com/product-detail/RFID-Syringe-embedded-12mm-microchip_1620554840.html) (Fig. 2A; see Table 1 for all parts) was then loaded with a sterilized RFID tag (soaked in 70% ethanol solution). Note we substituted the tag supplied with the injector for one available from Sparkfun (125 kHz glass RFID tag SEN-09416). The thoracic part of the torso was disinfected with betadine and the fur displaced using a pair of forceps to reveal a small patch of skin (Fig. 2C) that served as the target for the injection needle. An incision was made on the revealed skin using the injection needle and care was taken to ensure penetration of the dermal layers and not the underlying muscle. The needle was pushed anteriorly and parallel to the spinal cord until reaching the nape of the neck (Fig. 2D). We chose the nape of the neck as mice will be less able to scratch the site of the incision leading to a lower probability of tag-loss and infection. During insertion the tag was pushed out using the injector plunger and the needle retracted with the tag remaining in place. The mouse was given buprenorphine (2 mg/ml) and was allowed to recover from the injection within their home cage for at least three days before any behavioural training. Alternatively, the mouse can be given an oral tablet of carprofen at 5 mg/kg (Kendall et al., 2014) and allowed to recover from the injection within their home cage for at least three days before any behavioural training.

2.3. Electronics

The RFID tag glass cylinders with a diameter of 1.93 mm and a length of 12.25 mm were obtained from Sparkfun (SEN-09416). The tags operate at a frequency of 125 kHz and encode a 32-bit non-reprogrammable unique ID. The RFID tags were read using a USB based RFID reader (model ID-20LA SparkFun). The SEN-09416 tag was chosen since it is compatible with the ID innovations tag readers also from Sparkfun. We anticipate that other 125 kHz glass capsules from sites such as Alibaba.com that match the encoding type and frequency response to that of the SEN-09416 would be suitable.

The data was collected and organized using a Raspberry Pi Model 2B computer, using custom software written in Python (see code in Supplemental Material). The software logs the ID of the mouse and combines a precise date stamp and specific event with mouse ID. Although we employ a Raspberry Pi, the system will also run under any PC by installing a version of Python (3.4 or higher) and employing the Sparkfun USB based tag reader.

3. Results

3.1. Weighing Protocol

Recently several studies utilize water restriction protocols with mice to motivate them to do perform specific behavioral tasks (Guo et al., 2014; Murphy et al., 2016). Within these studies it is critical to track the weights of the animals to ensure they are in a healthy state, especially if the experiment calls for chronic water restriction. We provide sample software to track of the weights of mice using the implanted RFID tags for identification. The software (see commented Python code in Supplemental Material) is run and a mouse is placed within 25 mm of the tag reader until the program indicates a successful identification (see 3D-drawing of weighing stand in Supplemental Material). The weight of the mouse is measured using a balance and input when queried by the program. The program records the data to text files that depend on the mouse ID, new data is appended to end of file, and the files can be easily backed up automatically using self-served cloud services such as Syncthing. The software is written in Python and the code is open source and under the MIT license.

The software is written in a modular format. For example, the tag reader class can be imported into a more complex behavioral and/or imaging program. To run the software one has to install Python 3.4+ as well as the pyserial library. One must specify the COM port for Windows or the/dev/ttyUSBx port for Linux systems and then create a TagReader object with the port as an argument to the class constructor. At this point calling the read_tag() function which will return an integer representing the tag ID when a tag is detected. This function can be used in more complex behavioral tasks and can wait for a mouse to be detected before initializing a task parameters specific to that mouse's ID.

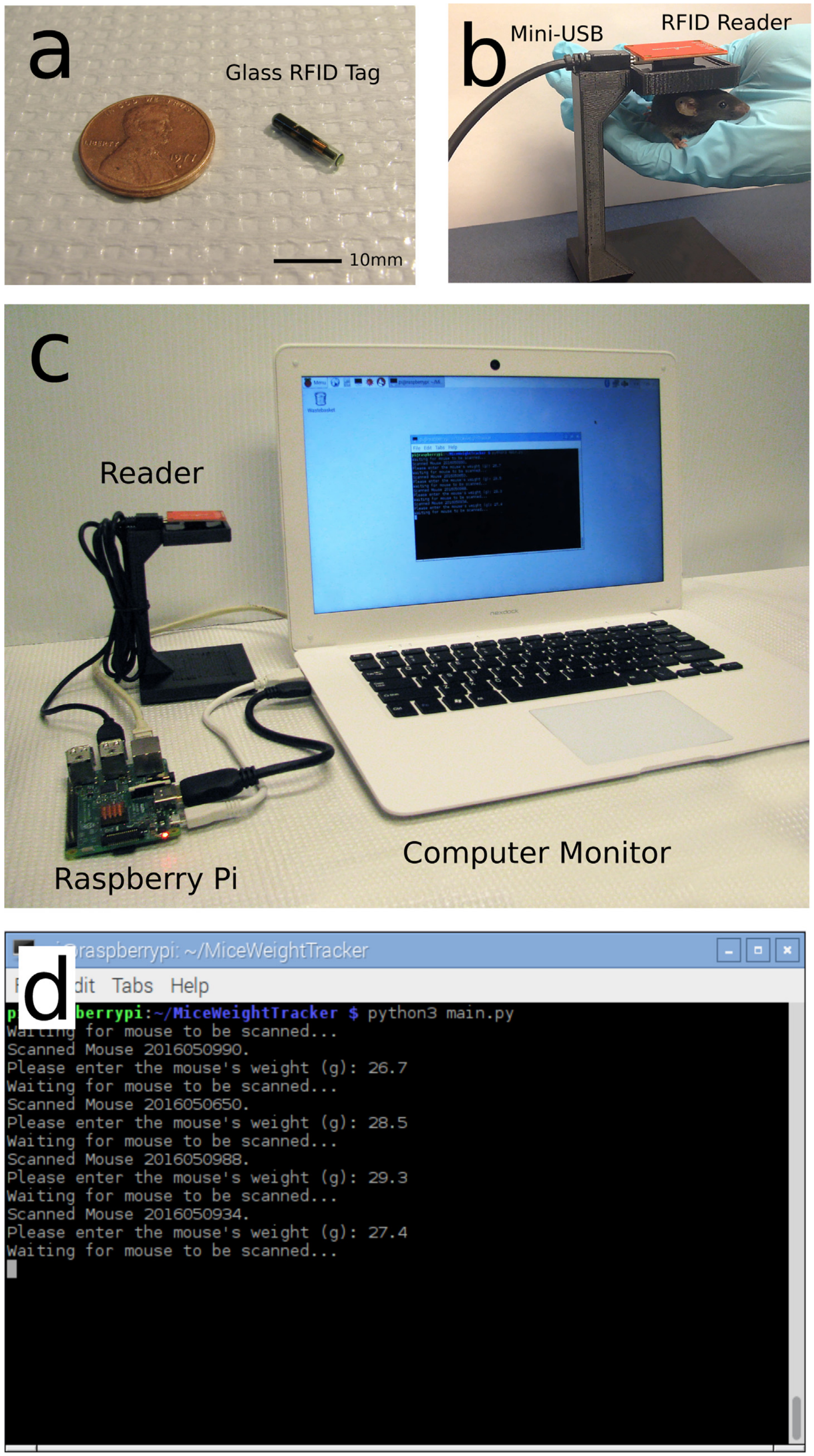

Fig 1. Cost effective radio frequency identification reader using a Raspberry Pi computer. (a) Glass RFID tag that is implanted under the skin over lying the neck. The tag stores a unique number that represents the mouse ID. (b) The USB RFID reader is mounted on a 3D printed platform to hold the reader above the mouse's body. (c) The Raspberry Pi computer interfaces with the RFID reader, while connected to a computer monitor and keyboard for manual aspects of data collection such as inputting weights. (d) Command line Python interface that is used to collect the data. Shown on screen are the mouse IDs and the current weight for that mouse.

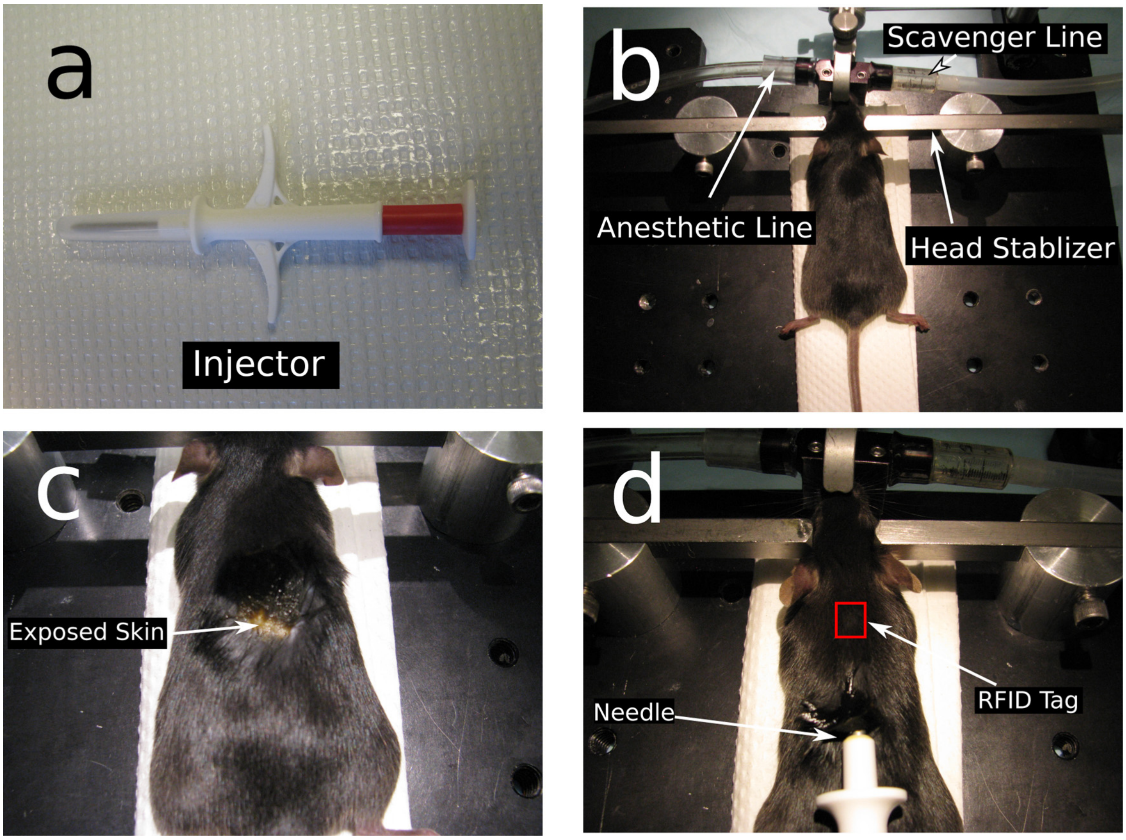

Fig. 2. Surgical procedure for tag implantation. (a) The injector used to implant the RFID tag. The needle can be sterilized to inject multiple tags with a single injector. (b) Surgical setup image. Anesthesia was induced in a separate chamber and maintained with 1.5% isoflurane in air using an anesthesia mask (Kopf 923B). The mouse is shown in a stereotaxic apparatus in anticipation of also being used for a cranial window surgery (Silasi et al., 2016), however it is not necessary to employ the stereotaxic frame. In this case the anesthetized mouse is placed on a surgical plate and the injection made while holding the body with the other hand. An experienced operator should take less than 5 min to inject a tag. (c) The incision area was created by displacing fur with a pair of forceps. (d) The RFID tag can be seen slightly protruding at the mouse's neck as it is being pushed out of the injector.

4. Discussion

An RFID based animal logging system has several advantages over traditional identification methods. By virtue of being entirely computer based one eliminates most human errors, since the tags implanted in each animal are all unique, one only has to ensure the correct mouse was the one initially selected. The system can reduce animal stress and associated confounds as less researcher contact and handling is required to determine identities. It is even possible to construct systems using RFID-tag coupled gates to completely automate sorting of animals (Santoso et al., 2006; Winter and Schaefers, 2011). The system we outline is cost effective, a full unit with five tags costs around $85USD. A single RFID reader system can read any number of different tags. One just needs the infrastructure to house many animals and a tag for each animal which cost $5 USD each. It is also possible to link multiple cages together where animals pass through a small chamber allowing their tags to be efficiently read.

The glass-encapsulated tags stay intact beneath the skin of the mouse, without any signs of infection discomfort, or decomposition of the tag. The tags can be recovered and, once sterilized, reused in future experiments when mice have reached experimental endpoints. In an automated imaging experiment (Murphy et al., 2016) the tags remained intact for more than three months with no failures in 16 mice studied.

Although we describe open-source software for weighing, the code can also be used for basis of complex behavioral, imaging, or optogenetic tasks. Recently, we have employed a similar tag reader and tags to trigger brain imaging in individual mice that were automatically head-fixed (Murphy et al., 2016). Our tagging software and hardware can also serve as the basis of more complex tasks where social interaction can be tracked using RFID tag reader grids, or animals sorted into task-specific compartments within their homecage (Winter and Schaefers, 2011). Assessment of social interactions in mice is an exciting frontier for neuroscience and has been employed using analysis of depth sensor data using the Kinect Sensor from Microsoft Corporation and the Senz3D depth and gesture sensor from Creative Technology Ltd. In this study individual mice we defined using contrasting coat colors (Hong et al., 2015). RFID tag reader grids within cages facilitate monitoring social interactions without the ambiguity associated with tracking multiple mice based on video analysis of coat color tags such as paint or bleach, or other strategies which could become obscured with more than two subjects. The RFID tag can also be used to dispense water as in our automatically headfixed imaging task, or extended to dispense animal specific quantities of drugs without the stress of repeated handling (Santoso et al., 2006). Currently, the Sparkfun reader will read tags within 30.2+-2.4 mm ($n = 10$) of the sensor, these distances can be modulated by use of shields to decrease efficacy, or a stronger external antenna to read from greater distances. As mice can be moving quickly as they pass the tag reader we estimated the minimal velocity the tag can move to still be read using the standard antenna to be 0.47 m/s. Given this speed in less than the peak running velocity of C57bl6 mice (Billat, 2005) one can take steps such as adding obstacles, barriers, or a dead-end chamber to increase read fidelity (Murphy et al., 2016). Another method to enhance signal is described by ID innovations datasheet (https://cdn.sparkfun.com/datasheets/Sensors/ID/ID-2LA,%20ID-12LA,%20ID-20LA2013-4-10.pdf). The datasheet describes that depending on the power supply used one can add a low pass filter to ensure the output voltage ripple does not affect the AC frequency across the antenna. The data sheet also describes employing a larger, home-made antenna which will also increase the distance read. However, we point that longer read distances are not always advantageous as this can increase cross talk between animals.

In other applications, RFID tags in combination with multiple tag readers that form a grid can be used to localize individual animals within a cage. One potential issue with multiple tag readers is interference between the antennas. However, a simple solution implemented in commercial systems is to intermittently pulse antennas so that reading is done rapidly but sequentially (Phenosys Social Video system).

5. Conclusions

We anticipate that RFID tag readers and surgical procedures will be the mainstay of any automated behavioral neuroscience experiment and can be used to facilitate the identification of individual animals for imaging assessments or any task needing repeated identification.

Contributions

F.B. performed animal experiments. T.H.M, F.B. and J.M.L. wrote the paper. F.B. developed all hardware and software.

Competing financial interests

The authors declare no competing financial interests.

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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.2016.11.011.

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