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An inexpensive wireless video recording system for continuous behavioral observations

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Abstract

Video recording technology is an important tool in the field of animal behavior, by

producing a record of experiments, interactions, and contextual information, and by reducing

observer effects; but video recording systems remain cost prohibitive for many researchers.

Here we present an inexpensive method for building a wireless video recording system to

continuously monitor behavioral or other biological experiments. Our system employs Raspberry

Pi computers and cameras, open-source software, and allows for remote operation, live-

streaming, and the capacity to simultaneously record from several cameras in an array. To

validate our system, we continuously monitored home-cage behavior in the California mouse

(*Peromyscus californicus*) in a laboratory setting. To record behaviors of this nocturnal species,

we captured video in both low- and bright-light environments. We then quantified mating

interactions of California mouse pairs by analyzing the videos with an open-source event

logging software. Our video recording platform offers users the flexibility to modify the

specifications for a range of tasks beyond animal behavior experiments. Moreover, this low cost,

flexible and scalable, wireless video recording system offers a tool to make research more

efficient and reliable to a larger population of scientists.

Introduction

Behavior often represents an animal's most immediate and dynamic response to internal or external cues [1]. Thus, behavioral data are invaluable indicators of change, yet the complexity and diversity of behaviors can be challenging to quantify. Traditional sampling methods from live observations (e.g., scan sampling, focal animal sampling, one-zero sampling [2-3]) frequently results in an estimate, rather than a precise measure of behaviors, or only provides data from a subset of subjects or interactions within a social group [4]. The behavioral record is therefore often incomplete with live observational data, and the presence of an observer can influence the behavior of study subjects, thus biasing the results [5–7]. Moreover, to fully understand an animal's response, it is imperative to understand the context and the environment in which the behavior was expressed. Video recording enables researchers to review behaviors multiple times to yield a more complete dataset, reduces observational biases, and can capture contextual information.

Video recording has become a nearly ubiquitous tool for behaviorists and useful in a wide range of studies. For example, video can improve data collection in manipulative behavioral assays from studies of visual recognition in cichlids [8] and foraging in fruit flies [9], to parental care [10] and anxiety in rodents [11]. Moreover, passive monitoring approaches, such as home-cage cameras, can be used to record social behaviors, such as pair bonding in marmosets [12], or mating in whiteflies [13], guppies [14], and gerbils [15]. Outside of the lab, passive monitoring with nest box cameras have revealed unexpected behaviors including paternal care in tree swallows, even when extra-pair copulations were evident to the male [16], personality traits and social dominance in zebra finch [17], and long-term social interactions in "near natural" enclosures in house mice [4]. Continuous video recording systems provide a record of behavior and contextual information in a variety of studies from controlled laboratory assays to passive monitoring in the wild.

With few exceptions, continuous video recording improves rigor and repeatability in all

behavioral studies, yet the technology remains cost prohibitive for researchers with limited

funding or for complex experimental designs requiring numerous cameras. Here we present an

inexpensive method for building a wireless video recording system using-source software that

allows for remote operation, live-streaming, and the capacity to simultaneously record from

several cameras to create an array. We then present validation of this system, in which we built

an array of modules to continuously record mating behavior in California mice, *Peromyscus*

californicus.

Materials and Methods

We constructed a video recording array using Raspberry Pi (RPi; Caldecote, Cambridge,

UK) modules and-source software to continuously record multiple mating pairs of captive

California mice (*Peromyscus californicus*) across several reproductive cycles. We then

transferred the video files to Google Drive (Google LLC, Mountain View, CA, USA) for cloud-

based storage, and later scored videos using the free open-source software, BORIS (Behavioral

Observation Research Interactive Software [18]).

Recording module assembly

Raspberry Pi computers are very small, inexpensive computers that offer a flexible

platform to build a video recording system. Some base units can be purchased with cameras

but storage and cables must be purchased separately. Table 1 describes the basic starting

materials we used and ordering information. At the time of purchase, each recording module

cost roughly $60.00 to build.

Table 1. Basic Materials

Product	Comments
64 Gb micro SD card	A larger card can be purchased if the budget allows; be sure the card comes with a SD card adaptor.
Raspberry Pi Zero(W)	The "W" version must be purchased for WiFi connectivity.
Raspberry Pi NoIR Camera	
Pi Foundation Official Pi Zero Case (Pi Zero Case)	This is the case for the assembled components.
Micro HDMI charging cable	
Charging dock	A multiple port dock is advised if using multiple recording devices.
USB Hub	

To build a recording module, we first loaded all application files, including an operating

system (OS), onto a micro SD card. We used 64 Gb micro SD cards, as roughly 8 Gb must be

allocated to the OS and our video files averaged 30 Gb in size. We formatted the card as MS-

DOS(Fat); the "Raspbian" OS used in this application was designed to be loaded onto cards

smaller than 32 Gb, therefore the 64 Gb card required reformatting. Once formatted, we loaded

the "Raspbian" OS (https://www.raspberrypi.org/downloads/noobs/) onto the card and then

installed it into the micro SD card port of a Raspberry Pi Zero(W) board (Figure 1A).

Next, we attached the Raspberry Pi NoIR camera to the R-Pi board (Figure 1B) and

placed the board into the Pi Zero Case (Figure 1C). This step requires extreme care, as there

are locking pins (see arrows in Figure 1D) that secure the board and camera to the case, thus it

was necessary to bend the case to prevent damaging the R-Pi board. Once the case was

closed, the recording module was complete (Figure 1D). We next attached the recording

module to a monitor, mouse and keyboard with a USB hub, and then connected the module to a

power source to activate the unit. The OS automatically initializes as soon as power is

connected, the activation process runs for approximately 10 minutes and then the Raspian

desktop appears.

Figure 1. Assembly of a recording module. (A) MicroSD card installed into a Raspberry Pi
Zero(W) board. Grey arrow indicates the location of the MicroSD card port. (B) Raspberry Pi

board is attached to the camera assembly. (C) Pi Zero Case. Grey arrows indicate locking pins
that the board and camera must fit underneath. (D) An assembled recording module.
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<u>Recording Module Programming</u>

On every module, the default username is "pi" and password is "raspberry", making them

susceptible to unauthorized off-network access. Therefore, we next reset the default username

and password in the "Raspberry Pi Configuration Menu". Under the interface tab of that same

menu, we then enabled the "Camera", selected "SSH" (allows for remote access) and "VNC"

(allows for remote file sharing) options, and then we rebooted the module. After reboot, we

established an internet connection by hovering the curser over the WiFi icon, selected the

appropriate network, and logged in. By hovering the curser over the WiFi icon after establishing

a connection, the IP address is revealed; with this IP address we could remotely log into the

module using RealVNC Viewer (https://realvnc.com/en/), an open-source software.

<u>Behavioral Observations</u>

To assess the routine use of the recording module to record social interactions, we

observed mating behavior in California mice, *Peromyscus californicus*. We originally obtained

four male and four female mice from the Peromyscus Genetic Stock Center at the University of

South Carolina (https://www.pgsc.cas.sc.edu/), and housed them at the University of Maryland,

in accordance with the Institutional Animal Care and Use Committee policies (protocol number:

R-Jul-18-38), and in standard transparent laboratory rodent cages lined with cedar shavings.

We provided food and water *ad libitum* and maintained all mice at 22°C in a 16:8 light-dark

cycle, simulating this species' breeding season [19].

To record the mating behavior, we placed a male and a female into a cage on a camera

rack. Camera stands can be easily purchased online or 3D printed, but we fashioned camera

racks from metal shelving on which we attached two blocks cut from recycled styrofoam at

either end to hold the two recording modules per cage (see below), in the middle of which we

placed a cage (Figure 2). We recorded from four pairs, each with their own pair of recording

modules. We recorded behaviors during the light period with one module and used a second

module to record behavior during the dark period while the room was illuminated with red lights

and inferred LED lights. We found that using two modules, each set at different parameters

optimized for each light environment, was the most effective way for us to obtain high quality

video across the entire light-dark cycle.

Figure 2. Assembled camera rack with animal cage.

Next, we remotely logged into the modules using a Python 3 shell (selected from the

programming menu on the Raspbian desktop) and executed the camera script (Table S-1; all

coding scripts described in this article can also be accessed at

https://github.com/wdavidweber/Pi-Recording-Module). To send video files directly to a Google

Drive folder, we used an additional script because the software is not included in the Raspian

OS. Using the terminal console in Raspbian, we uploaded the "rclone" (Nick Craig-Wood ©

2012) open-source software (Table S-2). We regularly monitored the modules and Google Drive

folders to make sure that data was uploaded correctly. To score the behaviors in BORIS, we

converted the .h264 format files (the default Raspbian filetype) into ".mp4" format using the

"ffmpeg" package, available in the Raspbian OS (the code for this is the last line of script in

Table S-1). We performed the file conversion using a High-Performance Computing Cluster at

the University of Maryland because our video files were too large to be converted on the

modules. When necessary, we live-streamed footage with a YouTube account

(https:www.youtube.com/) by loading "Docker" onto the module (Table S-3). With this method,

video recording and live-streaming cannot be accomplished simultaneously.

To quantify mating behavior, we scored videos using the open-source software, BORIS,

and developed an ethogram based on the work of Dewsbury [20]. We recorded the frequency of

mounts, intromissions, and ejaculations, as well as intromission latency (the time between the

start of a reproductive bout and the first intromission), ejaculation latency (the time between the

first intromission and the subsequent ejaculation), post-ejaculatory interval (the time between an

ejaculation and the beginning of the next copulatory bout), reproductive event (the total duration

of all mating events during a female's single reproductive period, beginning with the first

mounting event and concluding with the last ejaculation), copulatory bout (the duration between

the first mounting event and the first ejaculation).

Results

We continuously recorded home-cage behavior for three weeks from four pairs of

California mice. In total, we collected 2016 hours of video, which resulted in roughly 2 TB of

data. Recording one hour of footage during the light period (Figure 3A-B), at a resolution of

1280x768, a framerate of 15 frames per second, and a brightness value of 70, resulted in

recordings that were 440 Mb to 500Mb in size. Recording one hour of footage during the dark

period (under red lights and LED inferred lights; Figure 3C-D) at a resolution of 1280x768, a

framerate of 15 frames per second, and a brightness value of 80, resulted in recordings of

1.5Gb to 2Gb in size. Reducing the preset parameters for resolution, framerate, or brightness

resulted in smaller files, but less resolved footage.

Figure 3. Recording module array and output. (A) The array during the dark period with room
illuminated by red lights and inferred LED lights over the cages. (B) The same array in (A) but
from the other side while during the light period. Note the LED lights mounted on wall above
cages. (C) Still image of a video obtained during the dark period. (D) Still of a video obtained
during the light period.
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We scored mating behavior from the one pair that successful produced a litter after the

mating trial. The active mating period spanned a total of 5 hours, 24 minutes, and 3 seconds of

footage, all of which occurred during the dark period of the photocycle and included two

copulatory bouts. The first mating bout spanned 4 minutes, 15 seconds, and included 8 mounts,

3 intromissions and a single ejaculation; spanned 2 minutes, 45 seconds and included 6

mounts, 5 intromissions and a single ejaculation. We found that the mean intromission latency

was 1 minute 49 seconds and the mean ejaculation latency was 1 minute 42 seconds. The two

copulatory bouts were separated by a 36 minutes 39 second refractory period.

Discussion

Here we describe a wireless video recording system to remotely monitor animal

behavior. This tool permits user-determined specifications, including live-streaming and

multiplexing, and has the capacity to simultaneously record and upload data files to storage.

Moreover, this system allows for continuous behavioral monitoring on a flexible timescale. The

cost of the recording module that we describe is comparable to some pre-assembled

commercial surveillance cameras, which like the RPi recording module, often use SD cards to

store footage and may provide cloud storage for a fee with restricted allocations. Therefore,

while our system is not unique, we found that it offered a wider range of low-cost modifications

to suit a variety of research environments by incorporating open-source software add-ons.

For validation purposes, we used the video capture system we report here to record, and

subsequently quantify, mating behavior in the California mouse. Once we positioned the

cameras in place in our animal facility, we controlled the modules from our laboratory located in

another building. This design eliminated possible observer effects on the animals' behavior,

provided contextual information on the animals' environment, and allowed for off-hours

monitoring of the animal housing space. Furthermore, with the addition of red room lights and

inferred LED lights, we were able to record mating behavior of this nocturnal species without

interruption or disturbance.

In the research setting, our video recording system can be applied to tasks beyond

animal behavior experiments. For example, this system could be used to remotely monitor

chemical reactions or other assays in which timing is uncertain, or if housed within an incubator,

to monitor bacterial or embryo culture. The flexible platform, scalability and low cost of our "do it

yourself" wireless video recording system is a powerful lab tool to make research more efficient

and reliable to a larger population of scientists.

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Supplemental Material Captions

Table S-1. Camera operation and recording script

Table S-2. Rclone installation and GDrive connection script

Table S-3. Docker script for live-streaming

Figure 1

Figure 2

Figure 3