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An open source device for operant licking in rats

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Increasingly complex data sets are needed to fully understand the complexity in behavior. Credit card sized single-board computers with multi-core CPUs are an attractive platform for designing devices capable of collecting multi-dimensional behavioral data. To demonstrate this idea, we created an easy to-use device for operant licking experiments and another device that records environmental variables. These systems collect data obtained from multiple input devices (e.g., radio frequency identification tag readers, touch and motion sensors, environmental sensors) and activate output devices (e.g., LED lights, syringe pumps) as needed. Data gathered from these devices can be automatically transferred to a remote server via a wireless network. We tested the operant device by training rats to obtain either sucrose or water under the control of a fixed ratio, a variable ratio, or a progressive ratio reinforcement schedule. The lick data demonstrated that the device has sufficient precision and time resolution to record the fast licking behavior of rats. Data from the environment monitoring device also showed reliable measurements. By providing the code and 3D design under an open source license, we believe these examples will stimulate innovation in behavioral studies.

<http://github.com/chen42/openbehavior>.

1 An Open Source Device for Operant Licking 2 in Rats

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8 ABSTRACT

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22 Keywords: operant behavior, licking response, behavior testing device, environment variables, single
23 board computer, open source

24 INTRODUCTION

25 Quantitative measurement of rodent behavior is one of the cornerstones of neuroscience. Traditionally,
26 neuroscientists have favored the approach of vastly reducing the complexity of measurements. However,
27 more recently, many scientists have realized that in order to better grasp the complexity of behavior, data
28 sets capturing many more internal and environmental variables and with much higher time and spatial
29 resolution are required (Gomez-Marin et al., 2014).

30 One of the obstacles in obtaining such data sets is that most commercial behavioral measurement
31 equipment was designed decades ago and is not sufficiently flexible to be integrated with emerging
32 technologies. In rare cases when new technologies are used for behavioral measurements, such as
33 touch screen assays for rodents, the cost of the commercial equipment is prohibitive for most academic
34 laboratories. These factors have hindered the collection of “big behavioral data” from complex animal
35 behavior (Gomez-Marin et al., 2014).

36 On the other hand, Moore's Law has dictated the continued increase in computing power and the
37 reduction of its cost. Recent generations of single board computers are the size of a credit card and yet
38 are capable of controlling many behavioral tests. Furthermore, numerous sensors are available to be
39 connected to these computers. These sensors can be used to monitor a wide variety of environmental
40 variables as well as animal behaviors. Therefore, there exists an opportunity to exploit these readily
41 available electronic and computing resources for measuring multi-dimensional behavioral data.

42 Here, we describe a project where a Raspberry Pi® single board computer was used to study operant
43 licking in rats. Operant training in animals allows them to associate a response (e.g. peck a spot with the
44 beak, press a lever, or lick a spout) with a reward (e.g. food, water, or drugs), as well as reward-associated
45 cues (e.g. a light). Operant conditioning is used to study a wide variety of behaviors, especially those
46 related to the function of the reward system, such as food consumption or drug abuse. We also used

47 a motion sensor to track the locomotion of the rat and a radio-frequency identification (RFID) tag to
48 track the identity of the rat. In addition, we also designed an environmental sensor set that monitors the
49 temperature, humidity, barometric pressure, and ambient light levels.

50 METHODS

51 The operant licking device.

52 This system uses a touch sensor connected to the drinking spouts to record the licking behavior of rodents.
53 When the number of licks on a spout meets a predetermined criteria, a syringe pump is triggered to deliver
54 a fixed amount of solution to that spout. A visual cue (an LED) is turned on every time the solution is
55 delivered. A motion sensor is used to record locomotion of the rodent. An RFID reader is used to read the
56 glass ID tag embedded in the animal. All the data are transferred to a remote server via wireless internet
57 connection at the end of the sessions. All electronic devices are installed in a 3D printed frame and can
58 be placed in a standard rat cage. The syringe pump can be placed on top of the wire grid of the cage.
59 The design source files used for 3D printing, software, and instructions for assembly are available in our
60 github repository (<https://github.com/chen42/openbehavior>) under the Creative Commons Attribution
61 NonCommercial license (Version 3.0).

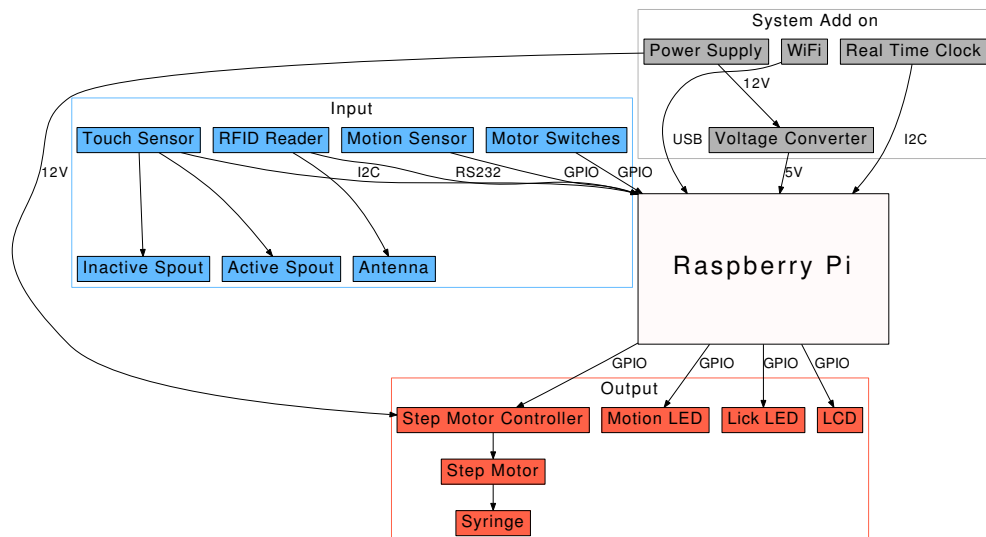


Figure 1. A Diagram of the Operant Licking System. A touch sensor is used to measure the number of licks by a rat on a spout. When the number of licks reaches a criteria, the Raspberry Pi computer advances a step motor, which in turn pushes a syringe to deliver a drop of solution. A motion sensor is used to record the movement of the rat.

62 Computer

63 We used the Raspberry Pi (RPi, model 2B) single board computer. The computer uses a Broadcom
64 BCM2836 Arm v7 quad core processor (900 MHz) and has 1 GB RAM. A total of 40 pins are available
65 on a header for user expansion, including 17 GPIO pins, an I2C connection, and a serial line. A diagram
66 of the peripheral components connected to the RPi is shown in Figure 1. Although sufficiently powerful,
67 the RPi is missing a few key components. Therefore, we added a WiFi module (Edimax) and a real time
68 clock (DS1307). The clock is connected to the RPi via an I2C interface and is needed to maintain the
69 system time when network connectivity is not available. The system power is provided by a 12 V AC-DC
70 converter (2 Amp). A DC-DC voltage step down module (LM2596) is used to provide 5 V power to the
71 RPi.

72 Input devices

73 The key component of the system is a capacitive touch sensor (Model MPR121, Adafruit), which
74 communicates with the RPi via the I2C protocol. The sensor can measure capacitances ranging from

75 10 pF to over 2000 pF with a resolution of 0.01 pF. It has a time resolution of 16 ms. Further, signal
76 filtering and debounce are all handled in the chip, which makes this sensor easy to use. We used only two
77 of the 12 channels of this sensor. Each channel is connected to one drinking spout to monitor the licking
78 behavior. A passive infrared motion sensor (HC-SR501) was used to monitor the activity of the animal.
79 We also connected a RFID reader (RDM6300) to the RPi. This reader can detect low frequency (125 kHz)
80 glass RFID tags that using the EM4100 standard. These glass tags can be implanted under the skin of the
81 rats to provide a unique identification code for each rat. Lastly, we installed two push buttons to provide
82 bidirectional manual control of the step motor. This allows the position of the motor to be adjusted when
83 loading the syringe.

84 **Output devices**

85 The main output device is a syringe pump modified from the design provided by Wijnen et al. (2014). A
86 step motor controller (model A4988, with a heat sink) is used to advance a NEMA 11 motor. The motor
87 is installed in a 3D printed frame, which also has a holder for a 10 ml syringe. The motor is calibrated to
88 push the syringe and deliver 60 μ l of solution to the tip of the spout (via a polyethylene tubing) each time
89 it is activated. A total of three LEDs are connected to the device. One is a cue light installed on top of the
90 active spout. Two other LEDs are turned on when licking or motion is detected, respectively. Lastly, an
91 LCD (16x2 characters) is connected to the GPIO pins. The LCD is the only display of the system and
92 provides information about the system before, during, and after the test sessions.

93 **Software and reinforcement schedules**

94 Our software is written in the Python programming language and runs on the Raspbian Linux operating
95 system (Jessie, released on 05-27-2016). Libraries for the touch sensor and the LCD are provided by
96 Adafruit. The main program is automatically launched at system start up. Once the program is loaded,
97 two push buttons can be used to adjust the pump for loading the syringe. The system then awaits input
98 from the RFID scanner. The session timer starts when the technician scans the glass RFID tag embedded
99 in the rat. The touch sensor records the timing of licking from two spouts, designated as the active and
100 the inactive spout, respectively. We implemented three reinforcement schedules. When the fixed ratio
101 (FR) schedule is used, a reward (i.e. fluid delivered to the active spout) is delivered when a predetermined
102 number of licks (e.g. 10) is recorded. A variable ratio (VR) schedule delivers the reward after a randomly
103 chosen number of licks, with a predetermined average (e.g. 10). When controlled by a progressive ratio
104 schedule, the number of licks required to obtain each subsequent reward is increased until the animal fails
105 to obtain a reward within a given time period. A time out period, when the number of licks is recorded but
106 has no programmed consequence, is enforced after each reward. Because there is no conventional input
107 device present, such as a keyboard or a pointing device, we use a few specific RFIDs to switch between
108 these reinforcement schedules (i.e. scanning one particular RFID tag will load the the progressive ratio
109 schedule).

110 A cue light located above the active spout is turned on for a fixed time (e.g. 5 s) after each reward.
111 The number of licks on the inactive spout is recorded throughout the session but has no programmed
112 consequence. Data from the motion sensor is recorded using a separate Python program. In addition to
113 the timing and type of each event (i.e., lick, movement), the start and finish times are recorded in the data
114 files. The LCD is used throughout the session to provide system status and real time data. The Linux
115 program rsync is used to automatically transfer data files to a remote server upon the completion of each
116 session.

117 **The environmental variable monitoring device**

118 The standalone environmental variable monitoring device also uses a RPi. Four sensors are connected
119 to the RPi via the I2C communication protocol. These are the TSL2561 for ambient light, HTU21D-F
120 for humidity, and BMP085 for barometric pressure. Both the HTU21D-F and the BMP085 contains a
121 temperature sensor. Thus the temperature we record is the average reading from these two sensors. The
122 Python libraries from Adafruit for these sensors are used to obtain data. We have observed that these
123 sensors can draw relatively large amounts of current when in use. Thus, we supplemented an additional
124 power source to the I2C bus. Further, instead of running the data logging program continuously, it was
125 activated once every 10 min as a cron job, which has increased system stability. The Linux rsync program
126 is used to transfer the collected data to a remote server automatically. The Python programs are available
127 in our github repository listed above.

128 **Operant licking for sucrose or water**

129 Ten female rats purchased from Harlan Laboratory were used. Animals were housed in a reverse light-
130 cycled room (lights off at 9:00 AM and on at 9:00 PM). Food and water were provided *ad libitum*. These
131 rats were tested under a FR10, a VR10, and a PR schedule to obtain a 10% sucrose solution (n = 5) or
132 water (n = 5). The FR10 and VR10 sessions were 1 h. The PR session ended 10 min after the last lick
133 on the active spout. These tests were run in a dark room with all lights turned off. All procedures were
134 conducted in accordance with the NIH Guidelines Concerning the Care and Use of Laboratory Animals
135 and were approved by the Institutional Animal Care and Use Committee of the University of Tennessee
136 Health Science Center.

137 **Statistical analysis**

138 Data are presented as mean \pm standard error. Student's t-tests were used to analyze the difference between
139 the licks on the active vs. the inactive spouts. Statistical significance was assigned for $p < 0.05$. The R
140 statistical analysis language was used for data analysis and plotting.

141 **RESULTS**

142 **Operant licking response for sucrose**

143 We tested two groups of rats for operant licking in five devices assembled as describe above. Rats obtained
144 either a sucrose solution (10%, n=5) or water (n=5). Each rat was tested using the FR10, VR10, and PR
145 reinforcement schedules. The number of licks and rewards for each data set are plotted in Figure 2. Rats
146 licked 4902 ± 1076 , 4525 ± 1072 , and 1081 ± 227 times on the active spouts when tested on the FR10,
147 VR10, and PR reinforcement schedules, and received 89 ± 12 , 101 ± 11 , 14 ± 1 drops of sucrose solution,
148 respectively. In contrast, rats licked 141 ± 38 , 241 ± 84 , and 115 ± 28 times on the active spouts when
149 tested on the FR10, VR10, and PR reinforcement schedules, and received 8 ± 2 , 9 ± 2 , 5 ± 1 drops of
150 water, respectively. The number of licks on the active spout was significantly greater than those on the
151 inactive spouts when sucrose or water was provided (2). These results are very similar to those reported
152 by Sclafani and Ackroff (2003), indicating that the devices are providing reliable data.

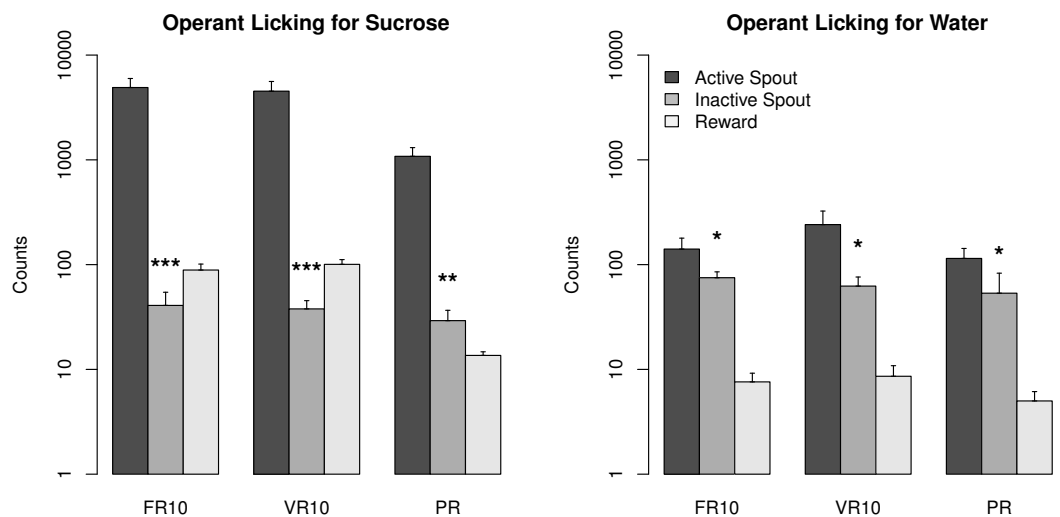


Figure 2. Summary of Lick Responses and Rewards. Ten rats were tested using the operant licking devices to obtain a sucrose solution (10%, n=5) or water (n=5) under the control of a fixed ratio 10 (FR10), a variable ratio 10 (VR10), or a progressive ratio schedule. A logarithmic scale is used for the Y-axis. *: $p < 0.05$, *** $p < 0.001$, compared to the active spout.

153 The time course of licks on the two spouts as well as rewards earned from the rat with the highest
154 number of licks in each of the six test conditions was plotted in Figure 3. Rats licked almost continuously
155 on the active spouts for the entire 60 min testing period for sucrose when the FR10 or the VR10 schedule
156 was used. The rate of licking was much lower when the PR schedule was used. Although they sampled

157 the inactive spout initially, licking activity became exclusive for the active spout near the end of each of
 158 the sessions. In contrast, when water was provided, the licks were much sparser, with large time lags
 159 between receiving rewards. Thus, these data better illustrate the number of licks between rewards. The
 160 number of licks was not even when the FR10 schedule was used. This is because rats continue to lick on
 161 the active spout during the 20 s time out period after the reward was delivered.

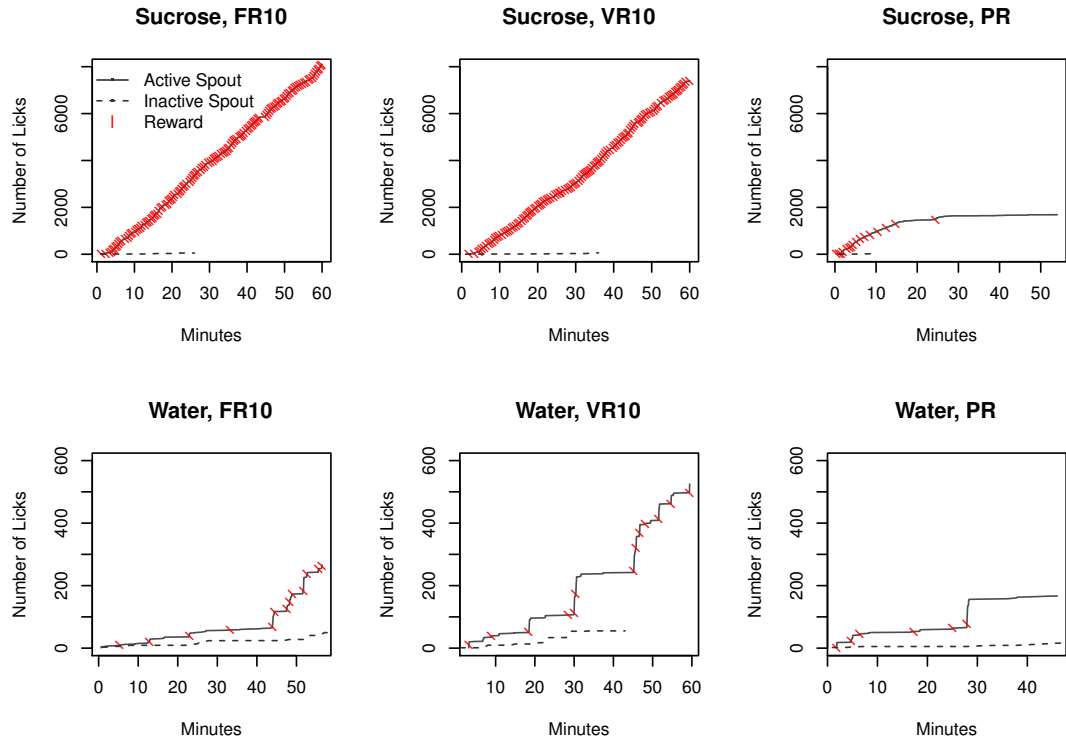


Figure 3. *Time Course of Licks and Rewards.* The cumulative number of licks on the active and inactive spouts as well as rewards (i.e. sucrose solution or water) earned during one test session are shown. Rats licked continuously on the active spouts for sucrose when the FR10 or VR10 schedule was used. The rate of licking slowed down dramatically under the PR ratio, where the workload for each subsequent reward increased rapidly. The rate of licking was much lower when water was provided.

162 The microstructure of licks is informative for the reward value of the taste substance (Davis and Smith,
 163 1992). As previously reported (Davis and Smith, 1992; Wang et al., 2014), we defined a cluster as licks
 164 that occurred within 0.5 seconds of each other. Clusters with fewer than two licks were excluded from the
 165 analysis. The size of a lick cluster is the number of licks it contains. Inter-licking interval is defined as the
 166 time between each lick within each cluster. The distributions of inter-lick intervals and the size of lick
 167 clusters for sucrose or water under the FR10 schedule are shown in Figure 4. The average size of lick
 168 clusters on the active spout for sucrose was 35.7 ± 6.4 for sucrose and 7.2 ± 1.1 for water ($p < 0.05$). In
 169 contrast, the average size of lick cluster on the inactive spout was 8.6 ± 4.0 for sucrose and 5.9 ± 0.4 for
 170 water ($p > 0.05$).

171 The inter-lick interval on the active spout was 0.15 ± 0.002 s for sucrose and 0.22 ± 0.01 s for water
 172 ($p < 0.01$). The inter-lick interval on the inactive spout was 0.20 ± 0.01 s for sucrose and 0.26 ± 0.02
 173 for water ($p < 0.01$). The size of the lick clusters for sucrose was in agreement with those reported in
 174 the literature (Davies et al., 2015). These data not only confirm that the subjective value of sucrose is
 175 significantly greater than that of water, but more importantly indicate that the device we designed has
 176 sufficient time resolution to accurately measure the rapid licking behavior of rats.

177 The locomotion data collected from the FR10 and VR10 session were presented in Figure 5. Data
 178 from the PR session was not shown because each rat had a different session length. The movement was
 179 combined into 1 min bins. The average number within each bin from all the rats were shown. These

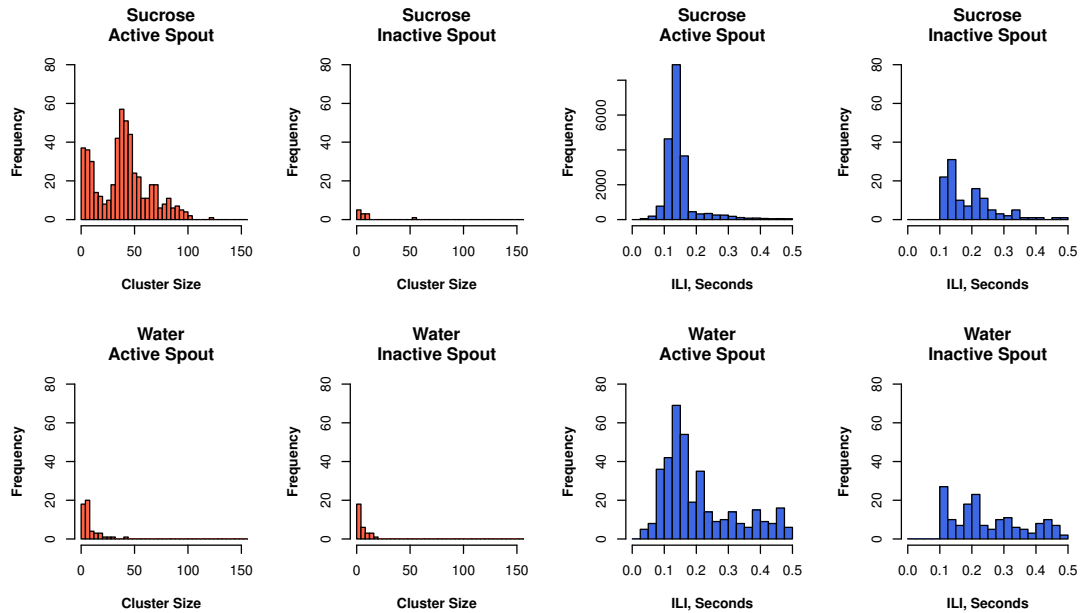


Figure 4. *Lick Microstructure Analysis of the FR10 Data Set.* We defined a cluster as a group of licks where the inter-lick interval (ILI) was less than 0.5 s. Data from all five rats were combined for the analysis.

180 data showed that although there was a general trend of reducing locomotion, rats remained active during
 181 the entire 1 hr session when sucrose was provided. However, the rats were less active when water was
 182 provided.

183 **Environmental data**

184 The data gathered by the environment sensor set from an animal housing room for the first week of
 185 July 2016 is plotted in Figure 6. The data showed that the animal facility is under tight climate control.
 186 However, after adjusting the airflow on July 5th, the temperature was reduced by $\sim 1^\circ\text{C}$ and the humidity
 187 was increased by 3%. The air pressure also shows fluctuation. The level of light recorded indicates that
 188 the light of the room was reverse cycled (On at 9 PM and off at 9 AM, and that technicians occasionally
 189 enter the room during the day.

190 **DISCUSSION**

191 We designed two devices using single board computers. We tested the operant licking device by training
 192 rats to obtain sucrose or water using several reinforcement schedules in regular housing cages. We also
 193 designed a device that can continuously record four environmental variables, including temperature,
 194 humidity, air pressure, and light levels. We used 3D printers to manufacture the frames of the devices. The
 195 designs of the devices, including all source files and software, are available under an open source license.

196 Since their inception, computers have been an integral part of quantitative behavioral studies. Desktop
 197 computers are used in most behavioral equipment produced in the past few decades. As predicted by
 198 Moore's Law, the number of transistors per square inch continues to double every two years. Computers
 199 the size of a credit card currently have processing power equivalent to desktop computers from 10 to 15
 200 years ago. Many behavioral researchers have discovered the relevance of these small computers for data
 201 collection projects and taken full advantage of their usefulness. For example, Escobar and Pérez-Herrera
 202 (2015) described the use of an Arduino microcontroller in conjunction with a laptop computer to conduct
 203 operant conditioning experiments. Pineño (2014) reported an operant conditioning device using an iPod
 204 Touch and an Arduino microcontroller. Most interestingly, Rizzi et al. (2016) designed an Arduino-based
 205 system that triggered the delivery of optogenetic stimulation from nose pokes of mice.

206 We chose to use single board computers over microcontrollers because they have much faster proces-

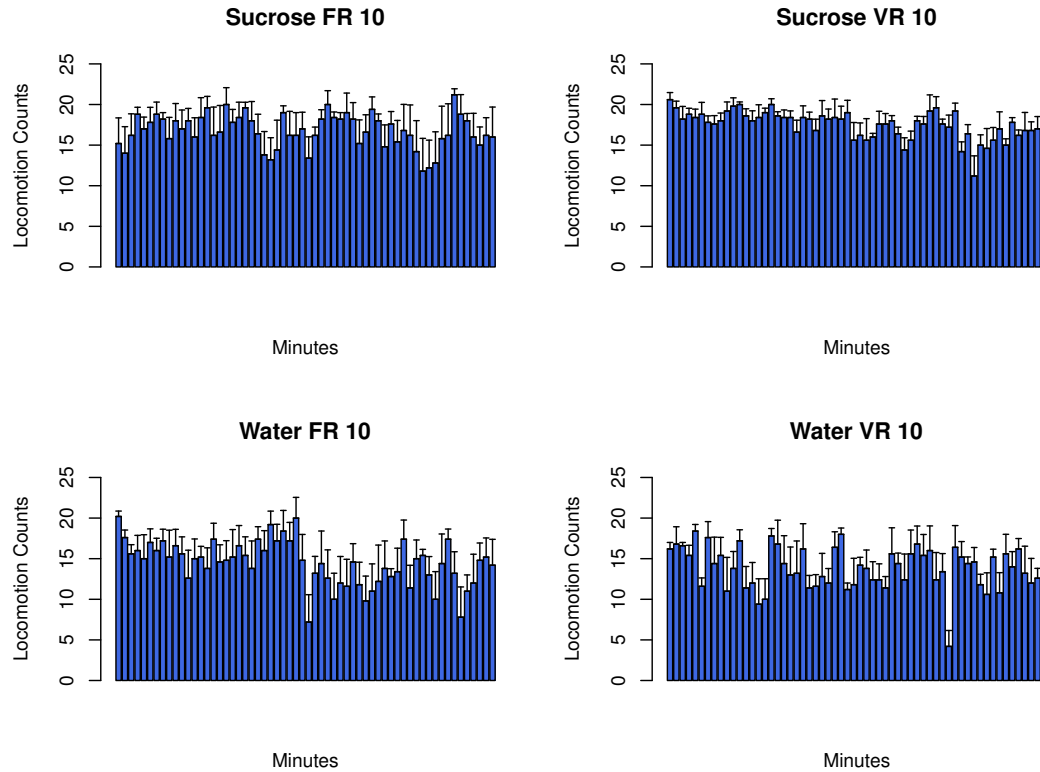


Figure 5. *Locomotor Response.* The locomotion data from each rat were combined into 1 min bins. Rats remained active during the entire 60 min when sucrose was provided, while fewer locomotor activity counts were recorded when water was provided.

207 sors, easy access to permanent data storage, and network access, and run on modern operating systems
 208 while still being very affordable. Although there are numerous single board computers available, we chose
 209 the Raspberry Pi (\$35) because it has a large user community and is the most likely to have sustained
 210 development, as demonstrated by the recent release of Raspberry 3, which includes a built-in WiFi
 211 module.

212 Our devices demonstrated the utility of RPi in studying rodent behaviors. The small size of the RPi
 213 allows the entire device to fit in a regular rat housing cage. Combined with low cost, this has the potential
 214 for breaking down two of the main barriers for many academic labs to conduct operant behavioral tests:
 215 the lack of funding for expensive equipment and limited space in the vivarium. Another advantage of
 216 shrinking the size of behavioral test equipment is that it allows rodent behavior to be recorded continuously
 217 in the home cage. Thus, the diurnal rhythm of the behavior can be recorded without incurring large costs
 218 for dedicated equipment.

219 RFID tags are starting to be used in animal research to provide unique identification codes for animals.
 220 The low frequency (125 kHz) glass tags (~\$1 each) usually encode a twelve character hexadecimal number
 221 and therefore can uniquely identify 2.8×10^{14} animals for a research project. Because of their small
 222 size, they can be inserted into a syringe needle and be injected under the skin of rodents without general
 223 anesthesia. Once embedded, they provide a permanent ID for each individual. These tags can even be
 224 placed with tissue samples once the animals are euthanized.

225 Integrating an RFID reader with the behavioral device allows each test subject to be unequivocally
 226 identified in the data files. Further, the device can be programmed to start the test session when an RFID
 227 is detected. This not only simplifies the workflow but also reduces potential noise in the data (e.g. when
 228 testing multiple animals, some technicians prefer to start the recording once all the animals are placed in
 229 the testing device. Thus some animals are exposed to the device while their behavior is not recorded).

230 Devices with small footprints can suffer from the limited choices of input methods. Although touch

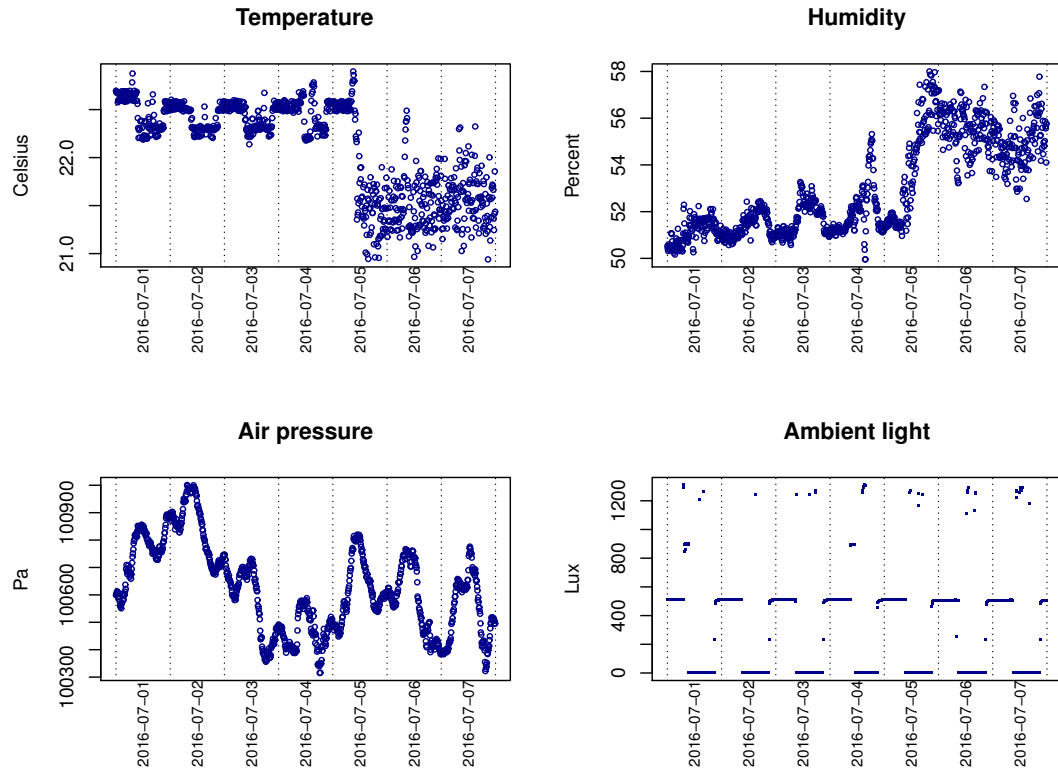


Figure 6. *Environmental data.* The temperature, humidity, air pressure and level of light in an animal housing room are shown. The shift in temperature and humidity on July 5th coincided with an adjustment of the airflow in the facility.

231 screens can be used, RFID tags provide a helpful alternative when the number of program options are
 232 limited. For example, while our program defaults to start the variable ratio 10 schedule, we encoded the
 233 value of several RFID tags in the program so that when these particular tags are detected, the fixed ratio
 234 or the progressive ratio schedules will be used. Although this is very simple to use and can be readily
 235 expanded to include other options, one shortcoming is that we need to use those particular tags to start the
 236 program. We remediated this by programming two alternative tags for each reinforcement schedule.

237 Another potential advantage of the RFID tags is that they allow for the possibility of multiple animals
 238 to be tested in a group housing setup. However, in our tests, we have found that the detection of RFID tags
 239 using the RDM6300 reader is not sufficiently reliable. It sometimes misses the tag even when the antenna
 240 is in close proximity to the tag (maximum sensitivity is found when the tag approaches the antenna in
 241 a perpendicular direction and at the edge of the antenna loop. One of the main future directions of this
 242 project is to improve the sensitivity of the RFID detection system, possibly by using a different RFID
 243 reader, such as those used by Howerton et al. (2012).

244 There are several commonly used methods for recording the licking behavior of rodents. The contact
 245 lickometer supplies a small voltage between the wire floor and the spout. It then detects the small current
 246 passing through the rat when it licks the spout. This requires a metal floor to be used. An alternative
 247 method is to set up an infrared beam to monitor the tip of the spout. The tongue blocks the light beam
 248 and allows the licks to be detected. This method requires the position of the light and the spout to be
 249 carefully calibrated. We used a capacitive touch sensor to monitor the licking events. Our analysis of the
 250 lick microstructure showed that this method has sufficient time resolution and sensitivity to reliably detect
 251 rodent licking behavior. One caveat is that these touch sensors are very sensitive. They can sometimes
 252 be triggered by environmental interference, especially after they are connected to a large piece of metal,
 253 such as a rodent drinking spout. We found that adjusting the threshold to touch=36 and release=18 in the
 254 Python library for the MPR121 touch sensor is sufficient to avoid the noise while still reliably recording

255 the licking events.

256 One of the main motivations in developing these devices is to study operant alcohol self-administration
257 in rats. Rats have many advantages for behavioral neuroscience research (Parker et al., 2013). However,
258 many of the widely used rat strains do not readily consume alcohol. We hope the device described here
259 will be helpful in establishing a robust model of oral alcohol self-administration. Operant licking provides
260 potential advantages over lever pressing behavior, such as its high response-reinforcer contingency.
261 Further, the lick microstructure analysis can provide insights into the subjective value of the reward (Davis
262 and Smith, 1992). The combination of low cost and small footprint of this device also provides a unique
263 opportunity to perform relatively large-scale studies on the diurnal rhythm of alcohol consumption.

264 Lastly, the environmental monitoring device allows the collection of several variables that could
265 potentially influence alcohol intake in the long run and improve experimental reproducibility. Although
266 commonly ignored, more and more recent data show that environmental factors such as temperature
267 (Chesler et al., 2002), humidity (Chesler et al., 2002), barometric pressure (Mizoguchi et al., 2011), noise
268 (Okada et al., 1988; Prior, 2002), illumination (Valdar et al., 2006), and vibration (Okada et al., 1988) have
269 a great impact on animal behavior. Our low cost system can capture large amounts of environmental data,
270 which can improve understanding of the complex effects of the environment on behavior and increase
271 research reproducibility (Collins and Tabak, 2014).

272 A potential disadvantage of our approach is that manufacturing these devices may require skills not
273 present in a behavioral neuroscience lab. For example, although 3D printers have become very affordable,
274 printing high-quality parts is still a trial and error process. For example, two parts of the syringe pump
275 that hold the stainless steel rods need to be printed with precise dimensions to ensure accurate delivery of
276 the solutions. It is possible that the design files need to be slightly modified according to the printer and
277 printing conditions (e.g. temperature, resolution, etc.) to achieve the precision needed. One alternative is
278 to use commercial 3D printing stores to manufacture these parts.

279 In summary, we developed two open source devices that can be used to collect multi-dimensional data
280 for behavioral studies. By providing the design and software under an open source license, we hope they
281 will stimulate the wider adoption of single board computers and innovation in behavioral measurements.

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