Integrative Systems

Development of an Open Face Home Cage Running Wheel for Testing Activity-Based Anorexia and Other Applications

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Abstract
Running wheels for mice residing in the home cage are useful for the continuous measurement of locomotor activity for studies testing exercise interventions or exercise-induced effects on brain and metabolism. Here, we have developed an open source, printable, open-faced running wheel that is automated to collect locomotor information such as distance traveled, wheel direction, and velocity that can be binned into epochs over 24 h or multiple days. This system allows for remote data collection to avoid human interference in mouse behavioral experiments. We tested this system in an activity-based anorexia procedure. Using these wheels, we replicate previous findings that food restriction augments wheel-running activity.

Key words: activity-based anorexia; locomotor activity; open behavior; open source; running wheels

Significance Statement
Anorexia nervosa (AN) is a psychiatric disease with few treatments and a high mortality rate. It is important to better understand the biology to accelerate the development of new therapies. The most used animal model to study AN is the activity-based anorexia model, which measures physical activity during food restriction. We have developed open source running wheels that allow for continuous measurement of activity for multiday experiments and demonstrated efficacy in the activity-based anorexia model.

Introduction
The prevalence of eating disorders such as anorexia nervosa (AN) has been escalating in recent years (Val-Laillet et al., 2015) and has increased over the covid-19 pandemic (Devoe et al., 2022). AN has a lifetime prevalence of 1% (Hudson et al., 2007; Smink et al., 2012; Bou Khalil et al., 2017). The mortality rate of these individuals is 5 times greater than a healthy individual (Arcelus et al., 2011; Bou Khalil et al., 2017) which is the highest mortality rate for any mental disorder (Arcelus et al., 2011; Smink et al., 2012; Val-Laillet et al., 2015). Furthermore, the current recovery rate for AN, 10 years after onset, is a meager 10% (Bergh et al., 2013). An improved understanding of the etiology and neurobiological underpinnings of this disorder will lead to improved treatments and outcomes.

The hallmark characteristics of AN are a restriction in energy intake that leads to low body weight, an intense fear of gaining weight, and a disturbed body image including overestimating body size (American Psychiatric

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Association, 2013). In addition, an increase in physical activity has been observed among many individuals with AN (Bergh et al., 2013). A common model used to study AN in rodents is the activity-based anorexia (ABA) model that replicates many symptoms seen in AN (Routtenberg, 1968; Watanabe et al., 1992; Bergh et al., 2013; Gutierrez, 2013). In this model, access to a running wheel is paired with food restriction (FR), resulting in an increase in activity, a decrease in food consumption, and a reduction in body weight. Mice and rats lose the ability to self-regulate their food intake and energy expenditure, eventually resulting in weight loss to the humane end point where they are removed from the study.

An important limitation to the ABA model is easy access to mouse running wheels with the ability to record mouse activity. Although commercial products are available, they are expensive and require proprietary software devoted to wheel use (Welch et al., 2018). Many commercial products also use a closed wheel that is not compatible with modern optical and recording techniques. Other open source wheel designs had features that were not ideal for our application (Bivona and Poynter, 2021; Zhu et al., 2021). For these reasons, we designed and constructed an open source running wheel system that runs independent from a central computer. The wheels are 3D printed and are operated using a Raspberry Pi zero W, a small but highly available microprocessor, and are programmed in Python. The data are transmitted to a personal computer via e-mail, where it is then automatically downloaded, parsed, and analyzed with Python and MATLAB programs. Our running wheel system is inexpensive, simple, adaptable, and completely open source. In this study, we demonstrated its utility with the ABA model.

Materials and Methods

Mice

Thirty-two female BALB/c mice (Charles River) 7–8 weeks old were used for the ABA model. Mice acclimated in the animal facility 3–5 d before the habituation period of the study. Before habituation, mice were housed in groups of 2–5, maintained on a 12 h light/dark schedule [lights on at 0800 h, zeitgeber time 0 (ZT0)], and given chow and water ad libitum (Ad lib). Mice were fed standard chow (Pico-Vac Mouse Diet 5062, Lab Supply), which is composed of the following (percentage of total kcal): 23% protein, 22% fat (ether extract), and 55% carbohydrate. The total density of this diet was 4.60 kcal/g. All experiments and procedures were performed in accordance with the ethical guidelines established by the Canadian Council for Animal Care and were approved by the University of Calgary Animal Care Committee (protocol no. AC21-0034).

Running wheels

Each running wheel consists of a 3D printed wheel and electrical components connected to a Raspberry Pi 0 W (Fig. 1A, www.PiShop.ca). The spinner part of the wheel contains a metal ball bearing at the center and is surrounded by three sets of magnets that are evenly spaced. Three hall effect sensors that protrude up through the base of the wheel detect changes in the magnetic field, which occurs when the magnets pass over top. Each hall effect sensor has three pins, with the first pin being connected to the 3.3 V Raspberry Pi power supply through the MCP3008 analog digital converter (ADC; Fig. 1A,B). The second pin connects to the Raspberry Pi ground. The third pin transmits magnetic field values to the Raspberry Pi through the ADC. To reduce noise, a 10 kΩ pull-up resistor is placed between the third pin and the ground. The ADC is soldered to a piece of printed circuit board (PCB) prototyping board and 20 gauge solid wires connect the board to the Raspberry Pi and the three hall effect sensors. The three hall effect sensors are inserted inside a 3D printed sensor holder, which fits through a gap left in the base of the wheel. All Raspberry Pi boards receive power through the micro-USB port. Copper tubing was bent and cut to serve as a protective sleave for the USB power cable inside of the mouse cage. The total cost of each wheel was approximately $65.00 CAD (Canadian dollars).

3D printing

All files for spinner parts were made as SLDPRT (SolidWorks Part) files in solid works and then converted to STL (stereolithography) files. Both SLDPRT and STL files are available at (https://github.com/borglandlab/RunningWheel) to allow for direct printing or modifying the wheel to individual needs. When assembled, the wheel is 15.24 cm (6 inches) l width, 10.64 cm (4.19 inches) in height, and 14.45 cm (5.69 inches) in depth. Using the STL files, 3D models were then sliced using Cura 4.9 in preparation for printing (https://ultimaker.com/learn/ultimaker-cura-4-9-seamless-and-efficient-with-digital-library-integration). For the wheels used in the ABA model, printing was done using either a Stock Eryone Thinker ER20 (ShenZhen Eryone Technology Co, Ltd) or FLSUN QQ-S-pro (Zhengzhou Chaokuo Electronic Co, Ltd) with 0.4 mm E3D V6 nozzle. All 3D printing used generic 1.75-mm-diameter PLA (polylactic acid). To demonstrate the universal nature of our design, all wheel parts were also sliced and printed with the Sindoh 3DWOX printer (Sindoh Co, Ltd) and supporting software with excellent results. More information on the printing process can be found within our 3D model files.

Raspberry Pi code

The Raspberry Pi is programmed to continuously check the values of all three hall effect sensors. When the value of one hall effect sensor is >30, the trigger time and values for all three hall effect sensors are recorded. New
values cannot be recorded until the values for all three hall effect sensors drop to $<15$, allowing the magnet to clear to ensure that the next trigger is indeed a separate incident. Thus, a single magnet passing over the three sensors will not accidentally trigger the sensors more than once. For this reason, because there are magnets in three different locations on the spinner top, each triggering of the hall effect sensors reliably indicates that the mouse has traveled one-third of the circumference at the running position of the spinner top. Every hour, or when the number of recorded entries is $>3000$, the entries are written to a text file. These data will be stored on the micro-secure RPi ADC.

1. Using Python, text files are downloaded, and values are parsed from the text and saved in excel files.
2. Data is imported to MATLAB and stored in a MATLAB structure.
3. Distance and velocity are calculated, and the total distance and average velocity are plotted immediately.
4. Data in MATLAB structure can be analyzed using a script to look at whatever is of interest.

Figure 1. Description of the running wheels and computer interface. A, Illustration indicating how data are collected and distance and velocity are calculated. B, Illustration of the Raspberry Pi motherboard connections. Image of the Raspberry Pi computer is adapted from https://en.wikipedia.org/wiki/Raspberry_Pi. C, Photograph of a mouse on the running wheel when connected to a fiber-optic patch cord. D, Photograph of a Green Line cage containing a mouse on the wheel with the power cord attached through the bottle holder. Water bottles are delivered via the food hopper for these experiments. Additional photographs of the wheel are located at https://github.com/borglandlab/RunningWheel.
digital (SD) card and can be accessed at the end of the experiment. Although writing to the text file is relatively fast, the Raspberry Pi will wait for a moment when no wheel movement is occurring to ensure that minimal data are lost during this process. In addition, if the Raspberry Pi detects an Internet connection with speeds >1 Mb/s it will also send an e-mail containing the text file information to the specified e-mail account. The frequency of data storage and transmittal can be adjusted for specific experimental needs (Fig. 1A). To connect the Raspberry Pi boards to the university network, we supplied the MAC (media access control) addresses from each Raspberry Pi to the IT services.

**Personal computer**

Using Python code (https://github.com/borglandlab/RunningWheel), the e-mails sent from the Raspberry Pi are downloaded (Fig. 1A,B). Trigger times and values for each hall effect sensor are parsed from the text, organized, and stored in a MS Excel workbook. Individual emails are stored as sheets within a workbook, with a maximum of 49 sheets per workbook before a new workbook is created. Each running wheel is assigned a folder that will contain all the excel workbooks for that specific wheel. A directory MS Excel workbook is also created to keep track of the location of each sheet. A MATLAB program imports the data from these excel workbooks and stores each individual time point, direction of wheel rotation, the cumulative distance, and the velocity for all running wheels in a MATLAB structure (www.Mathworks.com). MATLAB will also immediately create graphs showing the total distance and average velocity of the wheels. This code is run from a Python GUI (graphical user interface; www_python.org) automatically taking the data from a text file in your e-mail account to an organized MATLAB structure and viewable graphs. The MATLAB structure can be further analyzed by the code provided or by your own personal analysis. Furthermore, data can be binned into daily and hourly time frames, making analysis and data visualization more versatile (Fig. 1).

In addition to data collected from the running wheel, we also recorded body weight, food, and water weight each day on a workbook stored in Dropbox (www.Dropbox.com). This allowed us to automatically calculate changes in mouse weight, changes in food and water consumption, and the removal from study threshold with MATLAB. Graphs were automatically generated. Like the code for the running wheel, this can also be run from our Python GUI. However, because this code is specific to our experiment, it has been made optional to run in the experiment.

**Activity-based anorexia model**

The ABA model used in this study was based on models previously published (Ho et al., 2016; Achamrah et al., 2017; Welch et al., 2018). Mice were placed in individual cages that contained a spinning or nonspinning (dummy) running wheel, cardboard shelter, water bottle, and ad libitum chow in a feeding hopper, and were left unattended for 48 h to habituate to the novel cage. A 7 d baseline period immediately followed the habituation period. During the baseline, body weight, food, and water were weighed each day at ZT 0130 h. The spinner tops were cleaned, and 8–10 g of new food was weighed and left in the food hopper. Following baseline, mice were split into the following four groups: (1) ad libitum access with dummy wheel (n = 8); (2) ad libitum access with running wheel (n = 8); (3) food restriction with dummy wheel (n = 8); and (4) food restriction with running wheel (n = 8). Group 1 was paired with group 3, and group 2 was paired with group 4, such that measurements could be compared with a time-matched control. During this period, groups 3 and 4 were given access to food for 6 h (ZT 0130 h to ZT 0730 h) for 3 d (days 8–10). In a pilot study, mice did not lose weight to the humane end point when on 6 h food restriction for up to 10 d (Fig. 2B, Extended Data Fig. 2-1); therefore, we modified the protocol so that after 3 d of 6 h food restriction, they were food restricted to 3 h (ZT 0130 h to ZT 0430 h) for the next 6 d (days 11–17; Fig. 2A). Body weight, food, and water were weighed daily at ZT 0130 h. Spinner tops were cleaned at ZT 0130 h for nonrestricted mice, and after food restriction for restricted mice. Restricted mice, and their paired controls, were removed from the study if their body weight fell to 75% of their body weight recorded on the last day of baseline. Daily measurements of distance and velocity were recorded from ZT 1700 h (12:00 A.M.) to ZT 1700 h the next day. The anticipatory activity was measured in the 3 h before food access. Activity during food intake was measured during first 3 h of food access. Activity during the postprandial period was measured ZT 0730 h to ZT 1030 h. Mice begin to be removed from the experiment during day 11, and by day 13, after 6 d of food restriction, only one mouse remained in groups 2 and 4, obscuring comparisons beyond this point.

**Data analysis**

All values are expressed as the mean ± SEM and assessed for normality using a Shapiro–Wilk test. Statistical significance was assessed by using two-tailed unpaired Student’s t test for two comparisons. A two-way ANOVA followed by Sidak multiple-comparisons test was used for multiple group comparisons. GraphPad Prism 8.3 (GraphPad Software) was used to perform statistical analysis. Significance was defined at alpha = 0.05, p < 0.05 *, p < 0.01 **, p < 0.001 ***, P < 0.0001 ****.

**Results**

To validate the utility of our 3D-printed running wheels, we conducted a 3-week-long ABA model. During the 7 d baseline period, mice had access to active or inactive (dummy) running wheels and ad libitum access to food and water. On the final day of baseline, body weight was compared across the four groups: Ad lib access with dummy wheel (17.7 ± 0.2 g); Ad lib access with running wheel (17.4 ± 0.3 g); FR with dummy wheel (17.7 ± 0.3 g); and FR with running wheel (18.0 ± 0.3 g). There was no main effect of wheel running or food on body weight (running effect: F(1,28) = 0.003, p = 0.9; food effect: F(1,28) = 1.0,
p = 0.3), or wheel running × food interaction (interaction: $F_{(1,28)} = 0.8, p = 0.4$).

To examine the effect of FR on body weight, food and water consumption, and wheel running, we measured these parameters on day 11, the first day of the second food restriction phase. This was the time point when some mice first reached the humane end point and were removed from the study. The number of days to reach the humane end point whereby mouse body weight dropped to 75% of body weight from that measured on the last day of baseline was compared between FR with a running wheel and FR with a dummy wheel. FR mice exposed to wheel running had reduced probability of survival compared with FR mice with the dummy wheel (log-rank test: $\chi^2 = 5.1; df = 1; p = 0.02$; Fig. 2B). Thus, FR mice with access to a running wheel are removed from the experiment earlier.

To determine the effect of FR, mouse body weight was compared between groups on day 11. FR running wheel mice were at 78.1 ± 1.5% of their baseline body weight, whereas Ad lib dummy wheel mice were at 99.7 ± 0.9%. Ad lib running wheel mice were at 100 ± 0.8%, and FR dummy wheel mice were at 91.7 ± 1.9% of their original body weight. There were main effects of running and FR (running effect: $F_{(1,28)} = 9.8, p < 0.004$; food restriction effect: $F_{(1,28)} = 40.9, p < 0.0001$) as well as a running × FR interaction (interaction: $F_{(1,28)} = 7.3, p = 0.01$). A Sidak multiple-comparisons test showed that FR running wheel mice lost more weight than FR dummy wheel mice ($p = 0.002$) and Ad lib running wheel mice ($p < 0.0001$; Fig. 3A,B).

Daily food consumption was compared between groups (Fig. 3C,D). Twenty-four hour food consumption from day 10 demonstrated a main effect of FR (food restriction effect: $F_{(1,28)} = 34.6, p < 0.0001$) but no effect of running (running effect: $F_{(1,28)} = 3.4, p = 0.07$) on food consumption. Given that both wheel and dummy groups had reduced food consumption during the FR period, there was no interaction ($F_{(1,28)} = 0.2, p = 0.7$). However, a Sidak post hoc test on the main effect of food restriction indicated significant reductions in food intake in both dummy wheel groups (Ad lib dummy wheel mice, 3.1 ± 0.3 g; vs FR dummy wheel mice, 1.7 ± 0.2 g; $p = 0.0002$) and running wheel groups (Ad lib running wheel mice: 3.4 ± 0.2 g; vs FR running wheel mice, 2.2 ± 0.2 g; $p = 0.001$; Fig. 3D). Thus, access to the running wheel did not further restrict food intake in the FR groups.

We next measured daily water consumption (Fig. 3E,F). On day 10, there was a main effect of FR on 24 h water consumption (food restriction effect: $F_{(1,28)} = 46.4, p < 0.0001$) but no effect of running on water consumption (running effect: $F_{(1,28)} = 3.9, p = 0.06$) or running × FR interaction (interaction: $F_{(1,28)} = 0.1, p = 0.7$). A Sidak post hoc test on the main effect of food restriction indicated a significant

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**Figure 2.** Mice on an activity-based anorexia model with access to a running wheel have decreased probability of survival when food restricted. **A,** Description and time course of the following four groups: (1) Ad lib + dummy, (2) Ad lib + wheel, (3) FR + dummy, and (4) FR + wheel. **B,** FR mice with access to a running wheel (red) are removed from the study sooner when food restricted compared with Ad lib mice with a running wheel (black). Extended Data Figure 2-1 indicates that mice did not lose weight to the humane end point when on 6 h food restriction for up to 10 d. As such, we added a 3 h food restriction after 3 d of 6 h food restriction.
increase in water intake in both dummy wheel groups (Ad lib dummy wheel mice: 4.1 ± 0.2 ml; vs FR dummy wheel mice: 6.4 ± 0.3 ml, p < 0.0001) and running wheel groups (Ad lib running wheel mice: 4.6 ± 0.3 ml; vs FR running wheel mice: 6.9 ± 0.5 ml; p = 0.0002; Fig. 3F). Together, these data indicate that FR mice with access to a running wheel have reduced body weight compared with FR mice without the running wheel. However, food and water intake between these groups was similar.

We next recorded daily 24 h locomotor activity of Ad lib and FR mice with access to our 3D printed running wheels and supporting Python/MATLAB code (Fig. 4). Distance traveled between Ad lib and FR mice was measured daily. There were eight mice per group until day 11 when mice were removed from the study because of low body...
weight, and thus the number of animals per group varied on subsequent days (day 11, n = 6; day 12–16, n = 1; Fig. 4A). Thus, the averaged distance traveled during food restriction was measured the morning of day 11, which accounted for the preceding 24 h period before mice were removed from the study. There was a main effect of food restriction \((F_{1,28} = 21.1, p < 0.0001)\) and a main effect of time \((F_{1,28} = 12.5, p = 0.001)\), and a time \times food restriction interaction \((F_{1,28} = 12.5, p = 0.001)\). A Sidak post hoc test indicated no difference during the baseline period (day 7), but a significant increase in distance traveled after food restriction \((Ad\,lib\,groups, 7.7 \pm 1.2\,km; vs\,FR\,groups, 22.5 \pm 3.0\,km; p < 0.0001; \text{Fig. } 4B)\). Thus, FR increases wheel running in mice.

We next examined whether FR influences daily anticipatory activity in the 3 h period before food delivery (Fig. 4C,D). There was a main effect of food restriction \((F_{1,28} = 8.2, p = 0.008)\), a main effect of time \((F_{1,28} = 16.5, p = 0.0003)\), and a significant food restriction \times time interaction \((F_{1,28} = 5.2, p = 0.03)\). A Sidak post hoc test indicated no significant difference during baseline (day 7; \(p \approx 0.9\)), but it did indicate a significant increase in anticipatory activity during food restriction (day 11; \textit{Ad lib} groups, 0.9 \pm 0.4\,km; vs FR groups, 2.8 \pm 0.4\,km; \(p = 0.002; \text{Fig. } 4D\)). Thus, FR increases anticipatory wheel running.

Activity during the first 3 h of the food availability period was not different between groups (\textit{Ad lib} wheel mice, 0.6 \pm 0.2\,km; FR wheel mice: 0.4 \pm 0.1\,km). There was no main effect of food restriction \((F_{1,28} = 0.2, p = 0.6)\), time \((F_{1,28} = 2.0, p = 0.2)\), or food restriction \times time interaction \((F_{1,28} = 0.04, p = 0.8; \text{Fig. } 4E,F)\). These results suggest that, in our procedure, FR mice are making choices for food over wheel running during food availability.

We next examined daily wheel running activity during the postprandial period (Fig. 4G). There was a main effect of food restriction \((F_{1,28} = 7.9, p = 0.009)\), a main effect of time \((F_{1,28} = 10.5, p = 0.003)\), and a significant food restriction \times time interaction \((F_{1,28} = 7.1, p = 0.01)\). A Sidak post hoc test indicated that while postprandial activity was not different between groups during the baseline (\(p \approx 0.99\)), in the 3 h period after food access, the postprandial activity was greater in FR mice \((3.1 \pm 0.7\,km)\) than in \textit{Ad lib} mice \((0.7 \pm 0.3\,km, p = 0.001; \text{Fig. } 4H)\). Thus, FR mice increase their activity in the period after food availability compared with \textit{Ad lib} mice.

To examine the circadian pattern of activity, we compared the hourly distance traveled of \textit{Ad lib} or FR mice binned by hour and plotted across all of day 10 from 12:00 A.M. to day 11 at 12:00 A.M. There was a main effect of food restriction \((F_{1,14} = 20.9, p = 0.0004)\), a main effect of time \((F_{4,06,58,84} = 13.05, p < 0.0001)\), and a time \times food restriction interaction \((F_{23,322} = 4.03, p < 0.0001)\). A Sidak post hoc test revealed significant differences in activity between \textit{Ad lib} and FR groups in hours 18–24, the early part of their dark cycle (Fig. 5A). Furthermore, to demonstrate the full utility of the running wheels, the total daily activity of both \textit{Ad lib} and food-restricted mice was plotted, showing the direction of the rotation of the wheels [i.e., clockwise (CW) or counterclockwise (CCW); Fig. 5B,C]. We recorded wheel rotations in CW or CCW from \textit{Ad lib} and FR mice, with a greater amount of running in the CCW wheel direction. Together, FR mice travel greater distances on the running wheels over a 24 h period.

Given the high sensitivity of these running wheels, we calculated the average daily velocity of the mice from the recorded data (Fig. 6A). When comparing the baseline (day 7) to the final day before some mice were removed from the study (day 11), we found a main effect of food restriction \((F_{1,28} = 8.9, p = 0.006)\), but no main effect of time \((F_{1,28} = 0.2, p = 0.7)\) or significant time \times food restriction interaction \((F_{1,28} = 1.7, p = 0.2)\). We performed Sidak post hoc tests on the main effect of food restriction and found...
Figure 6. Averaged velocity is greater in FR mice. A, Daily velocity on the running wheel in Ad lib (open circles) or FR (filled circles) mice. B, Averaged velocity over 24 h measured on day 7 or day 11 from Ad lib (open bars) or FR (filled bars) mice. C, Daily velocity measured within the 3 h period before food delivery in Ad lib or FR mice. D, Averaged 3 h anticipatory velocity measured on day 7 or day 11 from Ad lib (open bars) or FR (filled bars) mice. E, Daily velocity measured during the 3 h food availability period from Ad lib or FR mice. F, Averaged velocity during the first 3 h of the food availability period on day 7 or day 11 from Ad lib or FR mice. G, Time course of daily postprandial velocity over 3 h after food access from Ad lib or FR mice. H, Averaged 3 h postprandial velocity on day 7 or day 11 from Ad lib or FR mice. Bars represent the mean ± SEM. Symbols represent individual mice.

Discussion

Our team has designed and built an open source running wheel system and validated the utility of our system through the ABA model. There are several advantages to our running wheels. First, they are highly economical compared with commercial products. The total cost to produce our wheels is approximately $65 CAD, and that production uses components that are readily available. This also means that replacement parts can be made should mice damage a part during the experiment. Second, the design of our running wheels makes them suitable for a variety of experiments and cage types. Like commercial and other open source products, these wheels have a low-profile design, making them ideal for mouse cages with low lids. Also, because of the open top design of the spinner these wheels are compatible with both optogenetics and fiber photometry. Third, with three sensors and three magnet locations, our running wheels can be used to reliably monitor the distance run by mice and monitor details such as wheel direction and speed. This increases their utility as a tool for data collection. Fourth, this system is adaptable for any laboratory without the need for an expensive computer and software for operation and data acquisition. These wheels only need power and an Internet connection. Even a good Internet connection has moments of instability, therefore these running wheels are built with safeguards to test the Internet connection before downloading data. The activity of the mice can then be monitored using our Python and MATLAB programs on your personal computer so that the experimenter is not in the room influencing the activity of the mice. Fifth, these running wheels can be replicated even without advanced expertise in electronics and computer science. All wheel parts are print ready. However, we have included SLDPRT files, which can be modified to your specific application. Although soldering is required for connecting the electrical components, the use of the PCB prototyping board makes this process straightforward. In addition, all the code to run this system, from the Raspberry Pi to your personal computer, is written in either Python or...
MATLAB, making this system adaptable to fit your specific needs. Furthermore, versions of this code are provided for both the macOS and Windows operating system.

Our running wheels have three limitations that are the result of design choices made to improve utility. First, our running wheels are not battery powered like some commercial products, but instead require connection to a USB power cable. This choice was made to avoid the need for battery changes throughout a long-term experiment, which we felt would become more disruptive. Our ABA protocol lasts for ~3 weeks. Avoiding the problem of dead batteries also avoids the potential of lost data. In addition, using an external power supply also allowed us to design a system that favors data collection speed and precision over energy efficiency. The USB power cable allows for utility despite the battery limitation.

Second, an added feature of our running wheels is their ability to autonomously collect and transfer data via e-mail in addition to saving the data to an SD card. This design removes the need for a nearby computer to act as a hub for data collection. However, according to our tests, this design requires a reliable Internet connection and Internet speeds of ≥1 mb/s for transmitting data via e-mail. However, if the Internet connection drops to <1 mb/s, we have programmed a fail-safe, such that our running wheels will determine whether the Internet speed is >1 mb/s before attempting to transmit an e-mail. If the Internet speed is too slow, the data will be saved only to the SD card and no e-mail will be sent. This is essential since attempting to transmit an e-mail when the Internet speed is too slow can result in the system freezing and data collection being interrupted. In most circumstances, this safeguard will prevent the Raspberry Pi from freezing. For the rare occurrence that the Raspberry Pi either freezes or becomes disconnected from the power source, our data download code also checks to see whether an e-mail has been sent from each running wheel in the past 3 h, sending an alert e-mail to your personal e-mail if this has not occurred. However, even with these safeguards in place, if the Internet connection is unreliable or <1 mb/s, we recommend using the no-Wi-Fi version of the spinner code that we have provided.

Third, our running wheels do not transfer data in real time. This means that, without modifications, they are not designed to be used if your need is to visualize mouse activity each second as it happens. In our design, the running wheel instead sends data either after 1 h or when the stored file reaches a specified capacity of 3000 entries. We found that this approach was more reliable for long-term multiday experiments. Together, our design is intended for long-term data collection of mouse activity with hourly data visualization.

Similar to other low-cost open source running wheel options (Mayr et al., 2020; Bivona and Poynter, 2021; Edwards et al., 2021), although some designs have used a magnet detected by a Reed switch (Zhu et al., 2021), with three hall effect sensors, we and others (Mayr et al., 2020; Bivona and Poynter, 2021) are able to record wheel directionality. Other wheels have features that we did not require for our experiments, such as wheel locking to limit running activity (Mayr et al., 2020; Edwards et al., 2021) and an RFID (radio-frequency identification) reader (Mayr et al., 2020), as our experiments required unlimited running and individual housing with food restriction. Further, we used a wired power setup as we were concerned about battery failure over the long duration of our experiments. Although other systems claimed that the lithium batteries could last up to a month, this was not tested directly (Zhu et al., 2021). One critical difference with our system compared with others is that we used a low-cost Raspberry Pi single-board computer instead of microcontroller feathers (main-boards) such as those made by Adafruit (Zhu et al., 2021) or Arduinos (Mayr et al., 2020; Bivona and Poynter, 2021; Edwards et al., 2021). While those systems have reduced power draw, the Raspberry Pi offers increased flexibility in customizability and programmability using common programming languages such as Python. Together, there is a variety of open-source running wheels available that have their advantages and disadvantages. Our system offers another option with increased flexibility in programming.

To verify that this application works using the ABA model, we have demonstrated that, consistent with other studies, FR mice have increased distance traveled, anticipatory, and postprandial locomotor activity compared with the nonrestricted control mice (Klenotich and Dulawa, 2012; Chowdhury et al., 2015; Beeler and Burghardt, 2021). While this did not alter self-induced food restriction, it did lead to a greater weight loss that restricted controls, suggesting that they could no longer match their food intake with their energy needs. Our model used a progressive food restriction of food availability. This method allowed for prolonging the activity of mice in our experiment and to delay the severe reductions in activity because of a loss of energy requirements. Given the reductions in quality of life, high mortality rate, and lack of effective treatments for AN, new models, such as ours are needed to explore the neurobiological underpinnings of this disease and to identify novel therapeutic targets.

In conclusion, we have developed a running wheel and running wheel system that is open source, economical, and highly versatile. Access to these running wheels will increase the ability of other laboratories to do research on AN, leading to improved treatments and outcomes. In addition, there are numerous additional experiments where these wheels can be implemented. Running wheel activity can be used to assess stress, hyperactivity, exercise-induced plasticity, and disruptions in circadian cycles. All of these are important aspects of numerous mental illnesses, increasing the translatability of experimental results.

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