TECHNICAL PAPER
Constructing an Inexpensive Elevated Plus Maze

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Introducing students to the challenges and rewards of legitimate experimentation has become an essential part of many undergraduate lab courses. However, this objective can be difficult to achieve if the students find the topic uninteresting and therefore do not take ownership of the project. Additionally, the budgets of most undergraduate courses do not allow for the purchase of new equipment for student-generated projects. Here we describe a lab project where students engaged in the process of designing and building their own inexpensive apparatus. Driven by their interest in anxiety research, students in a Neuroscience Methods course developed the following protocol to build an elevated plus maze (EPM) and optional data acquisition module, for less than $100 each. The project engaged students in work that required applied critical thinking and real-world problem solving, and produced a functional EPM that was used in multiple projects beyond this course.

Key words: anxiety; elevated plus maze (EPM); behavioral testing; Arduino, critical thinking

INTRODUCTION

Anxiety is a topic of interest among many undergraduates for both personal and academic reasons. Animal models of anxiety have been developed for a variety of species (Rodgers and Cole, 1993; Anderson et al., 2000), but rodents are commonly the model of choice in the undergraduate lab setting. Behavioral tests of anxiety include various maze configurations (Montgomery, 1955; Shepherd et al., 1994; Graeff et al., 1998) including the elevated plus maze (EPM) first introduced by Handley and Mithani (1984), then later modified by (Pellow et al.,1985), (Lister, 1987) and others. The EPM consists of a plus (or "X") shaped maze, elevated 50-70cm from the floor of the testing chamber. Typically, two arms of the maze are open and two are enclosed with walls and an open roof to permit observation or video recording. A trial is conducted by placing the animal in the center of the maze and allowing it to explore freely. Entry into each arm, time in the arm, and various other measures are then used as indirect measures of anxiety. Avoidance of the open arms is related to fear of open space (Pellow et al., 1985) and thereby a proxy for anxiety. Changes in the various behavioral measures that occur following administration of pharmacological or environmental agents can thereby indicate anxiolytic and anxiogenic effects (File, 1992; Leo et al., 2014).

While relatively simple in its design, commercial EPMs and the accompanying video equipment and software can easily exceed course (or departmental) budgets at small schools. Construction of the maze from wood or cardboard is an inexpensive option, but as these materials are porous, the data may be confounded by absorbed odors or urine. Coating the base material with paint or other sealants can also pose olfactory confounds or health concerns if consumed by the test animals. To address these challenges, students in an intermediate-level *Neuroscience Research Methods* course at Thiel College developed the following construction plans for an EPM. The original goals for this project were the development of an inexpensive (<$100) yet functional EPM that could be assembled, used for data collection, disassembled, and cleaned without specialized tools. After the construction of the maze, two of us (G. Fox and E. Torigoe) further modified it to include an optional data acquisition module using infrared (IR) break beam sensor connected to an Arduino microcontroller. This addition allowed for some automation of the data collection and brought the final cost of the EPM to approximately $140. Here we describe construction plans for the maze and detection system and discuss considerations for use in an undergraduate lab setting.

MATERIALS

The frame for the EPM was constructed from 2"x4" wood studs. As the animals (and their waste) would have no direct contact the frame, this material was selected for cost and ease of use. The boards were connected into an X shape using a half-lap joint located at the mid-point. In our case, the frame was then set on the tank of a Morris Water Maze. This elevated the final maze to the desired height of 50cm. If a base is not available, the boards can be cut to the desired length and connected with a basic butt joint to construct an elevated frame. Metal L-brackets were attached to the wooden platform to secure the maze and prevent lateral movement. The brackets were not physically attached to the maze for ease of disassembly. Rather, the arms of the maze fit between the brackets. The maze itself was constructed from non-porous, 4" PVC pipe (see Figure 1). Two, lengths of pipe (45.3cm each) were cut for the closed arms, which terminated with 4" PVC caps. A third piece of equal length was cut horizontally to produce the two open arms. This generated a "half-pipe" shape with a maximum depth of 4cm. The four arms were joined with a 4-way cross fitting, in which a fifth entrance hole was cut on the top using a 4" hole saw. The final arm length was 50cm when assembled. We chose not to cement the pieces together to facilitate storage and cleaning. See Table 1 for parts list and pricing.

The inferred (IR) break-beam sensors and accompanying wiring were attached to the outside of the

Part name	Part Number	Supplier	Quantity	Price each (USD)
4" PVC pipe	294919	www.lowes.com	4	$7.98
4" PVC caps	447-040	www.pvcpipesupplies.com	2	$3.99
4-way PVC cross	420-040	www.pvcpipesupplies.com	1	11.03
10' 2"x4" stud	7026	www.lowes.com	2	$6.36
2.5" flat-head screws (4ct)	333061	www.lowes.com	2	$0.70
L-brackets (2ct)	19165	www.lowes.com	4	$2.87
		Total		**$76.53**
Optional parts				
Arduino	50	www.adafruit.com	1	$24.99
Break beam sensors	2167	www.adafruit.com	6	$1.95
6-pin connectors	1665	www.adafruit.com	4	$1.50
LED lights	COM-09590	www.sparkfun.com	6	$0.35
Resistors	COM-1107	www.sparkfun.com	12	$0.95
Hook up wire 25 feet	PRT-o8022	www.sparkfun.com	3	$2.50
		Total with optional addition		**$140.22**

Table 1. A list of the electronics and materials needed to construct the elevated plus maze. Prices were obtained in the spring of 2017 and do not include shipping or tax.

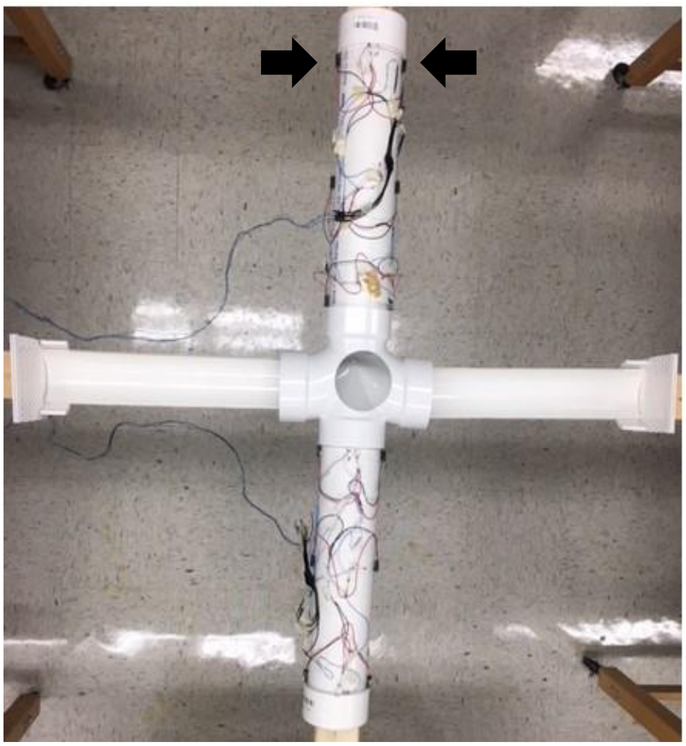

Figure 1. An overhead view of the EPM, mounted on tables for clarity. During data acquisition the maze was mounted above the tank of a Morris Water Maze. Three pairs of IR break beam sensors can be seen (black boxes on closed arms, one pair is denoted by arrows).

closed arms. Small holes (1/8mm in diameter) were drilled through the plastic with an inter-beam interval of 20.9cm (Figure 2). We chose to use an Arduino Uno microcontroller and wrote our control program using the open source Arduino integrated development environment available at https://www.arduino.cc/en/Main/Software. The Arduino code used to coordinate sensor signals, time stamps, and data logging can be obtained by contacting the corresponding primary author.

During data acquisition trials, the program continuously logged the times when the IR beams were broken and/or reconnected, denoting the location of the rat (see Figure 3

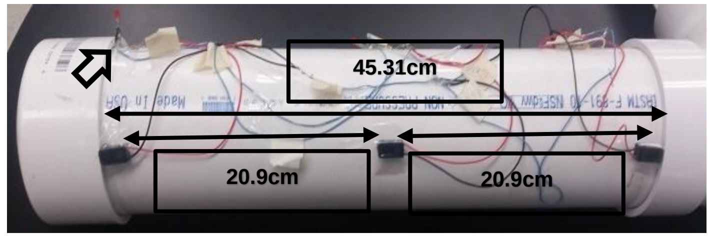

Figure 2. A closed arm of the EPM including electrical circuitry. Each closed arm had three IR emitters (black rectangles), break beam sensors (back side, not visible) and red LED lights (on top of the arm). The latter was used by observers to determine the location of the rat while in the closed arm. The terminal LED light on this arm, is indicated by the open arrow.

for a schematic representation of the Arduino circuitry). The Arduino board was programmed to send temporal data to a laptop computer. These data were then used to calculate total time in each arm and relative position. If desired, one could also use the data to generate a map the overall pattern of movement through the closed arms. Additionally, each beam was connected to a red LED light located on the top of the arm. This light was primarily used by observers to track the location of the rat. We chose not to include similar sensors on the open arms out of concern that they might distract the animals or incur damage.

It should be noted, the IR break-beam sensors used in this project emit light at a wavelength of 850 to 950 nm. Rats normally possess two types of photoreceptors; short wavelength "blue-UV" and mid-wavelength "green" cones, with a peak sensitivity around 510nm (Szél and Röhlich, 1992). Both of these photoreceptors respond minimally to infrared spectra emitted by the LED beams (Radlwimmer and Yokoyama, 1998). To adapt the maze for use in mice, we recommend placing the LED beams lower on the maze arm, and possibly reducing the diameter of the PCV pipe.

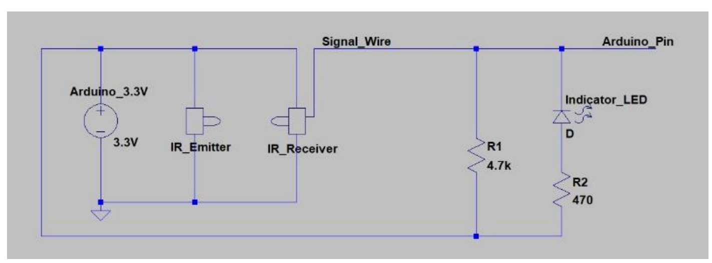

Figure 3. Schematic representation of the Arduino circuitry.

DATA COLLECTION

Following approval by the Thiel College IACUC, a cohort of eight adult male Long Evans rats were used for preliminary testing using the maze. This version did not have the Arduino circuitry or LED sensors. For these tests, animals were transferred into the center of the EPM and allowed to freely roam for a trial period of five minutes. Students manually recorded time using stop-watches and then calculated total time in each arm and in the center (Figure 4). For the manual test, an entrance into an arm was defined as the rats' front shoulders passing through the edge of the center cross. For the open arms, this standard was easily discernable. However, the preliminary tests quickly

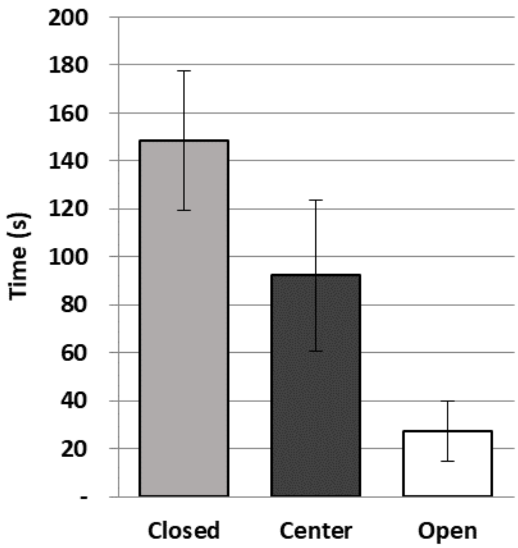

Figure 4. Preliminary testing of student-constructed EPM. Time spent in each of the three maze locations during a 5-min testing period for male adolescent Long Evan rats.

presented the students with a problem of tracking the animals' movement into and through the closed arms. Their stopgap solution was to observe the animal at an angle through the entrance hole in the center cross. However, the students correctly questioned the accuracy of the resulting data when they noted that the total recorded time for the three segments of the maze exceeded the five-minute trial time in some animals. This prompted an extended in-class discussion about the validity of their apparatus and method and led to a more general conversation about sources of and types of error.

The class was then asked to propose modifications to the device that would address these concerns. As most of the students in the class had little or no experience troubleshooting a device in this manner, the exercise provided a good opportunity for critical thinking and problem-solving in a real-world application. Their final solution was the addition of the IR break-beam sensors to the closed arms of the maze. Over the following semester, two students (G. Fox and M. Long) worked with a physics professor (E. Torigoe) to develop and construct the Arduino-based system. Although this work occurred as an independent project, one could easily incorporate it into a class setting. Soldering and assembling the detector array and programming the Arduino board would likely take two to three lab periods, depending on how much independence the students are given and their familiarity with the techniques.

Following completion and beta testing of the new detection array, a second trial of data collection was conducted using a cohort of five naive animals. As these animals were scrubs for another study, they were not age-matched to those used in our preliminary test. In this trial, the open arms and center were again monitored manually, while the Arduino software collected data for the closed arms (Figure 5, see Supplemental Material 1 for an example of raw output from the Arduino software). We used the output data to determine total time in each of the closed arms, however, the data could be analyzed to determine total path of the rat if desired.

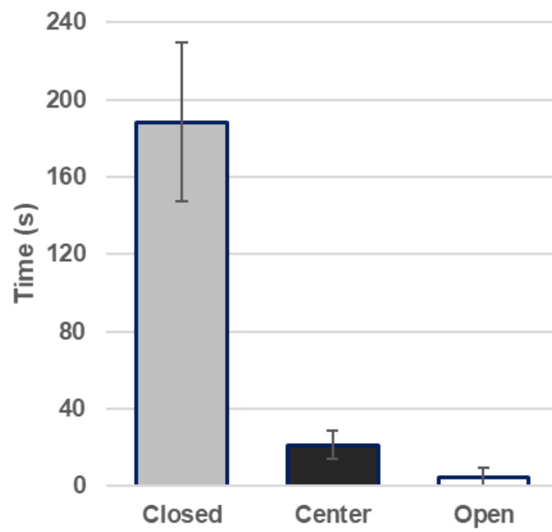

Figure 5. Data collected using the LED bem detectors. Time spent in each of the three maze locations during a 5-min testing period for male adult Long Evan rats.

DISCUSSION

From a design standpoint, the project met its original objectives. The students were able to produce a functional EPM for under $100, and only modestly exceeded that budget with the addition of the electronic detectors. While the final product may not be adequate for research-grade purposes, it would work well in a classroom setting and could provide legitimate data for subsequent statistical analysis. Additionally, construction of the maze required little technical experience beyond use of a drill and saw. An undergraduate lab group could assemble the maze within a single lab period as described above. Inclusion of electronic monitoring is slightly more challenging and would require an additional two lab periods and soldering equipment but is still feasible for students.

From a pedagogical perspective, the project accomplished the objective of engaging students with an activity that involved extensive critical thinking. Rather than simply working through a set of instructions and assembling pre-cut parts, this project required legitimate planning, design, and construction of the device. Additionally, the project provided opportunities to learn from failures and improve in an iterative fashion. For example, the class initially produced plans for three different versions of the maze. One built from rain-gutters was creative, but impractical and failed preliminary testing prior to use with animals. Another constructed from Plexiglass was similar to commercial devices but exceeded the $100 limit and was difficult to assemble/dissemble for cleaning. Yet each of these failed attempts contributed to improvements in the final maze. For some of the participating students, this was the first time they had actually built anything substantial. For others it provided the opportunity to apply knowledge of construction and serve as team leaders.

This method of instruction did force the instructor (G Butcher) to give up significant degree of control in the class. Apart from a few small safety and budgetary issues, essentially the students were free to try any design they could imagine. While some students were uncomfortable with this format, most enjoyed it as these selected quotes from their end-of-semester evaluations attest.

> *"…everything needs (to be) planned out more… we needed more time to [complete the maze] …"*

> *"I liked the behavioral studies…Keep the interactive parts!"*

> *"Being able to help with an ongoing research project was helpful."*

> *"Very interesting everyday"*

A growing body of evidence supports the need to engage students with the process of science rather than simple memorization of facts. Design and construction projects can provide students with such opportunities and have the added benefit of producing new equipment. In our case, the EPM since has been used in two additional classes to date and will be used in two more in the 2018-19 academic year. Additionally, this summer students not involved in the original design course will compare our EPM with a commercial version generously provided by San Diego Instruments through the Faculty for Undergraduate Neuroscience equipment loan program.

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