TECHNICAL NOTE
Design Plans for an Inexpensive Tail Flick Analgesia Meter

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While the pedagogical benefits of incorporating inquiry driven labs into an undergraduate curriculum are well established, often the prohibitive costs of providing equipment for such labs limits the types of experiences that can be offered. For example, the lab portion of *Advanced Neuroscience* at Centenary College of Louisiana consists of a semester-long research project developed by the students. Frequently, these junior- and senior-level students generate interesting research questions that must be culled or scaled back simply due to a lack of appropriate equipment. In the most recent iteration of the class, the students wanted to examine analgesia using the tail flick test, a measure of spinal nociception. In this test a rodent subject is restrained; its tail is exposed to a heat source; and the latency to flick its tail away from the noxious stimuli is recorded. As commercial devices were far beyond the lab budget, we sought to develop an inexpensive tail flick analgesia meter that was easy to use and generated reliable data. The prototype device was tested by students in the above-mentioned class and was found to consistently produce reliable data in agreement with the literature. Here we present plans for a tail flick analgesia meter that can be constructed for $50-75, roughly 100 times cheaper than commercial devices.

Key words: analgesia; nociception; tail flick test; behavioral testing; supraspinal pain; spinal reflex; arduino

Pain, the sensation of real or potential tissue damage and the subjective perception and interpretation of that sensation, is an inherently interesting and problematic area for inclusion in an undergraduate lab. Since at least the 1920s various objective measures have been developed to quantify nociception and analgesic responses using both human (Kiesow, 1928; Schumacher et al., 1940; Macht and Macht, 1940) and non-human models (D'Amour and Smith, 1941; Miller, 1948). While each of these tests has strengths and weaknesses from a research perspective, several have either become outdated due to modern animal welfare concerns or are simply impractical for use by undergraduates. In contrast, the tail flick test developed by D'Amour and Smith (1941) remains a commonly used test for the assessment of analgesia. In this measure, a rodent is loosely restrained while radiant heat is focused on the tail. The latency to flick the tail away from the noxious thermal stimuli is then recorded. This test is easy to learn, simple to conduct, produces only transient thermal pain and can be repeatedly applied to individual animals (however, see King et al., 1997). In the decades following its development, several groups reported that the response was primarily a spinal reflex (Irwin et al., 1951; Grossman et al., 1982). More recent reports have noted that supraspinal systems (Jensen and Yaksh, 1986; Blass et al., 1993) and learning (King et al., 1997) may modulate the response depending on the strength of the thermal stimulus.

The tail flick test can be conducted with minimal equipment (essentially a heat source and stop watch); although, the need for consistency has often necessitated the use of more elaborate devices that incorporate light sensors, internal and external thermometers, and various other features. While, commercial versions of such devices have been reliably used for decades, their costs are typically prohibitive for use in an undergraduate setting. For example, a recent survey of various commercial vendors found new devices averaged more than $5,000. Although, various groups have developed and published plans for self-built devices (Hillman, 1975; Owen et al., 1981), we found these devices to be more expensive than necessary and require a level of technical knowledge beyond the typical end user. To solve this problem, a Centenary physics major (A. Otto) was recruited to develop and construct a prototype device that 1) was inexpensive (<$100), 2) easy to assemble and operate, 3) utilized an open-source software platform and 4) produced consistent, reliable data when used by undergraduates. Here we present plans for construction of such a device and provide links to the operating software which are freely available for download.

MATERIALS AND CONSTRUCTION

We constructed the testing platform from 15/32" thick 2' x 4' sheet of plywood (Lowes Item #: 35663), Acrylic sheet ("plexiglass", Lowes Item #: 345710), and 2" x 2" x 8' wood (Lowes Item #: 204231). A photo of the platform is shown in Fig. 1. Details of the platform construction can be found in the supplemental materials and here www.centenary.edu/physics/tmessina/tailflick. Wood and acrylic were chosen because of their low thermal conductivity, providing a surface for the rodents that would not significantly increase in temperature during experiments. The platform was painted with Rust-Oleum spray paint to add to the device's longevity, seal the material to prevent absorption of body fluids and to make cleaning of the apparatus simple. The electronic circuitry was mounted under the wooden platform. Table 1 shows all of the items, their part numbers, vendor source, and cost (as of July 2011) used in this apparatus. The total cost shown is around $80; however, one may use "scrap" materials for the platform, and physics departments often

Figure 1. Photograph of the tail flick apparatus and controlling computer. The circuitry is attached under the mouse platform.

keep most of the electronic components on hand. In our case, we were able to construct the entire device for approximately $50 using recycled supplies. All of the materials were chosen because they are generic and comparable components may be found at many places, (e.g., any hardware store and Radio Shack). Two programs were written to control the apparatus. The first program was written in the Arduino language and loaded to the Arduino board through the free Arduino IDE.

During operation, this program runs as a continuous loop that monitors the status of a photo-sensing circuit similar to that shown in the top schematic of Fig. 2. The photo-sensing circuit incorporates an npn phototransistor which can be occluded by correctly positioning the rodent's tail over a small hole on the surface of the platform. The phototransistor acts as a switch that turns on when light is detected, i.e., the rodent's tail flicks. The looping Arduino program reads the state of this switch on digital pin 2. The Arduino then actuates the relay (on pin 12), disconnecting the 110 volt power from the heat lamp. The relay circuit is shown in the bottom schematic of Fig. 2.

The second program was written in Python 2.7, which is another freely available language for virtually all operating systems (http://www.python.org). PySerial is a separately downloadable module that is required to run the source code (http://pyserial.sourceforge.net). PySerial allows Python programs to interact with the Arduino through a USB port. This second program creates the graphical user interface (GUI). A screenshot of the interface is shown in Fig. 3. The user inputs a file name and sample name. Clicking "OK" starts the experiment, turning on the heat lamp and indicating the number of seconds that have elapsed until the rodent flicks its tail. After a tail flick is sensed, the heat lamp turns off and the sample name and total elapsed time are saved to the file. A single file may be used to save multiple samples by simply leaving the file name the same and changing (or not) the sample name. Every experimental run will append the sample name and elapsed time to the file.

USE AND FUNCTION

Prior to animal testing, we manually recorded the temperature of the platform to ensure that our heat source would not induce tissue damage within a 60 second testing window. As our manual testing wasn't able to produce the level of precision that may be required by certain experimental designs, we then tested the feasibility of incorporating temperature sensors directly into the Arduino

Part Name	**Part Number**	**Supplier**	**Qty**	**Price Each (USD)**
NPN Phototransistor	T-1 3/4 , CE30.0uA, EC5.0uA	www.jameco.com	1	0.35
Diode	1N4004	www.jameco.com	1	0.05
1000 and 10,000 Ω resistors		www.jameco.com	1, 2	0.09
SPDT 5V Relay	G5V-1-DC5	www.jameco.com	1	2.95
Jumper Wire Kit	2127718	www.jameco.com	1	7.95
Mini Self-Adhesive Breadboard	20601	www.jameco.com	1	5.95
Arduino (Uno)	2121105	www.jameco.com	1	29.95
Lumber	See supplement	Lowes or Home Depot		6.50
Acrylic Sheet (PLASKOLITE, 3' x 2'6")	345710	Lowes or Home Depot	1	7.98
250W Heat Lamp Bulb	76573	Lowes or Home Depot	1	5.98
110V Lamp Wire and Plug	40273	Lowes or Home Depot	1	5.98
660W Ceiling Socket	71140	Lowes or Home Depot	1	1.63
Rust-Oleum Spray Paint		Lowes or Home Depot	1	4.24
TOTAL (minus tax and shipping)				**79.60**

Table 1. Electronics and construction materials for the tail flick apparatus. The list includes items, part numbers, vendor information, and 2011 pricing.

circuitry. We added two independent thermistors (temperature-dependent resistors) for sensing the temperature of the environment around the mouse's body and at the surface of the platform adjacent to where the tail would be positioned during testing. The circuit used for a single temperature measurement is shown in Fig. 4. As noted in the figure, a second thermometer would use analog input A2. A third thermistor could easily be added for monitoring body temperature (via a rectal probe) using A3.

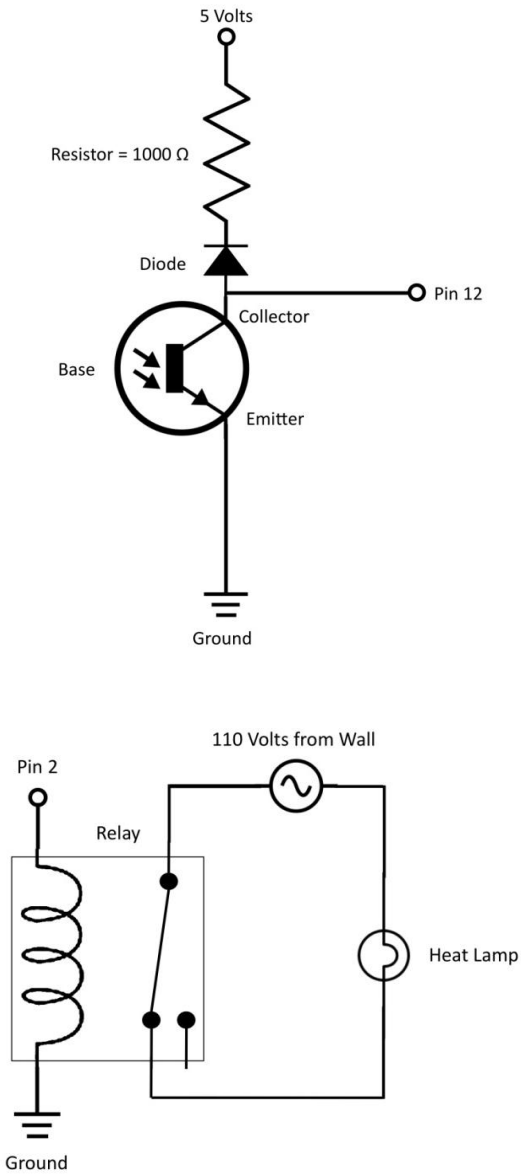

Figure 2. A schematic of the photo-sensing (top) and relay control (bottom) circuitry. The photo-sensing circuit utilizes a (photo)transistor as a binary switch to indicate to the Arduino pin 12 whether the transistor sees (1) or does not see (0) light from the heat lamp. The heat lamp powering circuit uses the Arduino pin 2 to supply 0 or 5 volts to a relay, turning the heat lamp on or off, respectively. Thus, the relay is a normally closed switch to a lamp cord plugged into a wall outlet. Photographs of the circuits may be found in the online materials.

The computer software and circuit diagrams for adding temperature monitoring are available with the supplemental information. These additions were made at no cost because the Arduino has 12 digital and six analog I/O ports that were not in use for the apparatus described above. The thermistors were obtained as free samples from Vishay Electronics (www.vishay.com). Alternatively, they may be purchased for less than $1 each.

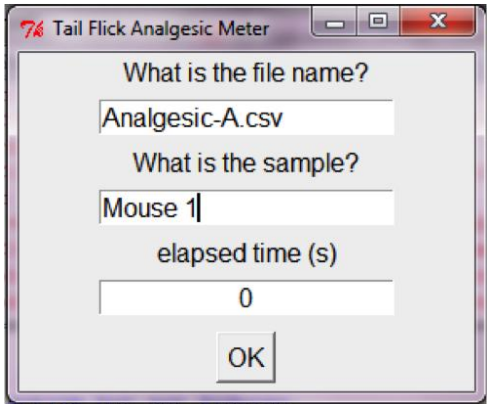

Figure 3. A screenshot of the user interface created in Python. The user inputs a file name to which sample name and elapsed time data will be saved. Multiple samples can be saved to a single file. The elapsed time displays the seconds as they pass while an experiment is in progress.

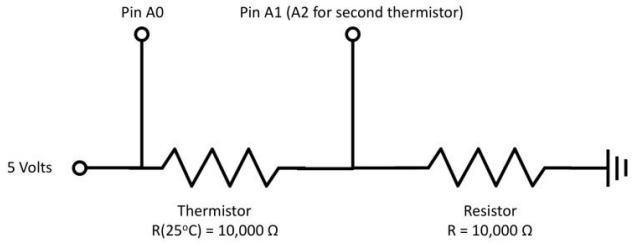

Figure 4. A schematic of the temperature-sensing circuit. The circuit is a simple voltage divider. Connection to analog pin A0 ensures that the measurement remains accurate when there are fluctuations in the 5 Volt Arduino output. More thermistors can be added using the other analog inputs. Only one connection from 5 Volts to A0 is necessary.

In Fig. 5, we show temperature data for the platform at the location of the tail and inside an insulated box where the rodent is placed during the experiment. The temperature of the rodent (ambient) stays nearly constant at just above room temperature, while the tail exposed to the heat lamp increases from 37°C to 60°C in just over one minute. Multiple trials showed the same characteristic rate of temperature increase at the tail. These tests indicate that the device produces a reliable heating curve appropriate for data collection by students in the context of a lab setting and within a timeframe applicable to most established research protocols. We envision that future users of the device may want to include add-ons (such as a tail-cuff for pulse rate and/or blood pressure monitoring). The flexibility of the Arduino platform makes this a relatively straightforward process.

Finally, we determined whether our device was capable of detecting changes in response latencies between controls and animals treated with ketamine, a commonly used rodent analgesic that has been noted to produce changes in tail flick latency (Banks et al., 1988). Intraperitoneal injections of saline or ketamine (200 mg/kg) were administered 10 minutes prior to tail flick testing (Fig. 6). The platform was pre-warmed to physiological temperatures to minimize loss of body temperature during testing, although as the platform is not metallic this is probably unnecessary in most cases. Relative to saline controls, animals injected with ketamine had significant longer latencies at the first two intervals tested (t=0 & 10, $P<0.05$). Additionally, analysis of variance indicated that animals injected with saline produced consistently stable responses that did not significantly vary across the hour long test ($F(4, 20) = 1.18$, ns).

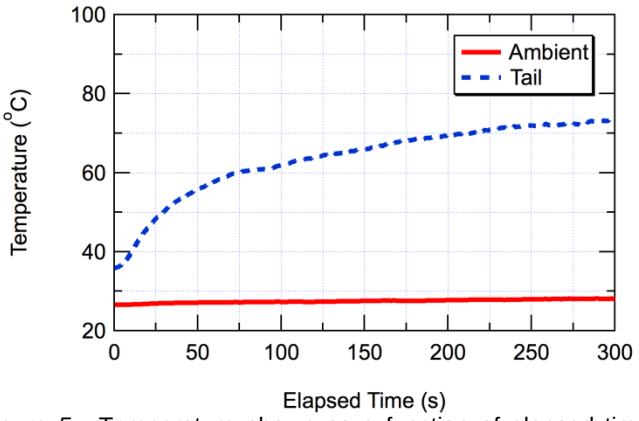

Figure 5. Temperature shown as a function of elapsed time in seconds. The ambient temperature inside the insulated box stays near room temperature over long periods (>5 minutes), while the temperature at the position of the tail shows a characteristic increase that plateaus near 70-75°C.

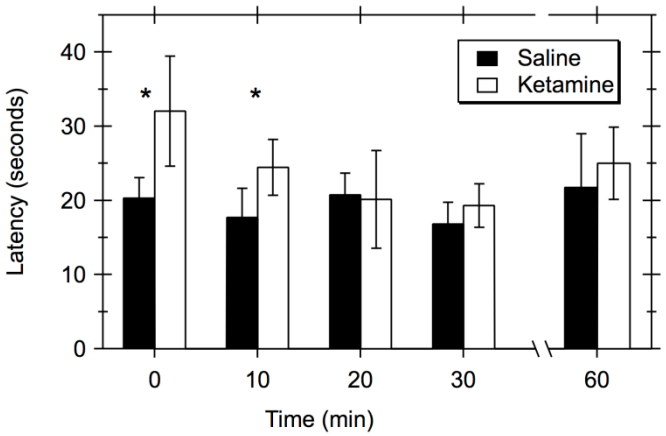

Figure 6. Latency data (in seconds) for adult male C57BL6 mice treated with saline or ketamine (as noted in the text). Tail flick latency was tested on five mice per group at the noted intervals. Time zero denotes the onset of general anesthesia in ketamine injected mice (10 minutes post treatment). * denotes a $P<0.05$.

DISCUSSION

From a technical standpoint, the prototype version of the described device accomplished all four of our original goals. From a pedagogical standpoint, we also feel that this project has been a striking success. The physics student (A. Otto) recruited to design the device gained valuable (and marketable) skills in developing and constructing scientific equipment and software. The seven neuroscience students who participated in beta testing the prototype were also able to contribute to its development as they provided direct feedback on pragmatic issues (notably platform size, intensity of lighting source, user interface and file format preferences). Furthermore the level of student engagement was markedly improved from previous years. Over half the students had strong favorable comments related to the development of the device in their end-of-semester evaluations. From those comments, it was apparent that students enjoyed working with equipment designed by a peer, and their excitement carried over to other students not involved in the project. Both projects (the design of the device and the data generated from the neuroscience class) were presented during an annual research forum where the student authors could each cite the others' work. This gave an additional sense of legitimacy to the project and provided all the participating students with direct experience in scientific collaboration.

The final device provides an inexpensive tool for use in either research or instructional settings. Construction of the device requires little technical experience and could easily be incorporated into an undergraduate lab or assembled by an individual in approximately one day. While originally designed for use in an undergraduate lab setting, the reliability of the data indicates that the device is also appropriate for use in a research setting.

In summary, we developed an inexpensive, computer-controlled tail flick analgesia meter. The apparatus functions comparably to commercial units that cost up to 100 times more. This apparatus has the same features as its commercial counterparts and may be easily extended with modules to be even more feature-rich. This apparatus is useful in undergraduate classrooms and laboratory settings for studying nociception, analgesia and interactions with other bodily properties such as temperature, pulse rate, and blood pressure. Our design greatly simplifies those found in the literature and offers a much greater capability for expansion.

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