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TECHNICAL NOTE
Design and Construction of a Two-Temperature Preference Behavioral Assay for Undergraduate Neuroscience Laboratories

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Behavioral assays in the undergraduate neuroscience laboratory are useful for illustrating a variety of physiological concepts. An example is homeostatic temperature regulation (thermoregulation). Many model organisms, from flies to mice, regulate internal temperatures in part by moving to suitable climates (thermotaxis). A particularly reliable method of quantifying temperature-dependent thermotactic behaviors is the two-temperature preference behavioral assay. In this preparation, an organism is free to move between two temperature-controlled surfaces, thus revealing its preferred thermal environment. Here we present the design and construction of a two-temperature preference assay chamber. The device uses Peltier-based thermoelectric modules (TECs) for heating and cooling, and is capable of precision control of temperatures from −5ºC to 60ºC. Our approach can be easily adapted for use in a variety of physiological and behavioral assays that require precise temperature control over a wide range of temperatures.

Key words: behavior, thermal preference, temperature control, cooling, heating, peltier, thermoelectric, physiology, thermoregulation, thermotaxis

Animals use a variety of homeostatic mechanisms to maintain an appropriate body temperature (thermoregulation). Physiological thermoregulatory mechanisms include metabolic, endocrine, vascular, and musculoskeletal changes**.** Thermoregulatory behavioral strategies include shelter-seeking (e.g., “burrowing”), re-positioning the body and limbs (e.g., “basking” and “sunning”), or simply moving to a more appropriate environmental climate (thermotaxis) (Montell and Caterina, 2007). Among these thermoregulatory behaviors, thermotaxis is ubiquitous among animal species (Thomas, 1995). Thermotaxis is also readily quantifiable using a number of simple assays (Daniels and McKemy, 2007). Together, these features make thermotaxis a useful behavior for experimentation in undergraduate laboratory settings.

A robust means of characterizing thermotactic behaviors is the two-temperature preference behavioral assay (Moqrich et al., 2005; Bautista et al., 2007). In this preparation, an organism is free to move between two temperature-controlled surfaces, thus revealing its preferred thermal environment. There are several methods of temperature control currently used in thermal preference behavioral assays, and they range widely in expense and complexity. The simplest and most inexpensive devices use radiant heat sources such as hot plates and incandescent light bulbs (Kingsley et al, 1976; Rose 1983). However, these systems do not permit the complete characterization of thermotactic behaviors in organisms that can discriminate temperatures below ambient.

Another option for temperature control in behavioral assays relies on perfusion of liquid through tubes or canals embedded within the floor of the assay chamber. Very simple systems, such as the excellent project described by Krans et al. are inexpensive and quite effective when used at moderate temperatures (Krans and Hoy, 2005). But simple perfusion-based systems are unable to sustain near-freezing temperatures, a necessity when examining some thermotactic and nocifensive behavioral responses. With commercially available perfusion equipment (such as low-temperature thermocirculators available from Harvard Apparatus) it is possible to maintain near-freezing temperatures, though at considerable expense; a single system’s cost is measured in the thousands of dollars, and two units are required for dual cold temperature comparisons.

A third option for heating and cooling in behavioral assays is to use Peltier thermoelectric modules (TECs). Peltier thermoelectrics act as heat pumps, operating on the principle that an electrical current flowing across a junction of dissimilar metals causes polarity dependent thermal absorption (or thermal discharge) (Riffat and Ma, 2003). Peltier TECs are inexpensive and available in a variety of sizes, and are used in many applications, including computer processor cooling, thermal cyclers, perfusion temperature control systems, refrigeration, and microelectromechanical systems (MEMS). Peltier TEC-based temperature control has two distinct advantages over the aforementioned methods for use in the two-temperature preference behavioral assay. First, Peltier TECs are capable of generating temperatures from -10°C to over 100°C, a range that exceeds the thermosensory capabilities of most animals. Second, temperatures can be precisely set and maintained using feedback control of the Peltier TEC elements, whereas perfusion-based systems rely on setting the temperature of the liquid perfusate. Thus, Peltier TEC-based temperature control is well-suited for thermal preference assays.

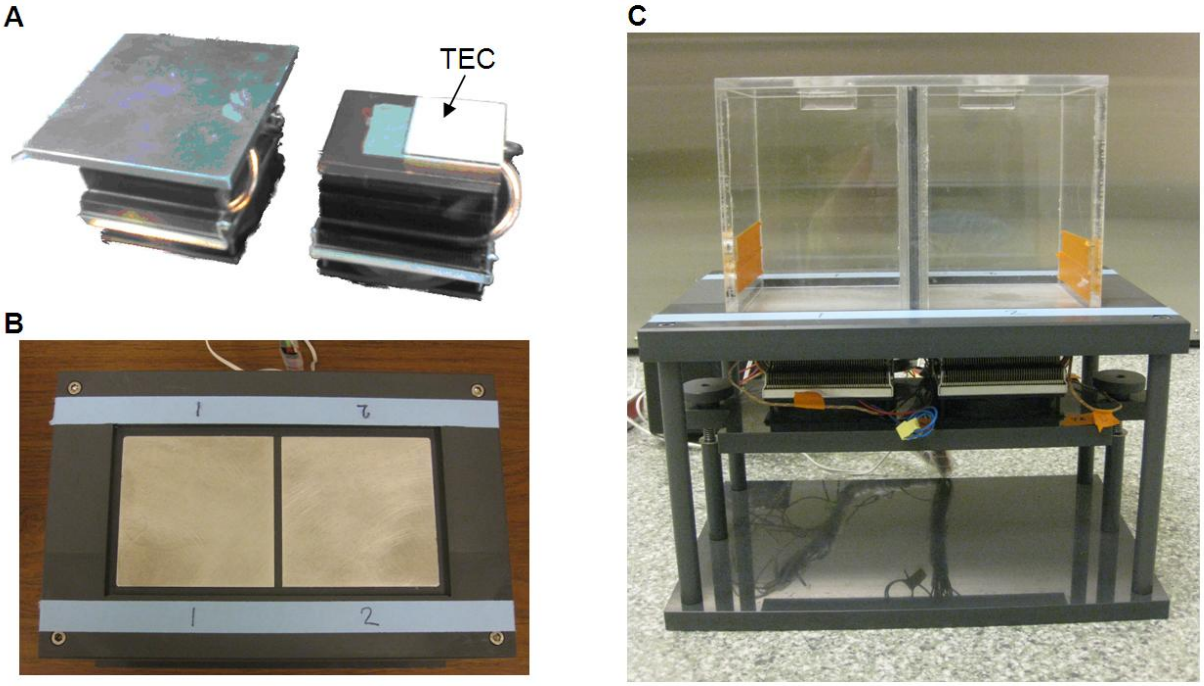

Figure 1. Enclosure and associated hardware. *A*. Thermoelectric Modules (TECs) are attached to heatsink/fan units using a thermal adhesive compound (blue). Aluminum plates are placed on top of the TEC, also attached with thermal adhesive. *B*. Top view showing two adjacent aluminum plates, surrounded by PVC enclosure. *C*. Device hardware, including plexiglass animal enclosure. The heatsink/fan units are supported by a platform so as to provide room for airflow beneath the fans. Thermocouples (marked with orange tape) measure the temperature of the aluminum floor plates. The free end of each thermocouple is inserted into a hole drilled into the underside of one of the aluminum plates.

There are limited options for obtaining thermal preference assay chambers and temperature control products. At the time of writing, one commercially produced product is available, item AHP-1200DCP, made by TECA Corp. (Chicago, IL). Aside from this ready-made solution it is possible, as some investigators have done, to use two, side-by-side hot/cold plates, such as those made by Columbus Instruments International (Columbus, OH) (Dkaka et al., 2007). However, the cost of these commercial devices is beyond what is available to most laboratory instructors, ranging from more than \$3000 for dual Hot/Cold plates to \$7500 for the TECA thermal preference assay. Because the essential features of these systems can be replicated using relatively inexpensive components, it may be more economical to build a custom device. The primary requirements for the assay are Peltier TECs, an appropriate power supply, and a means for feedback temperature control. Of these, feedback temperature control is the most complex aspect of such a project. Here again, a limited number of manufactured options are available, from such vendors as Accuthermo Technology Corp. (Fremont, CA), TECA Corp. (Chicago, IL), TE Technology, Inc. (Traverse, MI), Odgen Manufacturing Co. (Pittsburg, PA). These off-the-shelf controllers are quite suitable for thermal preference assays, but contain many extraneous features and add significant expense to a custom project (two units are required, at a cost of \$300-600/unit). Many controllers also rely on RS-232 (serial) computer ports, which are nearing obsolescence and are not generally found on current computers. Lastly, we find in the literature several informative reports of Peltier TEC-based temperature control devices for physiology and animal behavior experimentation (Forsythe and Coates, 1988; Sqalli-Houssaini et al., 1991; Corrèges et al., 1998). While very functional, these systems require the assembly of electronic circuits, adding cost and complexity. Therefore, Peltier TEC-based thermal preference assays remain inaccessible because of their high cost, incompatibility with current computer hardware, and/or difficult construction.

In the following technical note, we describe the design and construction of a Peltier TEC-based two-temperature preference assay chamber. Feedback temperature control is achieved using a laptop computer and appropriate computer interface devices from Phidgets, Inc. (Calgary, Alberta, Canada). Phidgets are low-cost control and sensing products that communicate via a standard Universal Serial Bus (USB) port, allowing any modern computer to serve as the user interface for the system. In brief, our system functions as follows. Phidget thermocouples register the temperature on each side of the assay chamber. Computer software allows the user to set, monitor, and record the temperature of each chamber surface. Finally, Phidget electrical relays control power to the Peltier TECs.

Testing of the unit has indicated an error of ±1.0ºC over the surface of the assay chamber when held at temperatures from −5ºC to 60ºC. The total cost of the

Part Name	Part Number	Supplier	Location	Qty	Price/ea. (USD)
Phidget Interface Kit 0/0/4	1014	Phidgets, Inc.	Alberta, Calgary (CAN)	1	60
Phidget Temperature Sensor	1051	Phidgets, Inc.	Alberta, Calgary (CAN)	2	60
K-type Thermocouple	3015	Phidgets, Inc.	Alberta, Calgary (CAN)	2	30
40MM X 44MM Thermoelectric module	CAT# PJT-7	All Electronics, Inc.	Van Nuys, CA (USA)	4	15
Thermaltake CL-P0075 80mm 2 Ball CPU Cooling Fan/Heatsink	N82E16835106055	Newegg.com	City of Industry, CA (USA)	2	25
12V DC Wall Adapter	273-028	RadioShack, Inc.	Fort Worth, TX (USA)	1	20
Arctic Silver CMQ-22G	N82E16835100012	Newegg.com	City of Industry, CA (USA)	1	10
Aluminum Plates	6061-T6	Onlinemetals.com	Seattle, WA (USA)	2	15
Mastech Power Supply 0-30V @ 0-5A	HY3005D	MultimeterWareHouse.com	Montclair, CA (USA)	2	90
Banana plugs for power supply	274-007	RadioShack, Inc.	Fort Worth, TX (USA)	1	5
Wire (20AWG)	278-1222	RadioShack, Inc.	Fort Worth, TX (USA)	1	5
Enclosure and Associated Hardware					50 (est)
TOTAL					**$650**

Table 1. Parts list for the two-temperature preference behavioral assay. Includes part numbers, vendor information, and current pricing information.

thermal preference assay, including the Peltier TECs, power supplies, materials for the assay chamber, and Phidgets devices (temperature sensors and electrical relays) is about $650, a figure that is especially low considering that we are controlling the temperature of two surfaces. We have found this design to be useful in controlling temperature for the two-temperature preference behavioral assay, and believe that the essential components may be useful in a variety of behavioral assays, physiological preparations, or other applications that require precise temperature control.

MATERIALS & CONSTRUCTION

Enclosure and associated hardware

We constructed a custom animal enclosure and supporting hardware such that the temperature of two adjacent 10cm x 10cm aluminum plates (the floor of the chamber) could be independently controlled (Figure 1). A complete listing of parts and vendors is given in Table 1. The supporting structure of the enclosure was built with polyvinyl chloride (PVC). Aluminum was chosen for the floor plates because of its low specific heat capacity (0.91 kJ/kg K), allowing for even and rapid temperature changes. The animal enclosure, made from clear ¼" plexiglass, was made to sit on top of the metal plates, held firmly in place by grooves in the PVC frame. The enclosure contains a hinged roof with holes to facilitate airflow. The supporting structure below the floor contains a platform for the heatsink/fan combination units. Peltier TEC modules are sandwiched between the heatsinks and the aluminum plates. Plexiglass and PVC cutting and assembly were performed at the University of Southern California Machine Shop (Los Angeles, CA).

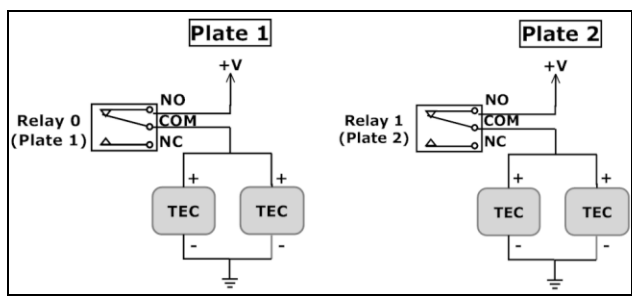

Figure 2. Schematic diagram of thermoelectric circuits. Under each plate, two thermoelectric modules (TECs), wired in parallel, are connected via the Normally Open (NO) contacts of a relay to a variable DC power source. When the computer closes the relay, current is supplied to the TECs and the device is cooled. Reversing the polarity at the power supply causes the TECs to warm upon activation rather than cool.

Temperature control of peltier thermoelectric modules

Heating and cooling was achieved using (2) 40x44mm thermoelectric modules (TECs, I_{max}=8.1 A, V_{max}=16.1 V (All Electronics, Inc., Van Nuys, CA) placed below each of the aluminum floor plates. Heat from the TECs was dissipated using a Thermaltake heatsink/fan combination that is commonly used in computer CPU cooling (Newegg.com, City of Industry, CA). The TECs were fixed to the aluminum floor plates and copper heatsinks using Arctic Silver 5, a thermally conductive adherent compound. The thermoelectric modules were driven by current from a benchtop power supply (Mastech, Inc., Montclair, CA). Power to the TECs is supplied as needed using computer controlled single pole double throw (SPDT) relays (Phidget Interface Kit 0/0/4, Inc., Alberta, Calgary, Canada). These electrical connections are summarized in Figure 2. Temperature readings were obtained with thermocouples. The exposed wire end of each thermocouple was inserted into a hole drilled into the underside of each plate 1 cm from the corner (Phidget Temperature Sensor and K-type Thermocouple, Phidgets, Inc., Alberta, Calgary, Canada).

Feedback temperature control of the device was carried out by a laptop computer running Microsoft Windows XP, connected to the Phidgets devices via USB ports. Phidgets, Inc. provides Application Program Interface (API) libraries and code examples for many commonly used computer programming languages, including MATLAB, C/C++, LabVIEW, Flash, Java, and Visual Basic. We have used both the LabVIEW 8.5 (National Instruments Corp.) and Visual Basic 2010 Express Edition (Microsoft Corp.) development environments to interface with the Phidget relay board and temperature sensors. Visual Basic 2010 Express Edition is available for free, and the source code for the project is provided as Supplementary Information. The user interface software has several principle elements, including inputs for desired temperature, readouts for the current plate temperature, and whether the plate is heating or cooling. It also displays information about the Phidgets devices that are attached.

Finally, we ensured that the software controlling the device temperature will not allow the plate temperature to exceed a safe operating range. The relays will open when plate temperatures exceed 60˚C or are cooled below −5˚C. This prevents the device from overheating, and more importantly provides a measure of protection for animal subjects.

COST

At the time of writing, the total cost of materials for the feedback temperature control system (which independently controls two aluminum plates) is about $600 USD. To be functional, the unit also requires an enclosure and a computer equipped with one or more USB ports. The assay chamber can be constructed using plastics such as PVC and plexiglass, which are readily cut and drilled. We used PVC for the base and body of the chamber, though 3/8" plexiglass plates (available from many online retailers) would be an appropriate substitute. For the animal enclosure, we used 1/4" clear plexiglass to construct a hinged box with holes in the top for airflow. We have estimated the cost of these materials at $50 (USD), though the actual cost will vary with the needs of the instructor. This brings the total cost of the device to approximately $650.

USE & FUNCTION

Device performance

We next determined whether the device could successfully control the temperature of the aluminum floor plates. We found that cooling from room temperature to a stable temperature of 0˚C required approximately 5 minutes while drawing 5 A (data not shown). This rate will differ between uses, as the exact rate of temperature change is a function of ambient temperature, the starting temperature of the plate, and the magnitude of current supplied by the variable power supply. We then tested whether the device could maintain a stable temperature. With each plate set at 20˚C, the device maintained the surfaces at temperatures very close to its target, oscillating around 20˚C with a minimum temperature of 19.6˚C, a maximum temperature of 20.1˚C, and an average temperature of 19.8˚C over a 70 second interval (Figure 3A). The device was similarly able to maintain stable holding temperatures at 1˚C and 50˚C (data not shown).

To check for even heating and cooling on each plate, we measured the difference in temperature between the center of the plate and the corner. We found that temperatures differed by no more than 0.5˚C from the center of the plate to the corner when the plate reached a steady-state temperature (tested at 0˚C, 20˚C and 50˚C (Figure 3B). Thus, the device is accurate to ±1.0ºC, which reflects both the ability of the feedback control to maintain steady-state temperature and the variability in temperature across the plate surface. Though more rigorously tested at temperatures between 0˚C and 50˚C, the device has been used at −5ºC and +60ºC with no problems.

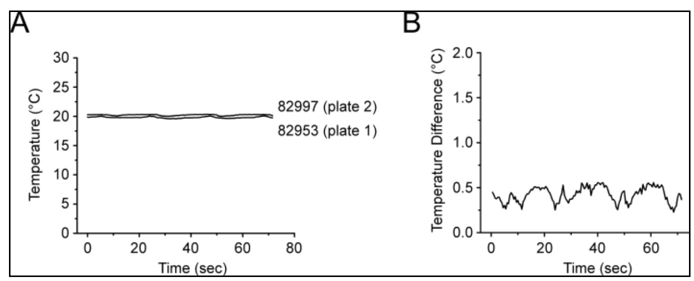

Figure 3. Steady-state temperature is maintained using computer control of thermoelectric modules (TECs). *A*. The temperature of the floor plates as measured by two centrally located thermocouples (serial numbers 82997 and 82953). The device is set at 20˚C, and maintains an average temperature of 19.8˚C over an interval of 70 seconds. Temperatures are stable over all time intervals tested (>4hrs). *B*. Temperature differences between two thermocouples placed in different locations (center, corner) on the same plate. The difference between thermocouples averaged 0.4˚C.

Use of the device in the classroom and laboratory

The two-temperature preference behavioral assay can be used to characterize the thermal preferences of a number of organisms. As a teaching tool, we have found this device to be useful in both introductory and upper-division neuroscience laboratory settings. For example, in an upper-division Neurobiology course at The College of Idaho, students examined thermotactic behaviors in fruit fly larvae (*Drosophila melanogaster*) as part of a series of four inquiry-based laboratory exercises that focused on the molecular basis of temperature-sensing. The first two labs investigated molecular and cellular aspects of thermosensation, specifically the functional properties of thermosensory ion channels in the Transient Receptor Potential (TRP) family. Many, if not all, animals (including *Drosophila*), express thermosensory TRP channels in subsets of sensory neurons; activation of these channels (and subsequent neuronal depolarization) is thought to underlie the ability to detect thermal stimuli (Julius and Basbaum, 2001; Jordt et al., 2003; McKemy, 2007).

Following these molecular and cellular investigations, students formulated hypotheses regarding *Drosophila* behavioral responses to environmental temperatures. Students assessed thermal preferences of fly larvae by observing their location after 5 minutes of roaming freely on an agarose-coated petri-dish that spanned the two floor plates of the device chamber (a sample laboratory handout for this exercise is given as Supplementary Information). The students found that *Drosophila* larvae avoid extremes of temperature (data not shown), findings which are in agreement with previous reports (Hamada et al., 2008; Rosenzweig et al., 2008).

In the final laboratory exercise, students tested hypotheses regarding several experimental manipulations. These included examining thermal preferences in a mutant fly strain developed by Paul Garrity and his colleagues at Brandeis University, in which dTRPA1 (a heat-gated ion channel) has been rendered non-functional. Student experiments demonstrated significant differences in the thermal preferences of wildtype flies as compared with this mutant strain (data not shown). These findings are in agreement with the results of several informative studies on the molecular basis of *Drosophila* thermosensation (Hamada et al., 2008; Rosenzweig et al., 2008; Kwan and Corey, 2009). In summary, the device has been an effective tool for illustrating the molecular basis of thermotactic behaviors in undergraduate neuroscience laboratory settings.

We have also used this device to characterize thermotactic behaviors in mice lacking functional copies of *TRPM8*, a gene required for proper detection of cold temperatures in mammals (Knowlton et al., 2010). Based on this usage of the device, we anticipate that mice would serve well in undergraduate laboratories as a model for mammalian thermotactic behaviors. Besides these uses, the system can serve as a simple hot or cold plate, useful in a variety of other behavioral assays or physiological preparations.

DISCUSSION

Several improvements would make this device more readily usable. First, bidirectional temperature control would simplify the operation of the device. Currently, the user is required to select an appropriate software program depending on whether the plate's temperatures will be warmed or cooled, and then appropriately determine the correct voltage polarity. One method that would enable the computer to select the direction of current flow is the use of an H-bridge, which allows current direction to be reversed by a series of switches or solid state switching devices (Scherz, 2007). In its current form, this solution is impractical for the device. Our device uses relays to control current to the TECs, and therefore to avoid short circuiting, a delay would need to be built into their opening and closing. This would likely cause significantly larger fluctuations in plate temperature. It would also be possible to combine an H-bridge design with a shift register-style control scheme, where all relays open before proceeding to their next state. However this solution, while causing an additional delay, would add significant audible noise, as each of the eight relays would open and close at least twice when switching current to the TECs.

A third significant improvement to the device would be incorporation of solid state switching devices such as metal–oxide–semiconductor field-effect transistors (MOSFETs), rather than relays. This would allow an easier implementation of an H-bridge to add bidirectional current control, and also increase the durability of the device by reducing its mechanical components.

In summary, we developed a computer controlled thermal preference assay chamber that utilizes solid state thermoelectric modules rather than liquid heating or cooling systems to uniformly control the temperature of adjacent aluminum plates to within ±1.0ºC. This system is useful in undergraduate laboratory settings for characterizing thermotactic behaviors, and our design provides the instructional community with an alternative method of Peltier TEC-based temperature control.

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