An Experimental Design for Examining Thermoregulatory Set Points in Ectothermic Animals

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As any student of physiology knows, body temperature has a profound influence on the physiology of organisms. This impact is revealed in the general phenomenon of the Q_{10} effect, where the proportional increase in a particular reaction rate per 10° C change in body temperature is typically 2 to 3 (i.e. $Q_{10} = (K_1/K_2)^{10/T_1 - T_2} = 2$ or 3). Physiological processes also tend to have narrow temperature plateaus over which they exhibit peak levels of performance. This is especially apparent in ectotherms, which tolerate much wider ranges of body temperatures than do endotherms. Performance plateaus and Q_{10} effects can be observed at many different physiological levels, ranging from the cellular and molecular levels (i.e. cellular metabolism) to the organismal level (sprinting ability and digestive efficiency).

The powerful impact of body temperature on physiological performance makes maintaining body temperature a high priority for ectotherms. It also provides a strong argument for examining thermoregulation in the labs of physiology courses. Despite its importance, thermoregulation has proven difficult to examine in much detail in student laboratories. For ectotherms, the standard experimental mechanism in the lab has been to use a thermal gradient to estimate a preferred body temperature, or PBT. The animal selects a comfortable environmental temperature in the gradient, and the average body temperature over time is termed the PBT (for examples, see Huey 1982). Although PBT is a good general indicator of the body temperature preferences of an animal, it does not actually represent the underlying thermoregulatory mechanism very well. It is not the set point for an animal's "thermostat." Instead, it masks the underlying mechanism for thermoregulation used by all vertebrates, the dual set point system (Barber & Crawford 1977, 1979; Crawshaw 1980). Animals utilizing a dual set point system will avoid body temperatures below a lower set point temperature (LSP) and body temperatures above an upper set point temperature (USP), rather than defend a particular PBT. When body temperature is between LSP and USP, the animal is indifferent to body temperature, so thermoregulatory effort does not need to be continuous.

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Examining set points will provide students and researchers alike with a more interesting, and accurate, view of the thermoregulatory mechanisms of animals. Using operant conditioning, it is possible to examine thermal set points. In the following discussion, I provide a protocol to examine the mechanisms of thermoregulation in a straightforward, but more sophisticated, manner than the thermal gradient. I present the methodology for conducting such operant studies, including design hints and troubleshooting, and finish with some discussion of how to interpret findings resulting from the methods used.

Methods

The Operant Conditioning Chamber

The idea behind operant conditioning is that the animal will learn by experience to manipulate its environment to achieve a desired goal, in this case a stable body temperature. In order for the experiment to have the most likelihood of succeeding, the behavior used by the animal must come easily. Given these considerations, you will have to design an appropriate experimental chamber in which to train and test the animal.

Perhaps one of the most straightforward animals to use in this type of study is a moderately large-bodied, diurnal, terrestrial lizard. Such an animal is active at the same time we are, does not require additional structures in the cage such as branches, and its size and mass will ease test chamber construction. I used the first chamber design I detailed below to examine the set points for such a species, the alligator lizard (*Elgaria multicarinata*, Kingsbury 1993). Species such as the larger skinks (*Eumeces*) or fence lizards (*Sceloporus*) would also be suitable.

The test chamber in which I tested the lizards was a 1.2 × 0.3 × 0.3 m runway with a 0.7 m seesaw treadle on the floor of one end (Figure 1). I called this device the "shuttlebox." A pressure-sensitive switch under the seesaw controlled a 150-watt light suspended above it. The switch was a normally open AC switch connected in series to the light. This switch did not influence the overhead fluorescent light cycle, so the chamber was always well lit during testing. Sievert and Hutchison (1988, 1991) showed that the availability of ambient light can have a potentially confounding effect on thermoregulatory behavior.

When the lizard moved beneath the lamp, the additional weight of its body closed the circuit and the lamp turned on. The lizard and its immediate surroundings then began to heat. Lamp intensity was adjusted so that the lizard's dorsal surface would heat at about 1° C/minute. Eventually, the lizard would achieve a body temperature

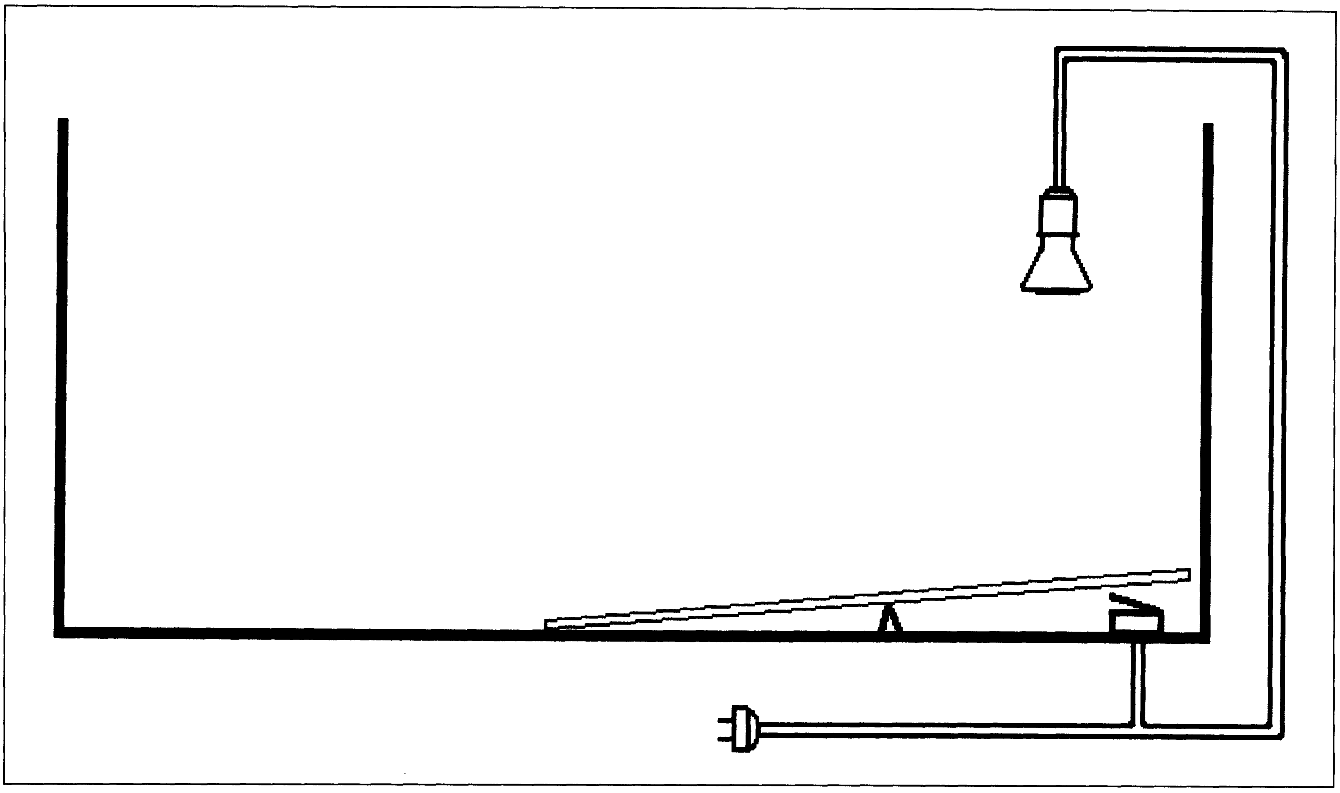

Figure 1. Diagram of a simple shuttlebox operant chamber. The animal can activate an overhead heat lamp by walking onto the seesaw switching mechanism at one end of the shuttlebox, and learns to do so within a few hours of exploratory activity. The light is turned on when the pressure of the seesaw (with the weight of the animal on it) presses down on the lever of the switch with enough force to close it. The switch is an AC type and is in series with the lamp. The animal can avoid heating by going to the other side of the shuttlebox.

above that voluntarily tolerated. To avoid further heating, the lizard moved toward the other end of the shuttlebox. This movement deactivated the lamp, and the shuttlebox cooled to background temperature. The importance of the light turning off at this time is that the animal cannot "cheat the system." It must either be heating or cooling, which will give us the temperature data we need to estimate our set points.

The only equilibrium temperature available to the animal is the background temperature to which the entire experimental apparatus would cool without the heating element activated, typically room temperature. Hopefully, this temperature is uncomfortably cool for your animal, ensuring that it will attempt to thermoregulate. However, if you select a background temperature which is too cool, you may unwittingly expand your experiment to include a demonstration of the incapacitating effects of low body temperatures on your species! If you do want to vary background temperature, consider housing the shuttlebox in an environmental chamber.

I also need to remind experimenters that AC current can be dangerous if not handled correctly. Make sure you understand the path of current flow in your apparatus, and avoid completing any circuits with your body, or that of your subject! The switch for the circuit should be placed below the seesaw, and the animal prevented from gaining access to that space. This protects the animal and saves you headaches caused by them fouling the wiring.

The beauty of this design is that it is very straightforward. A potential drawback is that the weight of the animal may be inadequate to reliably close the switch. Fine adjustment of the design will generally overcome this difficulty. On the other hand, the investigator may use a slightly more elaborate circuit that is not so reliant on the animal's mass. In this circuit (Figure 2A), a DC relay is added. It remains relatively simple to construct, and only requires that the seesaw move slightly to close the circuit. Thus, relatively small animals can be used.

In some cases, as when testing aquatic or large animals, the seesaw design is inappropriate. In those situations, infrared (IR) emitters and photo sensors may provide a suitable switching mechanism. The animal breaks the path of light traveling to a photosensor, which activates the heat source. Since the animal actually causes a break in the signal, a NAND gate is required in the circuit design to reverse the signal sent to the relay (Figure 2B). I have used the IR switch design in two situations. In one case, a study of the nocturnal lizard *Eublepharus macularius* (Kingsbury in preparation), the body of the lizard broke the IR beam when the animal rested inside a small thermoregulatory chamber, activating a heat source. In another case, in which I examined thermoregulation by the turtle *Chrysemys picta* (Kingsbury in preparation), the turtle broke a beam of light when it crawled onto a basking platform.

This latter experimental design relied on a computer (CR10 datalogger, Campbell Scientific Instruments, Inc.) to control environmental conditions and collect temperatures, so provides an example where more sophisticated equipment was used. A turtle could thermoregulate by activating a heat lamp over a basking platform. To use the lamp, a turtle had to climb onto the platform and center its body across the middle of it. In that position, the turtle's body would cover a photo transistor imbedded in the platform, preventing the light emitted by an infrared photo diode above the turtle from

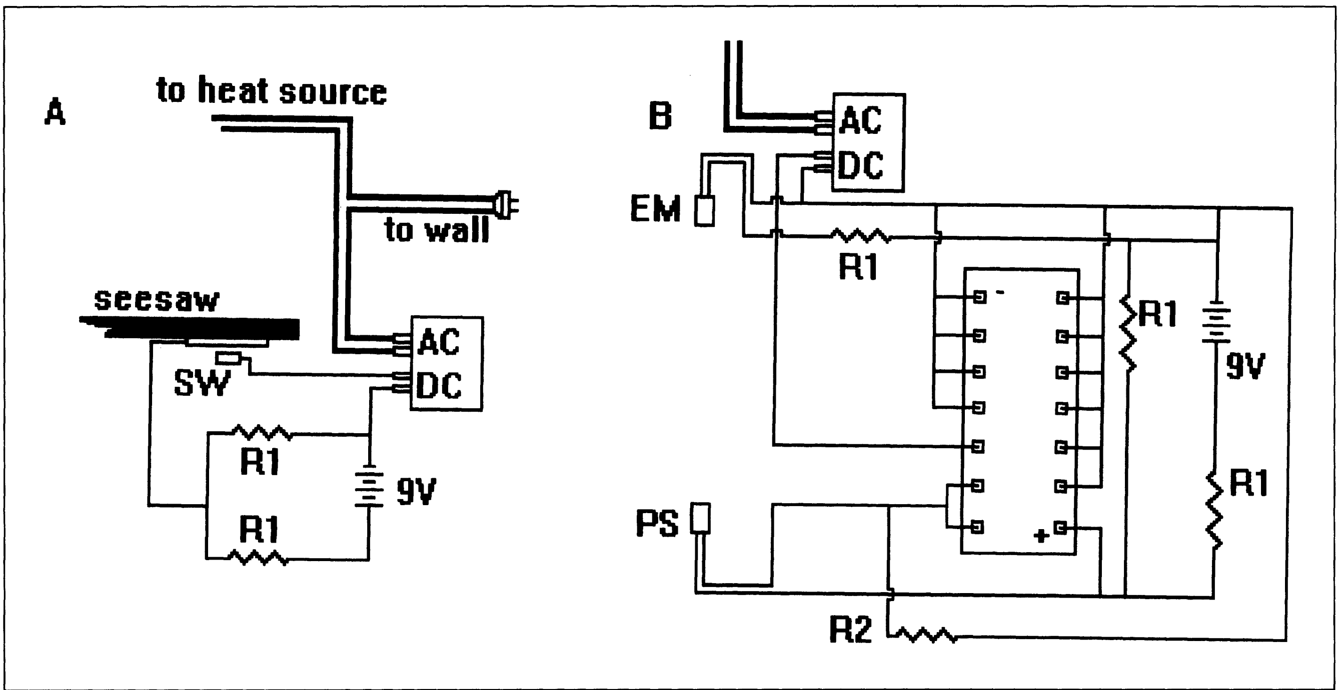

Figure 2. Sample cicuit diagrams for relay-controlled heat sources. A) A circuit for a seesaw type apparatus. B) A relay circuit incorporating an IR sensor and a NAND gate microchip. Abbreviations: SW = switch, AC = AC voltage connections on relay, DC = DC connections, R1 = 1000 ohm resistor, R2 = 2 megaohm resistor (or another large value), EM = infrared emitter, and PS = photosensor. The relay is a 5 volt DC, 110 volt AC, normally open (NO) solid-state relay, and the NAND gate shown is a four channel CMOS chip. Other types of relays and NAND gates can be used, but they may require a modified circuit. All unused pins on the chip must be grounded. The NAND gate inverts the signal sent to the relay, turning the heat source on when the light is blocked from reaching the photosensor. Since the chip has four channels, the circuit can be modified to provide up to four NAND gates. The switch (SW) shown can be any two metal contacts that come together when the animal moves under the heat source. If incident light (i.e. from lights in a room) is adequate to activate the photosensor, then EM and R2 can be removed from the circuit. Consider trying this option first, since it makes circuit construction easier.

reaching the photo transistor. This caused a voltage drop from the photo sensor, which was used to signal the computer to turn the light on. The existing circuitry of the computer, coupled with programmed commands, allowed the "NAND" operation required to turn on the light. The basking turtle would then heat until it began to get too warm, then move off the platform and begin to cool in the water. Once the turtle left the platform, the heat lamp would turn off (the photo sensor again received light), preventing the selection of any intermediate thermal regimes. A programmed two-minute deactivation delay insured that the turtle was forced off the platform and could not "cheat" the activation mechanism and achieve an intermediate thermal environment. As a result of this design, the turtle was either in the water cooling or under the lamp heating. As in the lizard example, the only equilibrium temperature that the turtle could choose was the background (water) temperature. The computer received and stored all of the temperature data (discussed below).

Two concerns regarding IR switches should be mentioned here. First of all, incident light can activate the IR sensor, bypassing the IR light produced by the emitter. Your design must either allow for this, or block it. One way to prevent incident light from reaching the sensor is to set it back in a black tube. Second, I would also caution circuit builders to avoid exceeding the voltage limit of the IR emitter. It may only be about one volt and is easily destroyed.

Measuring Body Temperature

Although designing and building the operant conditioning chamber relies more on ingenuity than expensive technology, the methods for measuring body temperature can vary substantially in cost. In most cases it will not be practical to use a simple thermometer. Serial samples of cloacal temperatures would be very disruptive to the experiment, as well as disturbing to the subject! However, placement of a small gauge thermocouple into the cloaca of a reptile or amphibian is generally tolerated quite well. After gentle insertion 2 to 3 cm into the anal opening, the lead can be taped to the animal's tail with cloth tape. The same method can be used with a thermistor lead if the wire is not prohibitively thick. Students can then monitor the screen of the thermocouple measuring device, videotape it over time, or have a computer store data as produced. This latter technique was used in the turtle experiment described above. Three thermocouples from the turtle, plus one for water temperature, delivered temperature information to the computer. The computer could store thousands of data points, as well as the times at which they were collected, and these could be downloaded at a later date to a PC for further processing and analysis (Figure 3).

Another technique to consider is radio telemetry. Implantable or surface-placeable temperature-sensitive radio transmitters may be purchased from vendors such as Holohil, Inc. or Mini-Mitter, Inc. They may also be constructed following published guidelines, as presented by researchers in Amlaner and MacDonald (1980), among others. If you implant the

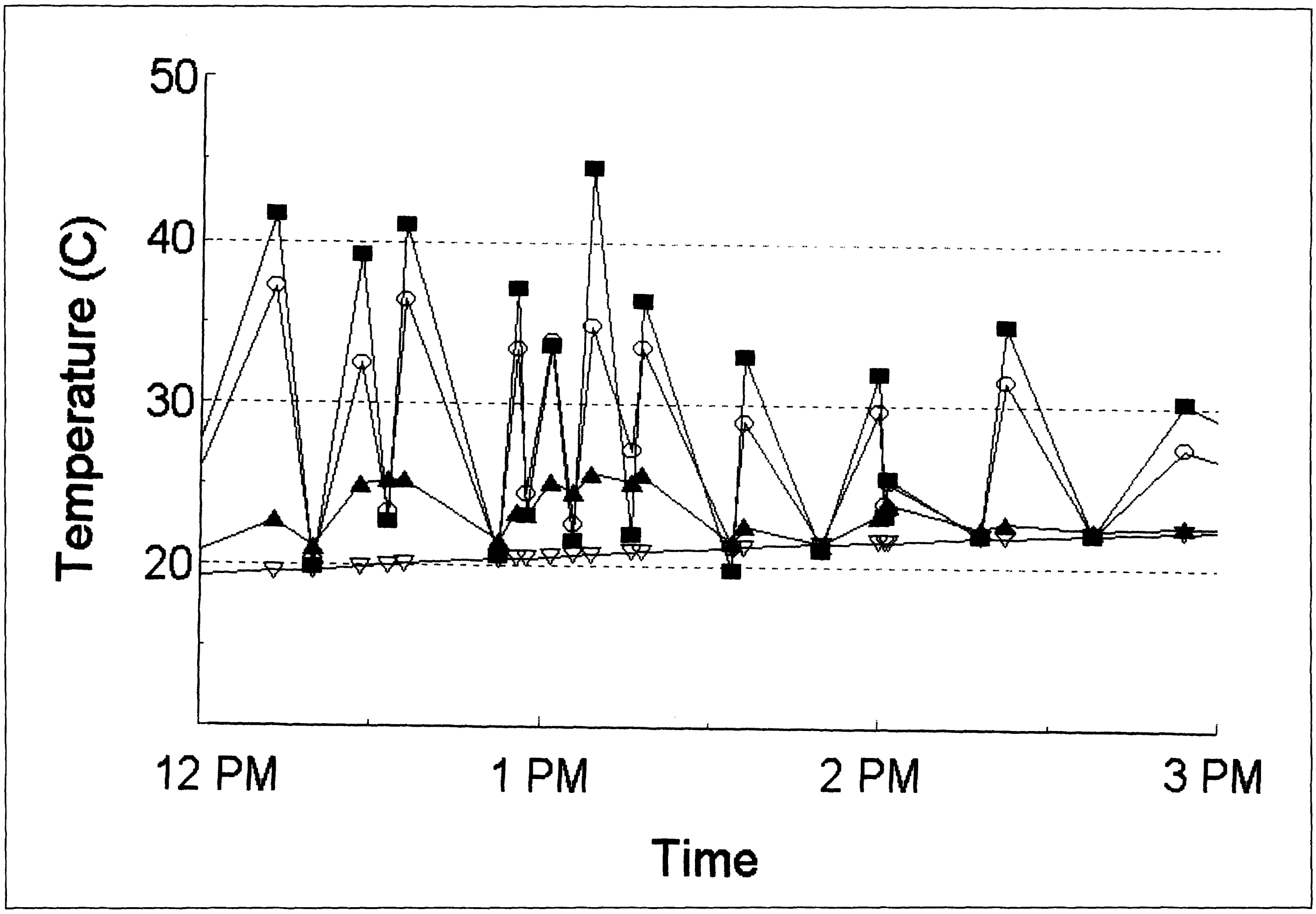

Figure 3. Sample data from a thermoregulatory trial with a painted turtle (*Chrysemys picta*). Four temperatures are shown: solid squares = the surface of the carapace, open ovals = the body temperature just beneath the carapace, solid triangles = body temperature in the turtle's intestine, and open triangles = temperature of the water in the experimental chamber. Data were collected via thermocouples leading to a Campbell CR10 datalogger. Only those temperatures collected when the turtle got on or off of the basking platform are shown.

transmitters, you will have to develop the necessary surgical techniques. In many situations, however, external placement is possible. Data recording options are the same as for thermocouples, although a special receiver may be required for receiving signals from the transmitters (some just need an FM radio as the receiver).

Another method to consider is the use of IR imaging systems (Clark et al. 1974; Jones & Avery 1989; Kingsbury 1993). Some of the more sophisticated devices are rather expensive, but handheld units may prove adequate in the classroom and are much less expensive. The way to use these is to measure the surface temperature closest to the heat source as the animal enters or exits a basking site. If a small animal is heating or cooling relatively slowly, then surface temperature and core temperature are about the same. Interestingly, it would appear that in some species of ectotherms, surface temperature is the more appropriate temperature to monitor if you are searching for the species' thermostat temperatures. In lizards, surface or "shell" temperature seems to be the principal input for thermoregulation (Barber & Crawford 1979; Crawshaw 1980), with brain temperature only being a contributing input (Myhre & Hammel 1969; Kluger et al. 1975). In mammals, the reverse seems to be true, where the hypothalamus seems to be the principal center of input as well as the thermoregulatory processing center (reviewed in Crawshaw 1980; Cassel & Casselman 1990). Not everything is figured out yet, however. The results of my *Chrysemys picta* study (Kingsbury in preparation) imply that turtles may have more "mammal-like" thermostats: shell temperatures are not the principal input for their thermoregulatory system.

Interpreting the Results

Depending on how body temperature was measured, you will have some form of a time course of temperature data (Figure 3). Although much of this data could be used and described in some way, it is the body temperatures at which our animals change their behavior that is most important. I have termed the body temperature of an animal when it shows heat avoidance behavior the upper turnaround temperature (T_u). Mean T_u for an individual is used as an estimate of its upper thermoregulatory set point temperature (USP). The body temperature upon seeking a heat source is the lower turnaround temperature (T_l), and mean T_l was used as an estimate of the lower thermoregulatory set point temperature (LSP). Using these data, students or researchers can arrive at estimates of the thermostat set points for their organism.

Conclusion

My intent in presenting this paper was to provide instructors of physiology courses with a mechanism for increasing the sophistication with which they examine thermoregulation. The techniques described can, of course, also be used by researchers investigating thermoregulatory behavior. There are numerous variations on the experiment, not only in terms of species used, but conditions imposed on the animals during the trials. Aspects of body condition, typically whether or not the animal has recently eaten (Lang 1979; Sievert 1989; Gibson et al. 1989) or is fully hydrated (DeWitt 1967), have been shown to influence thermoregulation. Another type of study might consider conflicting motivations during activity, such as perceived predation risk versus thermoregulatory effort. Other studies have examined precision of thermo-regulation as affected by the effort required (Campbell 1985; Withers & Campbell 1985). Whatever you try, good luck!

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