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# A technique to measure cold adaptation in freely behaving mice

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ABSTRACT

*Background:* Adaptation to environmental temperature is essential for survival in seasonal areas. The mechanisms of adaptation have been studied *in vitro*, but it has not been quantified *in vivo*.
*New method:* The extended Cold Plantar Assay (eCPA) cools the entire testing environment. Once the desired environmental temperature has been reached, a separate focal cold stimulus is applied to the hindpaw and the latency to withdrawal is recorded as a proxy for cold sensitivity.
*Results:* Using this technique, we can test the cold responsiveness of freely behaving mice at ambient temperatures ranging from 5 °C to 30 °C. The responses are consistent and unambiguous, and the environmental temperatures generated are reproducible. We are also able to measure cold responsiveness as animals are in the process of adapting to cold environments.
*Comparison with existing method(s):* Existing methods, such as the dynamic cold plate and the 2-plate preference assay test how mice respond to cold environments, but cannot assess how the thresholds for response are changed by acclimation in cold environments. Additionally, the eCPA requires very little specialized equipment, can test many mice at the same time on one apparatus, and has an objective readout.
*Conclusions:* The extended Cold Plantar assay is a significant methodological improvement, allowing the assessment of cold responsiveness in freely behaving mice at a wide range of environmental temperature conditions and during cold adaptation.



## 1. Introduction

### 1.1. Relevance of cold adaptation

Organisms that live in seasonal climates must be able to adjust to significant temperature shifts. One important aspect of this process is the ability to adapt their temperature sensitivity to levels suiting their environment, since organisms must be able to detect a cold stimulus whether the environmental temperature is hot or cold.

### 1.2. Previous techniques to assess cold adaptation

While the ability to modulate thermal sensitivity to match the environmental setting has been studied thoroughly *in vitro* (Rohacs et al., 2005; Daniels et al., 2009; Fujita et al., 2013), it has been difficult to test this phenomenon *in vivo*. Previously, the primary method for studying responses to ambient temperature changes was the "dynamic cold plate" (Yalcin et al., 2009; Descoeur et al., 2011), in which animals are put on a room temperature Peltier device which is then rapidly cooled (1 °C/min) until it reaches 1 °C. Behavioral responses including licking, rearing, and jumping are measured at different temperature ranges and used to estimate cold responsiveness. While this assay can dynamically test the responses of animals to cooling, it has some limitations. For one, it measures how a mouse responds to a cooling environment, but does not provide a way to test responsiveness to a discrete cold stimulus in the context of environmental cooling. It is also confounded by environmental exploration, since the animals are tested on the cold plate without the chance to acclimate to the environment unless they are acclimated for significant periods before testing, which increases the amount of time necessary for each mouse. It also requires expensive equipment, can only test one mouse at a time, and relies on subjective characterizations of the mouse behavior during the cooling.

*Abbreviation:* eCPA, extended Cold Plantar Assay.

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### 1.3. Utility of the extended Cold Plantar Assay

In order to complement this assay we have modified the previously described Cold Plantar Assay (Brenner et al., 2012) to test cold responsiveness at a wide variety of baseline temperatures. We are calling this new assay the extended Cold Plantar Assay (eCPA). The eCPA provides an easily quantified, objective measure of responsiveness to a uniform cold stimulus at different environmental temperatures. While our current apparatus can test six mice at a time, it has the potential to be easily and inexpensively scaled up for higher-throughput testing. We believe the eCPA is a significant methodological improvement for measuring cold adaptation and cold responsiveness.

## 2. Methods

### 2.1. Experimental animals

All mouse protocols were approved by the Animal Care and Use Committee of the Washington University School of Medicine (St. Louis, MO) and were in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were carried out with Swiss Webster mice acquired from Jackson Labs (Bar Harbor, ME) unless otherwise noted. Efforts were made to minimize the number of animals used, to minimize suffering. All mice used were male and 6–9 weeks old unless specifically noted. Mice were housed on a 12/12 h light/dark cycle with the light cycle beginning at 6 am. All mice had *ad libitum* access to rodent chow and water. Cage bedding was changed once a week, always allowing at least 48 h after a bedding change before behavioral testing was carried out. Mice were allowed at least 3 days between behavioral testing at any ambient temperature, and were tested first using at room temperature, followed by 17 °C, 12 °C, 5 °C, and 30 °C.

### 2.2. Behavioral analysis

All behavioral tests and analyses were conducted by an experimenter blinded to treatment. Behavioral tests were performed between 12 pm and 5 pm.

### 2.3. extended Cold Plantar Assay (eCPA)

.125 in., .1875 in., and .25 in. thick pyrex borosilicate float glass was purchased from Stemmerich Inc. (St. Louis, MO). Transparent plastic enclosures (4 in. × 4 in. × 11 in.) separated by opaque black dividers were arranged in one line along the center of the plate (Fig. 1A and B). The glass temperature was monitored with an IT-24P T-type filament thermocouple probe from Physitemp Instruments, Inc. (Clifton, NJ) that was secured in the middle of the plate with laboratory tape. Plate temperature data were collected from the thermocouple using an EA15 Data-Logging Dual Input Thermometer from Extech Instruments (Waltham, MA) (Fig. 1A and B). Dry ice or wet ice was piled on either side of the enclosures to uniformly cool the glass plate. The ice was contained either in packets made of heavy-gauge aluminum foil, or in custom-built aluminum boxes (Fig. 1A and B). The boxes are the same length as the glass plate, 4.5 in. wide and 3 in. tall with a lid. A drain with a stopcock was installed at the bottom of one short end of each box to allow easy drainage. The glass temperature can be adjusted by moving the ice containers closer to or further from the plastic enclosures (Fig. 2A and B), or by filling the container with hot water. After the plate reached the desired temperature, mice were acclimated in the enclosures for 3 h before testing. The time necessary for acclimation will vary with the distance and noise that mice travel from their housing facility to the testing apparatus. White noise was used to decrease noise disturbances.

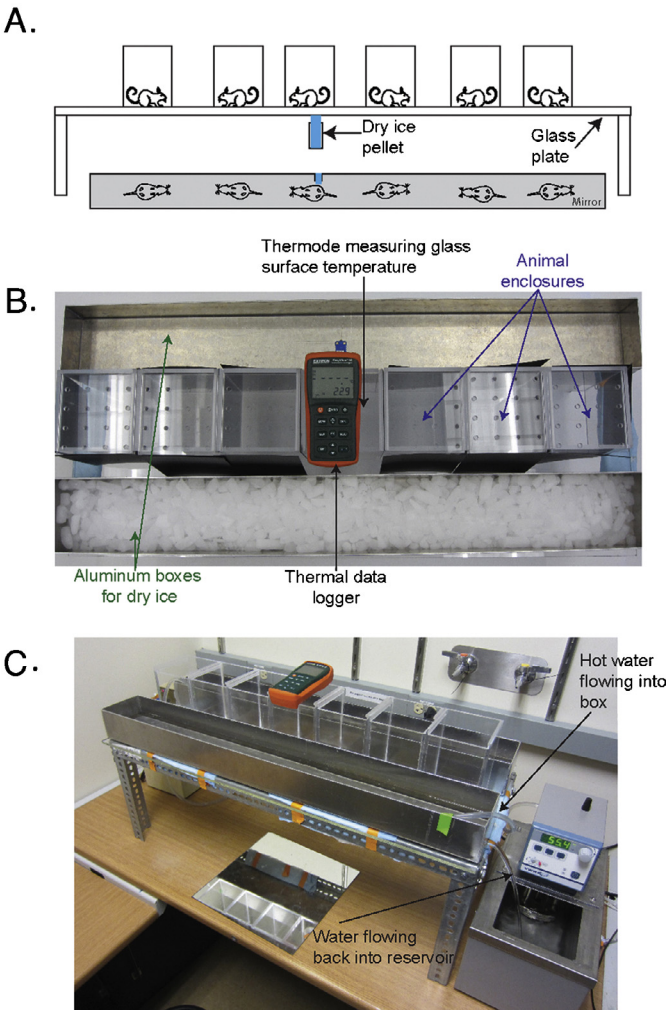


**Fig. 1.** How to perform the eCPA. (A) Schematic for performing the Cold Plantar Assay (CPA). Mice are isolated in plastic enclosures with black inserts on a glass plate. A compressed dry ice pellet is applied to the glass underneath the paw using a mirror for targeting. (B) The extended CPA (eCPA) assay. Aluminum boxes are positioned on both sides of the animal enclosures. A t-type thermocouple attached to a data logger is used to track and record the temperature at the center of the glass plate. (C) The eCPA assay configured for the 30 °C condition. Hot water circulators are positioned on either side of the glass plate. The water circulators pump fresh hot water into the aluminum boxes in order to warm the glass. The water drains out through holes in the sides of the aluminum boxes of the side directly into the reservoirs of the circulators.

Due to the $CO_2$ generated when using dry ice, we found that it is essential to have adequate ventilation over the apparatus or the mice may enter torpor. With proper ventilation, there were no signs of $CO_2$ intoxication in any condition.

#### 2.3.1. Testing mice at 30 °C

To warm the glass plate to 30 °C, the aluminum boxes were positioned approximately .25 in. away from the animal enclosures on either side. A VWR water circulator continually fed water heated to between 50 and 60 °C into the aluminum boxes in order to warm the plate up (Fig. 1C). The exact temperature of the water circulators was calibrated during each experiment to keep the plate at 30 °C. The water exited the aluminum boxes through the drain holes at the bottom of the short ends, and flowed directly back into the reservoir of the circulators for reheating. After approximately 90 min the