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An Undergraduate Laboratory Exercise that Demonstrates the Difference Between Peripherally and Centrally Mediated Measures

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One of the first concepts that students of neuroscience are exposed to is the overall organization of the nervous system and the two principle divisions of it: the Peripheral Nervous System (PNS) and the Central Nervous System (CNS). In sensory systems, this fundamental division plays a particularly prominent role in the information processing stream that integrates and processes information from the external environment to the CNS. To better understand the differences between the roles that the PNS and CNS play in information processing, we developed a relatively simple in-class laboratory exercise. The experimental methods used to determine several aspects of a subject's discriminative capacity (threshold detection, amplitude discrimination, duration discrimination) are described. These methods were used either under control conditions or after the students altered their skin sensitivity (i.e., the PNS) by cold water immersion.

At the conclusion of the lab exercise, students will thoroughly understand the principle of the PNS vs. CNS, as well as a fundamental understanding of quantitative sensory testing. This fundamental understanding of sensory testing provides a foundation for students to pursue or investigate other aspects of sensory information processing in either independent studies or subsequent lab exercises.

Keywords: Temperature and Sensitivity; Vibrotactile Amplitude Discrimination; Peripheral Mediation

INTRODUCTION

Sensory testing is frequently used in research and some clinical practices to make fundamental observations about an individual's neurological status. However, there is often a common misperception about the difference between sensory tests that are impacted by specific neurological disorders centrally and sensory tests that are impacted predominantly by peripheral factors. For example, vision, audition, and the sense of touch often degrade with age due to deterioration of the eye, ear, and skin physiology, respectively, while central information processing capacity remains intact (and often improves) under certain conditions of aging. Someone's hearing might degrade due to peripheral damage to their ears from excess exposure to loud noises. However, his/her central nervous system (CNS) would still be intact and functioning quite well regardless of changes in the peripheral nervous system (PNS).

There are a number of sensory based tests that are predominantly influenced by central mechanisms, and observations obtained from these tests have been demonstrated to be sensitive to neurological disorders. Our group has been developing sensory based tactile tests for over a decade that are sensitive to centrally mediated mechanisms, and the data from a number of populations with neurological disorders have shown significant deviations from healthy controls. These populations range from individuals with autism (Tommerdahl et al., 2008; Tannan et al., 2008; Francisco et al., 2013; Puts et al., 2014; Tassovolli et al., 2015), Tourette's (Puts et al., 2015), obsessive compulsive disorder (Güclü et al., 2015), different types of chronic pain (Zhang et al., 2011a; Nguyen et al., 2013a), alcohol abuse (Nguyen et al., 2013b), acute pharmacological effects (Folger et al., 2008), and concussion (Francisco et al., 2015; Tommerdahl et al., 2016). The aging population provides an interesting contrast in that peripherally mediated sensory perceptual metrics are impacted by age, but centrally mediated metrics are not (Zhang et al., 2011b).

The purpose of this laboratory exercise is to contrast the difference between centrally and peripherally mediated sensory metrics that are tactile based. Students will obtain sensory perceptual metrics on themselves before and after cold immersion – a process that should significantly impact performance on peripherally mediated metrics. Although there have been a number of laboratory exercises that expose students to the sense of touch, very few of those have focused on central information processing issues mediated by the sense of touch (with the exception of Holden et al, 2011 and Nguyen et al, 2013). The protocols used in this exercise have been described extensively in multiple publications that students can reference (e.g., Puts et al., 2013; Francisco et al., 2015).

Learning Objectives

Upon completion of the experiment, students should be able to:

1. Understand the difference between the PNS and the CNS.
2. Understand the difference between a centrally mediated and peripherally mediated sensory percept.
3. Understand the impact of cold on skin sensitivity and how to measure that impact.
4. Gain familiarity with the fundamentals of sensory

testing as well as be able to conduct future experiments involving sensory data collection and analysis.

5. Apply basic statistics, including formulating a hypothesis and deciding on the appropriate statistical tests to best analyze the data.

MATERIALS

Several multi-site mechanical stimulator devices (CM-6; Cortical Metrics Model #6; Figure 1), designed to optimally deliver vibrotactile stimuli to finger tips, were used in this lab exercise. Each stimulator interfaces with a laptop via an internal data acquisition box (DAQ), which is connected to the computer with a universal serial bus (USB) cable. An HTML5 application developed in-house for Google Chrome allows for a wide range of stimulus conditions to be delivered independently and simultaneously to each of the probes. The software also provides an interface for prompting user response and providing progress feedback. Stimulators are mounted on a drum that rotates and allows for independent positioning of each probe tip to best fit the hand of the individual. For a full technical description of the device, see Holden et al. (2012).

Figure 1. Cortical Metrics vibrotactile stimulator (CM-6).

Subjects were also provided with an ice water bath maintained at 7±2^{o}C by adding more ice as needed.

PROCEDURES

Subjects

Twenty healthy high school students age 15-18 were recruited into the study from Advanced Placement (AP) physics, chemistry and biology classes.

Experimental Setup

During the experimental session, each subject was seated comfortably in a chair facing a laptop that was connected to the tactile stimulator on one side of the laptop and a computer mouse on the other side. Each participant rested his/her right hand on the mouse and left hand on the stimulator, which was adjusted so that two probes made contact with the glabrous skin of the second (index, D2) and third (middle, D3) fingers. Participants were instructed to maintain fingertip contact with the probe tips throughout the duration of each trial.

Subjects were randomly assigned to either the control (N=12) or cold (N=8) treatment group. All subjects performed 3 protocols (threshold, amplitude discrimination, and duration discrimination), with the cold group placing their left hand in the ice bath for 45 seconds before each of the three tests.

Threshold Detection Protocol

A suprathreshold stimulus (25 Hz, starting amplitude 25 µm, duration 500 ms) was randomly delivered either to D2 or D3 and the participants were asked on which finger they felt the stimulus. A 1 up/1 down tracking paradigm (stimulus amplitude was decreased for a correct answer and increased for an incorrect answer) was used for the first 10 trials and a 2 up/1 down (two correct answers were necessary for a reduction in test amplitude) was used for the remaining 10 trials (ITI 5 s). For each subject, the Difference Limen (DL), or detectable difference between the two stimuli (second one being zero), was determined by averaging the tracking values obtained from the last five trials of each experimental run. Methods are previously described in Puts et al. (2013).

Amplitude Discrimination Protocol

Vibrotactitle flutter stimulation (25 Hz) was simultaneously applied to D2 and D3 of the left hand for 0.5 s during each of the 20 trials. A constant conditioning stimulus of 100 µm was randomly delivered to D2 or D3, with the other digit receiving the test stimulus. The participant was asked to determine which digit received the stronger stimulus. A 2AFC tracking protocol was used to determine the subjects capacity to discriminate between the amplitudes of the two simultaneously delivered vibrotactile stimuli such that the difference between the two subsequent stimuli of the next trial was increased or decreased based on subject response. A 1 up/1 down tracking paradigm (comparison stimulus amplitude was decreased for a correct answer and increased for a wrong answer) was used for the first 10 trials, and a 2 up/1 down (two correct answers were necessary for a reduction in the test stimulus amplitude) was used for the second 10 trials (ITI 5 s). The DL for each subject was determined by averaging the tracking values obtained from the last five trials of each experimental run (protocol previously described in Tannan et al., 2005a,b, 2006, 2007a,b; Tommerdahl et al., 2007a; Zhang et al., 2008; Puts et al., 2013; Francisco et al., 2015).

Duration Discrimination

Duration discrimination was assessed in a similar manner, using a 2-alternative forced-choice (2AFC) protocol, as described in Francisco et al. (2015). Briefly, each trial consisted of a vibrotactile test stimulus delivered sequentially either 500 ms before or after a vibrotactile standard stimulus of 500 ms. The order (standard followed by test or test followed by standard) and loci of the stimulus was randomly selected on a trial-by-trial basis. Stimulus amplitude was 300 µm. The subject was prompted on the

screen of the computer to ‘‘Choose the longer duration stimulus’’ along with buttons labeled ‘‘Left’’ and ‘‘Right.’’ The subject selected the skin site that perceived to be the longer duration stimulus by clicking the button on the screen and a 5 s delay interval followed before onset of the next trial. The test stimulus duration began 250 ms longer than that of the standard stimulus and was increased or decreased by a 25 ms step size according to a 1-up/1-down algorithm for the first 10 trials. The subjects were unaware that one of the stimuli was of fixed duration. Correct responses resulted in the decrease of the duration of the test stimulus, while incorrect responses increased the duration of the stimulus. After the initial 10 trials, the duration was varied using a 2-up/1-down algorithm. The subject’s DL was calculated by averaging the difference between the standard and the test from the final five trials of the 20 trial test. The rationale for implementing these algorithms was to initially expedite determination of vibrotactile discriminative range and then account for response bias. This method has been extensively reported (Tannan et al., 2006, 2007a,b; Tommerdahl et al., 2007a,b, 2008; Francisco et al., 2008; Zhang et al., 2008, 2009, 2011a,b).

Data Analysis

All calculations were made using SigmaPlot ver. 12.5. A one-tailed, two-sample Student’s t-test was used to assess differences between threshold control and cold group means, and two-tailed two-sample Student’s t-tests were used to assess differences in amplitude and duration discrimination group means. Choice of using a 1- vs. 2-tailed t-test was based on the original hypotheses that threshold would decrease with cold exposure, but there would be no difference in amplitude or duration discrimination following exposure to cold. For all tests, a p-value of 0.05 was used to determine statistical difference.

RESULTS

This lab exercise investigated the impact of cold immersion on three different sensory perceptual metrics by making comparisons between data obtained from a group of students who immersed their hand in cold water before being tested and a group of students who did not. A DL was obtained for each of the performance tasks on each individual: threshold detection, amplitude discrimination and duration discrimination. The DLs obtained from the individual performance tasks were then averaged and group comparisons were made.

A one-tailed two-sample Student’s t-test between control and cold groups shows that there is a significant difference in threshold level between the two groups (Figure 2A; P=0.026). Conversely, two-tailed two-sample Student’s t-test between the same treatment groups gives no evidence to suggest a difference in amplitude discrimination (Figure 2B; P=0.537) or duration discrimination (Figure 2C; P=0.295). Thus, as expected, results suggest that while cold exposure impacts the PNS (threshold), the CNS (amplitude discrimination, duration discrimination) remain unaffected.

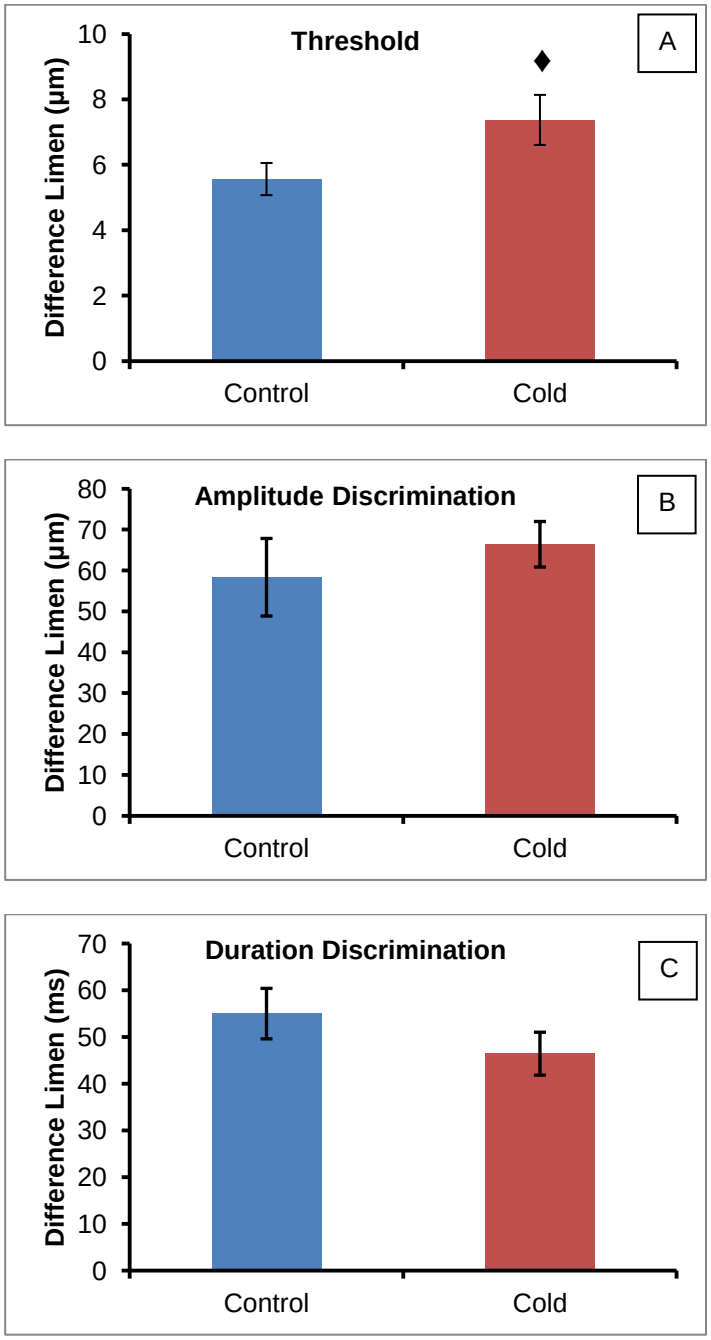

Figure 2. Mean±SEM of threshold (A), amplitude discrimination (B), and duration discrimination (C) from control and cold groups. ♦ Denotes significant difference.

DISCUSSION

Results from this exercise suggest that cold exposure has an impact on measures that are modulated peripherally (threshold detection) but does not impact metrics that are predominantly modulated centrally (amplitude discrimination and duration discrimination). The significance of this is that peripheral functions may be degraded while central functions remain intact. An important concept for students to learn is that some forms of sensory testing can be an effective means for evaluating the CNS, and some forms of sensory testing, such as

detection threshold, are influenced too much by peripheral factors to be an effective tool for evaluating changes in the CNS. For example, while detection threshold has been demonstrated to go up with age in healthy control subjects, amplitude discrimination remains constant across the same aging population (Zhang et al., 2011b). However, the same amplitude discrimination metric is impacted in some neurologically compromised populations (e.g., migraine, Nguyen et al., 2013a; concussion, Tommerdahl et al., 2016).

Cold immersion resulted in an increase in detection threshold and showed a subtle, though insignificant increase in amplitude discrimination capacity. This would be expected – increasing the detection threshold would be expected to shift amplitude discrimination in the same direction and remain consistent with Weber's Law (Francisco et al., 2008; Holden et al., 2011). Duration discrimination, or timing perception, on the other hand, requires that an individual only be able to detect the presence of the stimulus for a perceivable length of time. Cold immersion did not have an impact on this as subjects could still detect the presence of the stimulus. In fact, cold immersion appeared to have the impact of improving – although statistically insignificantly – duration discriminative capacity.

By the end of the exercise, students were familiar with sensory testing and had a fundamental understanding of the difference between the PNS and CNS. The students understood that exposure to cold affected their ability to feel the stimuli on their fingertip, but it did not alter their capacity for amplitude or duration discrimination, allowing for a deeper understanding of peripheral and central processing. Additionally, students gained a practical knowledge of conducting a relatively simple and easy to understand experiment whose results could be analyzed. This is the third laboratory exercise reported using these methods (previously reported in Holden et al., 2011 and Nguyen et al., 2013), and we anticipate that student based designs will lead to additional laboratory exercises that demonstrate concepts of centrally mediated information processing.

Methodological considerations

The students were broken up into two groups so that the impact of cold immersion could be assessed by comparing controls vs. non-controls. While doing a group study like this saves a significant amount of time (shortens the experimental time by a factor of 2), the study could also be done with all of the students doing the study both before and after cold immersion. The advantage of that procedure would be that students could assess individual data and determine the average change that occurs with each student. A disadvantage is that to do the study properly, an order effect would need to be taken into account, and either the sample size or the number of conditions would need to be increased. The additional time saved by doing a group study as we conducted allows for time to discuss the reason for doing the experiment, formulation of a hypothesis, and guiding the students to analyze the data in class.

While this study was done with Advanced Placement (AP) high school students, it could easily be used in an introductory college neuroscience lab, which would likely give students more time to complete the experiment. Each student should be able to complete their required set of protocols within 45 minutes.

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