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A portable tactile sensory diagnostic device

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Abstract

Current methods for applying multi-site vibratory stimuli to the skin typically involve the use of two separate vibrotactile stimulators, which can lead to difficulty with positioning of stimuli and in ensuring that stimuli are delivered perfectly in phase at the same amplitude and frequency. Previously, we reported a Two-Point Stimulator (TPS) that was developed in order to solve the problem of delivering two-point stimuli to the skin at variable distances between the sites of stimulation. Because of the success of the TPS, we designed and fabricated a new stimulator with 4 significant improvements over our original device. First, the device is portable, lightweight and can be used in a variety of non-laboratory settings. Second, the device consists of two independently controlled stimulators which allow delivery of stimuli simultaneously to two distinct skin sites with different amplitude, frequency and/or phase. Third, the device automatically detects the skin surface and thus allows for much better automated control of stimulus delivery. Fourth, the device is designed for rapid manufacture and, therefore, can be made readily available to other research (non-laboratory) settings. To demonstrate the device, a modified Bekesy tracking method was used to evaluate the simultaneous amplitude discrimination capacity of 20 subjects.

INTRODUCTION

The delivery of sinusoidal displacements to a single skin site via mechanical transducer has been used extensively for the study of flutter vibration in both psychophysical and neurophysiological settings for a number of decades (exemplary uses of such a device are described in (Mountcastle et al., 1969; Vierck and Jones, 1970; LaMotte and Mountcastle, 1975; Juliano et al., 1989; Goble and Hollins, 1993; Tommerdahl et al., 1993; Tommerdahl et al., 1998;Tommerdahl et al., 2002)). Typically, stimuli that can be delivered through mechanical transducers - vertical displacement stimulators such as the one originally described by Chubbuck (Chubbuck, 1966) – that are used for studies of somatosensation are very well equipped to deliver sinusoidal stimuli at a frequency range (1 - 250 Hz) with amplitudes of sufficient size (between 0 and 1000 μ m) to activate a broad range of mechanoreceptors. However, in order to stimulate more than one skin site – either during the course of human psychophysical testing or animal experimentation – it is necessary to position a second vertical displacement stimulator over a second skin site. Consequently, studying the effects of varying the distance between two stimulated skin sites can become cumbersome each time the investigator has to reposition the actual stimulators. A second problem that results from the use of two stimulators is that some effort must also be made in order to deliver the two stimuli perfectly in phase at the same amplitude and frequency. In order to allow for the development of experimental protocols that could compare the effects of delivering identical stimuli spaced

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at variably spaced distances on a trial-by-trial basis, we designed and fabricated a two point stimulator (TPS), a system that, when attached to the end of a vertical displacement stimulator, modified it from a single probe tip to dual probe tips (Tannan et al., 2005a). Both points of the TPS were driven by the single vertical displacement stimulator and distances between the two sites could be varied on a trial-by-trial basis. While this device was extremely useful in that a number of single and dual-site stimulus protocols could be effectively delivered to the skin to evoke measurable percepts (Tannan, et al., 2005a; Tannan et al., 2005b; Tannan et al., 2006; Tommerdahl et al., 2007), there were several limitations. First, we did not have independent control of the two stimuli, thus limiting our protocol development. Second, a significant amount of setup time was expended by the investigator to insure proper vertical positioning of stimuli over the skin. In other words, the stimulator itself could not detect the surface of the skin. Third, our studies were limited to those conducted in a laboratory setting. Fourth, the TPS was attached to a Chubbuck type stimulator which, though well ahead of its time in terms of design and performance in the 1960's (Chubbuck, 1966), had not been significantly improved upon. One of the goals of our current design was to make the stimulator both modern and portable. Portability would allow us to perform sensory testing on a number of subjects from virtually any location. A fifth restriction of previous stimulators was cost and availability. The utilization of modern rapid manufacturing techniques would allow us to build a large number of stimulators at a relatively low construction cost. An increase in availability of stimulators would allow for the distribution of the device to a number of clinical settings and allow for investigators to have much better access to tools for the tactile sensory evaluation of subjects with altered or impaired central and/or peripheral nervous systems.

The new device is described, and in order to demonstrate one aspect of the device's capability, a tracking protocol was used to generate human psychophysical data measuring the amplitude discrimination ability of subjects. Similar previous amplitude discrimination studies examined the ability of subjects to discriminate amplitudes of stimulation delivered to the same skin site sequentially. This study was unique in that amplitude discrimination was studied with two stimuli delivered simultaneously to two skin sites by a single, dual-probe stimulator system.

METHODS

Description of the Device

The Cortical Metrics (CM-1; see Figure 1) stimulator was developed in our laboratories for use in the series of experiments described in this report. The system was designed using stateof-the-art rapid manufacturing technology to allow multiple identical systems to be built and used in different locations. Also, the use of rapid manufacturing permitted very rapid design evolution, thereby potentiating the production of special fixtures and changes to geometry as needed for special applications, such as pediatric sizing or the use of special mounting hardware to adapt to existing equipment. The flat plates of the exterior housing and other components of approximately planar geometry are direct manufactured using laser-machined 6mm acrylic sheet, cut on a 120 Watt CO₂ laser engraving system, model number X660 (Universal Laser Systems, Scottsdale, AZ). The more complex housing and internal mechanism components are direct manufactured from polycarbonate (PC), by fusion deposition modeling (FDM) on a StrataSys Titan T-1 FDM (StrataSys, Inc., Eden Prairie, MN). All housing and mechanism components and assemblies were solid modeled prior to fabrication using SolidWorks solid modeling software (SolidWorks Corporation, Concord, MA).

The internal mechanism is comprised of two independent x-z positioning tables onto each of which is mounted a voice coil actuator (VCA) motor and position sensors. The VCA motors drive the plastic stimulator probe tips according to prescribed sinusoidal waveforms. The moving components of the stimulator tips are directly manufactured from PC by FDM as a single compliant mechanism component integrating a mounting flange, a thin-beam four-bar

linkage, a magnet coil bobbin, an optical displacement sensor vane, and the extension to the mechanical stimulator tip. These components are designed and manufactured so that they can be assembled in mirror-image configuration to allow the two internal tip-placement mechanisms to be mounted adjacent to one another and to allow the tips to be positioned horizontally in contact (distance = 0.0 mm) or separated linearly by a distance of up to 60 mm center-to-center. The compliant four-bar linkage mechanism allows the coil, optical position sensor vane, and tip to be vibrated vertically along a straight line for a distance of $\pm 1 \text{ mm}$. The 4-bar compliant mechanism also provides a very low hysteresis linear restoring force to center each tip vertically when no current is applied to the VCA coil. The VCA coil is 80 turns of 34 AWG magnet wire, wrapped in a rectangular bobbin permanently solvent bonded into the four-bar mechanism. The entire four-bar mechanism is 3.6 mm in thickness, and is positioned such that the VCA coils sit directly between two opposed rare-earth-element planar arc magnets of the type found in computer hard drives. The resulting VCA motors generate extremely linear force outputs as a function of drive current with very low hysteresis due to the "frictionless" nature of the single-piece bearing-less four-bar compliant mechanism.

The x-z positioning tables are each comprised of two orthogonal stacked linear slides driven by stepper motors and miniature precision ACME drive screws with embedded motor drive controllers for each motor, based upon the bipolar drivers we have employed elsewhere (Dennis et al., 2003). The x-position (horizontal movement) is detected using a linear slide potentiometer, allowing placement accuracy better than 0.1 mm over a horizontal movement range of 30 mm for each x-axis mechanism. Working in mirror opposition, the two x-axis slides thus allow a total tip separation up to 60 mm. The z-position (vertical movement) is similarly configured, but with an optical slit detector to determine the vertical "HOME" position, which is the maximum vertical position with the tips withdrawn to their greatest height within the device. The position of the vibrating tips is detected by non-contacting optical displacement sensors, one for each tip, similar in configuration to ones we have previously employed in precision optical force transducers (Dennis and Kosnik, 2002). When the tips are not being driven, the optical position sensors can act as a highly-sensitive contact sensor. By employing the optical position sensor, the tips can be driven to contact the skin, and the contact force of each tip can be adjusted so that they are either equal or different by a known amount, because the spring constant of the VCA four-bar linkage mechanisms is identical.

The electronics were designed using free CAD software from ExpressPCB

(www.expresspcb.com). The printed circuit boards were manufactured using the resulting CAD files, also by ExpressPCB. The electronics employ 5 Microchip microcontrollers; four as dedicated motor controllers for the stepper motors and one as a central controller for the entire system. The hybrid circuit includes signal amplifiers for the position sensors, an analog controller to allow either "force" or "position" control of each VCA motor and tip, a tunable analog PID controller for position control of each tip, and a bipolar push-pull high-current op-amp output stage to drive each VCA motor. This configuration allows each VCA motor to be positioned and driven independently, while coordinated in terms of relative position (x-axis separation between the tips), tip-to-skin mechanical preload, tip vibration amplitude, frequency content, and phase.

The user interface is flexible, allowing several modes of operation. In the simplest mode, used for this series of experiments, a 40-pin ribbon cable connects the internal control logic and analog waveform circuitry directly to a National Instruments data acquisition system (NI DAQ USB-6251). Tip x and z positions, feedback adjustment, and tip vibration waveforms are generated by a laptop operating NI LabVIEW 7.1 which interfaces to the device using the parallel data cable via the NI DAQ system. In the second configuration, not used in this study, the stimulator system interfaces directly with the laptop via USB, and the intervening NI DAQ system and parallel cable are not needed. The first, simpler configuration was employed in this

study because of the ease and convenience of developing tip stimulation waveform protocols using the NI DAQ analog output functions.

Sample plots of driving input voltages delivered by the NI DAQ analog output to the CM-1 vs. the displacement output, as detected by the optical sensors on the CM-1 unit, are shown in Figure 2. The optical sensors were calibrated using a high-resolution linear variable displacement transducer. Note that in panel A, the 10 Hz sinusoid driving input waveform is nearly identical to the displacement output waveform measured. Similar results are observed in panel B for a 25 Hz sinusoid (the frequency used in all of our amplitude discrimination studies). Panels C and D of Figure 2 illustrate the relationship between a 25 Hz driving input voltage and displacement output across a range of peak amplitude voltages when the CM-1 is operated in position mode. Note that although the relationship is close to linear for both loaded and unloaded conditions in Panel C (pre-calibration data shown for comparison), it is nearly identical for these two conditions after calibration of the device as shown in Panel D.

Experimental Procedure

Twenty subjects (20-32 years in age), who were naïve both to the study design and issue under investigation participated in this study. All procedures were reviewed and approved in advance by an institutional review board.

A two-alternative forced-choice (2AFC) tracking protocol was used to evaluate the amplitude discriminative capacity of each of 20 subjects. Initially, the two probe tips (5 mm diameter) were positioned 30 mm apart along a transversally oriented linear axis along the hand dorsum (Figure 3). At the start of each run, the two probe tips were driven towards the skin until each tip registered a force of 0.1 g, as determined by a closed-loop algorithm in the CM-1 stimulator feedback system. The tips were then further indented into the skin by 500 μ m to insure good contact with the skin. A vibrotactile test stimulus (ranging between 105-200 μ m peak-to-peak amplitude at 25 Hz; initial condition set at 200 μ m) was delivered simultaneously with a vibrotactile standard stimulus (100 μ m peak-to-peak amplitude at 25 Hz). The loci of the test and standard stimuli were randomly selected on a trial-by-trial basis. Stimulus duration was 0.5 sec, followed by subject response (subject was queried to select the skin site that received the most intense stimulus) and a 5 second delay before onset of the next trial.

During the initial experimental design, a run consisted of 30 trials with a 2 up / 1 down protocol throughout the block, indicating that two correct responses were required before the test amplitude was decreased by a step of 10 µm. The average performance across all subjects with the 2 up / 1 down paradigm is shown in Figure 4 (open square plot). In order to effectively reduce the experimental runtime, the protocol was modified to use a 1 up / 1 down tracking paradigm for the first 10 trials, allowing a single correct response to cause a reduction in test amplitude by a step. Tracking using the 2 up / 1 down algorithm was used after the first 10 trials, (requiring two correct answers for a reduction in test amplitude). The average plot for this modified protocol is also shown in Figure 4 (closed circle plot), and it demonstrates that modification of the protocol did not result in a significant difference in performance (ANOVA; p > 0.01). In addition, only 20 trials were required for a baseline performance level to be obtained, thus allowing an amplitude discrimination threshold to be determined much quicker. All subsequent tracking plots displayed in this report were acquired using the modified tracking protocol. Amplitude discrimination was tracked for four conditions of inter-stimulus probe distance (distance between the two probe tips): 5, 10, 20, and 30 mm. In addition, the results obtained from these different conditions of inter-probe distance tracking were compared with data obtained by experimental runs in which several conditions of inter-probe distance are delivered either simultaneously or sequentially. More specifically, after the first block of 20 trials of tracking with the stimulus probes 30 mm apart, a second block of tracking is continued with the probe tips at 25 mm. The initial conditions for the second block are the final conditions

of the first block. Subsequent blocks at 20, 15, 10 and 5 mm are delivered. The process for a second experimental run was repeated with the same conditions of inter-probe distance with the exception that the stimuli were delivered sequentially rather than simultaneously.

RESULTS

A two-alternative forced-choice (2AFC) tracking protocol was used to assess amplitude discrimination between two simultaneous 25 Hz vibratory stimuli under different conditions of inter-probe distance. Four distances were used: 5, 10, 20, and 30 mm.

The results averaged across all subjects are shown in Figure 5. At large inter-stimulus distances (20 and 30 mm; well beyond the two point limen for 25 Hz vibrotactile stimuli delivered to the hand dorsum; (Tannan et al., 2005a;Tannan et al., 2005b)), subjects were consistently able to track to an amplitude difference of approximately 25 μ m (125 μ m test versus 100 μ m standard amplitude). Note that when the distance was reduced to 10 mm and 5 mm, performance leveled off at much higher difference amplitudes of nearly 50 μ m and 65 μ m, respectively. ANOVA and two-sample t-testing indicate that the means for the 30 and 20 mm conditions are not significantly different from one another (p > 0.01) but are significantly different from the mean under the 10 mm condition, which is also significantly different from the mean under the 5 mm condition (p < 0.01). To summarize, simultaneous amplitude discrimination becomes worse when the two stimuli are positioned relatively closely (within or near the two-point limen).

With the intention of reducing experimental runtime as well as effectively demonstrating the functional capability of the CM-1 stimulator, the protocol was modified in which multiple inter-stimulus distances were tracked during a single run. The probe tips were initially spaced 30 mm apart. After the first 20 trials, the distance was reduced in steps of 5 mm every 6 trials until a minimum distance of 5 mm was reached (50 trials total). The results, shown in Figure 6, are consistent with the previous protocol in that amplitude discrimination tracks to values within each block for the different inter-probe distances that are very similar to those values shown in Figure 5. In short, as the probes are moved closer together, amplitude discrimination becomes progressively worse, presumably because the probes are within a subject's ability to discriminate between two points. ANOVA and two-sample t-testing indicate that the means obtained with the previous protocol (see Figure 5) and the modified protocol (see Figure 6), when compared at specific distances, are not significantly different (p > 0.01).

In order to directly determine whether or not the degradation of amplitude discrimination was due to the fact that the inter-probe distance was within or near a subject's two-point discriminative capacity, the experiment was repeated via sequential delivery of the two vibrotactile stimuli. In this experimental run, the test and standard stimuli were presented asynchronously with a 1 sec inter-stimulus interval (order and loci of the test and standard were randomized on a trial-by-trial basis). In the left panel of Figure 7, the averages of the tracking plots across all subjects for both the sequential and simultaneous conditions are compared. When the probe tips were positioned outside the typical two-point limen, subjects were able to discriminate amplitudes at similar levels for both simultaneous and sequential stimuli. However, while amplitude discrimination performance did not change at smaller inter-probe distances when the stimuli were delivered sequentially, performance significantly degraded in the same condition for the simultaneously delivered stimuli. In order to more directly compare performance between the two conditions at varying distances, tracking values from the last three trials at each distance were averaged, shown in the right panel of Figure 7. Note that amplitude discrimination did not change significantly with changes in inter-probe distance under the sequential condition, whereas performance was significantly reduced in a graded fashion under the simultaneous condition when the tips were 15, 10 or 5 mm apart. ANOVA

and two-sample t-testing for the simultaneous condition confirm that the means at distances of 20, 15, 10, and 5 mm are all significantly different (p < 0.01), whereas the means show no difference across all distances for the sequential condition (p > 0.01). For distances of 15, 10, and 5 mm, the means obtained for the simultaneous and sequential conditions are significantly different from one another when compared at the same distance (p < 0.01).

DISCUSSION

The CM-1 tactile stimulating device (described above) allows for simultaneous delivery of skin stimuli from two independently controlled stimulators that are mounted in a small, portable package that can be used on virtually any desktop. In this report, we demonstrated that simultaneous amplitude discrimination tracking is a task that can be completed reliably and efficiently with this system. When the probe tips were positioned outside the two-point limen, the subjects were able to discriminate between vibrotactile amplitudes at a level consistent with that obtained from stimuli delivered sequentially. However, when the probe tips were positioned much closer (inside the two-point limen), test results obtained from sequential delivery of the amplitude discrimination task indicated a significant improvement in performance compared to that with simultaneous delivery of the stimuli. The deviation from the baseline levels obtained for the amplitude discrimination performance task as the interprobe distance decreases could provide a basis for an objective measure of two-point discrimination. To date, two-point discrimination measures have relied on a subject's perceptual assessment of whether or not a two-point stimulus is perceived as one or two points. In other words, it has been based solely on subjective criteria.

The deviation point in amplitude discrimination performance between simultaneously and sequentially delivered stimuli is most likely impacted by the same GABAergic mediated mechanisms that are involved in contrast enhancement of repetitive stimuli (LaMotte and Mountcastle, 1975;Juliano et al., 1989;Mountcastle et al., 1967;Kohn et al., 2000;Simons et al., 2005) and show improvement in two-point discrimination with repetitive vibrotactile stimulation (Vierck and Jones, 1970;Tannan et al., 2005a;Tannan et al., 2005b). It is our current working hypothesis that conditions that increase the efficacy of lateral inhibition (e.g., longer stimulus durations) will improve a subject's performance at the amplitude discrimination task and would shift the deviation between the simultaneously vs. sequentially collected tracking responses to the right (e.g., Figure 6 at the point where the probe tips are more closely spaced). Decreasing the efficacy of lateral inhibition – either through pharmacological or neurological conditions – would most likely make the differences between the simultaneous and sequential response curves more distinct. Subsequent reports will describe conditions which alter the responses evoked by simultaneous amplitude discrimination.

It is anticipated that the practical use of two independently controlled stimulators – which allows for a significant range of inter-probe distances – should allow for protocol designs to largely focus on centrally mediated mechanisms. With the principle test site located on the hand dorsum (i.e., innervation density is distributed approximately equally between the two test skin sites), and with delivery of supra-threshold stimuli to the two skin sites, subject percepts will be most sensitive to conditions that directly relate to cerebral cortical mechanisms because the two stimuli are simultaneously evoking responses in adjacent and/or near adjacent cortical regions with contributions from the periphery that are, for the most part, equal. For example, although changing the inter-probe distance between simultaneously delivered stimuli increases the amplitude discrimination threshold, there is little difference, if any, on the impact of the stimuli on the skin surface as the probe tips are still engaging distinctly different regions of the skin. In other words, the delivery of supra-threshold simultaneous stimuli significantly reduces the necessity for evaluating the contributions of the periphery to the percept being evaluated because the impact of the stimuli being compared on the periphery is virtually

identical. Similarly, utilization of these supra-threshold stimuli negates the necessity for doing time-consuming and labor-intensive threshold testing as a pre-requisite for each subject before performing an amplitude discrimination task – note that the amplitude discrimination task results in remarkably robust similarities across a large number of subjects. It should be noted that the device can be applied to a number of skin sites other than the hand dorsum, and subtle changes in the housing would allow for application of the device on virtually any body part.

The CM-1 tactile stimulator system, by demonstrating its capability of performing simultaneous amplitude discrimination at various inter-probe distances, has the obvious capability of performing other types of stimulus discrimination tasks that have been described in the literature. Two point discrimination (Vierck and Jones, 1970; Tannan et al., 2005a;Tannan et al., 2005b), measures of spatial acuity (Tannan et al., 2006;Tommerdahl et al., 2007), frequency discrimination (LaMotte and Mountcastle, 1975), temporal order judgment (Fiorio et al., 2003), and changes in responsivity with adaptation (Goble and Hollins, 1993; Tommerdahl et al., 2005c) are all examples of methods which could be easily implemented and/or modified with this device and could prove useful as sensory diagnostics in a number of clinical and/or clinical research settings. A number of features of this tactile stimulator system make it unique from previously reported devices, and these features will have a positive impact on its potential use in such non-laboratory settings. First, the device's size and portability allow it to be located at sites convenient to the subject and/or the researcher (clinics, offices, retirement community centers, other research environments, etc.). Second, automated detection of the skin surface and monitoring contact force during an experimental session greatly reduces the setup time for an experimental session and removes the requirement for subjects' hands or forearms to be restrained. Third, it is anticipated that the practical use of two independently positioned and controlled stimulators should allow for unique protocol designs that could address issues previously difficult to investigate. Fourth, the rapidmanufacture design of the system will allow multiple systems to be fabricated and placed at a large number of test sites for relatively low construction cost. Fifth, the protocols that we are currently developing (such as the one described in this report) are designed with the objective of being both fast (1-5 minutes) and efficient. Implementation of such standardized protocols, developed with the above-described sensory diagnostic system, at a large number of research and clinical research venues would allow for not only data collection from a significant number of subjects, but from a diverse set of populations, many of which could have known neurological deficits. The knowledge obtained from mining data sets obtained with such standardized measures could have a very positive impact on current understanding of the cortical-cortical mechanisms involved in a number of neurological disorders.

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Fig 1.

Images of the Cortical Metrics (CM-1) stimulator. Left panel: Front view of the stimulator with a clear panel to allow visibility of internal components. Right panel: Probe tips detect the surface of the skin automatically (insert: close-up view).

Tannan et al.

Page 10



Fig 2.

Sample plots of driving input voltages delivered by the NI DAQ analog output to the CM-1 vs. the displacement output. **A**) The 10 Hz sinusoid driving input waveform is nearly identical to the displacement output waveform measured. **B**) Similar results are observed for a 25 Hz sinusoid, the frequency used for all stimuli delivered in this study. **C**) The relationship between a 25 Hz driving input voltage and displacement output across a range of peak amplitude voltages when the CM-1 is operated in position mode before calibration. **D**) Same as in Panel C, but after calibration of the device.



Fig 3.

Schematic of the protocols used in the amplitude discrimination experiments. Two modes of stimulus delivery were employed: simultaneous and sequential. In each trial of the simultaneous mode, two 25 Hz vibrotactile stimuli, the standard (S) and test (T), were delivered at the same time for 0.5 sec. A 5 sec delay (including subject response interval (RI)) was imposed before onset of the next trial. In the sequential mode, the standard or test was delivered to one of the two sites (randomly selected) for 0.5 sec. A 1 second inter-stimulus-interval preceded the next 0.5 sec stimulus and a 5 sec delay followed which preceded onset of the next trial.





Comparison of tracking paradigms. Subject performance is tracked with the 2 up / 1 down protocol (open square plot) and compared with a modified tracking protocol (closed circle plot; 1 up / 1 down tracking for the first 10 trials and 2 up / 1 down for the last 10 trials).

Tannan et al.



Fig 5.

Comparison of amplitude discrimination for different inter-probe distances. Average amplitude discrimination performance across all subjects for four different inter-probe distances (5, 10, 20, and 30 mm) are plotted. Note that there is no significant difference in performance between the 20 and 30 mm conditions (ANOVA, p > 0.01), but the means for the 20 and 30 mm conditions are significantly different from the mean under 10 mm condition. There is also a significant difference between the 5 and 10 mm conditions (*ANOVA and two-sample t-test, p < 0.01).



Fig 6.

Average of the amplitude discrimination tracking data across all subjects for an experimental protocol in which six inter-probe distances were tracked during a single run. The probe tips were initially spaced 30 mm apart for the first 20 trials after which the distance was reduced in steps of 5 mm every 6 trials until a minimum distance of 5 mm was reached. Note that the baseline performance remains relatively the same for 30, 25 and 20 mm.



Fig 7.

Direct comparison of simultaneous vs. sequential amplitude discrimination. Left panel: Average of the tracking plots across all subjects for the simultaneous condition (closed circles) and sequential condition (open circles). Right panel: The comparison of thresholds measured by averaging the tracking values from the last three trials at each inter-probe distance under sequential and simultaneous conditions. Note that there is a significant difference between the simultaneous and sequential conditions at distances of 15, 10, and 5 mm (*ANOVA and two-sample t-test, p < 0.01). Thresholds within the simultaneous condition are significantly different at distances of 20, 15, 10, and 5 mm (**ANOVA and two-sample t-test, p < 0.01).