NOVEL SENSORY TESTING METHODS FOR THE QUANTITATIVE ASSESSMENT OF CORTICAL-CORTICAL INTERACTIONS

by
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ABSTRACT

VINAY TANNAN: Novel Sensory Testing Methods for the Quantitative Assessment of

Cortical-Cortical Interactions

(Under the direction of Dr. Mark A. Tommerdahl)

Traditional tactile sensory testing has relied heavily on delivery of single-site stimuli to the skin and querying test subjects on various qualities of those stimuli. While these methods are effective in making measures that characterize the peripheral nervous system, they lack in quantitatively assessing centrally mediated disorders of the nervous system. Additionally, the models from which the developments of such peripherally-based protocols originate are based more on historical precedence of prior techniques than on a characterization of the central nervous system. This thesis describes the development of not only novel methods for delivering multi-site tactile stimuli, but a novel approach for sensory testing based on models derived from measures of neural population response yielded from *in-vivo* and *in-vitro* animal experimentation.

During the course of this study, two separate stimulators were designed and fabricated. The first, referred to as the "Two-Point Stimulator" (TPS), was a prototype developed to improve upon previously existing methods for delivering vibrotaction during psychophysical and physiological experimentation. To test the device, tracking protocols were used to assess the ability of human subjects to discriminate and localize between two

near-adjacent skin sites under stimulus conditions of varying amplitude, frequency, location, and duration. Data collected were consistent with previously published reports, suggesting that one possible use of the device would be to provide a means for improved measures of spatio-tactile acuity. These studies were repeated on subjects with autism resulting in significant differences in performance from that of the normal population. Correlating data obtained from these psychophysical experiments with cortical measures, acquired primarily with optical imaging and neural recording techniques in animal experimentation, has allowed us to develop a better understanding of the cortical dynamics involved in somatosensory processing. A second stimulator fabricated during this period, the CM-1 (Cortical Metrics – Model #1), improves considerably upon the TPS, most notably in portability, cost, and functional capability. Current ongoing experimentation using this novel device allows an improved means for measuring tactile sensibility and assessing differences in cortical information-processing strategies between normal healthy control populations and populations with various neurological disorders, in both research and clinical settings.

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CHAPTER 1

INTRODUCTION

Our systems neuroscience laboratory investigates mechanisms by which different regions of the cerebral cortex interact. The psychophysical experiments described in this thesis were designed based on the results of our other studies done in animal models (both *in-vivo* and *in-vitro*), in which a number of significant findings were made regarding the spatial-temporal integration of information processing in primary sensory cortex when stimuli are presented to the skin. These findings suggest that the cortical-cortical interactions observed in sensory cortex played a major role in sensory perception.

Breaking from decades of tradition, we have developed a new vibrotactile stimulus device (described in this report) that enables objective evaluation of the elaborate neuro-anatomical connectivity subservient to the neuronal communication between adjacent and near-adjacent regions within sensory cortex. Utilizing two vibrotactile stimulus points on the skin and controlling the distance between these points, as well as the stimulus frequency spectrum, amplitude and phase, we directly assess the intra-cortical functional connectivity between well-established regions in primary sensory cortex. This network is widely recognized to be essential to normal sensory function. The tests that we have developed appear exquisitely sensitive to the status of a number of mechanisms (i.e., neurotransmission mediated by the inhibitory neurotransmitter gamma aminobutyric acid (GABA) and by N-methyl-d-aspartate (NMDA) receptors, and interactions or interdependencies between

neurons and glial cells). These mechanisms currently are believed to play major roles in the disorders of sensory cortical information processing that all too frequently compromise the quality of life in a variety of subject populations.

The published scientific literature along with our preliminary findings suggest that neurological disorders such as Autism, Schizophrenia, Alzheimer's Disease, Chronic Pain and Traumatic Brain Injury exhibit altered cortical functionality which can be detected with dynamic sensory testing. Specifically, the fundamental building blocks necessary for normal cortical information processing are damaged in such a way as to limit the functional connectivity within and between the fundamental components of cortical circuitry. It is important to note that the methods developed in this work are a radical departure from the traditional "peripheral sensory threshold testing," which has been ineffective thus far as a general neurological diagnostic tool for the clinician.

Our preliminary data on human subjects has been carried out in parallel with animal studies, involving both *in-vitro* brain slices and direct cortical stimulation as well as *in-vivo* neurophysiological signals evoked by natural skin stimulation. This unique combination of experimentation has allowed us to develop and test mechanistic hypotheses (e.g., NMDA receptor antagonists show a dose-dependent effect and NMDA receptor functionality has been shown to change under a number of different conditions). Furthermore, preliminary studies have enabled us to verify the effectiveness of these new non-invasive methods for testing human subjects. Because the prototype testing approach that we have developed appears sensitive to a number of prescription drugs currently commonly prescribed, the quantitative assessment protocols may enable non-invasive and objective assessment of the impact that these drugs (or drug combinations) have on cerebral cortical function.

This thesis describes the process of developing a robust quantitative sensory testing device and protocols designed with several features in mind. All of which makes them completely non-invasive, quick to administer (~ 1-5 minutes), relatively simple for subjects to understand and requiring very minimal amount of training for the diagnostician. Additionally, the portable device has been designed for rapid manufacture, and can be made readily available at relatively low cost to a large number of researchers. The clinical research tool that we have developed will have a significant impact on a number of research areas which are currently being explored, and will hopefully be used by not only medical researchers, but also by health care providers for objectively and directly measuring central nervous system disorders while assessing the efficacy of various therapies.

CHAPTER 2

DEVELOPMENT OF A NOVEL METHOD FOR DELIVERING TWO-SITE VIBROTACTION TO THE SKIN

A large portion of the work presented in this chapter was completed as a collaborative effort with the following researchers: Dennis RG, Tommerdahl M.

2.1 Justification for Two-Point Stimulator Development

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. Typically, stimuli that can be delivered through mechanical transducers – vertical displacement stimulators such as the one originally described by Chubbuck (1966) – and 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 μm) 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 compare the effects of delivering identical stimuli spaced at variably spaced distances on a trial-by-trial basis, we designed and fabricated the Two-Point Stimulator (TPS) that attaches to the end of a vertical displacement stimulator. Both points of the TPS are driven by the single vertical displacement stimulator and distances between the two sites can be varied on a trial-by-trial basis. The device is described, and in order to test the device, a Bekesy tracking protocol was used to generate preliminary psychophysical data measuring the two-point limen under three different stimulus conditions. Data collected for this first study were consistent with previously published reports, in which the distance between two skin sites was varied manually and spatial acuity improved when the two probes were oscillated (Vierck, 1970; Solomonow, 1977).

2.2 Stimulator Design

The TPS is composed of two independently controlled devices. The first, the Cantek Metatron CS-525 vertical displacement stimulator (Cantek Metatron Corp., Canonsburg, PA), has been used in a number of previously reported studies and its use in our laboratory has been described recently (Tommerdahl, 2005a). The device itself is based on a device first described by Chubbuck (1966). The vertical displacement stimulator delivers a stimulus in the range of 1 - 250 Hz via cylindrical probe. A second device attaches to the vibrating tip of the Cantek, modifying it from the standard single probe tip to two probe tips, each capable of moving independently laterally to set the tip-to-tip spacing (see Figure 2.1).
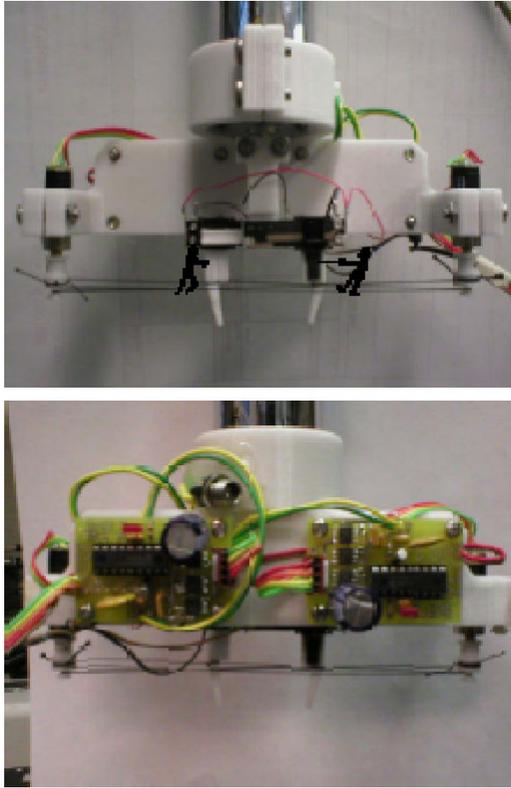
Figure 2.1 Images of the front and back of the Two-Point Stimulator as attached to a vibrotactile stimulator.

A program written in LabVIEW sends digital signals from a computer, through a data acquisition system (National Instruments PCI-6025E), to the TPS. For each of the two tips, two single-bit digital signals are required: one to specify direction of motion, and another to command the tip movement at a pre-set speed. These signals are sent to a custom step motor driver circuit board interfaced with PIC microprocessors that control each of two separate gear-head stepper motors (Micro-Mo model #: AM1020-V-6-65-08 10/1 64:1) mounted on the framework (see Figure 2.1).

As each stepper motor turns in the direction determined by the digital signals, the gear head turns a capstan wound with 2-0 braided silk suture to drive the corresponding probe tip in that direction. The suture runs on a pulley and the capstan on the motor gear

output shaft, and is affixed to the probe tip. A mirror image mechanical arrangement drives the other probe tip. This mechanical arrangement removes the motor mass from the vibrating tip to minimize the tip mass, thus maximizing the vibration bandwidth.
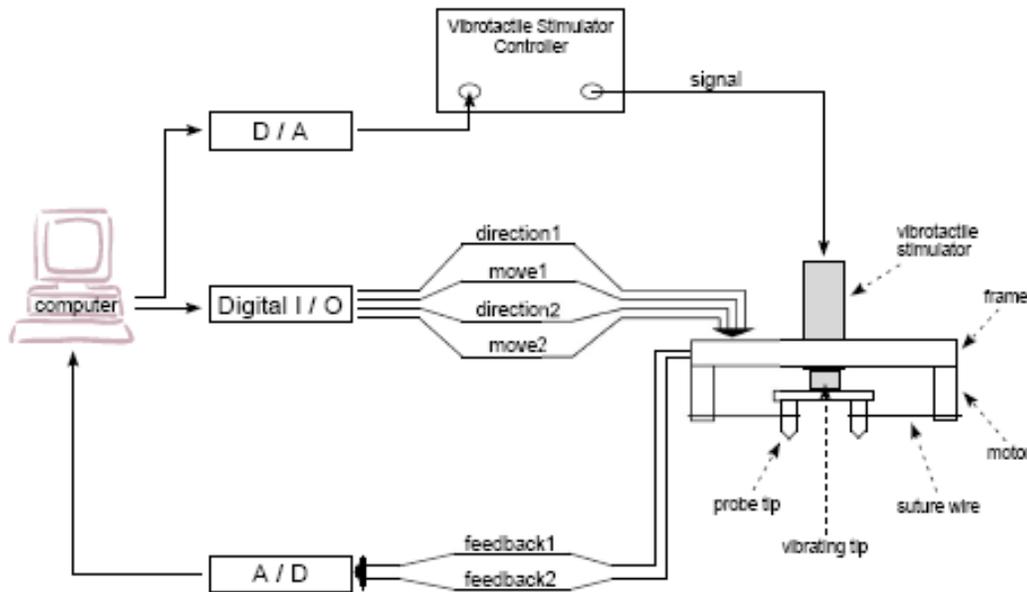
Figure 2.2 Block diagram of control system for the Two-Point Stimulator.

Each probe tip slides on a linear potentiometer (DigiKey P/N: CT2302-ND), which serves as both a linear slide and a linear position sensor (see Figure 2.3). Each linear potentiometer generates an analog voltage linearly proportional to the spatial position of the tip. This position voltage is sampled through a 12-bit A/D as feedback to the computer and processed by an algorithm to indicate the location, in mm, of a probe tip. The two tips are capable of a maximum separation of 40mm and a minimum separation of 0mm (at which the tips are flush). As a tip moves from 0 to 20 mm, the position voltage ranges from 0 to 5 V, respectively.
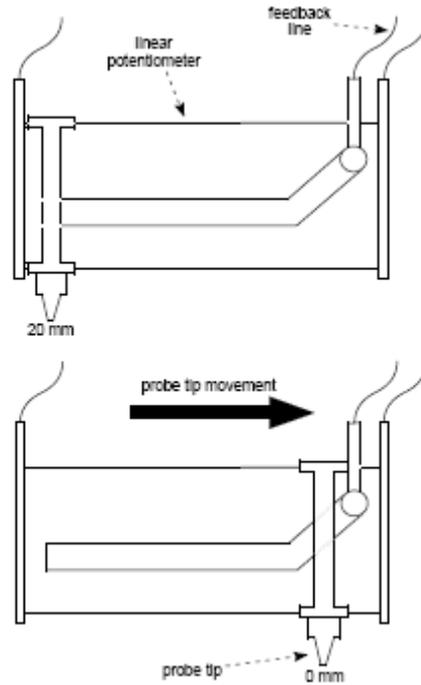
Figure 2.3 Linear potentiometer system. Each probe tip slides on a linear potentiometer, which serves as both a linear slide and a linear position sensor.

The mechanism hardware and attachment fixtures were built using a StrataSys Fusion Deposition Modeler (FDM) (StrataSys model Titan-T1) with polycarbonate material. Solid models were generated using SolidWorks 2004 (professional version) and converted to *.STL files for rapid manufacturing on the FDM. The Stratasys FDM was employed only because it allowed the rapid manufacture of this device. Several prototypes were designed, manufactured, assembled, and tested in less than one week. Though other methods of manufacture, such as the use of traditional chip-forming machining processes, could also produce a functionally identical device, the system development time would have been considerably longer.

Amplitude, frequency, and offset, which can be varied on a trial-by-trial basis, are delivered directly to the vibrotactile stimulator, allowing both probe tips to operate

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simultaneously and with identical displacement. For psychophysical applications, several methods currently used for testing spatial acuity require the investigator to apply stimuli to the subject by hand (e.g., the Two-Point Discrimination test – for recent review of its use, see Lundborg, 2004). This device improves the consistency of the stimulus intensities delivered to the skin, as the two stimuli are always displaced under a single stimulus condition. The separation of the probe tips ranges from 0 to 40 mm and min-to-max separation occurs in 2.5 sec. Percent error of separation, stemming from the voltage output variability of the linear pots, is measured to be ± 1% for any given displacement. Dual foil leaf contacts on each sliding contact of the linear potentiometers eliminates voltage dropout during tip movement as well as during vibration of the head. A key consideration during device design was the amount of weight placed upon the vibrotactile stimulator, as excessive weight on the vibrating tip results in irregular tip displacement. Therefore, all circuitry, including the motors, are mounted on a frame which attaches to the body of the vibrotactile stimulator. The only weight placed upon the vibrating tip is that of the probe tips and the linear pots. This weight load difference from typical probe tips does not significantly affect the performance of the vibrotactile stimulator, this being confirmed by monitoring of the vibrating tip displacement over the frequency range used.

CHAPTER 3

EFFECTS OF OSCILLATION ON TWO-POINT LIMEN

A large portion of the work presented in this chapter was completed as a collaborative effort with the following researchers: Dennis RG, Tommerdahl M.

3.1 Abstract

Current methods for applying two-site vibration 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. The Two-Point Stimulator (TPS) was developed in order to deliver two-point stimuli to the skin at variable distances between the sites of stimulation on a trial-by-trial basis. The apparatus attaches to a vibrotactile stimulator, modifying it from the standard single probe tip to two probe tips. Each of the two probe tips can be independently positioned to set the tip-to-tip spacing. Both points of the TPS are driven by the single vibrotactile stimulator and distances between the two sites can be varied on a trial-by-trial basis. To test the device, a modified Bekesy tracking method was developed and used for two-point limen testing under stimulus conditions of varying amplitude and frequency. Data collected were consistent with previously published reports, suggesting that one possible use of the device would be to provide a means for improved measures of spatio-tactile acuity.

3.2 Methods

Four naïve subjects (20 - 26 years in age) participated in this psychophysical study. All procedures were reviewed and approved in advance by an institutional review board.

Sinusoidal vertical skin displacement stimuli were delivered using the Cantek Metatron CS-525 vertical displacement stimulator (Cantek Metatron Corp., Canonsburg, PA). The stimulator made contact with the skin via the two tips of the Two-Point Stimulator attachment (2.5 cm long, diameter 2 mm) fitted to the terminal end of the moving shaft of the stimulator transducer. An adjustable mechanical arm with lockable joints mounted to a free-standing, rigid platform (fabricated locally) enabled convenient adjustment and maintenance of stimulus position.

The subject was seated in an adjustable dental chair and the right arm was placed on an X-ray bag filled with glass beads. The investigators molded the bag to fit the contours of the subject's arm, and when the subject was comfortable and the arm positioned appropriately to allow unimpeded access of the stimulator to the center of the dorsal surface of the right hand, the bag was made rigid by evacuating it of air (achieved by connecting the bag to a vacuum line). In this way the arm was maintained in a comfortable but stable position for the full duration of the experimental session. The subject was unable to see either the experimenter or the stimulator and stimulus control instrumentation. White noise presented via headphones eliminated potential auditory cues. A micrometer permitted the stimulator transducer and probe assembly to be lowered towards the predefined skin site. The micrometer position at which the digital display on the stimulator controller registered a 0.1 - 0.2 g change in resistive force was interpreted as the point at which the stimulator probes made initial contact with the skin.

A tracking protocol was used to conduct a two-point limen test, which determines the "least two-point separation at which the subject feels (has the subjective impression of) two points," (Johnson, 1981) at the dorsal surface of the right hand. For each run, the two probe tips were initially spaced 30 mm apart. The stimulus signal was delivered from a computer via a D/A (National Instruments PCI-6722) to the vertical displacement stimulator. The stimuli were presented to the skin for 1sec at an offset of 500 μm then completely removed from the skin for 1sec at an offset of -500 μm. The subject was given these two seconds to report feeling one or two points – no button press for one point; button press for two points. The response signal was transmitted back to the computer and processed with an algorithm written in LabVIEW. When two points were detected, the two probe tips moved closer together by a step (1 step = 1 mm); when only one point was detected, the two points moved farther apart by a step.
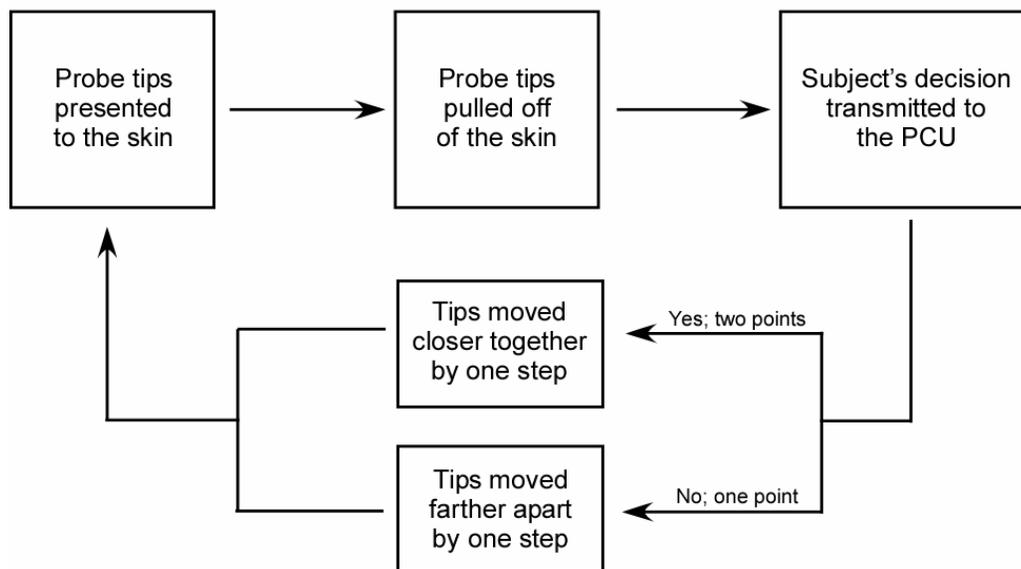
Figure 3.1 Flowchart of the modified Bekesy tracking protocol used to determine the two-point limen of a subject with the Two-Point Stimulator.

The computer controlled the positioning of the probes with a closed-loop control algorithm. The probe tips remained off the skin for the tip movement duration of 1 sec, thus the inter-stimulus interval lasted for a total of 2 sec. This process was repeated until a threshold could be determined, usually around 30 trials, hence a single run took approximately 90 sec. The inter-run interval was 60 sec in duration. The two-point limen was measured under three conditions of amplitude and frequency: static (no vibration), 25 Hz - 100 μm, and 200 Hz - 20 μm (see Figure 3.2). In a session, three runs were conducted, each with one of the aforementioned stimulus conditions. Order of stimulus conditions within a session was randomized and varied for each subject.
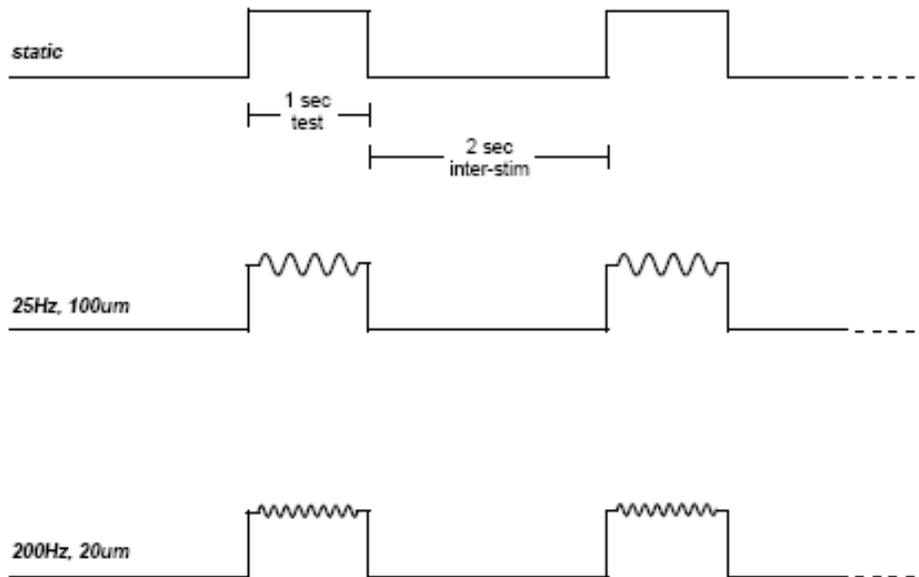
Figure 3.2 Tracking protocol conditions. A tracking protocol was used to conduct a two-point limen threshold test under three conditions of amplitude and frequency: static (no vibration), 25 Hz - 100 μm, and 200 Hz - 20 μm.

A module written in LabVIEW was used for full control of the TPS during experimentation (see Figure 3.3). The user has the ability to control several aspects of the

stimulus delivered by the vertical displacement stimulator, including stimulus amplitude, frequency, duration, inter-stimulus interval, and offset.   TPS controls include initial separation of the two probe tips, step size between trials, and a static/oscillating switch. Other controls on the graphical user interface include a file path and save button for data storage, numerical sampling rate controls, and an array of buttons for starting the program, terminating the program, and clearing graphs and arrays. Several indicators on the screen offer feedback during an experimental run, including a numeric indicator of current tip separation, a graph of separation versus time, a graph of the stimulus waveform, and an LED indicator for the feedback button status.
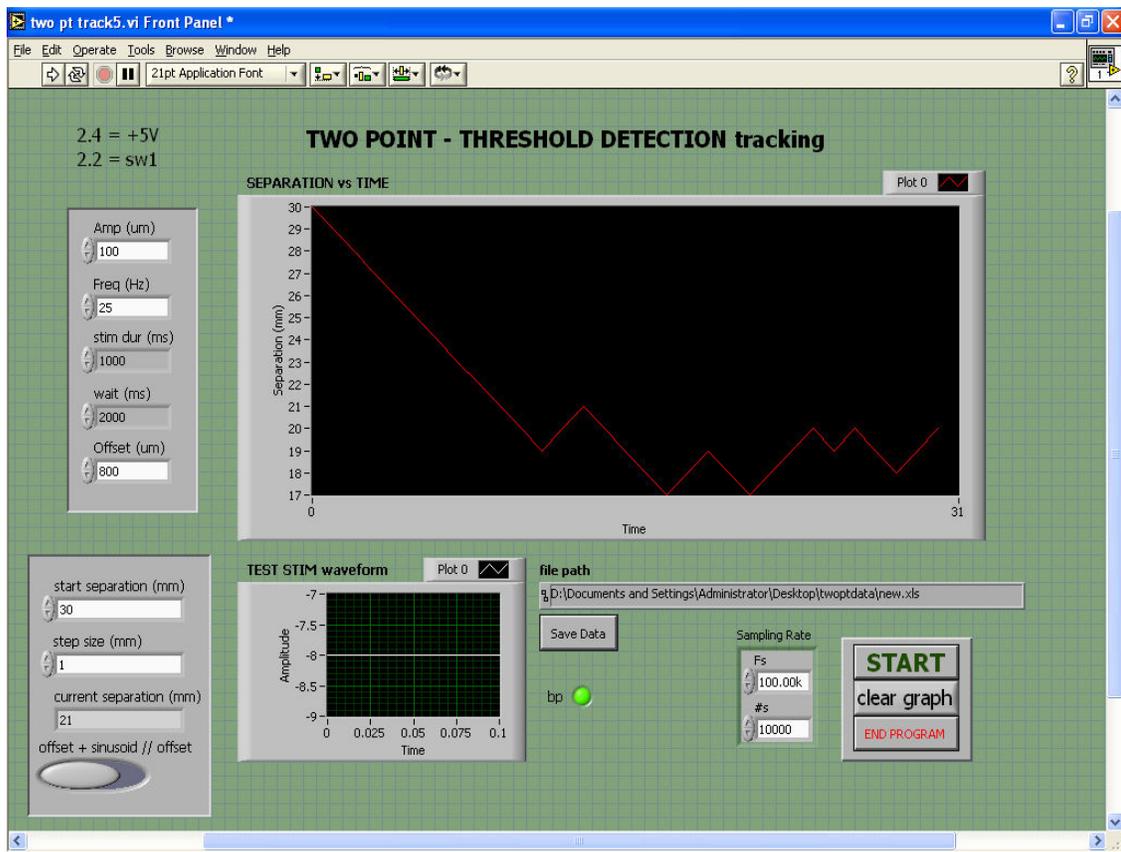
Figure 3.3 Graphical user interface used for two-point tracking throughout experimentation. All programming was done in NI LabVIEW.

3.3 Results

The Two-Point Stimulator (TPS) was used in preliminary psychophysical studies to determine the feasibility of simultaneously delivering vibrotactile stimuli at two independently positioned skin sites. Bekesy tracking algorithms were used to find a subject's two-point limen at the dorsal surface of the right hand. Figure 3.4 shows exemplary results of one session (three runs) for each of the four subjects. In one run, the subject was tracked to a "static" stimulus – a stimulus that did not vibrate at any frequency. In the other two runs tracking was observed while the probes were vibrated at 25 Hz and 200 Hz, respectively. For example, Subject 1 (see Figure 3.4) was unable to detect the presence of two points under the static condition until the separation was increased to around 35 mm, whereas for 25 Hz and 200 Hz vibration the separation required was around 16 mm and 28 mm, respectively. In each case, oscillating the probes (at the same phase) results in the two-point limen being reduced. Additionally, in all sessions of all subjects run thus far, the two-point limen for the 25 Hz run is always less than that acquired for the 200 Hz run, and both the 25 Hz and 200 Hz conditions always yield two-point limen that are less than that obtained in the static condition.
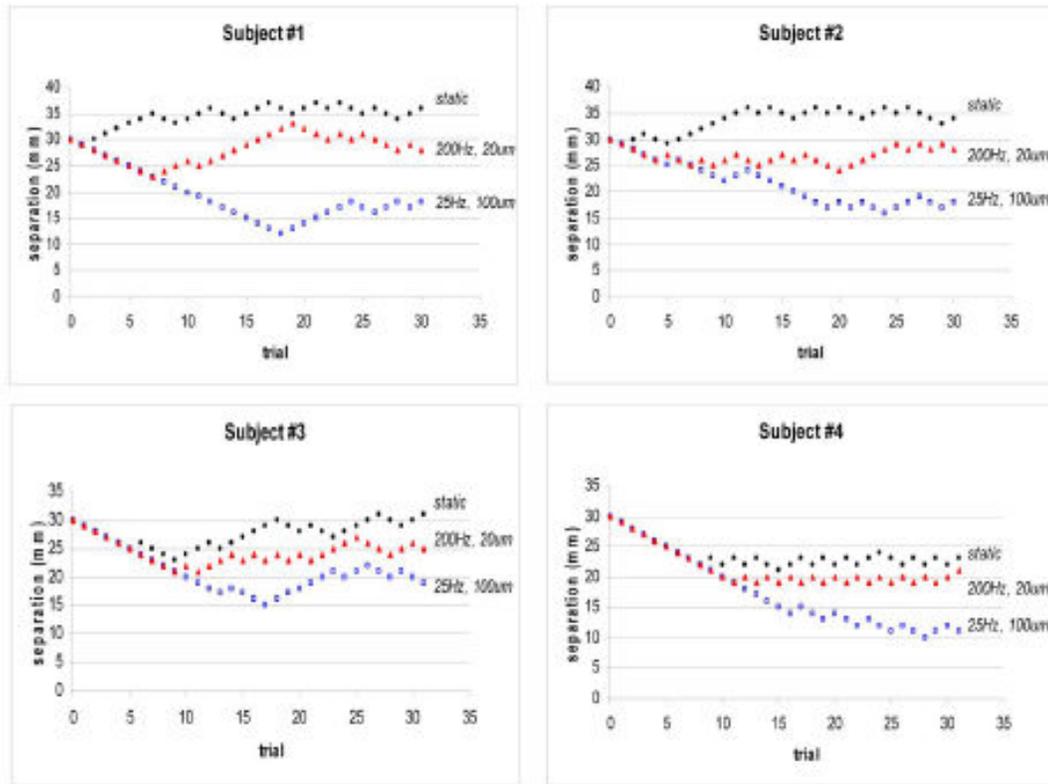
Figure 3.4 Tracking plots from one session for each subject. Tracking data from one session is shown as measured from four subjects. The three traces indicate change in separation (mm) between the two stimuli over time for the stimulus conditions of static, 25 Hz - 100 μm, and 200 Hz - 20 μm. Note that in each case, the 25 Hz condition yields a two point limen < 200 Hz condition < static condition.

To determine the across-subject consistency of the above findings, we averaged the tracking responses to the two point limen from the data acquired from all sessions. In order to adjust for individual differences in sensitivity, the data was normalized to the static condition, primarily because we are most interested in the effect caused by changing the stimulus condition from static to oscillating on the subject's response. Thus, the two-point limen for the static condition is always defined as a "1" and all other distances are plotted as a proportion of the values obtained under the static condition. Figure 3.5 displays the average of two-point limen for all four subjects. Note that the two-point limen was reduced

for the 200 Hz condition, but least (highest spatial acuity) for the 25 Hz condition. In the 25

Hz condition, the two-point limen tracks between 50 and 60% of the static condition,

indicating a 40 to 50% improvement that can be attributed to the oscillation of the probe.
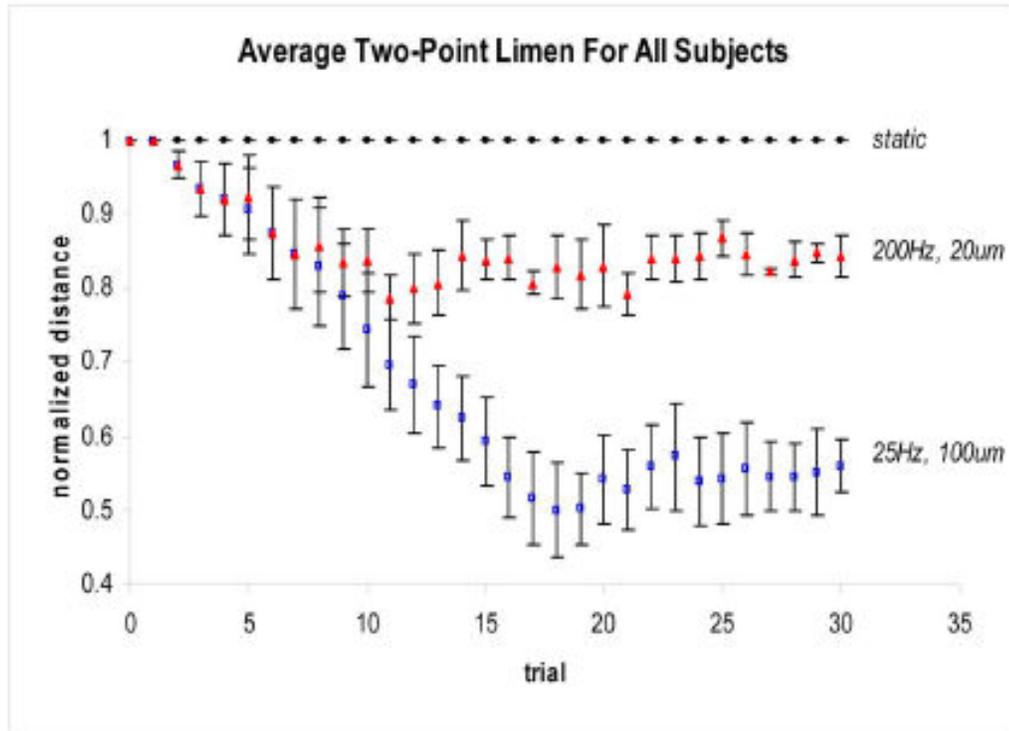
Figure 3.5 Average two-point limen across all subjects. All distances are normalized to the two-point distance recorded under the static condition. Standard error bars demonstrate that across-subject variability for the two-point limen tracking method is fairly consistent.

The results of the tracking experiments are summarized in Figure 3.6, in which the

average of the two-point distance of the last five trials for all subjects is compared. Clearly,

there is a significant reduction in the two-point limen under both the 25 Hz (45% reduction)

and 200 Hz (16% reduction) condition.
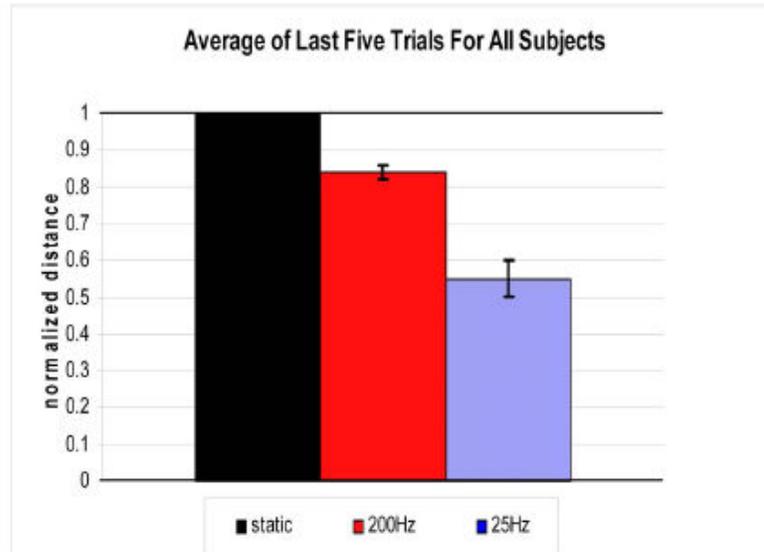
Figure 3.6 Average two-point limen (last five trials) across all subjects. All data is normalized and plotted as a proportion of the distances recorded under the static condition.

3.4 Discussion

The device described in this report has been designed to offer an improved method for the delivery of two-point stimuli to the skin. The apparatus attaches to a vibrotactile stimulator, modifying it from the standard single probe tip to two probe tips, each capable of moving independently laterally to set the tip-to-tip spacing. Both points of the TPS are driven by the single vibrotactile stimulator and distances between the two sites can be varied on a trial-by-trial basis. The device demonstrated capability of repositioning the two probes on a trial-by-trial basis with an inter-trial interval of 2 sec, and the probes were variably separated in increments of 1mm. Since the two probes can be independently positioned, studies that require vibrotactile stimulation to be delivered to dual skin sites at different positions, variable on a trial-by-trial basis, can now be performed. One other device that allows for multiple stimuli to be delivered to the skin was reported by Bensmaia et al. (2004), and the device that they report consists of 400 independently controlled probes arrayed in a

20 by 20 array. However, as spacing of the probes in that device are at 0.5 mm, center to center, the largest probe to probe distance that can be achieved is 10 mm. While the dense probe device is well suited for presenting multiple stimuli at high resolution to the skin, the 10 mm distance is a limitation for researchers who would prefer to deliver stimuli farther apart. Other devices have been described that stimulate multiple sites, but in general, these devices are not appropriate for studies of two-point discrimination (Rinker, 1994; Kaczmarek, 2003).

In order to test the device, a modified Bekesy tracking method was developed and used for two-point limen testing under stimulus conditions of varying amplitude and frequency. Data obtained was similar to that previously published on the subject of improvements of spatial acuity with frequency of stimulation (Vierck, 1970; Solomonow, 1977). Vierck and Jones found that two-point discrimination (TPD) was better when the two points, delivered simultaneously, were vibrated at 300 Hz than under the static condition and TPD was best when delivered at 10 Hz. Currently, the data we have collected from four subjects (back of the hand) has been consistent with the Vierck and Jones study (1970). The two-point thresholds for stimulus conditions are similar and robust for all subjects, as threshold was highest for the static condition, lower for the 200 Hz condition, and lowest for the 25 Hz condition. Thus, although the tracking algorithm in this study was much simpler and quicker than the protocol described by Vierck and Jones (1970) in their TPD assessment, primarily due to the automation of the stimulus localization, the results are in general agreement.

The TPD test is widely used for measuring spatio-tactile acuity and can be especially useful for assessing sensibility and innervation density. Currently, the common method of

applying a TPD test is through use of handheld instruments (i.e. two-point calipers, "two-point discriminator"), of which a user applies to the subject by hand during an experiment. Lundborg and Rosen (2004), in a recent review of TPD methods, suggest several concerns with current methods for assessing TPD testing, and these concerns originate primarily from human error in administering the TPD or applying inconsistent pressure to the two stimulus locations, inconsistent amplitude of stimuli and non-synchronous application of the two points. In addition to problems with administration of the TPD test, Craig and Johnson (2000) found a number of problems with both the subjective and objective methods of subjects reporting their responses. In this study, the subjects were presented with two points at varying separation and asked to report feeling one or two points. Through this subjective method, a tracking protocol allows two-point limen to be determined. It would also be possible, with this device, to conduct experiments that would utilize an objective method, using a two-alternative forced choice protocol (subject would be presented with two points in one interval and one point in the other, and then asked to report the interval that included two points). Regarding the subjective method, such as that used in this study, a severe problem is that perception of two points gradually changes as the point separation varies. Also, variability in discrimination is usually high within and between subjects. Two important drawbacks to using the objective method were noted by Craig and Johnson (2000) as well. First, a previous study by Johnson and Phillips (1981) had reported the two-point threshold on the fingertip to be zero, whereas a number of psychophysical studies using the objective method had reported threshold to be greater than zero. To explain this inconsistency, the objective method must be measuring a phenomenon other than spatial resolution. Second, other cues (mainly intensity cues) created by the objective method allow subjects to

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discriminate between one and two points, thereby confounding the actual measure of spatial acuity. Although these concerns are important to consider, our goal in designing the Two-Point Stimulator device was simply to offer a more efficient way to deliver two-point stimulation to the skin. We believe that better methods for testing spatio-tactile acuity can be derived from such stimulators, as a number of errors, particularly those that are human in origin, can be eliminated with this automated device.

CHAPTER 4

STIMULUS-DEPENDENT EFFECTS ON SPATIAL TACTILE ACUITY

A large portion of the work presented in this chapter was completed as a collaborative effort

with the following researchers: Dennis RG, Tommerdahl M.

4.1 Abstract

Background

Previous studies have shown that spatio-tactile acuity is influenced by the clarity of

the cortical response in primary somatosensory cortex (SI). Stimulus characteristics such as

frequency, amplitude, and location of tactile stimuli presented to the skin have been shown to

have a significant effect on the response in SI. The present study observes the effect of

changing stimulus parameters of 25 Hz sinusoidal vertical skin displacement stimulation

("flutter") on a human subject's ability to discriminate between two adjacent or near-adjacent

skin sites. Based on results obtained from recent neurophysiological studies of the SI

response to different conditions of vibrotactile stimulation, we predicted that the addition of

200 Hz vibration to the same site that a two-point flutter stimulus was delivered on the skin

would improve a subject's spatio-tactile acuity over that measured with flutter alone.

Additionally, similar neurophysiological studies predict that the presence of either a 25 Hz

flutter or 200 Hz vibration stimulus on the unattended hand (on the opposite side of the body

from the site of two-point limen testing – the condition of bilateral stimulation – which has

been shown to evoke less SI cortical activity than the contralateral-only stimulus condition) would decrease a subject's ability to discriminate between two points on the skin.

Results

A Bekesy tracking method was employed to track a subject's ability to discriminate between two-point stimuli delivered to the skin. The distance between the two points of stimulation was varied on a trial-by-trial basis, and several different stimulus conditions were examined: (1) The "control" condition, in which 25 Hz flutter stimuli were delivered simultaneously to the two points on the skin of the attended hand, (2) the "complex" condition, in which a combination of 25 Hz flutter and 200 Hz vibration stimuli were delivered to the two points on the attended hand, and (3) a "bilateral" condition, in which 25 Hz flutter was delivered to the two points on the attended hand and a second stimulus (either flutter or vibration) was delivered to the unattended hand. The two-point limen was reduced (i.e., spatial acuity was improved) under the complex stimulus condition when compared to the control stimulus condition. Specifically, whereas adding vibration to the unilateral two-point flutter stimulus *improved* spatial acuity by 20 to 25%, the two-point limen was *not* significantly affected by substantial changes in stimulus amplitude (between 100 - 200 μm). In contrast, simultaneous stimulation of the unattended hand (contralateral to the attended site), *impaired* spatial acuity by 20% with flutter stimulation and by 30% with vibration stimulation.

Conclusions

It was found that the addition of 200 Hz vibration to a two-point 25 Hz flutter stimulus significantly improved a subject's ability to discriminate between two points on the

skin. Since previous studies showed that 200 Hz vibration preferentially evokes activity in cortical area SII and reduces or inhibits the spatial extent of activity in SI in the same hemisphere, the findings in this paper raise the possibility that although SI activity plays a major role in two-point discrimination on the skin, influences relayed to SI from SII in the same hemisphere may contribute importantly to SI's ability to differentially respond to stimuli applied to closely spaced skin points on the same side of the body midline.

4.2 Introduction

LaMotte and Mountcastle (1975; 1979) asserted that the capacity of a subject to accurately localize a flutter stimulus on the skin is determined by the locus and clarity of the flutter-evoked neuron population response within the topographically organized SI network. If this is the case, then the ability of a subject to discriminate between two points would improve if the locus of the responses in SI to the stimuli at the two corresponding skin sites were more clearly defined – i.e., if the spatial extent of the response in SI to a point stimulus was limited or reduced. Previously, observations by Tommerdahl and colleagues demonstrated that the SI response to a 200 Hz vibration stimulus, and in particular a complex stimulus (one comprised of both flutter and vibration), is reduced when compared to the response to flutter alone (Tommerdahl, 1999a; 1999b; 2005a; Whitsel, 2001). Thus, based on the effect that same-site vibration has on the SI response to flutter, we were led to the prediction that vibration, if presented simultaneously at the same sites as two-point flutter stimuli (i.e., as a complex stimulus comprised of 25 Hz and 200 Hz components), would *improve* a subject's ability to discriminate between two points. Similarly, recent findings comparing the SI cortical activity evoked by different conditions of contralateral, ipsilateral and bilateral stimulus conditions in the cat (Tommerdahl, 2005b) and monkey (unpublished observations) led us to the prediction that a subject's two-point limen would increase, indicating reduced spatial acuity, with the addition of a stimulus to the unattended hand. In the second study discussed in this report, we directly test these predictions and find that vibration, when added to a flutter stimulus, does, in fact, improve spatial acuity, and additionally, the presence of a mechanical stimulus (either flutter or vibration) to the hand contralateral to the test site degrades a subject's ability to discriminate between two points.

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4.3 Methods

Five naïve subjects (21 - 32 years in age) participated in the second psychophysical study. All procedures were reviewed and approved in advance by an institutional review board.

Sinusoidal vertical skin displacement stimuli were delivered using the Cantek Metatron CS-525 vertical displacement stimulator (Cantek Metatron Corp., Canonsburg, PA). The stimulator made contact with the skin via the two tips of the Two-Point Stimulator (TPS) attachment (2.5 cm long, diameter 2 mm) fitted to the terminal end of the moving shaft of the stimulator transducer. The TPS is previously described in this report in detail (also see Tannan, 2005a). An adjustable mechanical arm with lockable joints mounted to a free-standing, rigid platform (fabricated locally) enabled convenient adjustment and maintenance of stimulus position. A second identical Cantek stimulator, implemented in trials that required bilateral stimulation, was fitted with a single 2 mm diameter probe tip and positioned on the hand opposite the TPS in a similar fashion.

The subject was seated in a chair with arms placed comfortably on a table surface. Both arms were placed on X-ray bags filled with glass beads. The investigators molded the bags to fit the contours of the subject's arms, and when the subject was comfortable and the arms positioned appropriately to allow unimpeded access of the stimulator to the center of the dorsal surfaces of each hand, the bags were made rigid by evacuating them of air (achieved by connecting the bag to a vacuum line). In this way the arms were maintained in a comfortable but stable position for the full duration of the experimental session. The subject was unable to see either the experimenter or the stimulator and stimulus-control instrumentation. White noise presented via headphones eliminated potential auditory cues.

A micrometer permitted the stimulator transducers and probe assembly to be lowered towards the predefined skin sites. The micrometer position at which the digital display on the stimulator controllers registered a 0.1 - 0.2 g change in resistive force was interpreted as the point at which the stimulator probes made initial contact with the skin.

As in the previous experiment, a tracking protocol was used to conduct a two-point limen test, which determines the "least two-point separation at which the subject feels (has the subjective impression of) two points," (Johnson, 1981) at the dorsal surface of the right hand. The hand dorsum was chosen because the innervation density at this site coincided with optimal resolution and separation capabilities of the TPS, and also because the surface is relatively flat, reducing confounds of skin curvature present at other potential sites of stimulation. Previous studies indicate that response to tactile acuity tests on the hand dorsum is similar to that on the fingertip, suggesting the dorsum to be a suitable site for such tests as well (Schlereth, 2001). The subject was instructed to attend to the two-point stimulus presented by the TPS on the tested hand throughout experimentation. For each run, the two probe tips were initially spaced 30 mm apart. The stimuli were presented to the skin simultaneously for 1sec at an offset of 500 μm then completely removed from the skin for 1sec at an offset of -500 μm. The subject was given these two seconds to report feeling one or two points using a footswitch – no press for one point; a single press for two points. When two points were detected, the two probe tips moved closer together by a step (1 step = 1 mm); when only one point was detected, the two points moved farther apart by a step. The probe tips remained off the skin for the tip movement duration of 1 sec, thus the inter-stimulus interval lasted for a total of 2 sec. This process was repeated until a threshold could be determined, usually around 30 trials, hence a single run took approximately 90 sec. The

inter-run interval was 60 sec in duration. The two-point limen was measured under four conditions of frequency and amplitude: unilateral 25 Hz – 100 μm, unilateral 25 Hz – 100 μm + 200 Hz – 20 μm ("complex"), bilateral stimulation of 25 Hz – 100 μm on both hands, and bilateral stimulation of 25 Hz – 100 μm on the attended hand and 200 Hz – 20 μm on the opposite unattended hand. In a session, four runs were conducted, each with one of the aforementioned stimulus conditions. In the complex and bilateral conditions, stimuli were applied by a single timing mechanism and thus were presented to the skin in phase and synchrony. Order of stimulus conditions within a session was randomized and varied for each subject.

4.4 Results

Bekesy tracking algorithms were used to find a subject's two-point limen at the dorsal surface of the right hand under four different stimulus conditions. Exemplary results for a single session (four runs) of a subject are shown in Figure 4.1. The two-point limen of the subject was tracked for two points delivered simultaneously and oscillated at 25 Hz on the attended hand (AH). The data presented indicates that under this condition the subject was able to detect the presence of two points at a separation of approximately 19 mm (average response for the last five trials). In a second run (the "complex" stimulus condition), the two-point limen was tracked under identical conditions as the first run, with the exception that the 25 Hz stimulus waveform was delivered with an additional 200 Hz vibration on the attended hand (see Methods). The addition of the 200 Hz vibration to the 25 Hz flutter resulted in a decrease in the two-point limen to approximately 16.4 mm. In the two other conditions, the two-point limen was tracked to a two-point 25 Hz flutter stimulus on the attended hand,

under identical conditions as the first run, but with the addition of a simultaneous 25 Hz flutter or 200 Hz vibration stimulus to the opposite, unattended hand (UH). Interestingly, in both cases, stimulation of the unattended hand impaired the subjects' ability to discriminate between two points on the attended hand, and thus, the two-point limen actually increased to values of approximately 22 mm and 24 mm for 25 Hz and 200 Hz unattended conditions, respectively. To summarize, the detection of two points presented simultaneously with flutter was *improved* with same-site vibration and *degraded* with the addition of either a flutter or vibration stimulus on the opposite, unattended hand.
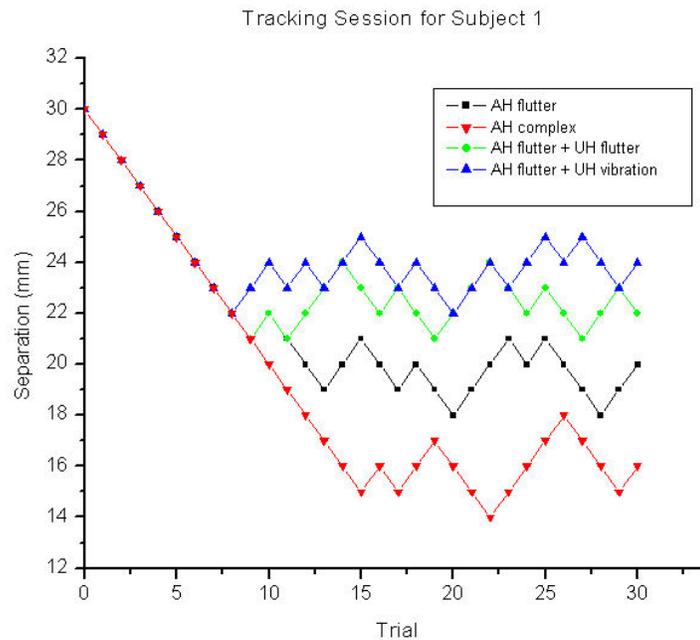
Figure 4.1 Tracking data for one session for an exemplary subject. A tracking protocol was used to conduct a two-point limen threshold test. Separation of the two probe tips on the attended hand (AH) versus time was observed under four conditions of stimulation. One condition consisted of 25 Hz flutter applied by the TPS on the AH. In a second condition, the two tips were applied to the AH by a complex stimulus (25 Hz + 200 Hz). For the other two conditions, flutter was applied to the AH with either a 25 Hz flutter or 200 Hz vibration stimulus applied simultaneously to the unattended hand (UH). A single trial consisted of stimuli presented to the skin for 1 sec, and then completely removed from the skin for an inter-stimulus interval of 2 sec. Each run consisted of 30 trials, or duration of 90 sec total.

To determine subject consistency of the above findings, the tracking data collected under each condition for an individual subject was averaged. The data was normalized to the flutter condition since the primary objective of this study was to determine the effect of vibration on the response normally evoked by two-point flutter stimulation. Thus, the two-point limen for the flutter condition is always defined as a "1" and all other distances are plotted as a proportion of the values obtained under the flutter condition (Tannan, 2005a). The normalized average two-point limen plot across one subject is displayed in Figure 4.2. Note that the two-point limen was *reduced* (i.e., spatial acuity is improved) for the complex condition - the two-point limen tracks at approximately 80% of the values measured under the flutter condition. In contrast, the two-point limen is larger (i.e., spatial acuity is worse) for both bilateral conditions. In the case in which the opposite or unattended hand is presented with a simultaneous 25 Hz flutter stimulus, the two-point limen tracks approximately 20% higher than the control (attended hand only) condition. Similarly, applying a 200 Hz vibration stimulus simultaneously to the unattended hand results in two-point limen values that are approximately 30% higher than the control condition.
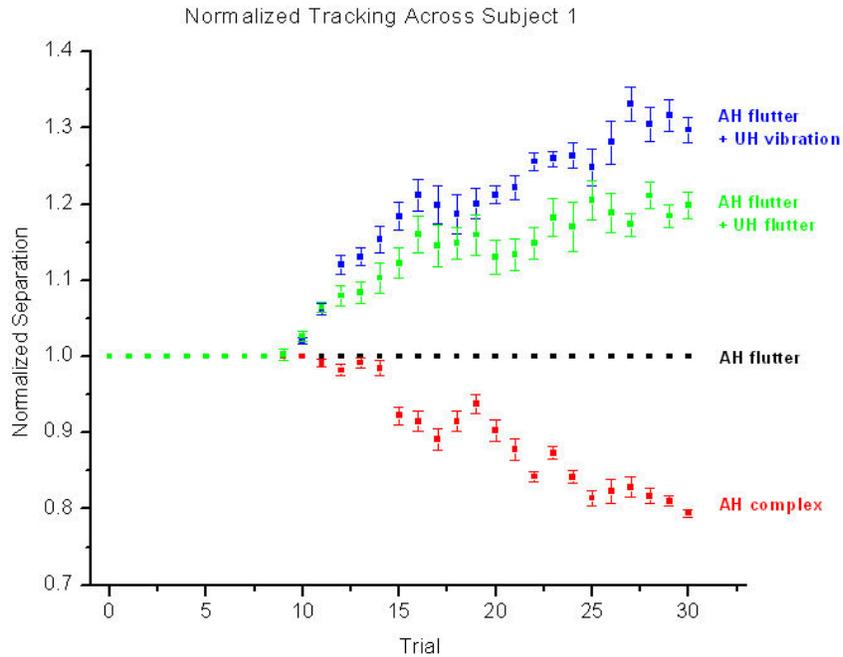
Figure 4.2 Average two-point limen for one subject. All distances are normalized to the two-point distance recorded under the attended hand (AH) 25 Hz flutter condition. Standard error bars demonstrate that across-session variability for the two-point limen tracking method is fairly consistent.

To determine the across-subject consistency of the above findings, the data normalization process applied to the single subject case, as shown in Figure 4.2 described above, was repeated for data collected under each condition across all subjects. Normalized and averaged data are shown in Figure 4.3. Similar to the data presented in Figure 4.3, the two-point limen for the complex condition tracked between 75 and 80% of that measured under the flutter condition, indicating a 20 - 25% improvement in spatial acuity resulting from the presence of vibration during the flutter stimulus driving the TPS on the attended hand. Alternatively, the two-point limen shows an increase of approximately 20% and 30% for the conditions in which the unattended hand was stimulated with flutter and vibration, respectively.
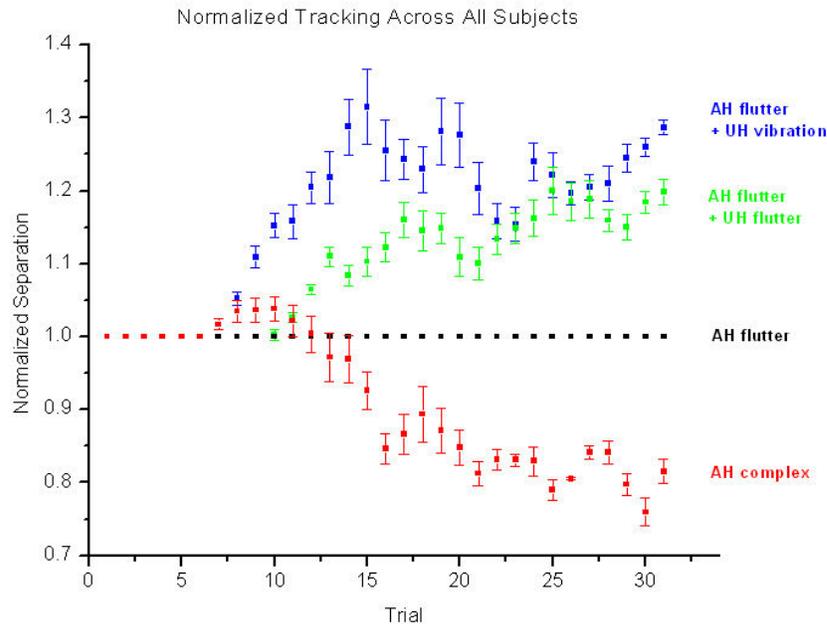
Figure 4.3 Average two-point limen across all subjects. All distances are normalized to the two-point distance recorded under the attended hand (AH) 25Hz flutter condition. Standard error bars demonstrate that across-subject variability for the two-point limen tracking method is fairly consistent.

In order to more directly compare the responses measured under each of the stimulus conditions, the tracking values obtained from the last five trials across all subjects was averaged and normalized to the flutter condition (Figure 4.4). Again, it is apparent that the two-point limen values decrease by approximately 20% under the complex condition, or when vibration is presented with flutter, by dual-site stimuli on the attended hand. Alternatively, the two-point limen increases when a second, simultaneous stimulus is added to the unattended hand – approximately 20% for the 25 Hz flutter condition and 30% for 200 Hz vibration condition. Standard error bars demonstrate that across-subject variability for the two-point limen tracking method is fairly consistent. ANOVA testing was conducted on this data with the null hypothesis that the mean under the control flutter condition is significantly different than the means obtained under the three test conditions. The means for

the bilateral conditions of unattended hand 25 Hz (F = 47.7; p < 0.00000001) and unattended hand 200 Hz (F = 76.3; p < 0.00000001), as well as the complex condition (F = 27.6; p < 0.00001) are significantly different from the mean under the flutter condition.
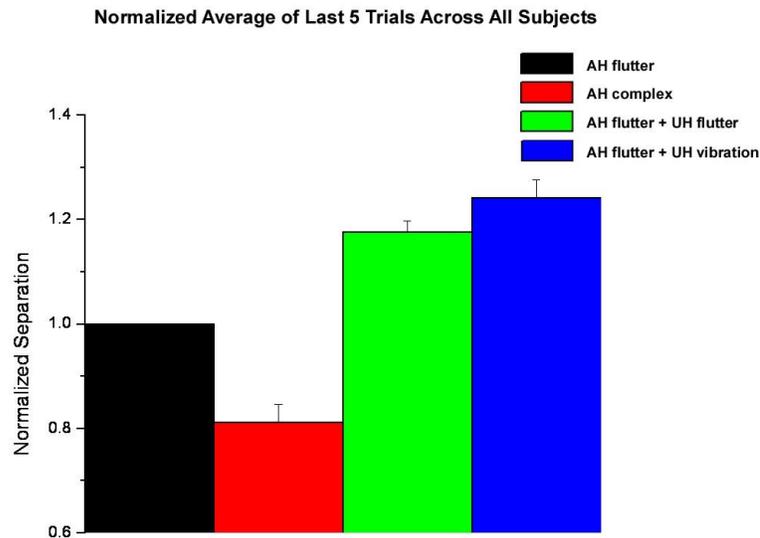
Figure 4.4 Average two-point limen (last five trials) across all subjects, with standard error bars. All distances are normalized to the two-point distance recorded under the attended hand (AH) flutter condition.

To ensure that the enhanced acuity of a subject under the complex stimulus condition was not due simply to the increased amplitude that resulted from adding vibration to a flutter stimulus (which resulted in a stimulus amplitude of 120 μm), the two-point limen was tracked on the attended hand at 25 Hz flutter of varying amplitudes. Specifically, a separate series of sessions were conducted to track and compare the two-point limen for the amplitudes of 100, 150, and 200 μm in the flutter-only condition. The results were normalized to the distances observed under the 100 μm condition and were plotted in the same manner as the previous results (see Figure 4.5). In both the 150 and 200 μm conditions, the two-point limen oscillated approximately within 10% of that observed at the

100 μm condition, suggesting that there was no consistent effect on the two-point limen due to the increased amplitude of the complex stimulus and that the effect seen under the complex condition was most likely attributable to the additional high-frequency component.
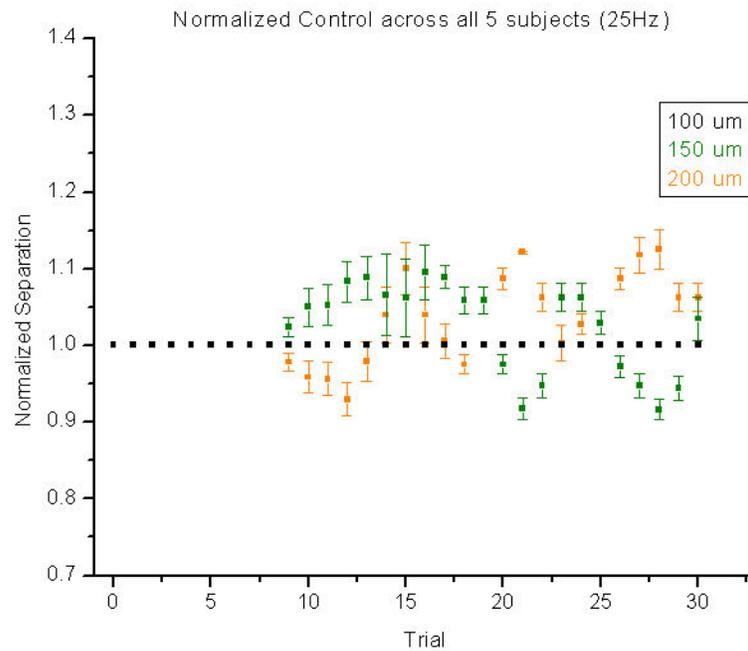
Figure 4.5 Average tracking data for control conditions. Two-point limen across all subjects for control conditions: 25 Hz flutter stimulus applied by the TPS to the contralateral hand at amplitudes of 100 μm, 150 μm, or 200 μm. All distances are normalized to the two-point distance recorded under the 25 Hz – 100 μm condition.

4.5 Discussion

In this study, we observed stimulus-dependent effects on two-point tracking of a flutter stimulus at the dorsal surface of the attended hand. The two-point limen was reduced (spatial acuity was improved) with a complex stimulus that consisted of flutter and vibration components (25 Hz flutter and 200 Hz vibration). Specifically, it was found that adding vibration to the unilateral two-point flutter stimulus *improved* spatial acuity by 20 to 25%. When the amplitude of the unilateral two-point flutter stimulus was varied (between 100,

150, and 200 μm), the two-point limen was *not* significantly affected.   Simultaneous stimulation of the hand contralateral to the attended site, however, *impaired or reduced* spatial acuity by 20% with a flutter stimulus and 30% with a vibratory stimulus.

Vega-Bermudez and Johnson (2004), using grating orientation studies, cited the importance of skin deformation as a factor affecting spatial acuity.   For this reason, we considered the possibility that enhanced spatial acuity with a complex stimulus may be due to the fact that adding vibration to the flutter stimulus introduces another amplitude component, thereby increasing the overall magnitude of the stimulus.   Results from our study showed that, within the amplitude range used, there were no significant differences in the two-point limen.   This result is also consistent with the idea that increasing amplitude of a stimulus does not increase the spatial extent of its response – a finding recently reported (Simons, 2005).   In that report, observations obtained from imaging the optical intrinsic signal in non-human primates showed that higher amplitudes of stimulation with a 25 Hz flutter stimulus in the amplitude range studied (50 – 400 μm at a frequency of 25 Hz) did not produce larger areas of cortical activation in primary somatosensory cortex (SI).   Rather, the spatial extent of the cortical patterns of activation evoked by the flutter stimulus was limited.   Simons et al. (2005) postulated that cortical response is sculpted or refined by lateral inhibition, thereby limiting changes in spatial extent.   Thus, these findings are consistent with the idea that the spatial extent of the SI cortical response evoked by each of the point stimuli plays a role in a subject's ability to discriminate between two stimulus sites on the skin, since changing stimulus amplitude has little effect on both the spatial extent of the SI cortical response and on the two-point limen.

Summers and Chanter (2002) reported results on tactile acuity in the fingertip in response to stimuli presented by a broadband tactile array. They found that localization of a 40 Hz target stimulus was improved with the addition of a 320 Hz background stimulus (which surrounded the target) compared to that with a 40 Hz background stimulus. However, Summers and Chanter also stated that this type of interpretation (that the addition of high-frequency vibration to a lower-frequency stimulus results in improvement in perception of that stimulus) was problematic because of "the known differences between mechanoreceptors" (Summers, 2002). Previous studies had established the fact that spatial acuity is worse at high frequencies (in the Pacinian range) than at low frequencies (RA/SA range) (Tannan, 2005a; Vierck, 1970; Sherrick, 1990). However, if spatial acuity is attributed to cortical activity as LaMotte and Mountcastle (1975) first proposed, then the cortico-cortical interactions that result from the condition of simultaneous flutter and vibration (Tommerdahl, 2005a) would undoubtedly have an effect on measures of spatial acuity. Flutter stimuli, such as the ones presented in this study, are known to evoke significant and sustained activity in SI cortex. On the contrary, 200 Hz skin stimulation has been shown to reduce the SI response normally evoked by a 25 Hz flutter stimulus (i.e., a complex stimulus evoked a response that was reduced in spatial extent and magnitude when compared to that of the response evoked by a flutter stimulus) (Tommerdahl, 1999a; 1999b; 2005a; Whitsel, 2001). Reducing the spatial extent of the cortical representation in SI evoked by stimuli at two points on the skin with a second, simultaneous stimulus would predictably increase the two-point limen.

Tommerdahl et al. (1999a) compared the intrinsic signal evoked in areas 3b/1 by 25 Hz skin stimulation to the intrinsic signal evoked by a same-site skin stimulus containing

both 25 and 200 Hz sinusoidal components (a "complex waveform stimulus"). Such experiments revealed that the increase in absorbance evoked in areas 3b/1 by a stimulus having both 25 and 200 Hz components was substantially smaller than the increase in absorbance evoked by "pure" 25 Hz stimulation of the same skin site. It was concluded that within a brief time after stimulus onset, 200 Hz skin stimulation evokes a powerful inhibitory action on area 3b/1 RA neurons. Inhibition due to same-site vibration may play a role in funneling the cortical activity due to flutter stimulation, creating a sharper and more finely tuned response, suggesting improved spatial acuity.

The finding in previous OIS imaging experiments in cats that high-frequency skin stimulation is accompanied by an absorbance increase in area SII and, simultaneously, by a decline in absorbance in SI in the same hemisphere led Tommerdahl et al. (1999b) to consider the possibility that activity in the corticocortical connections that link SII with SI in the same hemisphere (Burton, 1995; Alloway, 1985) leads to suppression/inhibition of SI during high-frequency skin stimulation. Insofar as the detailed mechanism by which SII might suppress/inhibit SI, the most straightforward possibility (first suggested by Hirsch and Gilbert – see Hirsch, 1991) is that long-range corticocortical (i.e., SII→SI) inhibition results from the distinct axonal termination patterns of the local inhibitory neurons in SI. That is, because the two major types of local inhibitory cells in the upper layers of somatosensory cortex (basket and chandelier cells – see Jones, 1975) terminate on cell bodies and initial segments of pyramidal cells, and either do not establish synaptic contacts with other inhibitory cells (this is the case for chandelier cells), or terminate only on the dendrites of inhibitory neurons (characteristic of basket cells), a strong excitatory input from another cortical area (e.g., the input that SI presumably receives from SII at only a brief delay after

the onset of high frequency skin stimulation) should evoke an inhibitory process in the SI region that receives the upper layer input, and the inhibition should be selectively expressed on pyramidal cells.

A recent report described that the ability to localize a stimulus on the fingertips of one hand may be impaired with the interference of a similar stimulus on a fingertip of the opposite hand (Braun, 2005), suggesting that spatial acuity may be worse with bilateral stimulation than with unilateral stimulation under certain conditions. In a recent study, we reported that the SI cortical response to contralateral skin stimulation was reduced when an identical stimulus was presented simultaneously to the ipsilateral (mirror image) skin site (Tommerdahl, 2005b). This finding led us to postulate that, since SI is recognized as the cortical region most responsible for spatial localization (LaMotte, 1975; 1979), a reduction in SI response – via ipsilateral stimulation - could cause a reduction in spatial acuity. Results from the present study support this hypothesis, suggesting that bilateral stimulation of two homologous body parts leads to a decrease in the percept of spatial acuity.

As mentioned earlier in this report, Vierck and Jones (1970) found that two-point discrimination is improved when the stimuli applied to the skin are oscillated versus steady (not oscillated). Consequently, they proposed a model of how spatial acuity improved with oscillating versus static probes. In their study, Vierck and Jones (1970) postulated that receptive fields in SI were smaller as a result of the oscillating stimulus condition, and that smaller receptive fields were less likely to overlap with one another, and thus, spatial acuity could improve as a result of changing stimulus conditions. We propose to extend that model by suggesting that the two-point limen is highly correlated with contrast between clusters of cortical activity in SI evoked by stimulation of two points on the skin. Figure 4.6

summarizes the effect that modification of the stimulus conditions, as reported in this paper, has on our proposed model of SI cortical activity. It should be noted that this conceptual model has been influenced by recent findings about the SI cortical response to skin stimulation (most notably, Tommerdahl, 2005a; 2005b; Simons, 2005).

Predicted SI Cortical Activity — **Tactile Stimulation Site**

Flutter *(distant)*

Flutter *(near)*

Complex *(near)*

Bilateral *(near)*
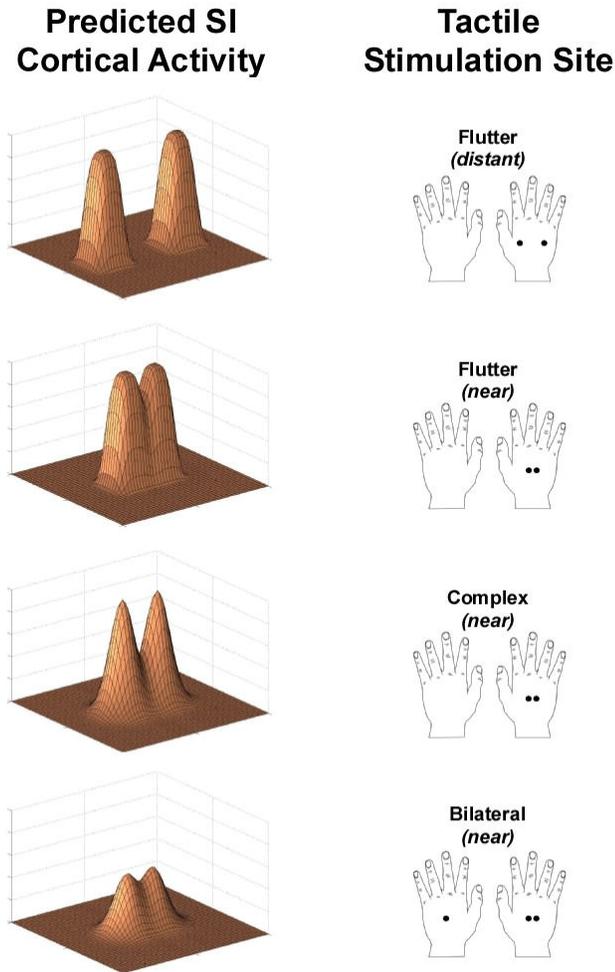
Figure 4.6 Model of predicted SI cortical activity in response to specific conditions of tactile stimulation. This model is an extension of the Vierck and Jones model (1970) on two-point receptive fields.

When stimuli consisting of two points are oscillated on the skin at low-frequency flutter at distant sites, the peaks of SI cortical response are distinct and non-overlapping

(Figure 3.6a). Thus the subject is easily able to discriminate between the two points. As the

points are positioned at stimulus sites that are closer together, the peaks of response begin to

overlap (Figure 3.6b), and because the peaks of activity are no longer easily distinguishable,

the two-point limen is increased (spatial acuity is worse). Reducing the spatial extent of the

response in SI would predictably make it easier to distinguish between two points on the

skin. Adding a same-site high-frequency vibration to the flutter stimuli ("complex" stimuli)

would be expected to reduce the spatial extent in SI (Tommerdahl, 1999a; 2005a) (Figure

3.6c). By presenting a stimulus at the same skin site on the opposite side of the body from

the two-point flutter stimulus, it is expected that bilateral interactions will reduce the

magnitude of activity due to flutter stimulation but will have no effect on spatial extent of

activation (Figure 3.6d). Tommerdahl and colleagues found that OIS response to bilateral

stimulation was 30 - 35% smaller than the response evoked due to contralateral flutter

(Tommerdahl, 2005b). As a result, spatial acuity will be degraded.

CHAPTER 5

EFFECTS OF ADAPTATION ON SPATIAL LOCALIZATION

A large portion of the work presented in this chapter was completed as a collaborative effort with the following researchers: Tommerdahl M, Whitsel BL.

5.1 Abstract

A two-interval forced choice tracking procedure was used to evaluate the effects of a pre-exposure to vibrotactile stimulation ("adaptation") on the capacity of human subjects to spatially localize a subsequent tactile stimulus. A 25 Hz flutter adapting stimulus was presented at a randomly selected position within a 20 mm linear array oriented transversely on the hand dorsum. Two flutter stimuli delivered subsequently were applied to different sites along the linear array; one to the same locus that received the adapting stimulation (the "standard" stimulus), the other to a distant site (the "test" stimulus). Following each trial, subjects were queried as to which of the two stimuli was delivered to the same skin site that received adapting stimulation. A correct response resulted in a reduced distance between the sites contacted by the standard and test stimuli in the following trial. Four subjects participated in 10 sessions each. A session consisted of two sets of 20 trials (one set at 0.5 sec and another at 5 sec adapting stimulus duration). For every subject, 5 sec adaptation resulted in an approximately 2-fold improvement in spatial discrimination performance over that achieved following 0.5 sec adaptation. It is proposed that the improved human

vibrotactile spatial localization performance following 5 sec of 25 Hz stimulation is due to enhanced spatial funneling of the global neuronal population response of primary somatosensory cortex (SI) that has been demonstrated to accompany increases in duration of 25 Hz flutter stimuli delivered to the skin.

5.2 Introduction

Extended exposure to continuous vibrotactile stimulation ("vibrotactile adaptation") at a discrete skin site not only elevates vibrotactile detection threshold, but decreases the subjective magnitude of suprathreshold stimuli whose physical attributes are similar to those of the adapting stimulus (Bensmaia, 2000; Burton, 1998; Delemos, 1996; Gescheider, 2004). Although the neural mechanisms that underlie these perceptual effects of a pre-exposure to vibrotactile stimulation remain to be established with absolute certainty, animal studies have demonstrated that such a pre-exposure is reliably accompanied by reductions in neuronal responsivity at both peripheral and central levels of the somatosensory nervous system. For example, multi-second vibrotactile stimulation is accompanied by a sustained decrease of the responsivity of skin mechanoreceptors located in the vicinity of the stimulated skin region (Leung, 1995), a long-lasting depression of the responsivity of neurons in the cuneate nucleus of the brainstem ipsilateral to the stimulus site (O'Mara, 1998), and a decrease of the spatial extent of the SI region activated by mechanical stimulation of a discrete skin site (Juliano, 1981; Juliano, 1983). Other studies have shown that, despite the above-described decreases in the responsivity of somatosensory afferents and CNS neurons, vibrotactile adaptation is followed by *significant improvement* of the capacity of subjects to discriminate the amplitude and frequency of vibrotactile stimuli when the frequencies of the adapting and standard stimuli are similar (Delemos, 1996; Goble, 1993; 1994; Tommerdahl, 2005c). When the frequencies of the adapting and standard stimuli are substantially different (e.g., 25 Hz adaptation followed by test frequencies in the vicinity of 200 Hz), however, human vibrotactile frequency discriminative capacity is significantly degraded following adaptation (Tommerdahl, 2005c).

Observations obtained in recent optical intrinsic signal (OIS) imaging studies which used different durations of vibrotactile stimulation have raised the intriguing possibility that the very different SI cortical activity patterns evoked by long- *vs.* short-duration contralateral skin flutter stimulation might support very different vibrotactile spatial localization capacities. More specifically, Simons et al (Simons, 2005) reported that the response of SI (squirrel monkey) recorded after 5 sec of 25 Hz skin flutter stimulation is characterized not only by an increase in activity in the region that receives short-latency input from the stimulated skin region, but also by a prominent decrease in activity in the surrounding region which is not observed when stimulus duration is 0.5 sec or shorter. This discrepancy between the responses evoked in contralateral SI cortex by long- *vs.* short-duration skin flutter stimulation, together with the prominent post-stimulus persistence of the decrease in activity a flutter stimulus evokes in the territory that surrounds the stimulus-activated region in SI (Simons, 2005) strongly suggested that the SI response (and presumably, therefore, the perceptual experience) evoked by a skin flutter stimulus applied to the same or a neighboring skin site would be significantly altered if the stimulus was applied within a few seconds after a preceding exposure (at least 5 sec in duration) to 25 Hz stimulation. Furthermore, if as is widely believed, the ability to spatially localize a tactile stimulus is determined by the locus of stimulus-evoked activation within SI, it seemed likely that pre-exposure to a 5 sec flutter stimulus would alter both the SI response to a flutter stimulus applied subsequently to a nearby skin site as well as the perceived location of that stimulus. This study evaluated the latter expectation using a two-interval forced-choice (2IFC) tracking paradigm to characterize the ability of human subjects to localize the site of skin flutter stimulation subsequent to 5 sec *vs.* 0.5 sec vibrotactile adaptation.

5.3 Methods

Four subjects (20-29 years in age) were studied who were naïve both to the study design and issue under investigation. All procedures were reviewed and approved in advance by an institutional review board.

Sinusoidal vertical skin displacement stimuli were delivered to the dorsum of the hand using a vertical displacement stimulator (Cantek Metatron Corp., Canonsburg, PA) fitted with a Two-Point Stimulator (TPS). The TPS and its use are described in detail in two separate reports (Tannan, 2005a; 2005b). A single probe tip (2 mm diameter) is positioned along a linear axis 20 mm long at incremental steps of 1 mm (step error of approximately 1%). The stimulator apparatus was mounted on an adjustable mechanical arm with lockable joints that was attached to a free-standing, rigid platform (fabricated locally) which enables convenient adjustment and maintenance of stimulus position. Spatial discrimination tasks generate similar psychometric functions at the fingertip and the hand dorsum, differing essentially only by an order of magnitude (Mahns, 2006; Schlereth, 2001). A transversally oriented linear array (20 mm in length) on the hand dorsum was selected to receive the stimulation because: 1) innervation density across this skin region remains relatively constant, 2) the surface is easily accessible and permits convenient stimulator placement, 3) the surface is relatively flat, reducing confounds of skin curvature present at other potential sites of stimulation, and 4) it permits positioning of the subject's arm and hand in a comfortable and stable position for the full duration of an experimental session.

The subject was seated in a chair with the right arm placed resting on an X-ray bag filled with glass beads. The investigator molded the bag to fit the contours of the subject's arm, and when the subject was comfortable and the arm positioned to allow unimpeded

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access of the stimulator to the center of the dorsal surface of the right hand, the bag was made rigid by evacuating it of air (achieved by connecting the bag to a vacuum line). The subject was unable to see either the experimenter or the stimulator and stimulus-control instrumentation. White noise presented via headphones eliminated potential auditory cues. A micrometer permitted the stimulator transducer and probe assembly to be lowered towards the predefined skin site. The micrometer position at which the digital display on the stimulator controllers registered a 0.1 - 0.2 g change in resistive force was interpreted as the point at which the stimulator probe made initial contact with the skin.

A two-interval forced-choice (2IFC) tracking protocol was used to evaluate spatial localization. The subject was instructed to attend to the percept evoked by 25 Hz (100 μm peak-to-peak amplitude) vibrotactile stimulus to the right hand. Each trial consisted of three stimuli: 1) an adapting stimulus (either 5 or 0.5 sec in duration), 2) a standard stimulus (0.5 sec) delivered at the same site as the adapting stimulus, and 3) a test stimulus (0.5 sec) delivered to a skin site different from the standard stimulus. Duration of the inter-stimulus (ISI) and inter-trial (ITI) intervals was held constant for all runs at 2 and 30 sec, respectively. All stimuli were superimposed on a pedestal of skin indentation (500 μm), and following each stimulus the probe was retracted to a position 500 μm above the skin surface.

Figure 5.1 Stimulus position and timing diagram of experimental protocol.

Timing and stimulus position diagrams in Figure 5.1 illustrate the sequence of stimuli for several trials. The adapting stimulus always was presented first during the Adapting Interval (AI) and was positioned randomly along the axis. Following the adapting stimulus was, in turn, either the standard stimulus or the test stimulus (order of presentation was randomly determined on a trial-by-trial basis) during Interval 1 (I1). The third stimulus

delivered in a trial (standard or test, whichever had not been presented), was applied during Interval 2 (I2). Subject feedback was provided during the Response Interval (RI).

In each trial the adapting stimulus was delivered at a randomly selected locus within the 20 mm array. The distance between the standard and test stimuli (10 mm in the 1st trial at the start of each run) was determined on the basis of subject performance. The subject was instructed to report the interval during which the test stimulus was perceived to have occurred at the same skin site contacted by the adapting stimulus. If the subject chose the correct interval, the distance between the skin sites contacted by the test and standard stimulus was reduced by 1 mm. If the incorrect interval was chosen, the distance was increased by 1 mm. This procedure was repeated for a minimum of 20 trials in an attempt to identify the minimally detectable separation (spatial localization threshold) between the test and standard stimuli under a given adaptation condition (5 sec *vs.* 0.5 sec). Order of the 2 adapting stimulus conditions (5 or 0.5 sec) within a session was randomized. Each subject completed 10 sessions (each session consisted of 2 runs).

5.4 Results

A two-interval forced-choice (2IFC) tracking protocol was used to determine spatial localization threshold under two different durations of adapting stimulation (5 sec *vs.* 0.5 sec). Exemplary results for one session (two runs) for each of the four subjects are shown in Figure 5.2. Note that under the condition with a 0.5 sec adapting stimulus, the subjects were able to correctly localize the points at a distance of approximately 8 - 9 mm as indicated by the tracking plots.
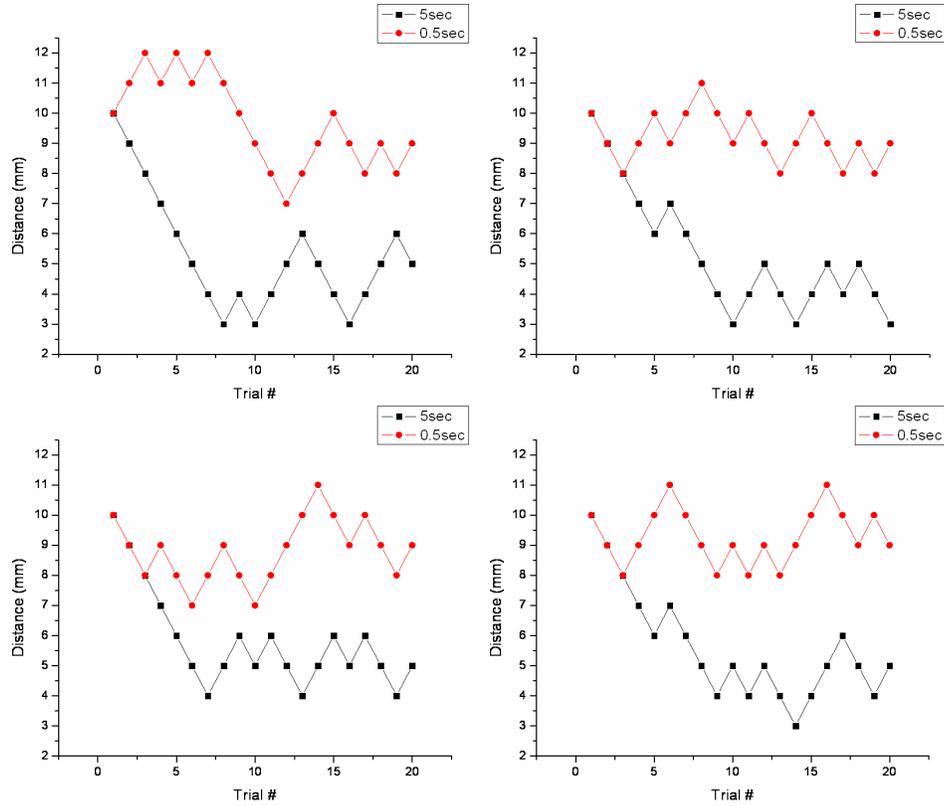
Figure 5.2 Exemplary spatial localization tracking data for one session. Two runs from each of the four subjects. Distance between the standard and test stimuli versus time was observed under two conditions of adapting stimulus duration (0.5 or 5 sec).

When the adapting stimulus duration was increased to 5 sec, the subjects were then able to localize at much smaller distances, generally between 4 and 5 mm. The tracking data collected under the two conditions were averaged for each individual subject. Average plots for each subject are shown in Figure 5.3. Standard error bars indicate a high degree of repeatability for each subject across all 10 sessions.
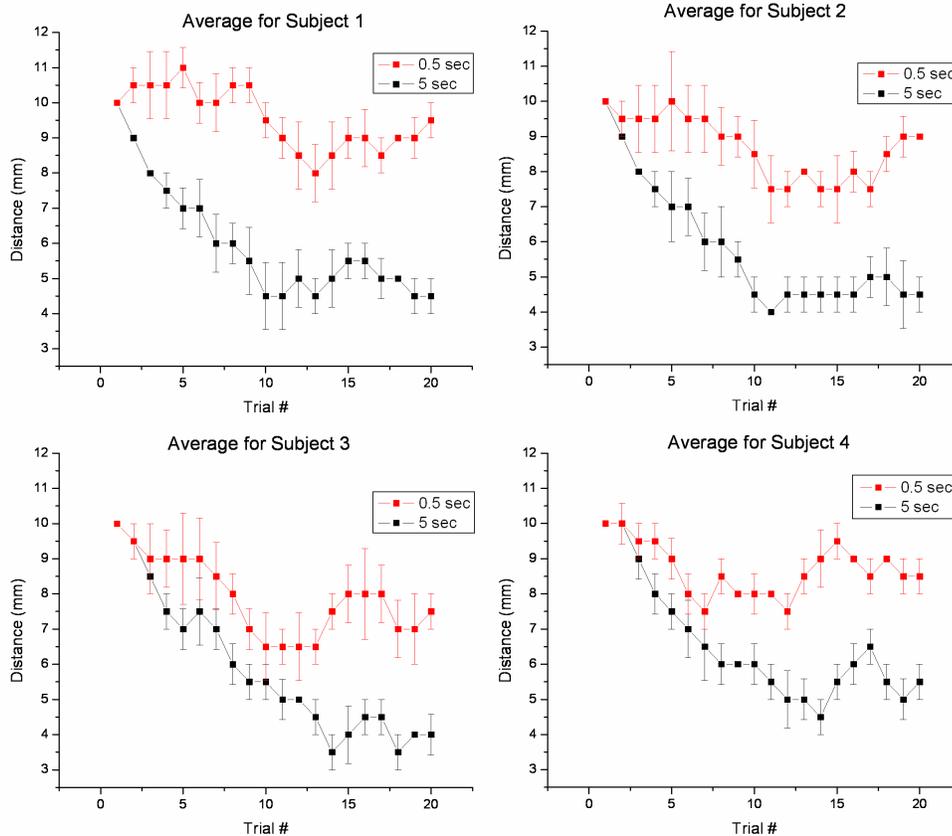
Figure 5.3   Average tracking plots for each individual subject.   Standard error bars demonstrate that across-session variability for the spatial localization tracking method is fairly consistent.

To determine the across-subject consistency of the above findings, the tracking data collected under each condition for all subjects (40 sessions in total) were averaged. As seen in the left panel of Figure 5.4, the discrepancy between the two adapting conditions was remarkably robust. On average, localization distance was tracked to 8 - 9 mm with the 0.5 sec adapting stimulus and 4 - 5 mm with the 5 sec adapting stimulus. To summarize, the localization of tactile stimuli presented with flutter was *improved* with a 5 sec adaptor compared to that with a 0.5 sec adaptor. In order to more directly compare the responses measured under each of the stimulus conditions, the tracking values obtained from the last

five trials across all subjects were averaged, shown in the right panel of Figure 5.4. These values were 8.400 ± 0.324 mm for the short-duration adaptor and 4.850 ± 0.293 mm for the long-duration adaptor (mean ± std err).
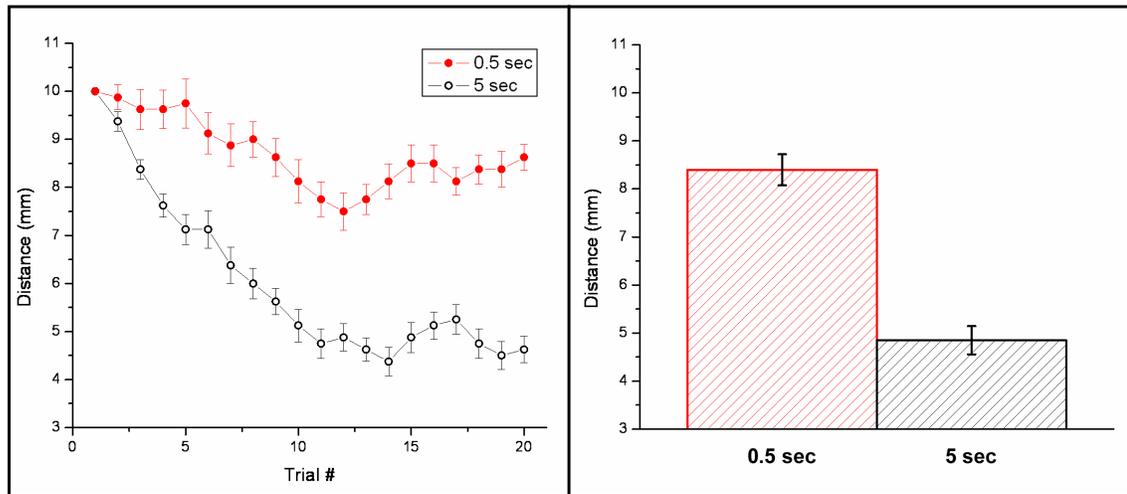
Figure 5.4 Average tracking plots across all subjects. Left Panel: Average tracking plots across all subjects. Standard error bars demonstrate that across-subject variability for the spatial localization tracking method is fairly consistent. Right Panel: Average of the last five trials across all subjects.

ANOVA testing was conducted on the data averaged across all subjects with the null hypothesis that the mean obtained with the longer adapting stimulus duration condition is significantly different than the mean obtained under the short adapting stimulus duration condition. The means for the two conditions ($F = 41.82859$; $p < 0.01$) are significantly different.

One of the concerns expressed by the reviewers of an early version of this paper was that using a simple 1up-1down tracking protocol was inappropriate as it would lead to bias and could account for the differences observed between the 0.5 sec and 5 sec adapting conditions. We originally used the 1up-1down protocol because it was consistent with our overall goal of developing perceptual metrics that can be made quickly and efficiently in out-

of-laboratory environments. The argument against our selection of protocols given was that the protocol favored "good guessing" and that the differences observed between the two conditions in this study could be accounted for by subject bias alone. Although we considered this argument as quite weak, given the consistency of the findings (during 40 sessions, there was never a single case in which the condition of 5 sec adaptation showed worse spatial localization than 0.5 sec), we repeated the study with a 2up-1down protocol, which requires a subject to answer correctly 2 times before reducing the distance between the stimuli (thus significantly increasing the amount of time necessary to complete each session). Though some bias may lead to subtle differences in the absolute values obtained for the different conditions, the relative difference between the results obtained from the two different conditions remained consistent. The average tracking data collected with this revised protocol are displayed in the left panel of Figure 5.5. Conditional differences are again shown by averaging the last 5 trials across all subjects, as displayed in the right panel of Figure 5.5. The results obtained under the 2up-1down protocol differ from the previous protocol essentially only in the overall testing period, as additional trials are required for tracking distances to stabilize. ANOVA testing indicates that the means for the two conditions are significantly different (F = 4284.9; p < 0.01).
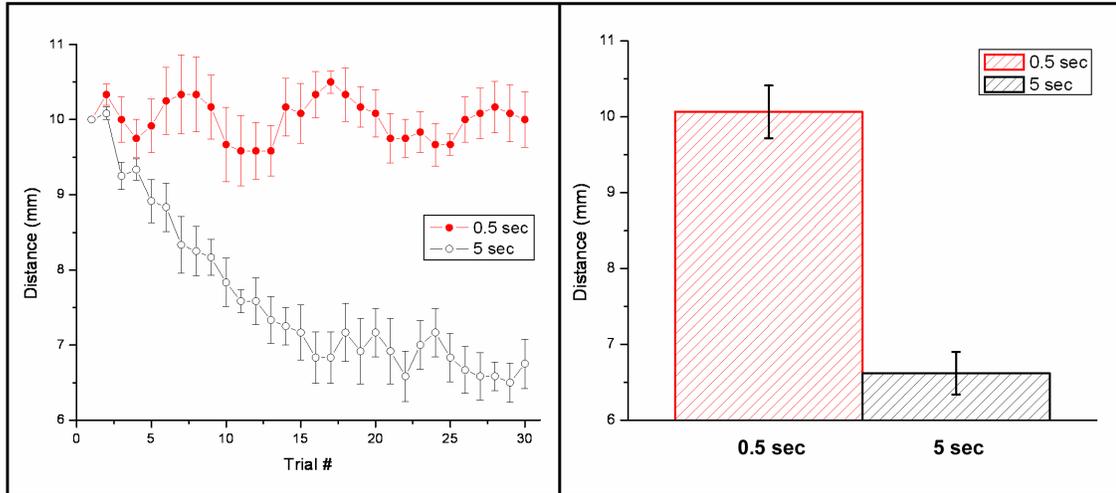
Figure 5.5 Average tracking plots across all subjects with increased bias. Left Panel: Average tracking plots across all subjects obtained with the 2up-1down tracking protocol. Standard error bars demonstrate across-subject consistency. Right Panel: Average of the last five trials across all subjects under the 2up-1down protocol.

5.5 Discussion

In this study, we observed the effects of adaptation on spatial localization of a 25 Hz flutter stimulus on the dorsal surface of the attended hand. The localization tracking distance was greatly reduced (i.e., spatial acuity was improved) with a 5 sec adapting stimulus compared to that with a 0.5 sec adapting stimulus. Specifically, it was found that long-duration adaptation resulted in *improved* spatial acuity by nearly 2-fold relative to the short-duration adaptation condition. To our knowledge, this phenomenon has not been previously reported, most likely due to the difficulty of delivering stimuli capable of extracting this measure. Similar adaptation-enhancing effects have been described for amplitude and frequency discrimination by others in the supra-threshold range of vibrotactile stimulation in circumstances in which the stimulator position was not changed (Delemos, 1996; Goble, 1993; Goble, 1994; Tommerdahl, 2005c), and it appears that spatial localization can now also be considered as a parameter that improves with adaptation.

Mountcastle and Darian-Smith (1968) proposed that a subject's ability to spatially discriminate between two points on the skin would be dependent on the lateral inhibition that enables the formation of the peaks of neuronal activity in SI cortex. Additionally, LaMotte and Mountcastle (1975; 1979) asserted that the capacity of a subject to accurately localize a flutter stimulus on the skin is determined by the locus and clarity of the flutter-evoked neuron population response within the topographically organized SI network. If this is the case, then the ability of a subject to discriminate between two points would improve if the locus of the responses in SI to the stimuli at the two corresponding skin sites were more clearly defined – i.e., if the spatial extent of the response in SI to a point stimulus was limited or reduced. Tannan et al (2005b) made observations about the stimulus-dependent effects on the two-point tracking of a vibrotactile stimulus presented to the dorsal surface of the hand. Subjects' two-point limens were reduced (i.e., spatial acuity was improved) when a complex stimulus that consisted of 25 Hz flutter and 200 Hz vibration components was delivered instead of a 25 Hz flutter-only stimulus. Specifically, it was found that adding vibration to the two-point flutter stimulus improved spatial acuity by 20 to 25%. When the amplitude of the two-point flutter stimulus was significantly varied (between 100 - 200 μm), the two-point limen was *not* affected. Findings from parallel neurophysiological studies of primate SI response provided additional confirmation of the idea that the SI cortical representation of a tactile stimulus has a significant impact on a subject's spatial acuity. Tommerdahl and colleagues demonstrated both that the SI response evoked by a complex stimulus is smaller in spatial extent than that evoked by 25 Hz flutter alone – leading to the improved clarity between two cortical loci that predicts the improved spatial acuity that was observed in human subjects – and that increasing the amplitude of a 25 Hz flutter stimulus does not lead to a change in the

spatial extent of the SI cortical response, which led to the tested (and proven) prediction that the two-point limen would not be affected by changing the stimulus amplitude (Chiu, 2005; Simons, 2005; Tannan, 2005a; 2005b; Tommerdahl, 1999a; 1999b). These observed relationships between SI cortical patterns of response and percepts such as spatial localization lead us to the hypothesis that the differences in spatial localization that occur with different durations of adapting stimulation could be accounted for by changes that occur with repetitive stimulation that have been observed in SI cortex.

Several other studies have described changes in spatial localization with extended exposure to stimulation. However, the resolution of these studies was significantly different in regards to either the spatial resolution that was being tested or the temporal duration of the adapting stimulus (i.e., these tests were done with much longer adapting periods). Schweizer et al (2000) tested spatial localization across digits and found that, after 20 hrs of tactile training (1 hr/day over 4 weeks), mislocalizations were more likely to occur on digits nearby rather than remote to the stimulated digit, possibly due to training-induced changes in somatosensory cortex. Other studies (Pilz, 2004; Pleger, 2003) observed changes in spatial discrimination on the fingertip after applying a same-site passive coactivation stimulus for several hrs, those changes being correlated with reorganization of cortical representations of the coactivated fingers. Weimer et al (2003; 2000) proposed a model, based on Hebbian learning and cortical dynamics, that stressed the correlation of the temporal stimulus structure with neural topography and psychophysical performance. Although the above-cited reports indicate changes in spatial acuity as well as cortical representation after adaptation, the adapting stimulus durations used to achieve such perceptual changes were significantly

longer than those used in this study, and thus the mechanisms responsible for the results found in those studies could be quite different from those suggested in this report.

The adapting stimulus durations in the protocol tested in this report were predictions based on recent experimental observations of SI activity evoked by variable length stimulus durations (Simons, 2005). In those studies, it was found that brief vibrotactile stimuli (0.5 sec) evoked a very transient positive response that was tightly coupled to the stimulus onset and offset, while longer duration (5 sec) stimuli evoked responses in SI that persisted several seconds after the stimulus offset. Thus, although stimulus offset of a brief skin stimulus leads to an almost immediate recovery of the evoked response back to baseline levels, the responses evoked by longer stimuli persisted at above-background levels for 5 - 15 sec after stimulus offset (Simons, 2005; Tommerdahl, 1998; 1996). Additionally, and perhaps more significantly, the SI response evoked in the surrounding below-background cortical region by the longer stimulus duration exhibited an even slower time course of recovery, while there was no below-background response (or formation of a surround) evoked by 0.5 sec stimuli (Simons, 2005). What are the implications of the slow recovery of these responses in the surround evoked by longer stimulus durations? It appears that after a 5 sec vibrotactile stimulus, SI cortex is conditioned to optimally respond to a stimulus that is most similar to the adapting stimulus. This optimization does not mean that a particular region of cortex will maximally fire in conjunction with a stimulus that has the characteristics of an adapting stimulus. Rather, it should be considered that, as thresholds go up with adaptation (and neural responsivity goes down), and percepts such as amplitude and frequency discrimination *improve* with adaptation in the supra-threshold range (Delemons, 1996; Goble, 1993; 1994; Tommerdahl, 2005c) this optimization could be indicative of the fact that the adapted SI

cortical network would be most sensitive to detecting *changes* in stimulus conditions. Stimuli similar to an adapting stimulus delivered to the skin would be predicted to evoke a pattern of response very similar to the cortical pattern of activity evoked at the end of the adapting period. On the other hand, the same adapted cortical network will *not* be optimized to respond to a stimulus in a location significantly different from the adapting stimulus. Stimuli presented on the skin in a location different from the adapting stimulus will result in a more pronounced change in the cortical pattern of response. Long duration (5 sec) adaptation could be providing cues to the subject both by optimizing the cortical response to stimuli that are at the same or nearly the same locus as the adapting stimulus and by non-optimally altering the way that the cortex responds to stimuli which are positioned very differently from the adapting stimulus. Whether or not such an optimization could explain improvements in perception that accompany adaptation remains to be seen, and this interesting possibility is currently being more directly explored.

CHAPTER 6

ALTERED SPATIO-TEMPORAL INTEGRATION IN AUTISM

A large portion of the work presented in this chapter was completed as a collaborative effort with the following researchers: Tommerdahl M, Cascio C, Baranek G, Whitsel BL.

6.1 Abstract

A recent study (Tannan, 2006) showed that pre-exposure of a skin region to a 5 sec 25Hz flutter stimulus ("adaptation") results in an approximately 2-fold improvement in the ability of neurologically healthy human adults to localize mechanical stimulation delivered to the same skin region that received the adapting stimulation. Tannan et al. (2006) proposed that adaptation improves tactile spatial discriminative performance because adaptation enhances the spatial contrast in the response of contralateral primary somatosensory cortex (SI) to mechanical skin stimulation – an effect identified in previous imaging studies of SI cortex in anesthetized non-human primates (e.g., Simons, 2005; Tommerdahl, 2002; Whitsel, 1989).

In the experiments described in this report, a paradigm identical to that employed previously in the above-described study was used to study adults with autism. The results demonstrate that although cutaneous localization performance of adults with autism is significantly better than the performance of control subjects when the period of adapting stimulation is short (i.e., 0.5 sec), tactile spatial discriminative capacity remained unaltered in

subjects with autism when the duration of adapting stimulation was increased (to 5 sec). Both the failure of prior history of tactile stimulation to alter tactile spatial localization in adults with autism, and the better-than-normal tactile localization performance of adults with autism when the period of adaptation is short are concluded to be attributable to the deficient cerebral cortical GABAergic inhibitory neurotransmission characteristic of this disorder.

6.2 Introduction

In a recent paper (Tannan, 2006) we reported that in healthy adult subjects spatial localization of a 25 Hz flutter stimulus on the skin of the hand improves substantially following adaptation. In that study, we delivered an adapting stimulus (0.5 sec or 5 sec) to a randomly selected location on the skin; and in 2 subsequent temporal intervals either the standard or a test skin flutter stimulus (the 25 Hz standard and test stimuli were equal in amplitude) was delivered at either the locus of the adapting stimulus, or at another randomly located skin site. Subjects then were queried as to which interval contained the standard stimulus. The observations revealed that long-duration (5 sec) adaptation *improved* spatial acuity by nearly 2-fold relative to performance under the short-duration (0.5 sec) adaptation condition. We hypothesized that this considerable improvement in performance was due to adaptation-induced pericolumnar lateral inhibitory interactions that lead to a more focused ("spatially funneled") primary somatosensory cortical response to mechanical skin stimulation (Simons, 2005; Tommerdahl, 2002; Whitsel, 1989; Juliano, 1989).

The above-described human psychophysical findings appear fully consistent with previously published theoretical accounts of the effects of repetitive skin stimulation on the SI cortical response to stimulation. More specifically, observations obtained in the pioneering neurophysiological recording studies led Mountcastle and colleagues to advance the proposal (Mountcastle, 1968; LaMotte, 1975; 1979) that a subject's ability to localize a mechanical stimulus on the skin depends on stimulus-evoked dynamic (time-dependent) pericolumnar lateral inhibitory interactions which increase the spatial contrast between regions of SI cortex activated differentially by stimulus-evoked afferent drive. The idea that a subject's ability to accurately perceive the spatial locus of a mechanical skin stimulus depends on the spatial

contrast of the activity profile that a stimulus evokes in the responding region of contralateral SI cortex has received wide acceptance, but the continuing absence of direct experimental support for this concept stimulated us to use the method of intrinsic optical imaging (OIS imaging; Simons, 2005; 2006) to investigate the effect of different durations of repetitive mechanical stimulation of a skin site on the spatial profile of SI activation. Briefly summarized, we (Simons, 2005; 2006) found that exposure of a skin site to a temporally extended (>0.5 sec) 25 Hz vibrotactile stimulus is consistently accompanied by a dramatic but stereotypical sequence of changes in the global SI activity pattern – activation in SI initially occurred in much of the extensive region that receives input from the stimulated skin site, and with continuing stimulation not only did the activated region shrink in size, but the surviving region of activation came to be bounded by one or more regions where activity was reduced to significantly below-background values. In addition, evidence obtained in multiple *in-vivo* and *in-vitro* imaging studies indicates unambiguously that GABA-mediated cortical inhibitory processes are responsible for the time-dependent spatial contraction of the SI activation pattern that accompanies the delivery of repetitive thalamocortical afferent drive (e.g., as can be achieved using temporally extended vibrotactile stimulation (Juliano, 1989), or repetitive electrical stimulation of thalamocortical afferents (Kohn, 2000)).

Additional findings by other workers led the authors to consider the possibility that individuals diagnosed with autism would not exhibit the adaptation-induced enhancement of tactile spatial localization characteristic of neurologically normal subjects. First, Casanova and colleagues have reported that neocortical minicolumnar size in autism is significantly reduced in ways that could reduce within-network communication (Casanova, 2006). Second, suppressed GABAergic inhibition has been suggested to be a common feature of the

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autistic brain (Hussman, 2001); and parietal cortex in subjects with autism exhibits an ~ 50% reduction in protein levels of the enzymes that synthesize GABA, glutamic acid decarboxylase (GAD) 65 and 67 (Fatemi et al., 2002) – observations that appear fully consistent with the frequently reported hypersensitivity (Casio, et al., 2007; Blakemore et al., 2006; Grandin 2000, 1992) and abnormally widespread (Belmonte et al., 2004) cerebral cortical response to skin stimulation in autism. If parietal cortical pericolumnar lateral inhibitory interactions are, in fact, deficient in subjects with autism, and if adaptation-induced spatial funneling of the primary somatosensory cortical response to repetitive skin stimulation is achieved via GABA-mediated postsynaptic inhibitory mechanisms intrinsic to primary somatosensory cortex, subjects with autism should not be expected to exhibit the adaptation-induced improvement of tactile spatial localization capacity that occurs reliably in neurologically normal subjects. Evidence bearing on this possibility was obtained using the same human psychophysical procedures and instrumentation described in Tannan et al. (Tannan, 2006).

6.3 Methods

Four adult male subjects clinically diagnosed with autism (i.e., Autistic Disorder or Asperger Disorder; DSM-IV-TR; APA, 2000), who were naïve both to the study design and issue under investigation, were participants in this study. The subjects were recruited from the University of North Carolina Neurodevelopmental Disorders Research Center Subject Registry. These subjects were tested with the Autism Diagnostic Interview – Revised (ADI-R; LeCouteur, et al., 2003), the Autism Diagnostic Observation Schedules (ADOS; Lord et al., 1999) as well as the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) to characterize the sample. All subjects in this sample were high functioning adults with

autism (IQ >80). Participants were screened for comorbid psychiatric diagnoses, peripheral injury, and other conditions that would affect somatosensation. The subjects gave informed consent and were paid $20/hour for their time. All procedures were reviewed and approved in advance by an institutional review board.

Sinusoidal vertical skin displacement stimuli were delivered to the dorsum of the hand using a vertical displacement stimulator (Cantek Metatron Corp., Canonsburg, PA) fitted with a Two-Point Stimulator (TPS). The TPS and its use are described in detail in two separate reports (Tannan, 2005a; 2005b). A single probe tip (2 mm diameter) is positioned along a linear axis 20 mm long at incremental steps of 1 mm (step error of approximately 1%). The stimulator apparatus was mounted on an adjustable mechanical arm with lockable joints that was attached to a free-standing, rigid platform (fabricated locally) which enables convenient adjustment and maintenance of stimulus position. Spatial discrimination tasks generate similar psychometric functions at the fingertip and the hand dorsum, differing essentially only by an order of magnitude (Schlereth, 2001; Mahns, 2006). A transversally oriented linear array (20mm in length) on the hand dorsum was selected to receive the stimulation because: 1) innervation density across this skin region remains relatively constant, 2) the surface is easily accessible and permits convenient stimulator placement, 3) the surface is relatively flat, reducing confounds of skin curvature present at other potential sites of stimulation, and 4) it permits positioning of the subject's arm and hand in a comfortable and stable position for the full duration of an experimental session.

The subject was seated in a chair with the right arm placed resting on an X-ray bag filled with glass beads. The investigator molded the bag to fit the contours of the subject's arm, and when the subject was comfortable and the arm positioned to allow unimpeded

access of the stimulator to the center of the dorsal surface of the right hand, the bag was made rigid by evacuating it of air (achieved by connecting the bag to a vacuum line). The subject was unable to see either the experimenter or the stimulator and stimulus-control instrumentation. White noise presented via headphones eliminated potential auditory cues. A micrometer permitted the stimulator transducer and probe assembly to be lowered towards the predefined skin site. The micrometer position at which the digital display on the stimulator controllers registered a 0.1 - 0.2 g change in resistive force was interpreted as the point at which the stimulator probe made initial contact with the skin.

A two-interval forced-choice (2IFC) tracking protocol was used to evaluate spatial localization. The subject was instructed to attend to the percept evoked by 25 Hz (100 μm peak-to-peak amplitude) vibrotactile stimulus to the right hand. Each trial consisted of three stimuli: 1) an adapting stimulus (either 5 or 0.5 sec in duration), 2) a standard stimulus (0.5 sec) delivered at the same site as the adapting stimulus, and 3) a test stimulus (0.5 sec) delivered to a skin site different from the standard stimulus. Duration of the inter-stimulus (ISI) and inter-trial (ITI) intervals was held constant for all runs at 2 and 30 sec, respectively. All stimuli were superimposed on a pedestal of skin indentation (500 μm), and following each stimulus the probe was retracted to a position 500 μm above the skin surface. Timing and stimulus position diagrams of the experimental protocol are reported in a recent study (Tannan, 2006). The adapting stimulus always was presented first during the Adapting Interval (AI) and was positioned randomly along the axis. The adapting stimulus was, in turn, followed by either the standard stimulus or the test stimulus (order of presentation was randomly determined on a trial-by-trial basis) during Interval 1 (I1). The third stimulus

delivered in a trial (standard or test, whichever had not been presented), was applied during Interval 2 (I2). Subject feedback was provided during the Response Interval (RI).

In each trial the adapting stimulus was delivered at a randomly selected locus within the 20 mm array. The distance between the standard and test stimuli (10 mm in the 1st trial at the start of each run) was determined on the basis of subject performance. The subject was instructed to report the interval during which the standard stimulus, delivered to the same skin site contacted by the adapting stimulus, was present. If the subject chose the correct interval, the distance between the skin sites contacted by the test and standard stimulus was reduced by 1 mm. If the incorrect interval was chosen, the distance was increased by 1 mm. This procedure was repeated for a minimum of 20 trials in an attempt to identify the minimally detectable separation (spatial localization threshold) between the test and standard stimuli under a given adaptation condition (5 sec *vs*. 0.5 sec). Order of the 2 adapting stimulus conditions (5 or 0.5 sec) within a session was randomized. Each subject completed 5 sessions (each session consisted of 2 runs).

6.4 Results

A two-interval forced-choice (2IFC) tracking protocol ("2 up – 1 down"; requires 2 correct answers, not necessarily consecutive, before decreasing the distance between the standard and test stimuli; 1 incorrect answer increases the distance) was used to determine spatial localization threshold under two different durations of adapting stimulation (5 sec *vs*. 0.5 sec) for subjects with autism. Five sessions (two runs in each session) were conducted for each of the four subjects with autism. To determine the across-subject consistency, the tracking data collected under each condition for all subjects (20 sessions in total) were

averaged. As seen in the middle panel of Figure 1, localization distance was tracked to 8 - 9 mm with both the 0.5 sec and 5 sec adapting stimulus. To summarize, the localization of tactile stimuli presented with flutter did not change with a 5 sec adaptor compared to that with a 0.5 sec adaptor. In order to more directly compare the responses measured under each of the stimulus conditions, the tracking values obtained from the last five trials across all subjects were averaged, shown in the right panel of Figure 1. These values were 8.983 ± 0.534 mm for the short-duration adaptor and 9.133 ± 0.511 mm for the long-duration adaptor (mean ± std err). Spatial localization thresholds obtained with control subjects from the previous study (see Tannan, 2006 #30) are shown for comparison, which were 10.283 ± 0.280 for the short-duration adaptor and 6.983 ± 0.366 for the long-duration adaptor using the same protocol.

ANOVA testing was conducted on the data averaged across all subjects with autism, with the null hypothesis that the mean obtained with the longer adapting stimulus duration condition is not significantly different than the mean obtained under the short adapting stimulus duration condition. The means for the two conditions ($p < 0.01$) are not significantly different. In addition, the means obtained under the long adapting stimulus duration condition for the control and autism samples ($p < 0.01$) are significantly different.
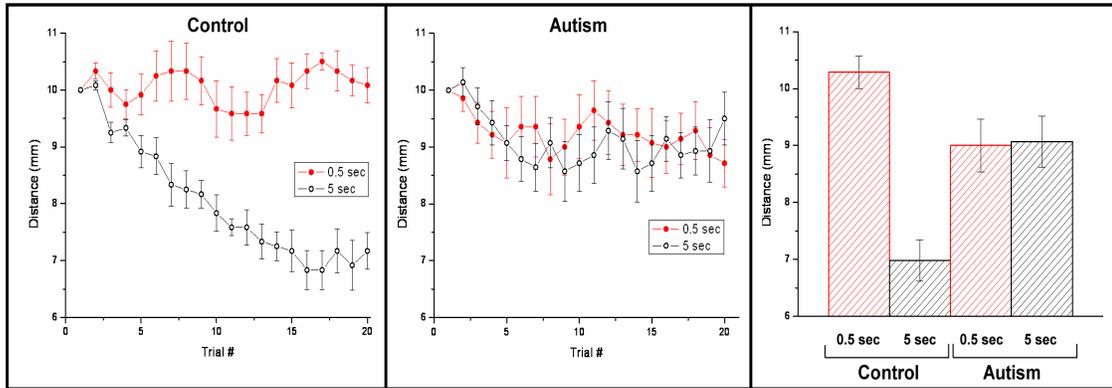
Figure 6.1 Spatial localization for adults with and without autism. Data displayed from the control subjects (left panel; previously reported (Tannan, 2006), contrasts markedly from the data displayed in the middle panel which was obtained from observations of subjects with autism. Note that subjects with autism, although they clearly outperformed the controls in the 0.5 sec adapting condition, did not improve with the 5.0 sec adapting condition. Panel at far right summarizes the findings with averages of the last 5 trials.

6.5 Discussion

In the present study, we observed the effects of adaptation on spatial localization of a 25 Hz flutter stimulus on the dorsal surface of the attended hand in adults with autism. The localization tracking distance did not change (i.e., spatial acuity was the same) with a 5 sec adapting stimulus compared to that with a 0.5 sec adapting stimulus. Thus, although it was found that long-duration adaptation resulted in *improved* spatial acuity by nearly 2-fold relative to the short-duration adaptation condition in the control population (Tannan, 2006), spatial acuity did not change with adaptation for subjects with autism. One finding of note is that subjects with autism actually *outperformed* subjects without autism in the short conditioning stimulus condition. This superior performance is notable in that it demonstrates that the subjects with autism could clearly understand and perform the tracking task and that the main difference between subjects with autism and with the control subject population was that there was no improvement with the longer duration conditioning (adapting) stimulus.

Given that improvement in spatial localization with increased stimulus duration most likely occurs within primary somatosensory cortex as a function of local functional connectivity, we regard this finding as indicative that local cortical-cortical functional connectivity in subjects with autism may be very different from that of control subjects. While a number of findings have demonstrated that long-range functional connectivity in subjects with autism is very different from that of the general population (for review, see (Polleux, 2004; Herbert, 2005), it is unlikely that the differences in the observations reported here could be accounted for by such differences in long-range connectivity. Improvements with adaptation in spatial localization would most likely occur as a result of local cortical-cortical interactions that improve the contrast of the cortical response to a repetitive stimulus through lateral inhibition. Thus, the measures described in this paper appear to be a measurable perceptual correlate of the changes in short-range connectivity predicted by the work of Casanova and colleagues. Such changes in connectivity could lead to an imbalance in the excitation and inhibition that has been predicted to result in hyperexcitability and unstable activity in cortical networks that could play a significant role in autism (Rubenstein, 2003; Polleux, 2004).

There is substantial evidence that in autism, cerebral cortical information processing not only is abnormal in those "high-order" neocortical areas that underlie language and social interaction skills, but also in the "lower-order" areas which receive and carry out the initial processing of the thalamocortical afferent activity evoked by sensory stimulation. Abnormalities of the stimulus-evoked response of primary sensory cortex in subjects with autism have been demonstrated using psychophysical testing procedures and neurophysiological recording methods. For example, some subjects with autism exhibit

hyperarousal to natural sensory input, and a decreased ability to select among competing sensory inputs (Belmonte, 2003; Gomot, 2002; Kootz, 1981; Plaisted, 2003). Furthermore, regions of cortex devoted to stimulus-driven sensory processing display excessive responsivity in subjects with autism, and exhibit little-to-no specificity in response to either the location or modality of sensory stimulation (Belmonte, 2004; Baron-Cohen, 2005). Although many reports acknowledge a widespread neocortical dysfunction in autism, it is less widely recognized that the dysfunction is non-uniform – e.g., the excessive responsivity of primary sensory cortex to peripheral stimulation is accompanied (in the same subject) by abnormally *low* activation / responsivity in cortical regions devoted to higher-order information processing (Belmonte, 2004; 2003).

Consideration of the above-described observations, together with the relatively recent demonstration that autism is associated with mutation in regions centered around the $GABA_A$-$\beta 3$ receptor subunit gene, led researchers (Belmonte, 2004; Polleux, 2004) to suggest that the neocortical dysfunction in this disorder may be attributable to a deficiency during early development in GABA-mediated synaptic neurotransmission. According to this view, the excessive excitatory neurotransmission within low-order neocortical processing areas that accompanies insufficient GABA-mediated neocortical inhibition leads to the experience-driven abnormalities in neocortical functional connectivity characteristic of autism. More specifically, in subjects that develop autism, abnormal neocortical information processing strategies attributable to excess excitation in low-order processing areas are viewed to direct the acquisition (via activity-dependent neuroplasticity) of a long-range neocortical inter-areal connectivity incapable of supporting the elaborate between-area communication and neuronal synchronization that underlie the social interaction and

language processing skills of normal subjects (Belmonte, 2004; Just, 2004). Consistent with this perspective are: (i) the recommendation that drugs that promote GABAergic CNS synaptic neurotransmission be considered for early intervention and as potential therapeutic agents for autism (Belmonte, 2004; Bethea, 2007); and (ii) the neuromechanistic proposal that an increased ratio of excitation to inhibition in CNS information processing systems accounts for the principal features of autism (Rubenstein, 2003).

The results described in this paper used an approach (Tannan, 2006) that enables convenient and rapid acquisition from both neurologically normal subjects and high-functioning adult subjects with autism of quantitative measures of somatosensory tactile spatial localization capacity – a well-defined somatosensory perceptual capacity that animal functional neuroimaging evidence strongly suggests reflects the status of GABA-mediated inhibitory neurotransmission in primary somatosensory cortex (Simons, 2005; Tommerdahl, 2002; Whitsel, 1989; Juliano, 1989). Additionally, the findings reveal statistically highly significant differences between the tactile localization performance of normal subjects and those with autism. The discrepancies in the tactile spatial localization performance of normal subjects and subjects with autism appear consistent with: (i) the proposal that somatosensory cortical GABAergic inhibitory neurotransmission is deficient in autism, and (ii) the widely-known, but poorly understood tendency for subjects with autism to perform worse (relative to control subjects) on tasks requiring spatial and/or temporal integration, but better than normal when the task emphasizes appreciation of local detail (for review, see Mottron, 2007). To our knowledge, this is the first objective metric that clearly defines a difference between the centrally-mediated somatosensory discriminative capacities of a sample with autism from the general population. Further studies such as these may provide valuable insights into

improving experimental models used for studying not only autism, but other neurological disorders as well.

CHAPTER 7

A PORTABLE TACTILE SENSORY DIAGNOSTIC DEVICE

7.1 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 on 20 subjects.

7.2 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, 1969; Vierck, 1970; LaMotte, 1975; Juliano, 1989; Goble, 1993; Tommerdahl, 1993; 1998; 2002). Typically, stimuli that can be delivered through mechanical transducers – vertical displacement stimulators such as the one originally described by 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 μm) 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 at variably spaced distances on a trial-by-trial basis, we designed and fabricated a two point stimulator (TPS) that attached to the end of a vertical displacement stimulator (Tannan, 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

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stimulus protocols could be effectively delivered to the skin to evoke measurable percepts (Tannan, 2005a; 2005b; 2006; Tommerdahl, 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 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

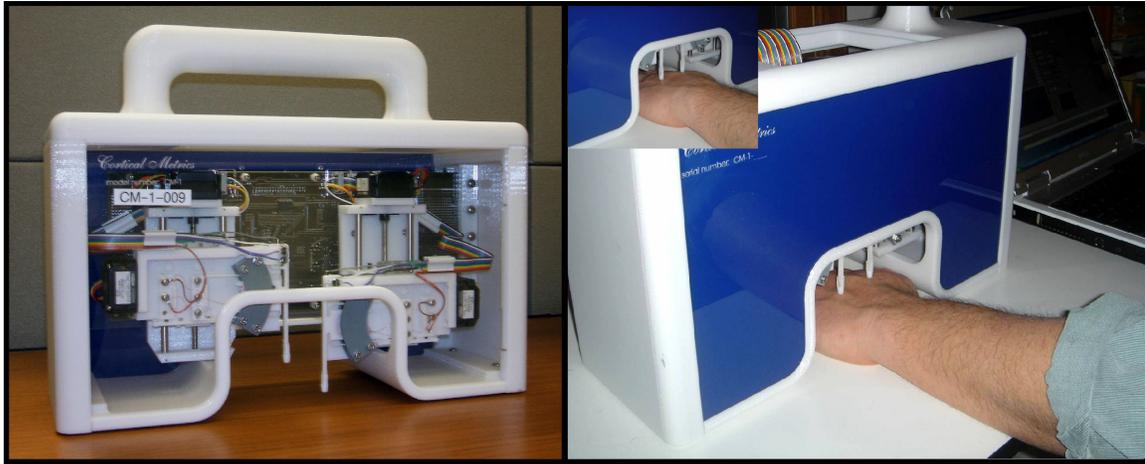studied with two stimuli delivered simultaneously to two skin sites by a single, dual-probe stimulator system.

Figure 7.1 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).

7.3 Methods

Description of the Device

The Cortical Metrics (CM-1; see Figure 7.1) stimulator was developed in our laboratories for use in the series of experiments described in this report. The system was designed using state-of-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 CO_2 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 ± 1 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, 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, 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.
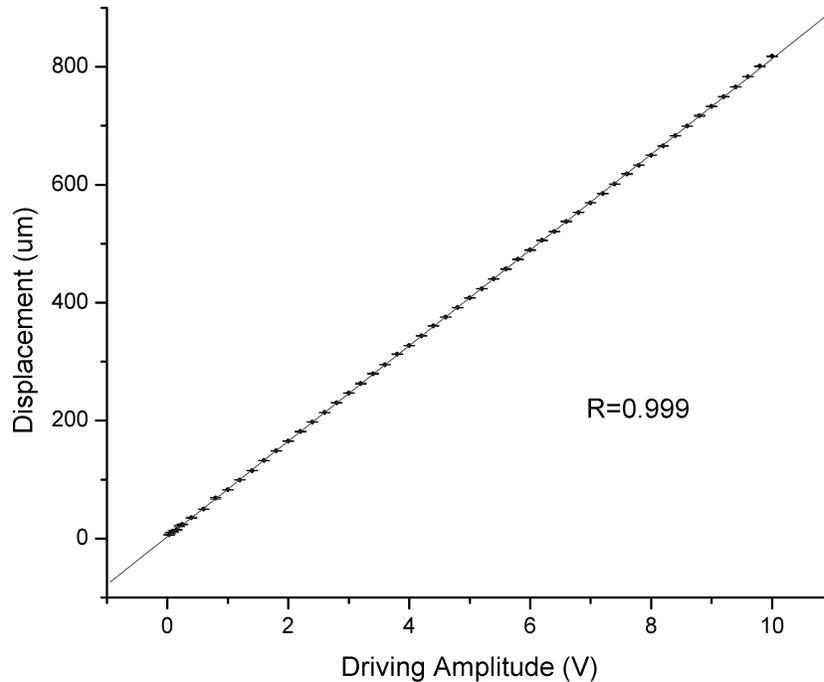
Figure 7.2 Amplitude calibration and range of the CM-1. During system calibration, 25 Hz sinusoidal signals with variable peak-to-peak amplitude (driving voltage) were delivered by the NI DAQ analog output to the VCA coil of the CM-1. In this graph, the driving input voltage is plotted against the displacement (in μm) of the tip of the CM-1.

A sample plot of driving input voltage for a 25 Hz sinusoid (the frequency used in all of our amplitude discrimination studies), delivered by the NI DAQ analogy output to the CM-1 vs. the displacement output, as detected by the optical sensors on the CM-1 unit is shown in Figure 7.2. The optical sensors were calibrated using a high-resolution linear variable displacement transducer.

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.
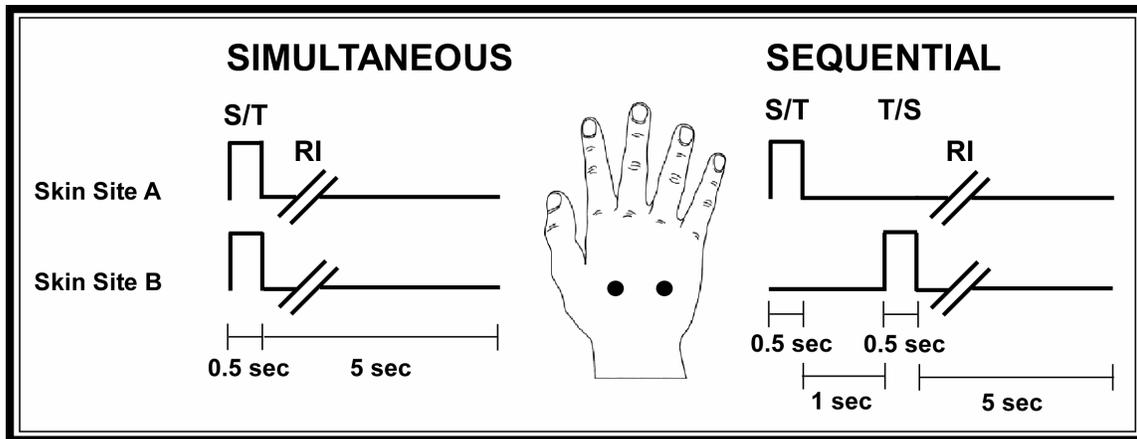
Figure 7.3 Schematic of the protocols used for amplitude discrimination. Two modes of stimulus delivery were employed: simultaneous and sequential. In each trial of the simultaneous mode, two 25 Hz vibrotactile stimuli, the standard (S) and test (T), were delivered at the same time for 0.5 sec. A 5 sec 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 sec inter-stimulus-interval preceded the next 0.5 sec stimulus and a 5 sec delay followed which preceded onset of the next trial.

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 7.3). 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 μm to insure good contact with the skin. A vibrotactile test stimulus (ranging between 105-200 μm peak-to-peak amplitude at 25 Hz; initial condition set at 200 μm) was delivered simultaneously with a vibrotactile standard stimulus (100 μm peak-to-peak amplitude, 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 larger stimulus) and a 5 sec delay before onset of the next trial.
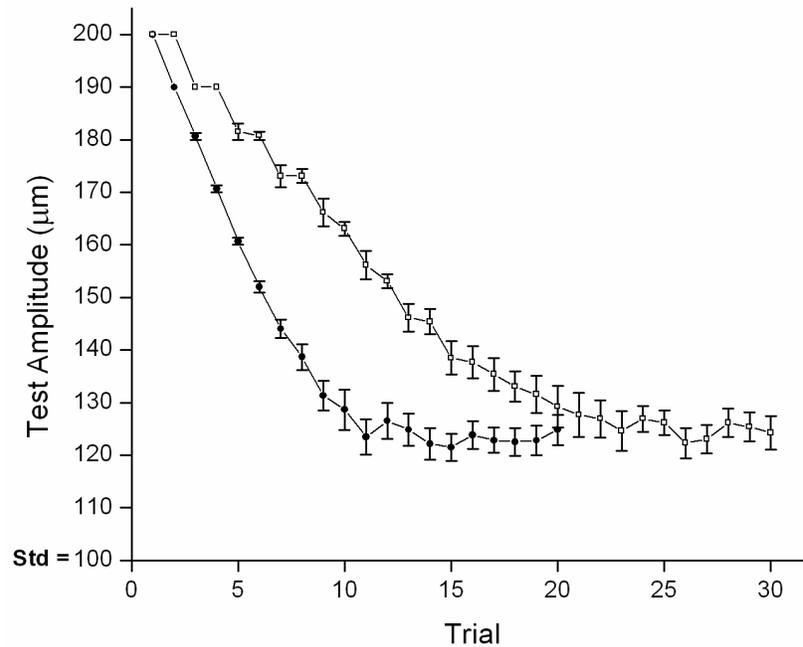
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Figure 7.4 Comparison of tracking paradigms. Subject performance is tracked with a 2 up / 1 down protocol (open square plot) and compared with a modified tracking protocol (closed circle plot; 1 up / 1 down tracking for the first 10 trials and 2 up / 1 down for the last 10 trials).

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 7.4 (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 7.4 (closed circle plot), and it demonstrates that modification of the protocol did not result in a significant difference in performance. 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.

7.4 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.
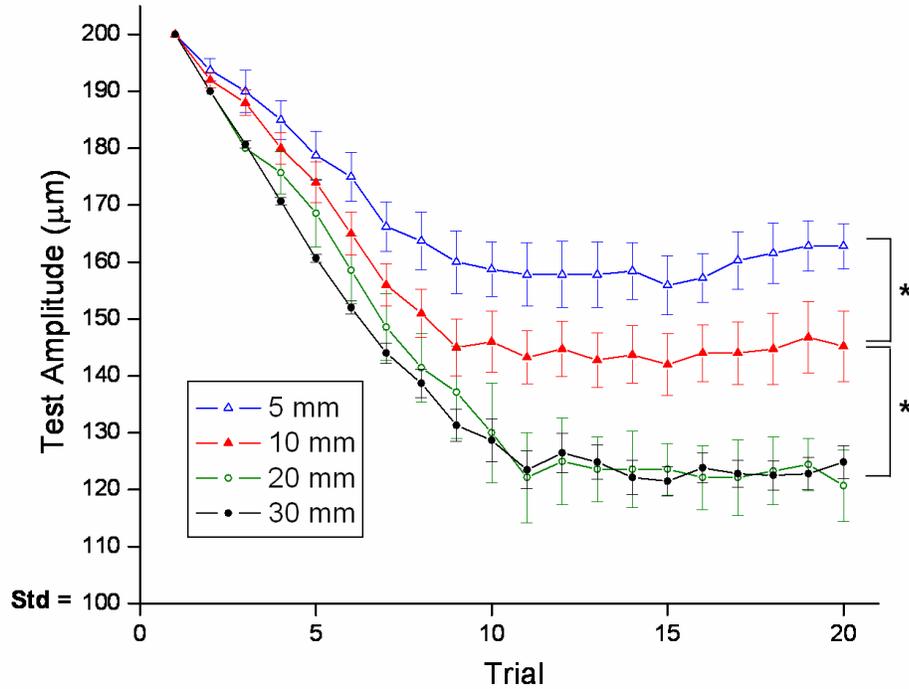
Figure 7.5 Amplitude discrimination for different inter-probe distances. Average amplitude discrimination performance across all subjects for four different inter-probe distances (5, 10, 20, and 30 mm) are plotted. Note that there is no significant difference in performance between the 20 and 30 mm conditions, 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, $p < 0.01$).

The results averaged across all subjects are shown in Figure 7.5. 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 2005a; 2005b), subjects were consistently able to track to an amplitude difference of approximately 25 μm (125 μm test versus 100 μm 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 μm and 65 μm, respectively. ANOVA and two-sample t-testing indicates that the means for the 30 and 20 mm conditions are not significantly different from one another 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).
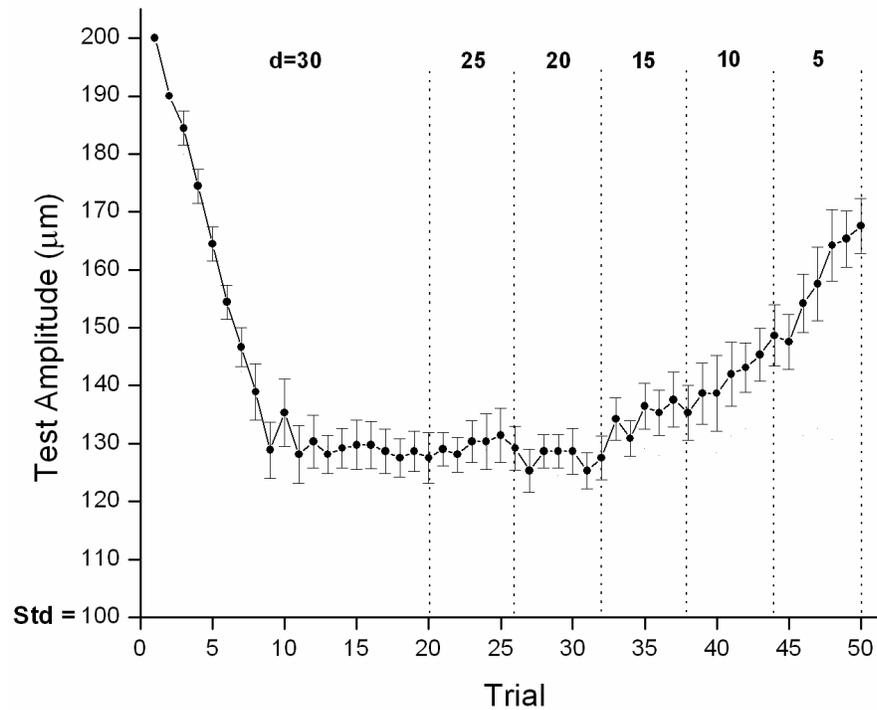
Figure 7.6 Average of the amplitude discrimination tracking data across all subjects. An experimental protocol was implemented in which six inter-probe distances were tracked during a single run. The probe tips were initially spaced 30 mm apart for the first 20 trials after which the distance was reduced 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.

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 7.6, 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 7.5. 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.
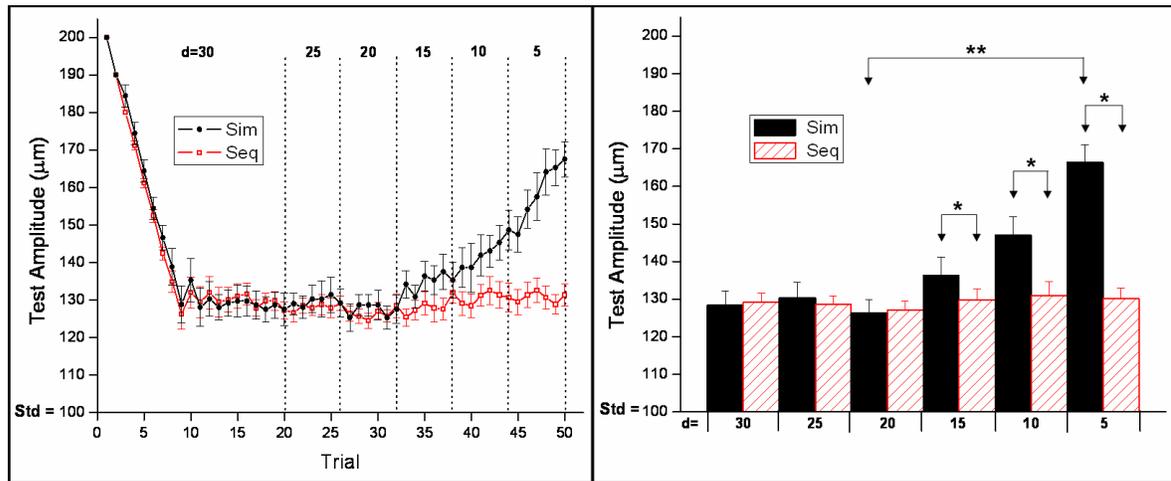

Figure 7.7 Direct comparison of simultaneous vs. sequential amplitude discrimination. Left panel: Average of the tracking plots across all subjects for the simultaneous condition (closed circles) and sequential condition (open circles). Right panel: The comparison of thresholds measured by averaging 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, $p < 0.01$).

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 7.7, 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 7.7. 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, whereas the means show no difference across all distances for the sequential condition ($p < 0.01$ for all tests). 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.

7.5 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 inter-probe 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, 1975; Juliano, 1989; Mountcastle, 1967; Kohn, 2000; Simons, 2005) and show improvement in two-point discrimination with repetitive vibrotactile stimulation (Vierck, 1970; Tannan, 2005a; 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 7.6 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.

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, 1970; Tannan, 2005a; 2005b), measures of spatial acuity (Tannan, 2006; Tommerdahl, 2007), frequency discrimination (LaMotte, 1975), temporal order judgment (Fiorio, 2003), and changes in responsivity with adaptation (Goble, 1993; Tommerdahl, 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, the practical use of two independently controlled stimulators – which allows for a significant range of inter-probe distances – allows 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. Fourth, the rapid-manufacture design of the system will allow multiple systems to be fabricated and placed at a large number of test sites for relatively low 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.

CHAPTER 8

EFFECTS OF ADAPTATION ON SIMULTANEOUS AMPLITUDE DISCRIMINATION

8.1 Abstract

In a separate report based on work in our lab, it was reported that different durations of vibrotactile stimulation had a significant impact on the optical image evoked from SI cortex. In particular, it was found that increasing stimulus durations, in the range between 0.5 sec and 5.0 sec, led to a much more pronounced and persistent below-background surround. In order to ascertain the effects of such differences in short duration vibrotactile stimulation on an objective sensory percept, we conducted several amplitude discrimination procedures. In all procedures, a two-alternative forced-choice tracking task was used to determine a subjects' ability to discriminate between two different amplitudes of vibrotactile stimulation simultaneously delivered 30 mm apart on the hand dorsum. In the first block (first 20 trials) of each tracking procedure, the two simultaneous stimuli were delivered to the skin for 0.5 sec. In the second block (second set of 20 trials), the two simultaneous 0.5 sec vibrotactile stimuli were preceded by different conditions of adapting stimulation. In the first experiment, the duration of adaptation at one of the stimulus sites was varied in order to determine the relationship between the duration of a conditioning stimulus and the impact that it has on the intensity percept. This altered percept at the single site was measured by direct comparison with the stimulus presented at the non-conditioned site via amplitude discrimination of the two skin sites. In the second experiment, an adapting stimulus at two

sites preceded delivery of the amplitude discrimination task, and as predicted (by previous findings in the literature), amplitude discrimination was improved. A comparison between these findings and our previously published findings on the SI cortical response to similar conditions of vibrotactile stimulation is discussed, and due to this correlation, as well as the speed and efficiency at which these perceptual measures can be obtained, leads us to conclude that similar measures may be useful in clinical and/or clinical research settings for the assessment of cerebral cortical health.

8.2 Introduction

Adaptation – the process by which neuronal response is influenced by prior history – can have a pronounced effect at a number of time scales. Additionally, there is evidence that the functional connectivity of sensory cortical networks can change significantly and reversibly under conditions of stimulation that occur in everyday life rather than only under conditions of prolonged stimulation. For example, visual cortical pyramidal neurons exhibit prominent, fully reversible, use-dependent modifications of both RFs and response properties that develop within tens of milliseconds of stimulus onset, and disappear within seconds of stimulus termination (for review see Kohn, 2002).

Recently, we characterized the SI cortical response evoked by both different amplitudes (in the supra-threshold range) and different durations of vibrotactile stimulation (Simons, 2005; 2006; Chiu, 2005). The results of these optical intrinsic signal imaging studies demonstrated a strong correlation between not only the amplitude of 25 Hz vibrotactile (flutter) skin stimulation and the response magnitude evoked in SI, but that the spatial pattern of response in SI changed systematically with increases in the amplitude and the duration of the stimulus. Additionally, responses in the same region of SI elicited by flutter stimuli of different duration demonstrated not only differences in their peak magnitude, but also in the relative rates of rise and decay of the evoked intrinsic signal. Of particular note was that although the short duration stimuli evoked transient responses which decayed to baseline within 1-2 seconds, the responses evoked by longer duration stimuli remained elevated for several seconds following stimulus offset. From these studies, we predicted – due to the changes observed in SI cortical response with different durations of repetitive stimulation – that the sensory percept of different amplitudes of vibrotactile

stimulation in the supra-threshold range could be affected by prior exposure to a stimulus delivered to the same skin site systematically and progressively with different durations of stimulation. In this chapter, we demonstrate such an effect with a previously unreported method – simultaneous amplitude discrimination in the presence and absence of adapting stimuli – and show a parallel between the responses evoked in SI by different durations of repetitive stimulation and the percepts evoked by similar stimulus conditions.

8.3 Methods

Twenty subjects (20-29 years in age) were studied who were naïve both to the study design and issue under investigation. 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. The subject's hand was placed under a dual-site portable stimulator (CM-1; for full description, see Tannan, 2007). The two probe tips (5 mm diameter) were positioned 30 mm apart along a transversally oriented linear axis along the hand dorsum. 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 μm to insure good contact with the skin.
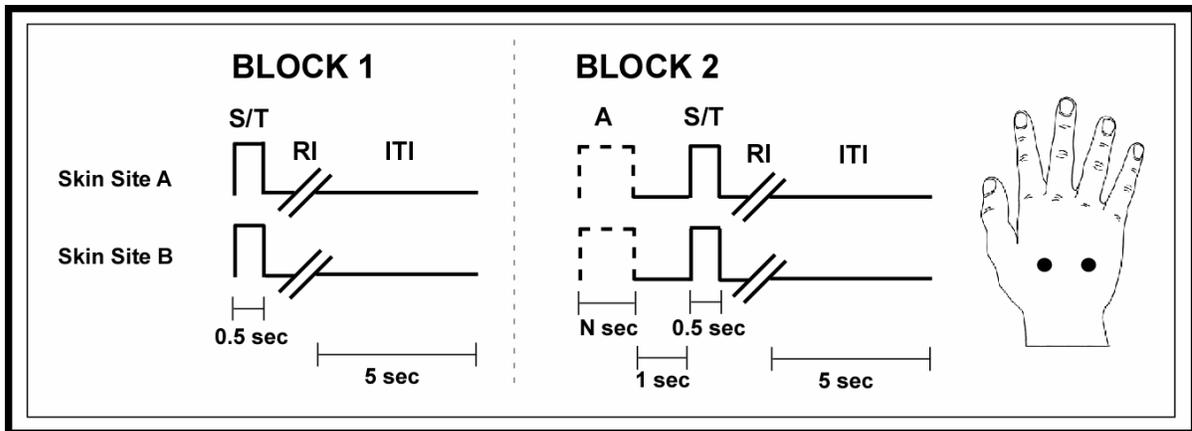
Figure 8.1 Schematic of the protocols used for amplitude discrimination. Two blocks of stimulus delivery were employed. In the first block, two 25 Hz vibrotactile stimuli, the standard (S) and test (T), were delivered at the same time for 0.5 sec. A 5 sec delay (including subject response interval (RI)) was imposed before onset of the next trial. In the second block, varying conditions of single-site or dual-site adapting stimulation were first delivered, followed by a 1 sec inter-stimulus interval, and then the standard and test stimuli.

The tracking protocol used to obtain the amplitude discrimination data in Figure 8.1 consisted of 2 sequential blocks. The first block of all experimental runs, in this report, was performed identically. In the **first block**, a vibrotactile test stimulus (ranging between 105 - 200 μm at 25 Hz) was delivered simultaneously with a vibrotactile standard stimulus (always 100 μm, 25 Hz). The two stimuli were delivered to the hand dorsum 30 mm apart. Previous studies have shown that this distance is well outside a subject's two point limen (Tannan, 2005a; 2005b; 2006) and that subjects perform nearly identically at simultaneous amplitude discrimination as they do at sequential amplitude discrimination (Tannan, 2007). 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 larger stimulus) and a 5 sec delay before onset of the next trial. Initially, the test stimulus was 200 μm (peak-to-peak amplitude) and the standard was 100 μm. Stimulus amplitude was modified based on subject response with a 1 up / 1 down forced

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choice protocol for the first 10 trials, and responses for the remaining trials (first and second block) were tracked with a 2 up / 1 down forced choice protocol in which the subject reported the location of the larger stimulus and 2 correct answers resulted in a decrement in the amplitude difference between the test and standard stimuli. Using a 1up / 1down tracking protocol for the first 10 trials is an efficient way to quickly move the amplitude discrimination task into a subject's discriminative capacity range without significantly impacting the results (Tannan, 2007).

In the second block, delivery of the test and standard stimuli was preceded by one of 6 different conditions of adapting stimulation. In the first condition, no adapting stimulus was delivered (control condition). In 4 of the conditions, a 100 μm adapting stimulus at the location of the test stimulus was delivered 1 sec prior to the presentation of the test and standard stimuli. Four different durations of adapting stimuli were delivered: 0.2, 0.5, 1 and 2 sec. In the final condition, a 100 μm adapting stimulus was delivered for 1 sec to both skin sites before the test and standard stimuli were delivered to the skin. In all conditions, a 2 up / 1 down protocol was used in Block #2 to track the subject's ability to determine the stimulus with the larger amplitude. The initial conditions of Block #2 were the final conditions of Block #1.

8.4 Results

A two-alternative forced-choice (2AFC) tracking protocol was used to determine amplitude discrimination threshold under different conditions of adapting stimulation. Each experimental run consisted of two blocks. In Block #1, the subject was instructed to choose the larger of two simultaneous stimuli. In *Experiment 1,* the task in Block #2 was the same

except that an adapting stimulus was first presented to the same site as the test, or larger, stimulus. Four conditions of adapting stimulus duration were used – 0.2, 0.5, 1, and 2 sec. In *Experiment 2,* adapting stimuli were presented simultaneously to both the standard and test sites for a duration of 1 sec.
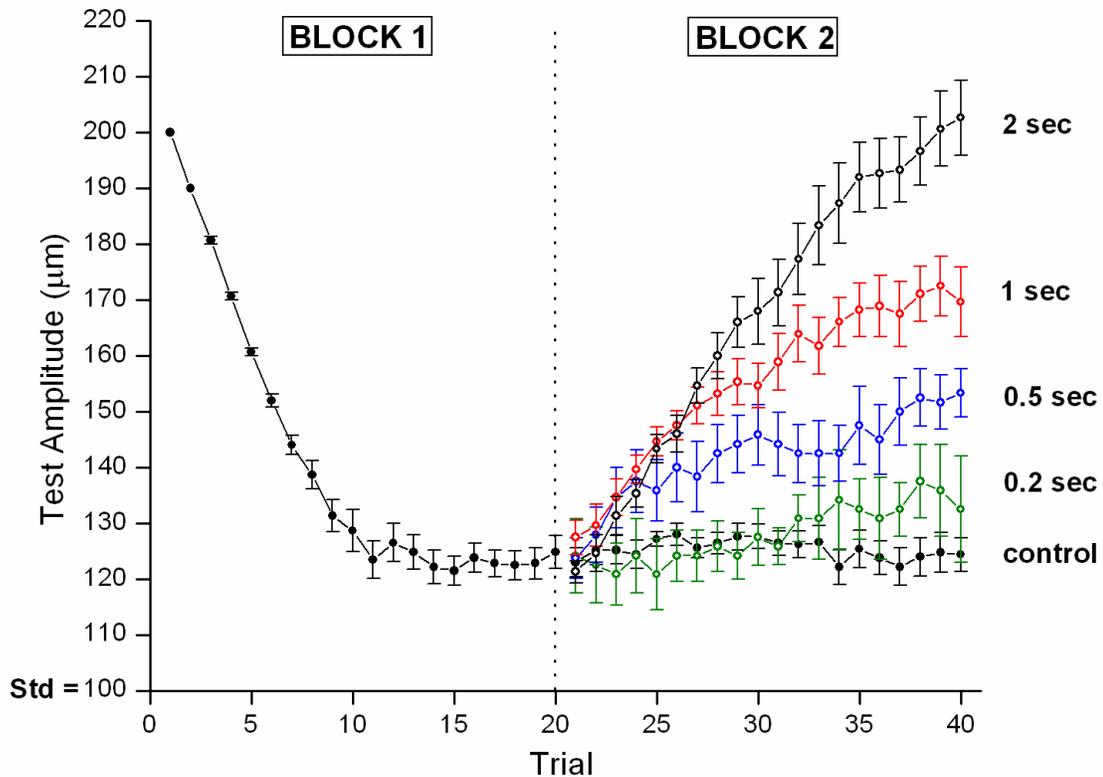

Figure 8.2 Comparison of amplitude discrimination for different adaptor durations. Average amplitude discrimination performance across all subjects for a control condition and four different single-site adapting stimulus durations (0.2, 0.5, 1, and 2 sec) are plotted. Note that there is no significant difference in performance between the Block #1 and control conditions, but the means for the adapting conditions are significantly increasingly different from the Block #1 condition in a graded fashion dependent on adapting stimulus duration (ANOVA and two-sample t-test, $p < 0.01$).

Experiment 1:

The results from the first experiment are shown in Figure 8.2. As seen in the plot in Block #1, on average, subjects tracked to an amplitude difference of approximately 25 μm (125 μm test vs. 100 μm standard). In all cases of Block #2 (except the control condition –

no adapting stimulus), the adapting stimulus had a clear effect on the subsequent tracking results such that the perceived intensity was lower at the test site even though the amplitude was much higher than that of the standard. Furthermore, note that as the duration of the adapting stimulus was increased (from 0.2 sec to 2 sec), the perceived test stimulus intensity was reduced in a graded fashion. Performance was increasingly worse at the amplitude discrimination task as the adapting stimulus duration was lengthened.
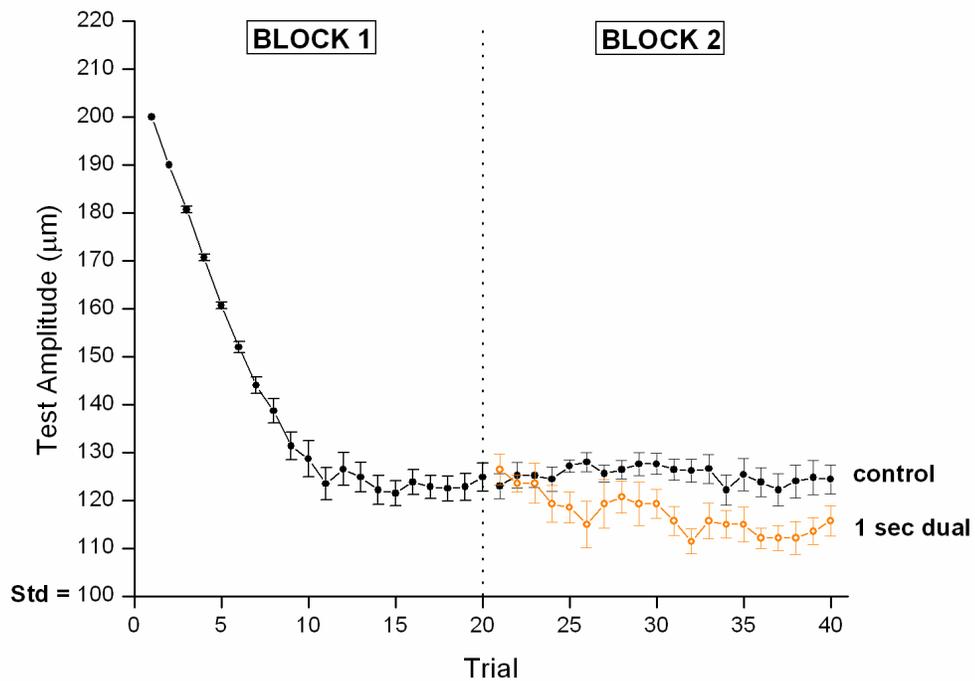
Figure 8.3 Comparison of amplitude discrimination for dual-site adapting stimulation. Note that in Block #2, amplitude discriminability is improved significantly over the control condition with pre-exposure to a 1 sec dual-site adapting stimulus (ANOVA and two-sample t-test, $p < 0.01$).

Experiment 2:

What would happen if an adapting stimulus were delivered at both stimulus sites prior to the amplitude discrimination task? Based on Experiment 1, the expectation is that the perceived intensity at each stimulus site would be reduced – and if there were little or no

cortical-cortical mediated adaptive response to the stimuli (such as the funneling described by Lamotte), the reduced perceptual intensity evoked at the two sites could lead to a decrease in performance of the task simply by a reduction in the signal to noise ratio of the evoked (cortical) responses. However, prior studies (Goble, 1993) demonstrated that amplitude discrimination is enhanced with single site adaptation followed by a sequentially delivered vibrotactile amplitude discrimination task. In order to directly address the question of whether or not the amplitude discrimination enhancement would occur with dual-site adaptation, the effects of an adapting stimulus on both skin sites prior to delivery of the test/standard stimuli was assessed, and the results from the this second experiment are shown in Figure 8.3. In this experiment, in which the initial conditions were determined by Block #1 performance tracking (as in Experiment 1), adapting stimuli were presented simultaneously to both the standard and test sites for 1 second and was followed by a 1 sec inter-stimulus interval which preceded the simultaneously delivered test and standard stimuli. Observation of the tracking performance in Figure 8.3 appears to demonstrate that amplitude discrimination performance improves when the stimulus loci are pre-exposed to an adapting stimulus.
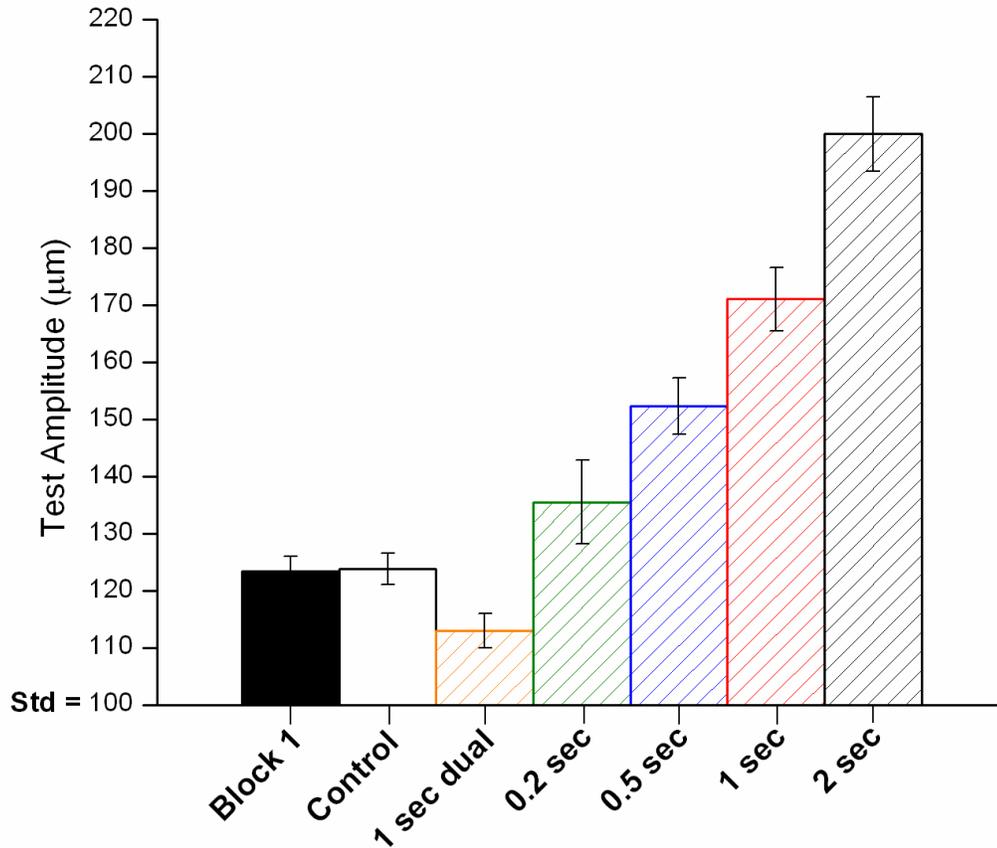
Figure 8.4 Amplitude discrimination for all conditions of adapting stimulation. The average of the last five trials for each condition across all subjects is plotted for direct comparison.

In order to more directly compare the responses measured under each of the stimulus conditions, the tracking values obtained from the last five trials across all subjects were averaged, shown in Figure 8.4. In the control condition, no adapting stimuli were present in Block #2, and therefore amplitude discrimination threshold did not change from Block #1. However, performance was significantly improved when the standard and test stimuli were preceded by dual-site 1 sec adapting stimulation. In contrast, when a single adapting stimulus was presented in the test location, subjects were less able to discriminate between amplitudes of the standard and test stimuli. A greater reduction in performance was observed in a step-wise fashion as the adapting stimulus duration was increased from 0.2 to 2 sec. To

summarize, the amplitude discrimination threshold of tactile stimuli presented with flutter was *improved* with dual-site adaptation and *worsened*, as a function of adapting stimulus duration, with single-site adaptation in the site of the test stimulus.

8.5 Discussion

In this study we have described the impact of adaptation on the ability of a subject to discriminate two flutter stimuli, on the basis of amplitude, presented simultaneously to the dorsal surface of the hand. Our results demonstrate that subjects were able to discriminate stimuli which differed by ~23 μm in the absence of an adapting stimulus. When adapting stimuli were delivered at both sites subjects demonstrated an improved ability to discriminate the amplitudes of a pair of subsequent test stimuli tracking to amplitude differences of ~13 μm. All subjects tested demonstrated a decreased ability in the amplitude discrimination task when the adapting stimulus was delivered to only one skin site prior to the presentation of the pair of test stimuli. Furthermore, the ability of each subject to correctly discriminate amplitude progressively worsened as the duration of the adapting stimulus (single-site) increased. We view these findings in strong agreement with a large body of literature detailing not only the perceptual but also neurophysiologic effects of adaptation. Additionally we believe that the methodology used in this study presents a number of advantages to standard methods for psychophysical assessment which could prove useful for the efficient and accurate measurement of perceptual metrics of cortical function.

A large number of studies have reported that individual SI neurons as well as the SI population response is systematically increased with an increase in the intensity of stimulation (Simons, 2005; Whitsel, 2003; Chen, 2003). These findings suggest that the

cortical mechanism responsible for coding the amplitude of a stimulus may be the magnitude of the population response in somatosensory cortex. A separate body of evidence describes a progressive decrease in the responsivity of neurons in somatosensory cortex during periods of repetitive and prolonged stimulation (Whitsel, 2003; Chung, 2002; Khatri, 2004; Sachdev, 2002; Laskin, 1979). Changes in the responsivity of neurons have been proposed to underlie the cortical mechanisms for stimulus feature extraction and may be important to behaviors involving discrimination. Decreases in responsivity evoked by periods of prolonged stimulation (such as the adaptation intervals used in this study) lead to a reduced response in the same neurons following a subsequent stimulus and may account for the decreases in a subject's capacity for amplitude discrimination, such as that observed during experiments with single site adapting stimuli.

The effects of delivering an adapting stimulus, sometimes referred to as a masking stimulus, on the perception of subsequent test stimuli has been characterized in some detail. Many psychophysical studies have reported that the presentation of an adapting stimulus causes an increase in the detection threshold, and thus a decrease in the perceived intensity, of a subsequent stimulus (for review, see Verrillo, 1985; 1977; Gescheider, 1995; Goble, 1993). More specifically, Gescheider et al. showed that the threshold shift which occurred after the presentation of an adapting stimulus systematically increased with increasing duration of the adapter (1995). Fewer studies have examined the impact of an adapting stimulus on the perceptual task of amplitude discrimination. Goble and Hollins reported that while an adapting stimulus increased the threshold for detection of a subsequent stimulus it also enhanced a subject's ability to discriminate subtle differences in the intensity of two subsequent (suprathreshold) stimuli which were presented serially to the same site (1993).

These studies suggest that the decline in subjects' performance on the amplitude discrimination task during single site adaptation observed in our study can at least in part be explained by a decrease in the perceived intensity of the test stimulus delivered at the same site of the adapter. Conversely, when adapting stimuli were delivered simultaneously to both sites, although it is likely that the perceived intensity of the subsequent test stimuli was diminished, the subjects' demonstrated improvement on the discrimination task.

What mechanisms are responsible for the changes that occur with repetitive stimulation? There is significant evidence that many of the moment-to-moment changes that occur with repetitive stimulation are NMDA receptor dependent (Whitsel, 1991). Observations of the spatial patterns of SI cortical response within an activated cortical region, such as those evoked by flutter stimulation of the skin, suggest that evoked cortical activity within such a territory is not evenly distributed. Furthermore, the cortical activity patterns changed in a manner that is dependent upon stimulus conditions – in particular, Chiu et al. demonstrated that these changes at the mini-columnar resolution level were systematically and progressively altered with increases in both stimulus amplitude and duration (2005). Very few, if any, studies have made systematic observations of the effects of a conditioning stimulus on the impact of a sensory percept evoked by two simultaneously delivered stimuli. What are the advantages of such a technique? One overriding goal that we have in the development of all of our psychophysical protocols (Tommerdahl, 2007) is the ultimate utility of providing not only the accurate assessment of sensory cortical function, but an efficient process that could be useful in a clinical and/or clinical research environment. It should be noted that the simultaneous amplitude discrimination task that we report here takes approximately 3-4 minutes to complete. This duration contrasts sharply with traditional

psychophysical techniques which usually require significantly longer periods of time and are difficult, if not impossible, to implement in a clinical setting. Demonstration of measures – such as the one described in this report - that are sensitive to changing conditions of adaptation, could prove useful for subject populations that have systemic alterations of the cerebral cortex that compromise factors which contribute to the cortical process of adaptation.

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