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14. ABSTRACT There are two specific aims or categories of deliverables to be accomplished as tasks in this DoD sponsored research: The first specific aim is the hardware design and fabrication of a portable tactile diagnostic stimulator that can be used for the assessment of the cerebral cortical health of neurologically compromised subjects – in particular, those subjects with autism. The second specific aim is the development of tactile discriminative protocols that will be used for the evaluation of the differences in cerebral cortical function between subjects with and without autism. In this first year of research, all the milestones listed for Y01 in the Statement of Work were met.					
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Introduction

There are two specific aims or categories of deliverables to be accomplished as tasks in this DoD sponsored research: The first specific aim is the hardware design and fabrication of a portable tactile diagnostic stimulator that can be used for the assessment of the cerebral cortical health of neurologically compromised subjects – in particular, those subjects with autism. The second specific aim is the development of tactile discriminative protocols that will be used for the evaluation of the differences in cerebral cortical function between subjects with and without autism. In this first year of research, all the milestones listed for Y01 in the Statement of Work were met. To summarize, two versions of the two-point vertical displacement vibrotactile stimulator were built and tested, and these devices were controlled by a standard PC with a DAQ interface. Additionally, after human subject research protocols were approved, the two-point vertical displacement vibrotactile stimulator prototype was used to assess and establish baseline values of simultaneous amplitude discrimination in healthy subjects. Subjects with autism were also recruited and studied using the same protocol. The final milestone - to initiate a protocol to establish baseline measures of the effects of vibrotactile adaptation in healthy subjects – was also achieved. The methods that have been and are being developed via tactile sensory diagnostics allow for objective assessment of neurophysiological functional connectivity and could prove to be effective tools for noninvasive assessment of cerebral cortical function.

Body

Milestone #1: *Design and fabrication of a portable diagnostic stimulator*

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. Prior to grant submission, 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. Based on the original TPS, we designed and fabricated a new stimulator with four 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.

Description of the Device

The Cortical Metrics (CM-1; see Figure 1) stimulator was developed in our laboratories for use in the series of experiments described in this report. The system was designed using 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 CO₂ laser engraving system, model number X660 (Universal Laser Systems, Scottsdale, AZ). The more complex housing and internal mechanism components are direct

manufactured from polycarbonate (PC), by fusion deposition modeling (FDM) on a StrataSys Titan T-1 FDM (StrataSys, Inc., Eden Prairie, MN). All housing and mechanism components and assemblies were solid modeled prior to fabrication using SolidWorks solid modeling software (SolidWorks Corporation, Concord, MA).

The internal mechanism is comprised of two independent x-z positioning tables onto each of which is mounted a voice coil actuator (VCA) motor and position sensors. The VCA motors drive the plastic stimulator probe tips according to prescribed sinusoidal waveforms. The moving components of the stimulator tips are directly manufactured from PC by FDM as a single compliant mechanism component integrating a mounting flange, a thin-beam four-bar linkage, a magnet coil bobbin, an optical displacement sensor vane, and the extension to the mechanical stimulator tip. These components are designed and manufactured so that they can be assembled in mirror-image configuration to allow the two internal tip-placement mechanisms to be mounted adjacent to one another and to allow the tips to be positioned horizontally in contact (distance = 0.0 mm) or separated linearly by a distance of up to 60 mm center-to-center. The compliant four-bar linkage mechanism allows the coil, optical position sensor vane, and tip to be vibrated vertically along a straight line for a distance of ± 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.



Figure 1: First Version of Portable Tactile Diagnostic Stimulator

The x-z positioning tables are each comprised of two orthogonal stacked linear slides driven by stepper motors and miniature precision ACME drive screws with embedded motor drive controllers for each motor, based upon the bipolar drivers we have employed elsewhere (Dennis et al., 2003). The x-position (horizontal movement) is detected using a linear slide potentiometer, allowing placement accuracy better than 0.1 mm over a horizontal movement range of 30 mm for each x-axis mechanism. Working in mirror opposition, the two x-axis slides thus allow a total tip separation up to 60 mm. The z-position (vertical movement) is similarly configured, but with an optical slit detector to determine the vertical “HOME” position, which is

the maximum vertical position with the tips withdrawn to their greatest height within the device. The position of the vibrating tips is detected by non-contacting optical displacement sensors, one for each tip, similar in configuration to ones we have previously employed in precision optical force transducers (Dennis and Kosnik, 2002). When the tips are not being driven, the optical position sensors can act as a highly-sensitive contact sensor. By employing the optical position sensor, the tips can be driven to contact the skin, and the contact force of each tip can be adjusted so that they are either equal or different by a known amount, because the spring constant of the VCA four-bar linkage mechanisms is identical.

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

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

Milestone #2: *Development of tactile discriminative protocols*

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.

In order to systematically investigate the spatial discriminative capacity of human subjects, a two alternative forced-choice (2AFC) tracking procedure was used to assess a subject’s ability to discriminate the amplitude difference between two stimuli positioned at near-adjacent skin sites. Two 25 Hz flutter stimuli, identical except for a constant

difference in amplitude, were delivered simultaneously to the hand dorsum. The stimuli were initially spaced 30 mm apart, and the inter-stimulus distance was modified on a trial-by-trial basis based on the subject's performance of discriminating the stimulus with higher intensity. The experiment was repeated via sequential, rather than simultaneous, delivery of the same vibrotactile stimuli. Results obtained from this study showed that the performance of the amplitude discrimination task was significantly degraded when the stimuli were delivered simultaneously and were near a subject's two-point limen. In contrast, subjects were able to correctly discriminate between the amplitudes of the two stimuli when they were sequentially delivered at all inter-probe distances (including those within the two-point limen), and improved when an adapting stimulus was delivered prior to simultaneously delivered stimuli. Subjects' capacity to discriminate the amplitude difference between two vibrotactile stimulations was degraded as the inter-stimulus distance approached the limit of their two-point spatial discriminative capacity. This degradation of spatial discriminative capacity lessened when an adapting stimulus was used. Performance of the task, as well as improvement on the task with adaptation, would most likely be impaired if the cortical information processing capacity of a subject or subject population were systemically altered, and thus, the methods described could be effective measures for use in clinical or clinical research applications. The details of these methods and results can be found in Zhang et al, 2008 in the Appendix.

A number of neurophysiological characteristics demonstrated in autism share the common theme of under-connectivity in the cerebral cortex. One of the prominent theories of the cause of the dysfunctional connectivity in autism is based on distinct anatomical structures that differ between the autistic and the neurotypical cortex. The functional minicolumn has been identified as occupying a much smaller space in the cortex of people with autism as compared to neurotypical controls, and this aberration in architecture has been proposed to lead to under-connectivity at the local or within-macrocolum level, which in turn leads to dysfunctional connectivity globally across cortical areas in persons with autism. Numerous reports have indicated reduced synchronization of activity on a large scale in the brains of people with autism. We hypothesized that if the larger-scale aberrant dynamics in autism were due – at least in part – to a widespread propagation of the errors introduced at the level of local connectivity between minicolumns, then aberrations in local functional connectivity should also be detectable in autism. In order to assess the impact that between local cortical-cortical connectivity could have on information processing in autism, the temporal order judgment (TOJ) and temporal discriminative threshold (TDT) of 10 adult autism subjects and 10 healthy control subjects were assessed both in the absence and presence of synchronized conditioning vibrotactile stimuli. The study demonstrated that delivering simultaneous and synchronized vibrotactile stimuli to near-adjacent skin sites decreases a healthy subject's ability to determine temporal order by 3 to 4-fold. However, results obtained from autism subjects demonstrated that subjects with autism do not demonstrate such decreased capacity in temporal order judgment (TOJ) in the presence of synchronized conditioning stimuli, although these same subjects do have TOJ thresholds well above that of controls. This finding leads to the speculation that the differences in sensory perceptual capacities in the presence of synchronized conditioning stimuli in autism are due to local under-connectivity in cortex at the minicolumnar organizational level, and that the above-average TOJ thresholds in autism could be attributed to structural differences that have been observed in the frontostriatal system of this population. These findings are summarized in Tommerdahl et al, 2008 in the Appendix.

Key Research Accomplishments

- Design & fabrication of a portable two-point stimulator
- Development of novel protocols of cortical information processing assessment
- Collection of baseline data from healthy subjects for spatial discrimination tasks
- Collection of baseline data from healthy subjects for amplitude discrimination tasks
- Initiation of data collection from subjects with autism

Reportable Outcomes***Peer Reviewed Manuscripts, accepted for publication:***

Zhang Z, Tannan V, Holden J, Dennis RG, Tommerdahl M. (2008) A quantitative method for determining spatial discriminative capacity. *BioMedical Engineering OnLine* 2008, 7:12 (10 Mar 2008).

Tommerdahl M, Tannan V, Holden JK, Baranek GT. (2008) Absence of stimulus-driven synchronization effects on sensory perception in autism: Evidence for local underconnectivity? *Behavioral and Brain Functions*, 2008, 4:19 (24 April 2008).

Manuscripts in Review:

Tannan V, Holden JK, Zhang Z, Baranek GT, Tommerdahl M. (2008) Perceptual metrics of individuals with autism provide evidence for disinhibition, *In Revision*.

Tommerdahl M, Tannan V, Favorov OV (2008) The role of cortical modularity in tactile information processing: an approach to measuring information processing deficits in autism.

Presentations:

Tommerdahl, M. The role of cortical modularity in tactile information processing. *Cortical Modularity in Autism*, Oct 14, 2007. Louisville, Ky.

Abstracts:

Zhang Z, Tannan V, Holden J, Tommerdahl M. A quantitative method for determining two-point discriminative capacity. *Neuroscience Abstracts* 74.8, 2007.

Conclusion:

The development of unique quantitative sensory testing methods was made possible by the design and fabrication of a portable two-point vibrotactile stimulator. The sensory testing methods and apparatus that were developed were designed to enable objective evaluation of the elaborate neuroanatomical connectivity that subserves the neuronal communication between adjacent and near-adjacent regions within sensory cortex that is widely recognized to be essential to normal sensory function. There have been several significant findings in the early stages of applications of these methods in our autism research. First, results comparing the spatial localization ability of subjects with autism vs. controls demonstrated that although cutaneous localization performance of adults with autism is significantly better than the performance of control subjects, tactile spatial discriminative capacity remained unaltered in the same autism subjects when examined after the duration of adapting stimulation was increased although significant improvement was observed in controls. Second, results comparing the ability of subjects with autism to discriminate between the intensity of two simultaneously delivered stimuli demonstrated that although autism subjects were equal to or better than control subjects at short duration discrimination tasks, conditioning stimuli delivered prior to a task had no impact on the autism subjects' ability to discriminate although this conditioning had significant impact on control subjects' perception. Both the failure of prior history of

tactile stimulation to alter sensory percepts in adults with autism, and the better-than-normal perceptual performance of adults with autism in these tasks, were concluded, in the above-mentioned studies, to be attributable to both the smaller than normal minicolumn width observed in autism subjects and the deficient cerebral cortical GABAergic inhibitory neurotransmission characteristic of this disorder. A third study examined the effective short-range functional connectivity of subjects with autism vs. neurotypical controls, and significant differences were found between controls and autism subjects in the influence that synchronizing stimuli have on sensory perception. An important emerging concept in autism research is the role of dysfunctional neural synchrony, and it was speculated from these recent findings that the local functional connectivity that normally subserves long range connectivity and synchrony could be a result of the abnormal minicolumn architecture that has been previously reported by others. One unifying theme of these findings is the role that cortical modularity plays in sensory information processing, and in autism, cortical modularity is disrupted to an extent that significant quantifiable deficits in sensory information processing can be detected. In terms of practical application, future work could mean that the methods that we are developing could be used for both basic diagnostic applications as well as determination of efficacy of intervention.

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- Tommerdahl M, Tannan V, Holden JK, Baranek GT. (2008) Absence of stimulus-driven synchronization effects on sensory perception in autism: Evidence for local underconnectivity? *Behavioral and Brain Functions*, 2008, 4:19 (24 April 2008).
- Zhang Z, Tannan V, Holden J, Dennis RG, Tommerdahl M. (2008) A quantitative method for determining spatial discriminative capacity. *BioMedical Engineering OnLine* 2008, 7:12 (10 Mar 2008).

Appendices:

The following papers are included in the Appendix:

- Zhang Z, Tannan V, Holden J, Dennis RG, Tommerdahl M. (2008) A quantitative method for determining spatial discriminative capacity. *BioMedical Engineering OnLine* 2008, 7:12 (10 Mar 2008)
- Tommerdahl M, Tannan V, Holden JK, Baranek GT. (2008) Absence of stimulus-driven synchronization effects on sensory perception in autism: Evidence for local underconnectivity? *Behavioral and Brain Functions*, 2008, 4:19 (24 April 2008)

Research

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A quantitative method for determining spatial discriminative capacity

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Abstract

Background: The traditional two-point discrimination (TPD) test, a widely used tactile spatial acuity measure, has been criticized as being imprecise because it is based on subjective criteria and involves a number of non-spatial cues. The results of a recent study showed that as two stimuli were delivered simultaneously, vibrotactile amplitude discrimination became worse when the two stimuli were positioned relatively close together and was significantly degraded when the probes were within a subject's two-point limen. The impairment of amplitude discrimination with decreasing inter-probe distance suggested that the metric of amplitude discrimination could possibly provide a means of objective and quantitative measurement of spatial discrimination capacity.

Methods: A two alternative forced-choice (2AFC) tracking procedure was used to assess a subject's ability to discriminate the amplitude difference between two stimuli positioned at near-adjacent skin sites. Two 25 Hz flutter stimuli, identical except for a constant difference in amplitude, were delivered simultaneously to the hand dorsum. The stimuli were initially spaced 30 mm apart, and the inter-stimulus distance was modified on a trial-by-trial basis based on the subject's performance of discriminating the stimulus with higher intensity. The experiment was repeated via sequential, rather than simultaneous, delivery of the same vibrotactile stimuli.

Results: Results obtained from this study showed that the performance of the amplitude discrimination task was significantly degraded when the stimuli were delivered simultaneously and were near a subject's two-point limen. In contrast, subjects were able to correctly discriminate between the amplitudes of the two stimuli when they were sequentially delivered at all inter-probe distances (including those within the two-point limen), and improved when an adapting stimulus was delivered prior to simultaneously delivered stimuli.

Conclusion: Subjects' capacity to discriminate the amplitude difference between two vibrotactile stimulations was degraded as the inter-stimulus distance approached the limit of their two-point spatial discriminative capacity. This degradation of spatial discriminative capacity lessened when an adapting stimulus was used. Performance of the task, as well as improvement on the task with adaptation, would most likely be impaired if the cortical information processing capacity of a subject or subject population were systemically altered, and thus, the methods described could be effective measures for use in clinical or clinical research applications.

Background

The capacity of a human subject to spatially resolve tactile stimuli delivered to the skin has traditionally been investigated by measuring the smallest distance between two tactile stimuli at which they evoke two distinct percepts [1]. Typically, the two-point discrimination (TPD) test has been widely used in clinical diagnoses as well as scientific studies. Along with its popular applications, however, TPD has been criticized as being imprecise for several reasons. First, it has been discussed that as the distance between two points varied, the perceptual patterns may gradually change. Tawney [2] stated that there were some intermediate sensations between the perception of one point and that of two points. As a result, the "first perception" of two points measured as TPD might provide an inaccurate measure of the minimum space of tactile spatial resolution whereas the "middle sensations" may represent the actual consciousness of spatiality [2,3]. Second, since different subjects adopted distinct criteria for defining two points, the responses were based to a great extent on the subject's experience. As a result, a large variability between subjects has been observed. Craig and Johnson [4] quoted a study in which Valentin and collaborators found that the TPD measures were highly inconsistent across all subjects, with nearly a four-fold difference in thresholds observed on the same region of the body. Third, traditional TPD tests involve a number of non-spatial cues which confounded subject discrimination. For instance, Tichener [5] found that in the objective TPD tests which employed one-point as well as two-point stimulation, subjects felt that the perceived intensity of one point was always stronger than that of two points. The above-described arguments suggest that the subjective TPD threshold might not provide a consistent and reliable measure of tactile spatial resolution. For these reasons, we sought to develop a more objective measure of spatial discrimination capacity.

Alternative methods have been developed to substitute for the traditional TPD test. Tannan et al. [6-8] presented a novel Two-Point Stimulator (TPS) which was capable of delivering two identical vibrotactile stimuli simultaneously at two discrete skin sites with variable distances on a trial-by-trial basis. By way of automated stimulus control and delivery, the TPS enabled a faster and more accurate administration of two-point measurement than previous TPD devices. However, in these particular studies, the discrimination test was still based on personal subjective criteria. Similarly, a number of other studies have demonstrated that grating orientation discrimination is a well-established and reproducible measurement of tactile spatial acuity on the finger pad [9-11]. However, it was argued that there might be substantial anisotropy on the finger pad which was related to spatial sensitivity and might permit subjects to discriminate grating orienta-

tion on the basis of intensive rather than spatial cues [10]. Additionally, a subject's orientation discrimination capability is typically assessed by interpolating the groove width with 75% correct responses [12,13]. Thus, in order to have enough values for interpolation, the percentages of accurate responses of several gratings with different groove widths need to be measured for each subject.

Recently, Tannan et al. [14] measured subjects' amplitude discrimination between two simultaneous 25 Hz vibratory stimuli delivered to the dorsum surface of the hand. The result indicated that amplitude discrimination became worse when the two stimuli were positioned relatively close together and was significantly degraded when the probes were within a subject's two-point limen. This impairment of amplitude discriminative capacity with decreasing inter-probe distance led the authors to hypothesize that the metric of amplitude discrimination could provide a means of objective and quantitative measurement of spatial discrimination between two-point on the skin. Such a measure could be used for objective evaluations of subject populations whose cortical information processing capacity is systemically altered or different from healthy control populations. In addition to assessing simple spatial discriminative capacity, slight modifications of stimulus conditions could reveal other aspects of a subject's central nervous system, based on predicted cortical-cortical interactions that result from these different stimulus conditions.

To investigate the above-described hypothesis, a modified Békésy protocol was used to assess a subject's ability to discriminate a constant amplitude difference between two 25 Hz flutter stimuli as the stimuli were tracked to more proximal skin sites on the hand dorsum. Although comparable to an amplitude discrimination task which measures the minimum discriminable amplitude difference between two simultaneously delivered stimuli [14], the current protocol was unique in that the amplitude difference was constant and well above the average threshold amplitude difference limen (reported in previous studies [14,15]), and the inter-stimulus distance was modified on a trial-by-trial basis based on the subject's performance. The inter-stimulus distance metric obtained from the study appears to be fairly robust across the subjects studied thus far (i.e., low variance between individual performance) and can be obtained relatively quickly (about three minutes).

Methods

Ten subjects participated in this experiment. They were naïve both to the study design and issue under investigation. All experimental procedures were reviewed and approved in advance by an institutional review board.

The tactile stimuli used in this study were sinusoidal vertical skin displacements delivered by a novel dual-site vibrotactile stimulator (details about the CM-1 stimulator are described in a recent report; [14]). The CM-1 dual-site stimulator is capable of delivering two tactile stimuli simultaneously or sequentially at discrete skin sites with independent control of vibration frequency, amplitude, and phase, while providing accurate control of stimulus's timing and location.

During the experiment, the subject was seated in a chair with his/her left forearm on the table positioned comfortably to allow unimpeded access of the stimulator to the center of the dorsal surface of left hand (Figure 1). To ensure a stable hand position for the duration of the experiment, the subject was instructed to place their palm on the table surface as flat as possible, and a bead bag was applied to immobilize the wrist. The reasons that we selected the hand dorsum to receive the stimulation are: 1) the innervation density across this skin region remains relatively constant; 2) the surface is easily accessible and permits convenient stimulator placement; 3) use-dependent plasticity is minimized (i.e., the hand dorsum is, for the most part, used the same amount in daily activity by all subjects); 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.

A two alternative forced-choice (2AFC) tracking procedure was used to assess a subject's ability to discriminate between the amplitudes of two simultaneously delivered

stimuli positioned at near-adjacent skin sites. Each run consisted of 20 trials. At the start of each trial, the two probe tips, 5 mm in diameter, were driven to the skin surface together and automatically stopped after skin detection. The tips were indented 500 μm further to ensure good contact with the skin. The stimulus position and timing diagram of the protocols are shown in Figure 2. Two 25 Hz flutter stimuli, identical except for a constant difference in amplitude (standard stimulus: 100 μm vs. test stimulus: 140 μm peak-to-peak amplitude), were delivered (see Figure 2a). After each trial, the subject was queried as to which skin site received the more intense stimulus. Subjects were instructed to indicate their selection with a switch box with their free hand.

The stimuli were initially spaced 30 mm apart (see Figure 1; well above two-point discrimination limen on the hand dorsum; [6-8]), and the inter-stimulus distance was modified on a trial-by-trial basis based on the subject's performance. During the first 10 trials, a 1 up/1 down tracking paradigm was used, allowing a single correct answer to cause a 10% reduction in inter-stimulus distance in the subsequent trial. After one inaccurate response, the probe tips were moved 10% further apart. In the last 10 trials, a 2 up/1 down tracking algorithm was used in which two correct responses were required to decrease the inter-stimulus distance by 10%. The combination of two tracking algorithms in this manner allows the threshold to be determined much faster without compromising the results [8,14].

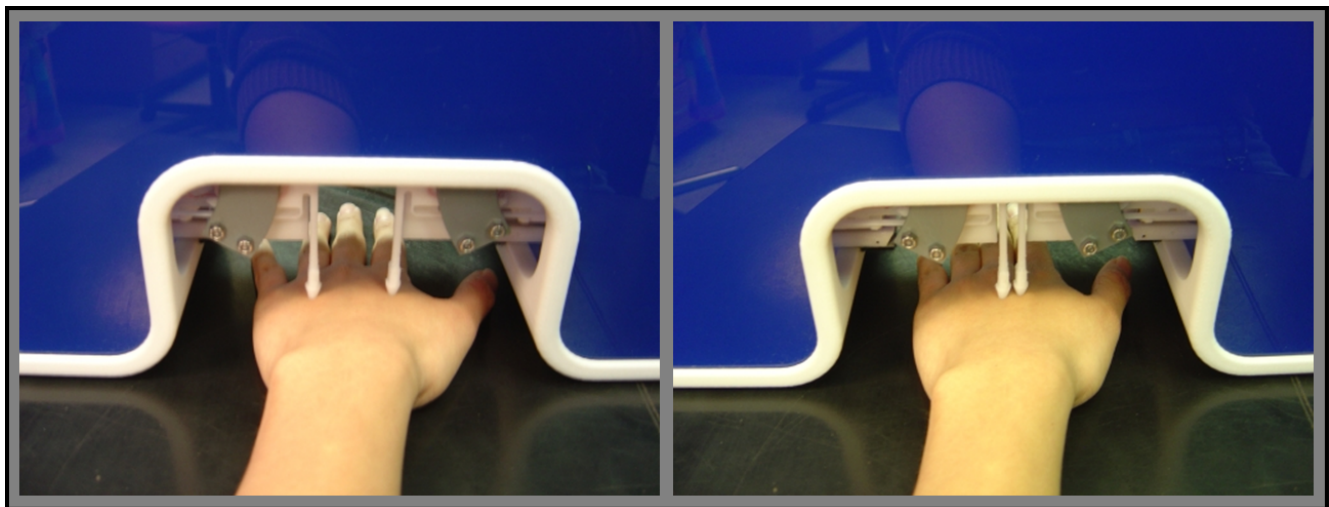
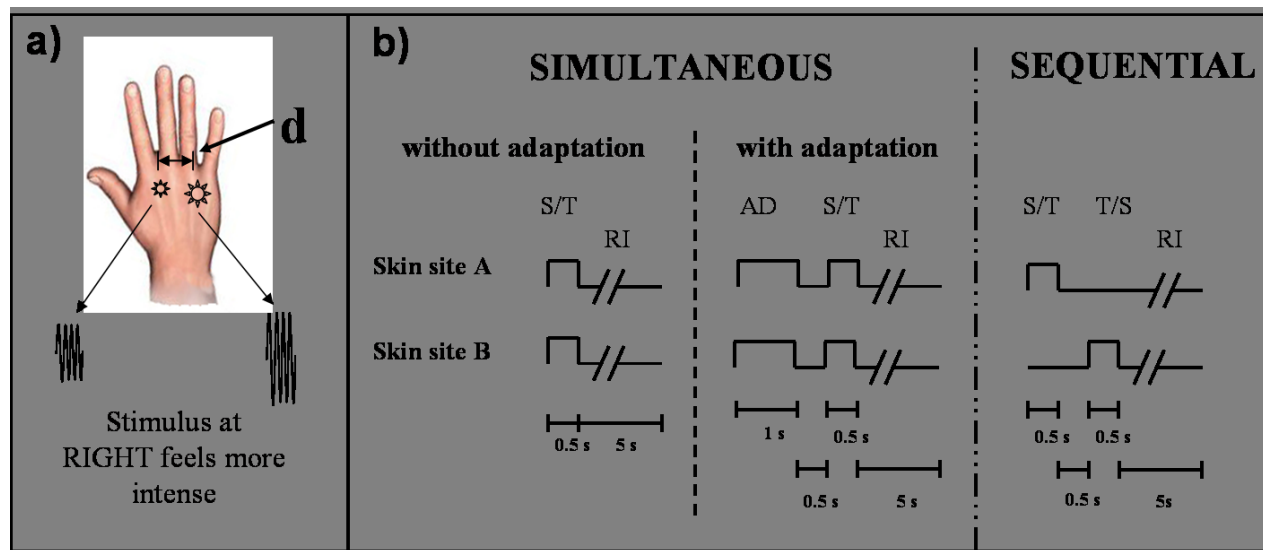


Figure 1
Stimulus position on the dorsal surface of the left hand. Probe tips detect the surface of the skin automatically. The stimuli were initially spaced 30 mm apart (left panel of figure) and the inter-stimulus distance was modified on a trial-by-trial basis based on the subject's performance. The minimal inter-stimulus distance possible was 5 mm with 5 mm diameter probe tips (right panel of figure).

**Figure 2**

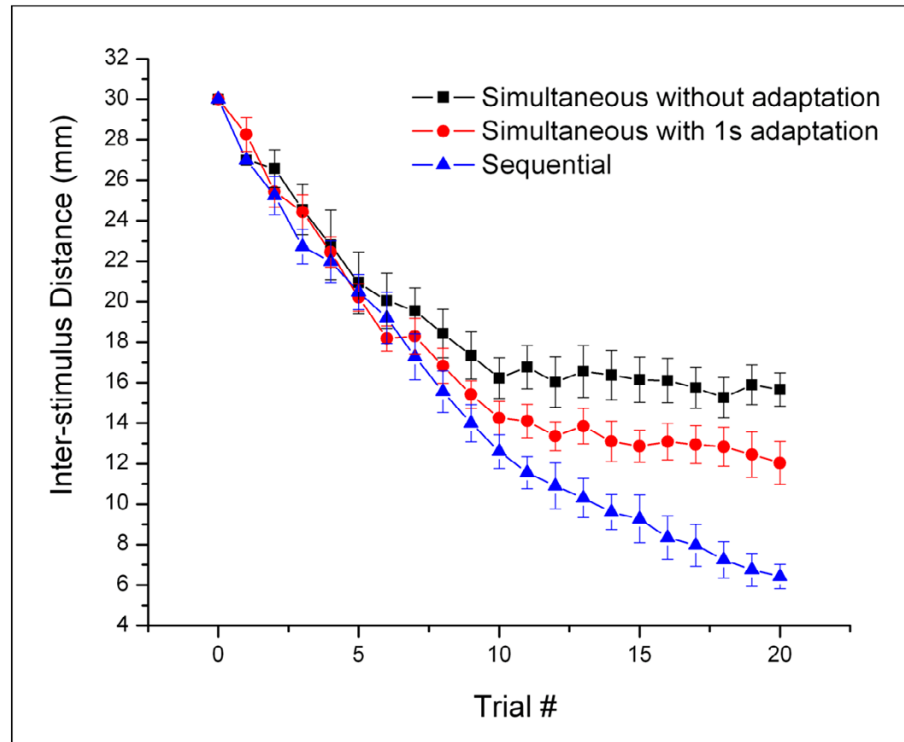
Timing diagram of the protocol. a) Two 25 Hz flutter stimuli, identical except for a constant difference in amplitude (standard stimulus (S): 100 μ m vs. test stimulus (T): 140 μ m peak-to-peak amplitude) were delivered. The stimuli were initially spaced 30 mm apart, and the inter-stimulus distance (d) was modified on a trial-by-trial basis based on subject performance. b) The task was performed under three conditions: 1) *Simultaneous without adaptation*: in each trial, the standard (S) and test (T) stimuli were delivered at the same time for 0.5 s. A 5 s delay including the subject response interval (RI) was imposed before onset of the next trial; 2) *Simultaneous with dual-site adaptation*: a pair of adapting stimuli (AD) (identical to the standard stimulus) was delivered first for 1 s at the same pair of sites as the test and standard stimuli. After a 0.5 s inter-stimulus interval, the test and standard stimuli were presented simultaneously; 3) *Sequential*: the standard and test stimuli were presented sequentially with a 0.5 s inter-stimulus interval. The order and loci of standard and test stimuli were randomized on a trial-by-trial basis.

The task was performed under three conditions (see Figure 2b): 1) *Simultaneous stimulation without adaptation*: in each trial, the standard (S) and test (T) stimuli were delivered at the same time for 0.5 s. A 5 s delay including the subject response interval (RI) was imposed before onset of the next trial; 2) *Simultaneous stimulation with dual-site adaptation*: a pair of adapting stimuli (identical to the standard stimulus) was delivered first for 1 s at the same pair of sites as the test and standard stimuli. After a 0.5 s inter-stimulus interval, the test and standard stimuli were presented simultaneously; 3) *Sequential Stimulation*: the standard and test stimuli were presented sequentially with a 0.5 s inter-stimulus interval. The order and loci of standard and test stimuli were randomized on a trial-by-trial basis. The three run conditions were randomized on a subject-by-subject basis.

Repeated measures analysis of variance (ANOVA) was used to evaluate the difference of the subject's performance under three conditions. Data are presented as means and standard errors (SE). A probability of less than 0.05 was considered statistically significant.

Results

A subject's ability to discriminate the intensity difference between two vibrotactile stimuli of fixed amplitudes at varying distances between stimulus sites was tracked to approach the inter-probe distance limit at which subjects could not reliably discriminate between the two stimuli. Figure 3 is a plot of the averaged response of tracking performance under three different conditions of stimulation. Each condition resulted in a significant change in tracking performance. Comparison of the data obtained in the sequential stimulation condition and the simultaneous stimulation condition demonstrates that the subjects' performance was degraded as the stimuli were moved closer together in the simultaneous condition, but not in the sequential delivery of stimuli. Note that when the inter-stimulus distance was decreased to approximately 16 mm (near the two-point limen for 25 Hz vibrotactile stimuli on the hand dorsum; [6-8]), discrimination performance became much worse. In contrast, for the sequential condition, subjects were able to correctly discriminate at all inter-stimulus distances, until the separation became 5 mm (minimal inter-stimulus distance possible with 5 mm diameter probe tips). Additionally, subjects' performance under the third condition – the simultaneous stimulation

**Figure 3**

The averaged response of tracking performance under three conditions. The subjects' performance was degraded as the stimuli were moved closer together in the simultaneous condition but not in the sequential delivery of stimulation. Under the third condition – the simultaneous stimulus condition with adaptation – pre-exposure to a pair of flutter stimuli (adaptation) at the same locations as the standard and test stimuli improve a subjects' discriminative capacity.

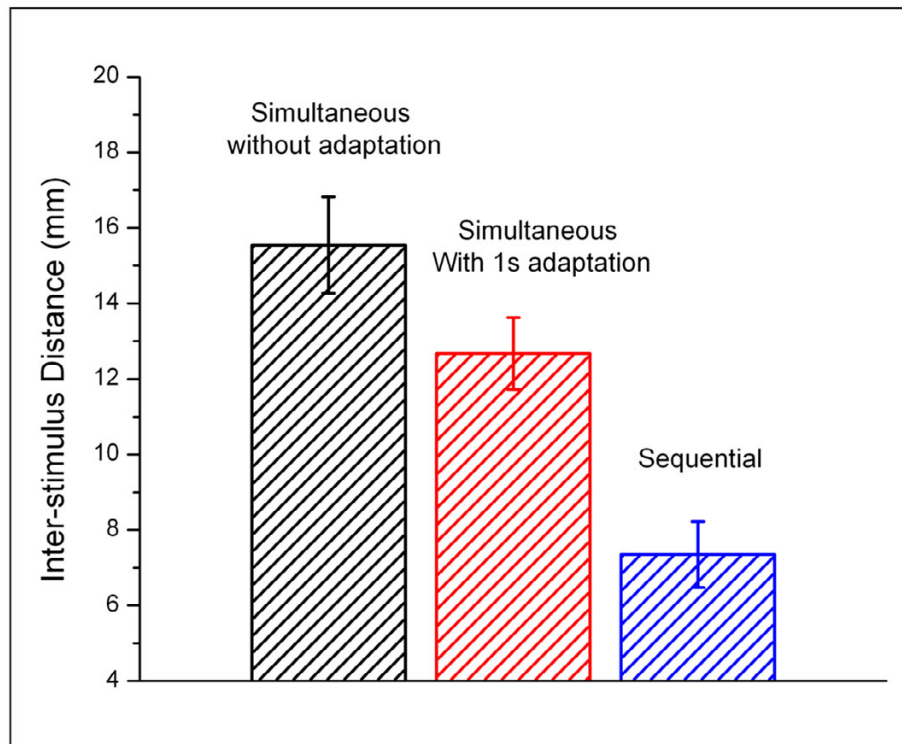
condition with adaptation – shows that pre-exposure to a pair of flutter stimuli (adaptation) at the same locations as the standard and test stimuli improve a subject's discriminative capacity. The data demonstrates a certain degree of consistency across subjects, as variability in the averaged plots of Figure 3 is relatively low (note error bars in plots).

In order to more directly compare the responses measured under each of the stimulation conditions, the tracking values obtained from the last five trials across all subjects were averaged (Figure 4). A significant difference was observed in performance between the simultaneous without adaptation and sequential conditions ($p < 0.001$). Additionally, when compared to the simultaneous non-adapting condition, subjects' performance in the simultaneous discrimination task with adaptation was significantly improved by ~20% ($p = 0.034$).

Discussion

In the present study, we investigated the effects of spatial acuity on amplitude discrimination between two flutter stimuli (25 Hz) delivered to the dorsal surface of the

hand. The results show that subjects were able to discriminate the amplitude difference between two sequentially delivered stimuli at all inter-stimulus distances from 30 mm to 5 mm (the diameter of the probe tip). When stimuli were presented simultaneously, however, the subjects' ability to discriminate the same amplitude difference was significantly impaired as the inter-stimulus distance was reduced to 16 mm (near the two-point limen). These results are consistent with a previously published report that demonstrated that amplitude discrimination capacity was significantly worse when inter-stimulus distances were reduced from 30 mm to 5 mm [14]. In a task that tracked only a subject's ability to discriminate amplitude differences, Tannan et al found a significant difference in amplitude discrimination capability when the stimuli were delivered simultaneously vs. sequentially at near adjacent skin sites (10 mm or less). Additionally, the results were consistent with the two-point discriminative capacity previously reported for the hand dorsum (16 mm, 17 mm, 20 mm, and 12 mm respectively for four subjects) by Tannan et al [6]. However, in that study, the inter-subject variability was reported to be much higher

**Figure 4**

Average of the distances tracked in the last five trials across all subjects. A significant difference was observed in performance between the simultaneous stimulation without adaptation and the sequential conditions ($p < 0.001$). Adaptation resulted in a significant improvement (~20%) on simultaneous amplitude discrimination at small inter-stimulus distances ($p = 0.034$).

(20% vs. 10% of the threshold value), and we suspect that the increased variability in that task was due to the subjective nature of the task. In other words, variability for the findings in this report were lower principally due to the increased objectivity of an amplitude discrimination task that fails due to a decreased spatial discriminative capacity rather than delivering two points to the skin and challenging the subject to only determine whether they felt one or two points.

Sequential and simultaneous test conditions were delivered in order to directly assess the impact that inter-stimulus distance had on a subject's amplitude discrimination capacity. The comparison between sequential and simultaneous stimulus conditions demonstrated that the degradation of amplitude discrimination capacity in the simultaneous stimulus condition was possibly solely due to the subject's inability to discriminate between two points when they were located in near proximity. LaMotte and Mountcastle [16,17] stated that the ability of a subject to accurately localize a flutter stimulus on the skin is determined by the locus and clarity of the neuronal pop-

ulation response within the topographically organized SI network. When two stimuli are positioned close together on the skin, the activity in the two neuron populations evoked by the two stimuli in the cortex may tend to overlap. As a result, subjects may perceive only one, instead of two distinct sensations. If this is the case, the distance between two stimuli tracked in the simultaneous stimulus condition may be equivalent to the spatial metric that traditional TPD tests were intended to measure.

An important distinction between the protocol used in this study and the traditional two-point discrimination tasks is that the amplitudes of the two stimuli were significantly different, and it is important to consider the spatial extent that larger amplitude stimuli may (or may not) occupy. Simons et al [18,19] imaged the optical intrinsic signal of the SI responses evoked by vibrotactile stimulation with different amplitudes in non-human primates. They found that as the stimulus amplitude was increased, the activity within the activated region of SI cortex progressively increased although the spatial extent of the activated region remained relatively constant. Rather, with

increasing stimulus amplitude and duration, the region surrounding the activated cortical field became less active (or more inhibited), suggesting that more intense and longer duration stimuli would result in more spatially resolved stimuli. Results of the present study appear to be consistent with the findings of Simons and colleagues such that all subjects demonstrated improved discrimination in the simultaneous stimulus condition when the stimulus sites were pre-exposed to 1 s adapting stimulation.

The effects of an adapting stimulus on the perception of subsequent stimuli – particularly the reduction in sensation – have been characterized in some detail [20-25]. However, only a relatively small number of studies have assessed the impact that prior exposure to vibrotactile stimuli has on spatial localization or the spatial acuity necessary to discriminate between two points on the skin, and all of these studies demonstrated that adaptation improved spatial acuity [7,8,26,27]. This improvement was originally proposed to be due to the improved spatial clarity between topographically distinct regions of SI cortical activity [16,17]. Two recent reports have examined the effects of stimulus duration-dependent changes on a subject's ability to spatially localize a stimulus. Tannan et al [8] demonstrated that the performance of neurologically healthy human adults on a spatial localization task undergoes a prominent change with pre-task exposure to an adapting stimulus. In that study, it was determined that adaptation with a longer duration (5 s) vibrotactile stimulus resulted in an approximately 2-fold improvement in spatial localization performance over that achieved with a shorter (0.5 s) stimulus. It was proposed that this observed improvement in spatial localization was due to the enhanced spatial funnelling of the population-level response of contralateral primary somatosensory cortex (SI) – a robust phenomenon that is at least in part due to GABAergic inhibitory neurotransmission [28] and has been demonstrated using comparable stimulus conditions in neuroimaging studies of anesthetized non-human primates [18,19,29]. A subsequent report strengthened this argument by demonstrating that neurologically compromised subjects with a known GABAergic deficiency (adults with autism) showed no such improvement at the same spatial localization task with adaptation [30]. Thus, there seems to be some evidence that spatial acuity does improve in a stimulus-dependent and GABA-mediated manner that undoubtedly impacts the spatial contrast of cortical activity evoked by vibrotactile stimuli. Changes in the responsivity of neurons have been proposed to underlie the cortical mechanisms for stimulus feature extraction and may be important in the improvements observed in spatial discrimination such as those described above (for review see [31]). This enhancement of discrimination capacity could be due, at least in part, to

the moment-to-moment changes that occur in the spatio-temporal patterns of response with repetitive vibrotactile stimulation.

We speculate that the observed improvement of subjects' performance in this study with adaptation is solely due to the effects of adaptation on spatial acuity. It is important to note that in this study, instead of tracking an amplitude difference (as in more commonly performed amplitude discrimination tasks), a constant amplitude difference, which is well above normal subject's amplitude discrimination threshold [14], was maintained while the inter-probe distance was tracked. The subjects' excellent performance under the stimulus condition in which stimuli were delivered sequentially suggests that discriminative capacity (in the simultaneous stimulation condition) was predominantly impacted by the spatial parameters imposed by the inter-stimulus distance. As a result, when two stimuli were delivered simultaneously and in near-proximity, the effects of pre-exposure to dual-site adapting stimuli would be to facilitate the discriminative aspect affected by spatial acuity, but not necessarily facilitate what would normally be an easy amplitude discriminative task. Thus, any adaptive effects on the amplitude discriminative task – which have been reported in several studies [15,32-34] – could most likely be regarded as having little impact on the results in this study.

Conclusion

Subjects were not able to discriminate between two amplitudes of vibrotactile stimulation simultaneously delivered to the skin as the inter-stimulus distance approached the limit of a subject's spatial acuity. The inter-stimulus distance metric obtained from the study is robust across the subjects studied thus far (i.e., low variance between individual performance) and can be obtained relatively quickly (about three minutes). The strongest candidate responsible for the improvement in performance observed with adapting stimulation appears to be GABAergic mediated lateral interactions. Performance on the task, as well as improvement on the task with adaptation would most likely be impaired if GABAergic function in a subject (or subject population) were systemically altered, and thus, the methods described could provide an effective set of measures for assessing systemic cortical alterations in such subject populations.

Abbreviations

TPD: two point discrimination; 2AFC: two alternative forced-choice; TPS: two-point stimulator; ANOVA: analysis of variance.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

ZZ participated in the design of the study, carried out the data collection and analysis, and drafted the manuscript. VT participated in the design of the study and revision of the manuscript. JH assisted in design of the protocol and in the data collection. RD contributed to the design of the experiment. MT played a role in the design of the study, the coordination of the study and drafting the manuscript. All authors read and approved the final manuscript.

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Absence of stimulus-driven synchronization effects on sensory perception in autism: Evidence for local underconnectivity?

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Absence of stimulus-driven synchronization effects on sensory perception in autism: Evidence for local underconnectivity?

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Abstract

Background

A number of neurophysiological characteristics demonstrated in autism share the common theme of under-connectivity in the cerebral cortex. One of the prominent theories of the cause of the dysfunctional connectivity in autism is based on distinct anatomical structures that differ between the autistic and the neurotypical cortex. The functional minicolumn has been identified as occupying a much smaller space in the cortex of people with autism as compared to neurotypical controls, and this aberration in architecture has been proposed to lead to under-connectivity at the local or within-macrocolumn level, which in turn leads to dysfunctional connectivity globally across cortical areas in persons with autism. Numerous reports have indicated reduced synchronization of activity on a large scale in the brains of people with autism. We hypothesized that if the larger-scale aberrant dynamics in autism were due – at least in part – to a widespread propagation of the errors introduced at the level of local connectivity between minicolumns, then aberrations in local functional connectivity should also be detectable in autism.

Methods

Recently, we reported a method for measuring the perceptual changes that are impacted by the presence of synchronized conditioning stimuli on the skin. In this study, the temporal order judgment (TOJ) and temporal discriminative threshold (TDT) of 10 adult autism subjects were assessed both in the absence and presence of synchronized conditioning vibrotactile stimuli.

Results

Our previous report demonstrated that delivering simultaneous and synchronized vibrotactile stimuli to near-adjacent skin sites decreases a subject's ability to determine temporal order by 3 to 4-fold. However, results presented in this report show that subjects with autism do not demonstrate such decreased capacity in temporal order judgment (TOJ) in the presence of synchronized conditioning stimuli, although these same subjects do have TOJ thresholds well above that of controls.

Conclusions

It is speculated that the differences in sensory perceptual capacities in the presence of synchronized conditioning stimuli in autism are due to local under-connectivity in cortex at the minicolumnar organizational level, and that the above-average TOJ thresholds in autism could be attributed to structural differences that have been observed in the frontostriatal system of this population.

Background

Autism is a pervasive developmental disorder that affects many aspects of the central nervous system, including sensory and motor deficits. For example, a number of autism studies have described Parkinson-like motor characteristics and/or postural control problems which could be attributed to deficits of the basal ganglia portion of the frontostriatal system [1, 2]. These deficits in sensorimotor control could be derived, in part, from the role that the frontostriatal system plays in an individual's timing perception as well as the coordination that is required between cortical regions during sensorimotor tasks. One relatively simple measure that can be used for the evaluation of a subject's timing perception is Temporal Order Judgment (TOJ). TOJ is a measure obtained from determining the minimal inter-stimulus interval necessary for a subject to detect the temporal order of two sequentially delivered peripheral stimuli. This metric of timing perception has been shown to be sensitive to lesions to the supplementary motor area (SMA), posterior parietal cortex, and basal ganglia [3, 4]. Additionally, these cortical areas have been implicated from significantly elevated TOJ thresholds (worse performance) in subjects with dyslexia [5], dystonia [6-8], and Parkinson's Disease [9]. One goal of our study was to determine if timing perception in subjects with autism would be elevated in a similar fashion.

Although the sensory aspect of an individual's timing perception could play a distinct role in sensorimotor coordination, the lack of larger scale across-cortex integration and coordination of activity across multiple cortical regions has been demonstrated as being characteristic of autism [2, 10-12]. Recently, the role of synchronization (or lack of synchronization) in autism has gained a certain degree of prominent attention. Uhlhaas and Singer [13] recently reviewed the experimental evidence that suggests that functional connectivity is reduced in autism, primarily based on fMRI studies [10-12, 14-16] that examine the coordinated activity between different areas of the cerebral cortex. Uhlhaas and Singer [13] argued that these data predict that measures of neural synchrony in subjects with autism should be reduced, yet they also pointed out that there are only a small number of studies that actually address such comparisons of synchronization between neurotypical adults and individuals with autism (e.g., [17, 18]). From another perspective, there is a large body of evidence that the cerebral cortex of subjects with

autism is significantly modified at the minicolumnar level [19]. Casanova and colleagues suggest that this aberrant minicolumnar structure results in the disruption of the inhibitory architecture [20] that is required for normal function in local neural circuitry. They suggest that disruption of functional connectivity at the local minicolumnar level could be responsible for, or strongly correlated with, the dysfunctional connectivity that has been observed across large scale cortical areas, as described in the neural synchrony studies noted above. In this study, we sought to obtain measures addressing the impact that coordinated somatosensory activity in a local cortical region has on subjects with autism.

We recently investigated the impact that stimulus-driven neuronal interactions, evoked by vibrotactile stimuli at dual skin sites which project to adjacent and near-adjacent cortical ensembles, could have on TOJ [21]. In that study, it was reported that delivering weak intensity (low amplitude) but synchronized and periodic vibrotactile stimuli unilaterally to two adjacent digit tips (D2 and D3) significantly and robustly (3-4 fold) degraded a subject's TOJ performance. However, delivery of the same stimulus conditions to bilateral skin sites showed that there was little or no impairment in TOJ performance. One of the conclusions that was drawn from that study was that the stimulus-driven effect of the synchronized conditioning stimuli coordinated the activity of near-adjacent cortical ensembles (such as those representing two fingers used in normal everyday tasks) and consequently, made it more difficult to distinguish one cortical locus from the other as the two stimulus sites were effectively perceptually bound by the stimulus-driven synchronization.

The above-described method that we recently reported involves “forcing” adjacent cortical regions to become synchronized (via stimulus-drive), and then measuring the impact that the cortical-cortical interactions generated by such synchronized activity has on sensory percepts known to be modulated by activity in those same cortical regions. In other words, if the activity in the cortical regions that represent D2 and D3 in somatosensory cortex become synchronized and/or coordinated, it should be more difficult to perform a TOJ task – assuming normal functional connectivity (as was observed in our previous report). If neurologically compromised individuals – such as those with autism – have distinct systemic cortical deficits, and that these deficits extend to local neuronal circuitry connectivity, then the

abnormal functional connectivity between adjacent and/or near adjacent cortical ensembles would decrease the effect that stimulus-driven synchronization has on the TOJ task (i.e., performance on the task would not degrade). Therefore, one goal of this study was to determine if synchronized conditioning stimuli would have an impact on TOJ performance in subjects with autism.

Methods

The subjects were ten males clinically diagnosed with autism (i.e., Autistic Disorder or Asperger Disorder; DSM-IV-TR; [22]), all naïve both to the study design and issue under investigation. Control data used for comparison has been reported in a previous study [21]. Autism subjects were recruited from the University of North Carolina Neurodevelopmental Disorders Research Center Subject Registry. All ten individuals had been previously tested with the Autism Diagnostic Interview – Revised (ADI-R; [23]), the Autism Diagnostic Observation Schedule - Module 4 (ADOS; [24]), as well as the Wechsler Abbreviated Scale of Intelligence (WASI; [25]), and met the diagnostic criteria for autism on the ADI-R. Education levels were as follows: one subject completed the 11th grade, and the remaining nine subjects completed high school. Participants were screened for co-morbid psychiatric diagnoses, peripheral injury, and other conditions that would affect somatosensation. The average ages were 26.1 ± 6.3 yrs for the autism group and 24.2 ± 6.1 yrs for the control group (mean $\pm$ stdev). The average IQ scores were as follows: for the autism group, Verbal = 102.3 ± 17.8 , Performance = 103.5 ± 18.7 , Full-4 = 102.8 ± 17.7 ; for the control group, Verbal = 112.0 ± 11.0 , Performance = 115.3 ± 8.2 , Full-4 = 115.6 ± 7.1 . No statistical differences were observed between the two groups for either age or IQ. The subjects gave informed consent and were paid \$25/hour for their time. The study was performed in accordance with the Declaration of Helsinki, all subjects gave their written informed consent, and 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 temporal order judgment (TOJ) and temporal discriminative threshold (TDT) capacity of each of the ten right hand dominant subjects. The protocol implemented in this study is described in full detail in a previous report [21]. The subject's right arm was rested comfortably on a table surface, and the hand was placed under a portable vibrotactile dual-site stimulator (CM-1; for full description, see [26]). The two probe tips (5 mm diameter each) were positioned at one of two sets of stimulus sites: (1) on the glabrous pads of digits 2 and 3 of the same hand (unilateral condition) or (2) on the glabrous pads of digit 2 of both hands (bilateral condition).

At the start of each run, the two probe tips were driven towards the skin sites until each tip registered a force of 0.1 g, as determined by a closed-loop algorithm in the CM-1 stimulator feedback system. The tips were then further indented into the skin by 500 μ m to ensure good contact with the skin. The tracking protocol used to obtain individual TOJ and TDT data consisted of 2 separate runs. In one run (20 trials), used for TOJ assessment, two single-cycle vibrotactile test stimuli (“pulses”; 1 mm peak-to-peak amplitude, 25 Hz) delivered to the skin were initially temporally separated by an inter-stimulus interval (ISI) of 150 msec. The locus that received the first of the two pulses was randomly selected on a trial-by-trial basis. The time allocated for stimulus duration was 1 sec (the two 40 ms pulses, separated by the variable ISI, were delivered at the center of this interval), followed by subject response (subject was queried to select the skin site that received the first stimulus) and a 5 sec delay before onset of the next trial (see Panel A of Figure 1). The ISI between the two pulses was modified based on subject response with a 1up / 1down algorithm for the first 10 trials and responses for the remaining trials of the run were tracked with a 2up / 1down algorithm in which two correct subject responses resulted in a decrement in the ISI. Using a 1up / 1down algorithm for the first 10 trials is an efficient way to quickly move the tracking task into a subject’s discriminative capacity range without significantly impacting the results [26]. A separate run (also 20 trials) of a similar 2AFC tracking protocol was used for TDT assessment. The TDT protocol differed from the TOJ protocol such that during the stimulus interval, the two pulses were delivered either at the same time or separated temporally by the ISI. Subject response was not dependent on the order in which the two stimuli were delivered, but rather on whether the pulses were felt to be simultaneous or not.

TDT assessment was observed unilaterally and TOJ assessment was observed at both the unilateral and bilateral stimulus sites. In the first condition, there was no concurrent stimulation (control; see Panel B of Figure 1). In the second condition, a 25 Hz concurrent stimulus was delivered (Panel C of Figure 1). During all cases of concurrent stimulus delivery, the concurrent stimulus was delivered for a minimum of 400 msec before the first of the two pulses was delivered and lasted for the entire duration of the allotted interval (1 sec) with the exception of the two 40 ms intervals during which the 1 mm pulses

were being delivered. A previous study has reported results obtained from the same described perceptual test with neurotypical adult subjects under multiple conditions of concurrent stimulation [21]. The effects of concurrent stimulation were observed for the unilateral conditions of TDT and TOJ assessment. The order in which the conditions were run was randomized.

Results

In order to compare the timing perception of individuals with autism and controls, a two-alternative forced-choice (2AFC) tracking protocol was used to assess discriminative capacities to determine the temporal order of two sequentially delivered tactile stimuli (temporal order judgment; TOJ) and to temporally resolve two sequential stimuli, regardless of order (temporal discrimination threshold; TDT), in individuals with autism. These results were compared with data obtained using an identical protocol from healthy neurotypical controls (control data previously reported in [21]). Figure 2 summarizes the TDT and TOJ measures obtained at the unilateral D2-D3 and bilateral D2-D2 paired skin sites. As was expected, both populations performed significantly better at TDT vs. TOJ (one-way repeated measures ANOVA; $p < 0.01$). Furthermore, significantly elevated thresholds were observed for the autism group when compared to the controls under both the TDT and TOJ unilateral conditions ($p < 0.01$). In the bilateral TOJ condition, although the control group appeared to perform slightly better at the task, there was no significant difference between the two populations ($p = 0.2836$).

While the TOJ and TDT measures provide an assessment of a subject's timing perception, they do not provide a measure of the impact that the coordinated or synchronized behavior of the adjacent cortical ensembles has on subsequent responses to tactile stimulation. In order to assess whether or not synchronized conditioning stimuli would have an impact on TOJ and TDT, conditioning stimuli were delivered before (a minimum of 400 msec) and concurrently with the TOJ and TDT tasks (see Methods; also [21]). Figure 3 summarizes the TOJ and TDT performance metrics obtained under the unilateral conditions in the presence and absence of 25 Hz conditioning stimulation. Note that for the control group, TDT was significantly elevated ($p < 0.01$) and TOJ increased 3 to 4-fold ($p < 0.01$) with 25 Hz conditioning when compared to the respective conditions when no conditioning stimulus was present. In contrast, for both TDT and TOJ measures, conditioning stimulation had no significant impact on the autism group (TDT, $p = 0.2561$; TOJ, $p = 0.4362$).

In order to determine whether the differential effects of conditioning observed between the groups were consistent within subjects, the data was normalized to the condition during which no conditioning stimulus was present (shown in Figure 4). The 25 Hz conditioning stimulus significantly impaired TDT by ~240% ($p < 0.01$) and TOJ by ~360% ($p < 0.01$) for the control group, whereas the autism group showed no significant change for either measure ($p = 0.1986$ and $p = 0.4329$, respectively). The small error bars in the normalized plot confirm that the change in performance due to conditioning was consistent within groups across all the subjects who participated in the study. To summarize, the results suggest two important outcomes: 1) Individuals with autism demonstrated impaired performance on unilateral TDT and TOJ tasks when compared to the control group, and 2) Subjects with autism showed no significant decrement in performance, as do neurotypical controls, on these tasks in the presence of 25 Hz conditioning stimuli.

Discussion

Degraded performance of TOJ and TDT in autism

In this study, we made the initial observation that individuals with autism perform significantly worse than neurotypical adults on the TOJ and TDT tasks. The results from our previously reported study [21] demonstrated that neurotypical subjects had TOJ thresholds that were in the range between 30 and 40 msec for both the unilateral (33 ± 4 msec) and bilateral (38 ± 8 msec) conditions. TDT thresholds were found to be around 20 msec for the unilateral (17 ± 3 msec) and bilateral (24 ± 4 msec) conditions, and both measures were well within the range of previously reported values (e.g., [6-8, 27-29]). However, the thresholds obtained from subjects with autism were elevated for both unilateral TOJ (57 ± 9 msec) and TDT (37 ± 3 msec), indicating that timing perception in individuals with autism is below normal. Bilateral TOJ measures obtained from subjects with autism and controls were not significantly different, although controls appeared to have lower thresholds (38 ± 8 msec vs. 47 ± 7 msec). Interestingly, within the autism group, performance of the TOJ task in the bilateral condition appears to be slightly better than the unilateral condition, though again – not significantly different.

Degradation in TOJ could be accounted for by abnormalities in the frontostriatal system

Timing perception – as measured by TOJ and TDT – is most often accounted for by the frontostriatal system largely as a result of these timing measures being sensitive to lesions to the supplementary motor area (SMA), posterior parietal cortex, and basal ganglia [3, 4], and also because of the fact that above-average TOJ thresholds occur in subjects with known damage to these same cortical areas (dyslexia [5], dystonia [6-8], and Parkinson's disease [9]). In subjects with autism, a number of structures, particularly in the frontostriatal system, have been reported to be compromised and could be responsible for the above average TDT and TOJ thresholds (or below average timing perception) demonstrated in our study. Specifically, several studies implicate the basal ganglia, or disproportionate changes in basal ganglia volume, in autism [30-36]. Langen et al [30] demonstrated an enlargement in the caudate nucleus volume that was disproportionate to increases in total brain volume in subjects with

autism. These findings were consistent with the work of others who found increases in basal ganglia volume in individuals with autism [31-33]. Additionally, differences in thalamus volume [37, 38], and impaired white matter connectivity in the frontal lobe [39] also implicate the frontostriatal system in the etiology of autism and are consistent with the impairments observed in timing perception in individuals with autism.

TOJ degrades with synchronized conditioning stimuli - but not in autism

Results from our previous report ([21]; also see Figure 3) showed that when 25 Hz synchronized conditioning stimuli were delivered concurrently at two pairs of unilateral test sites (D2 and D3), neurotypical adult subjects demonstrated a marked decrease in their ability to discriminate temporal order. The results of that study gave support to the theory that synchronization of cortical ensembles in SI could significantly impact the topography of temporal perception. In other words, co-activation of adjacent and/or near-adjacent cortical assemblies makes it more difficult for a subject to perceptually differentiate between the regions of skin that are receiving identical stimulation. However, in the present study, it was observed that there was no degradation in performance in the individuals with autism on the TOJ task in the presence of the same synchronized dual-site conditioning stimuli, and the topography of temporal perception was not impacted. While all control subjects in the previous study tested demonstrated a decreased ability in TOJ in the presence of synchronized conditioning stimuli delivered to unilateral stimulus sites, none of the autism subjects showed a significant alteration in TOJ with the same synchronized conditioning stimuli. The conclusion from the previous study was that the degradation in TOJ observed in controls in the unilateral stimulus condition resulted from the synchronization of activity in adjacent or near-adjacent cortical ensembles which led to those cortical ensembles becoming functionally connected or bound [21]. In the autism subjects, this stimulus-driven synchronization did not lead to degradation of TOJ, suggesting that the engaged cortical ensembles – though in near proximity topographically – are not functionally connected or do not bind. The lack of local functional connectivity between these two topographically proximal regions could be responsible for the absence of perceptual

binding that normally occurs with synchronized conditioning stimuli. Recognition of two independent, but identical stimuli, as a single stimulus, could be an indicator of integration on the local cortical level that is important for coordination of sensory input that might play an important role in normal sensorimotor function. However, if individuals with autism do not experience such perceptual binding – or coordination of sensory input - then this “dysfunctional” connectivity could explain a number of enhanced feature extracting capabilities that are often associated with autism [40].

Changes in local cortical circuitry could lead to changes in under-connectivity and synchronization.

The degradation in TOJ performance with stimulus-driven synchronization that is observed in control subjects (but absent in individuals with autism) is presumed to reflect the responses of near-proximal cortical ensembles in primary somatosensory cortex evoked by dual-site skin stimulation. If this presumption is correct, the findings obtained in the present study raise the possibility that local cortico-cortical functional connectivity in subjects with autism may be substantially abnormal. While a number of findings have demonstrated that long-range functional connectivity in subjects with autism is very different from that present in the general population (for example, see [41, 42]), it is unlikely that the different observations obtained from individuals with autism and control subjects reported in this study are attributable to differences in long-range cortico-cortical connections. Rather, the measures of the impact that synchronization of topographically proximal cortical ensembles described in this paper appear to reflect the deficit in short-range parietal corticocortical connectivity identified in subjects with autism by Casanova and colleagues [43]. Casanova and colleagues have reported minicolumnar reduction in a number of areas in the parietal cortex, primarily in the peripheral neuropil space surrounding the minicolumn structures [19]. The peripheral neuropil space, being the area that provides the "strong vertical flow of inhibition" described by Mountcastle [44], is the region populated by (inhibitory) double bouquet cells. If the lack of degradation of TOJ in the presence of stimulus-driven synchronization is, in fact, an indicator of altered local circuitry in autism, then it would fit well with the minicolumnar hypothesis of autism that has been put forth by Casanova and colleagues. Such changes in connectivity

could lead to the imbalance in excitation and inhibition that others have predicted underlies the neocortical hyperexcitability and unstable activity in cortical networks characteristic of autism [41, 45].

Long range vs. short range functional connectivity

The primary significance of this study is that the lack of perceptual degradation that does not result from stimulus-driven synchronization in autism demonstrates that there is an element of under-connectivity between cortical ensembles at the local regional level. A number of reports have focused on long range functional connectivity – or synchronization between cortical regions across large territories of cortex. These studies that propose long-range cortical under-connectivity in autism predict that there are lower than normal regions of activation that result from a reduction in the number of long range interactions between these areas [14, 18, 46, 47] and that a principle deficit in autism is the coordination of the activity of cortical ensembles across the entire cortex [46]. While the majority of these synchronization studies have been done with fMRI and PET (e.g., [14, 46, 47]) – which only provides an indirect measure of the dysfunctional neuronal synchrony [48] – a few studies have employed methods, such as MEG, that allow for higher temporal resolution. MEG and EEG studies have found that gamma oscillations, which are considered to be important in the process of coordinating cortical activity, to be below normal in subjects with autism [18, 49].

It could be argued that the results from this study have little to do with the synchronization effects that are often considered in functional neuroimaging studies. In those studies, multiple cortical areas become activated in response to a single simple task and the activity of those areas is determined to be highly correlated (or synchronized). However, individuals with autism typically do not exhibit the same degree of connectivity or synchronization as control subjects, and it is from this evidence that a number of under-connectivity theories initially arose [50]. In this study, the perceptual impact that synchronizing adjacent or near-adjacent cortical ensembles was evaluated in order to ascertain functional connectivity at the local cortical level. Proposals of over-connectivity at this local cortical level in autism have been put forth, but much of the evidence for local cortical over-connectivity is anecdotal. Belmonte

and colleagues suggested the co-morbidity with epilepsy that is highly prevalent in autism is evidence for over-connectivity [51]. Other reports have suggested over-connectivity or over-processing at the local level, principally because some individuals with autism exhibit hyper-responsive sensory symptoms and/or have enhanced feature processing skills [40, 52]. We suggest that perhaps over-connectivity is the incorrect term, and also suggest that our findings strongly support the anatomical findings of Casanova and colleagues. That is, many of the differences – such as those described above – could be accounted for by a higher density of minicolumns and a reduced neuropil surrounding those minicolumns. Although the increase in minicolumn density could account for some enhancements in perception, the reduction in neuropil surrounding adjacent minicolumns would lead to some below-normal perceptual metrics. For example, spatial localization of a stimulus on the skin is much better in individuals with autism than in controls [53], and this could be due to the increased resolution afforded by the higher density of minicolumns. However, because of the lack of GABA-mediated inhibition between those minicolumns, adaptation of the stimulus delivered to the skin – which normally (in healthy adults) results in a nearly 2-fold improvement in spatial localization performance – does not lead to an improvement in spatial localization in autism subjects [53]. Additionally, a degraded adaptive response in autism was recently demonstrated in autism in an amplitude discrimination task, and it was concluded that a generalized GABA deficiency could also account for this behavior [54]. The lack of or reduction of normal inhibitory connectivity between minicolumns in the cortex could, as Casanova has suggested, be responsible for the decrease in larger scale connectivity observed in autism. In other words, if the minicolumn is considered the smallest functional unit of the cortex – which significant neurophysiological evidence suggests [20, 44, 55-60] – then it stands to reason that the functional connectivity between macrocolumns (which are made up of minicolumns) and aggregates of macrocolumns would lead to a deficiency in larger scale cortical-cortical interactions. We view this idea as consistent with the data presented in this paper that differentiates the impact that synchronizing stimuli have on the perceptual metrics of individuals with autism and control subjects. The method we have developed for detecting the influence of stimulus-driven synchronization, or in the case of autism – the absence of the influence of stimulus-driven

synchronization, could be argued to be influenced predominantly by cortical structures at the macrocolumnar, and perhaps the minicolumnar, level. Assessment of the influence of such stimuli on local cortical ensembles is currently being more directly addressed.

One obvious shortcoming of this study that will be addressed in subsequent reports is the question of the relationship of the variability of the measures within the autism subject group and how well this variability correlates with clinical assessments. For example, is the impact of synchronization on TOJ reduced with a decrease in ADOS measures of the subject population? Although the distributions of our current subject populations are essentially non-overlapping in terms of influence of stimulus-driven synchronization (note comparison of normalized conditions in Figure 4), it will be interesting to see if a pattern of stimulus-driven synchronization effectiveness emerges with a larger sample size. Other future studies could include subject populations with abnormalities in somatotopic cortical organization such as those with dystonia, which are known to have disordered digit topographies [61], with the implication that predictions about digit topography could be made from the differential outcomes in TOJ with and without concurrent stimulation. Thus, the metric could be used to complement already existing technologies that are capable of resolving digit topography at high resolution (e.g., [62]) as well as technologies that have examined and detected differences in event-related synchronization/desynchronization in disorders such as Parkinson's, dystonia, physiological aging, degenerative dementia, and obsessive-compulsive disorder (for review, see [63]).

Conclusion

In our previous report, we concluded that our results suggested that in neurotypical adult subjects – in which functional connectivity between adjacent and/or near-adjacent cortical columns is intact or not impaired – the TOJ measure would be significantly impacted in the presence of a synchronizing stimulus that simultaneously engages paired cortical ensembles. In the case of autism, the results show that the same TOJ measure is not impacted by such synchronizing stimuli, suggesting what a number of reports of cortical microarchitecture have previously suggested in autism: a disruption of local functional connectivity. This seemingly robust measure, which can be obtained relatively quickly and non-invasively, could prove useful in future research assessing the efficacy of treatments for persons not only with autism, but for members of subject populations with other abnormalities in cortical organization.

Competing interests

The authors certify that the information listed above is complete to the best of our original research. The authors declare that they have no competing interests.

Authors' contributions

MT and VT participated in the design and conduct of the experiments and the drafting of the manuscript. JH participated in the conduct of the experiments. GB participated in the design of the experiments and the draft of the manuscript. All authors read and approved the final manuscript.

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Figure legends

Figure 1. Protocol details. **Panel A:** Two sequential vibrotactile pulses were delivered during the Stimulus Interval, one to each of either skin site A or B. Subject was queried as to which skin site received the first pulse (TOJ) or whether the pulses were synchronous/asynchronous (TDT) during the Response Interval, and this was followed by a 5 sec delay before the onset of the subsequent trial. **Panel B:** Pulse delivery sequence for the TOJ and TDT tasks during each 1 sec Stimulus Interval. Order of delivery (skin site A or B) was randomized on a trial-by-trial basis, and inter-pulse interval was decreased or increased, depending on subject response. **Panel C:** Exemplary 25 Hz conditioning stimulus delivered concurrently with TOJ/TDT task.

Figure 2. TDT and TOJ measures obtained at the unilateral D2-D3 and bilateral D2-D2 paired skin sites. In the unilateral condition, both groups demonstrated lower thresholds for TDT than TOJ (ANOVA; $p < 0.01$). Furthermore, for each task the control group performed significantly better than the autism group ($p < 0.01$). In the bilateral TOJ condition, although the control group appeared to perform slightly better at the task, there was no significant difference between the two populations ($p = 0.5836$).

Figure 3. TDT and TOJ performance metrics obtained under the unilateral conditions in the presence and absence of 25 Hz conditioning stimulation. Note that for the control group, TDT was significantly elevated ($p < 0.01$) and TOJ increased 3 to 4-fold ($p < 0.01$) with 25 Hz conditioning when compared to the condition when no conditioning stimulus was present. In contrast, for both TDT and TOJ measures, conditioning stimulation had no significant impact on the autism group (TDT, $p = 0.2561$; TOJ, $p = 0.4362$).

Figure 4. Data was normalized to the condition during which no conditioning stimulus was present. The 25 Hz conditioning stimulus significantly impaired TDT by ~240% ($p < 0.01$) and TOJ by ~360% ($p < 0.01$) for the control group, whereas the autism group showed no significant change for either measure ($p = 0.1986$ and $p = 0.4329$, respectively).

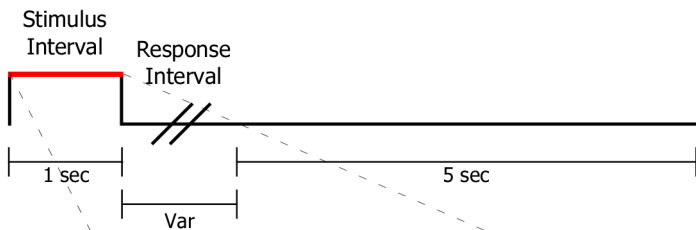
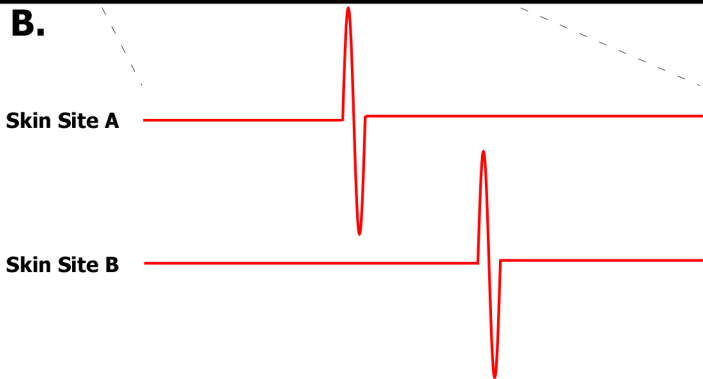
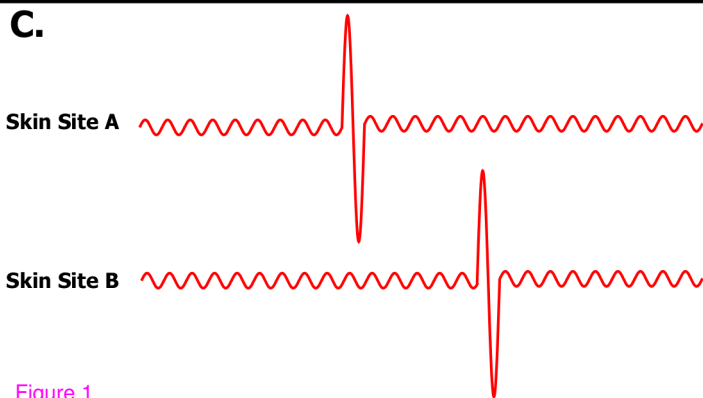
A.**B.****Skin Site A****Skin Site B****C.****Skin Site A****Skin Site B**

Figure 1

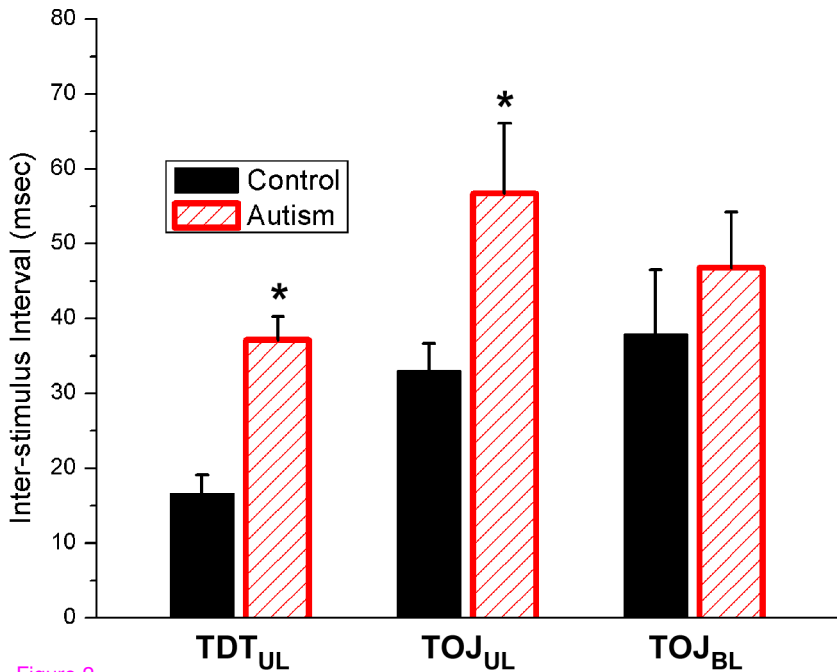


Figure 2

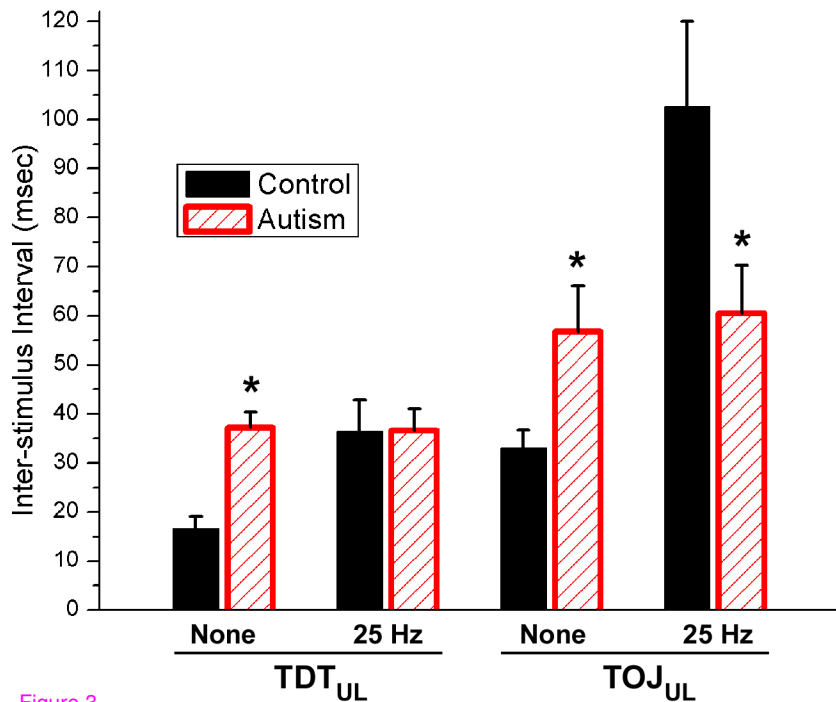


Figure 3

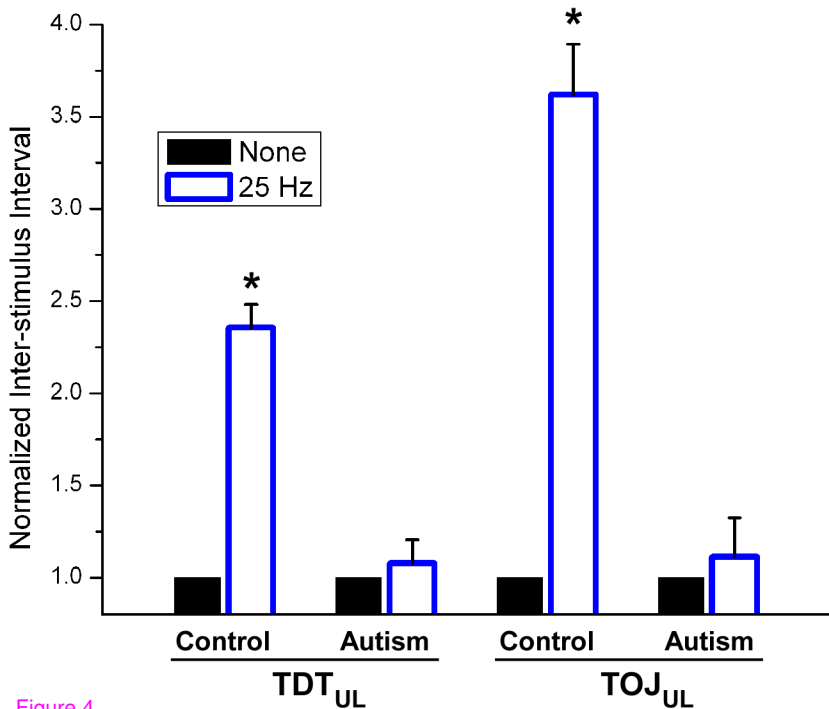


Figure 4