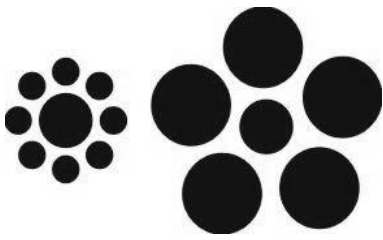


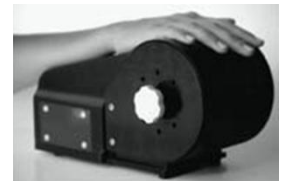
## Using illusions to quantify CNS information processing capacity.



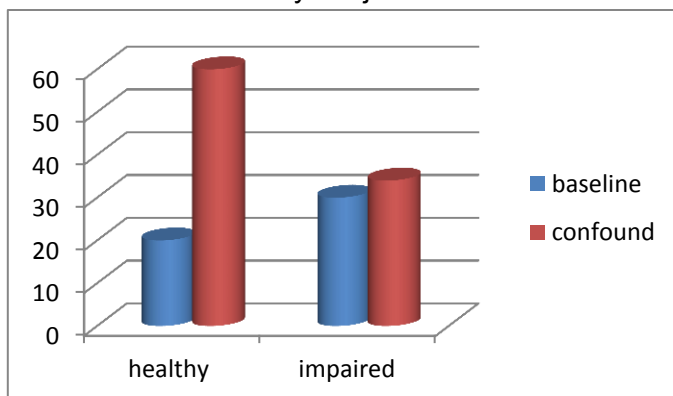
In the illusion pictured at the left, the center circles of each cluster appear to be different sizes even though they are not. This context dependent illusion results from a number of neural mechanisms being intact. In other words, if there was something wrong with an individual's CNS health, then they would not observe the illusion, and thus, their assessment, via visual percept of those 2 spheres, would be *more accurate* than that of someone who was not impaired. Thus, this would be a condition in which a CNS impaired individual could *outperform* a healthy individual. With this idea in mind, we designed a diagnostic system that could quantitatively assess the degree to which different types of illusions impacted an individual's perception.

The somatosensory system is ideally suited for the design of a CNS diagnostic system for a number of reasons. First, the organization of the system is such that adjacent skin regions project to adjacent cortical regions (i.e., it is somatotopic), and stimuli can be precisely controlled and delivered to these regions. Second, decades of neuroscience research have yielded a great deal of information about the nature of the interactions within and between the adjacent cortical regions that are being activated by tactile stimuli. Third, ambient environmental noise in the system can be easily controlled (i.e., it is less likely that a patient will be exposed to distracting tactile input than auditory or visual input). Fourth, the somatosensory system is the only sensory system that is highly integrated with the pain system, and this is often an important aspect of a patient's diagnosis.

**Percepts of tactile stimuli can be measured quantitatively.** We have developed several tests, made possible by our state-of-the-art tactile stimulator system, that differentiate healthy from impaired individuals by demonstrating that impaired individuals *outperform* healthy controls. This is accomplished by delivering stimuli to adjacent finger tips with a portable stimulator (pictured at right) and "tracking" subject responses. "Tracking" simply means that the subject answers questions about the stimuli that he/she feels on their fingers (e.g., "which one is larger?", "which one came first?", etc.) in the same manner that an eye chart exam is performed. With an eye chart, a subject is queried with progressively more difficult or demanding questions – when the subject can no longer accurately determine what is on the chart, a very accurate assessment of their visual acuity has been obtained.

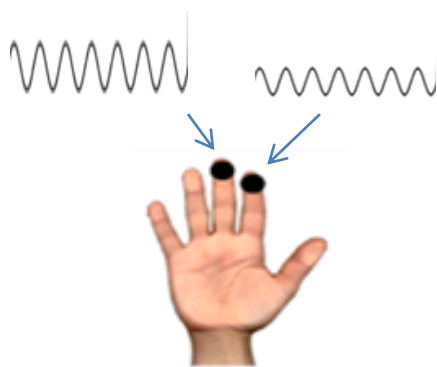


**Impact of illusory conditioning on sensory percepts are baseline independent.** One of the biggest problems with using any type of measure – whether it be via modern medical imaging technologies, sensory perceptual metrics or some type of psychosocial questionnaire, is that it requires a baseline measure to be obtained before whatever CNS systemic alteration occurs. Not only is this impractical – the majority of patients seeking health care do not have baseline measures on them – but it is often inaccurate because of neurological shifts that occur naturally with aging, training, and/or experience. Ideally, a measure could be derived that is independent of a baseline shift. For example, consider the graph of such a hypothetical situation at left. Healthy subjects are found to have "baseline" vision of 20/20, but when an illusory confound is introduced, they get much worse (20/60). Impaired subjects – whose vision might have gotten worse for a number of reasons (baseline of 20/30) do not get significantly worse with the introduction of an illusory confound. Thus, the critical measure is how much the illusory confound impacts the baseline measure. Several tactile illusory confounds have been developed and found to be sensitive to CNS health. Although all of these tests undoubtedly utilize multiple CNS mechanisms involved in interactions between adjacent and near-adjacent cortical regions, some play a more prominent role than others in each of the tests. This



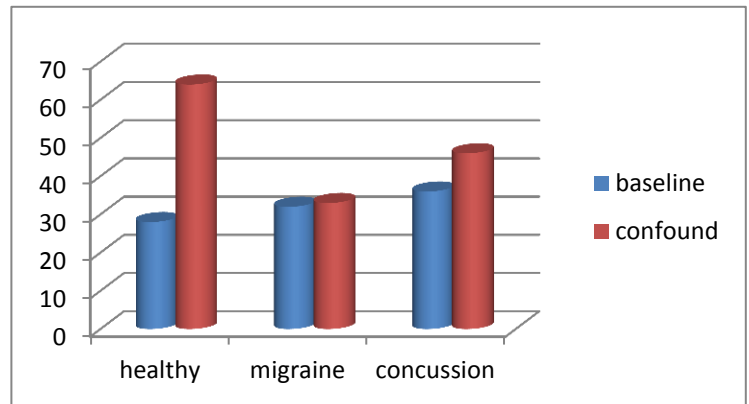
allows for a more powerful multi-parametric approach to be used in the analysis stage of profiling the CNS status of each individual. Each of these sets of tests takes approximately 2-3 minutes to complete. Due to space limitations, only 4 of these protocols are thoroughly described.

**Sensory Metric Category #1: Neuroadaptation metrics.**



**Baseline Test: Which one is larger?** Two stimuli are delivered to the two fingertips, D2 and D3, and the subject is asked which stimulus is larger. Among healthy subjects, this is a fairly robust measure across the age spectrum (Zhang et al, 2011b), but it is significantly impacted by an **illusory conditioning** stimulus delivered to one of the two digits before the test ((Tannan et al., 2006, 2007a, 2008; Tommerdahl et al., 2007, 2010b; Folger et al., 2008; Francisco et al., 2011; Zhang et al., 2008, 2009, 2011a, 2011b). For example, stimulating D3 in the image at the left **before** performing the test has the effect of making the larger stimulus feel smaller than it really is; this results in healthy subjects

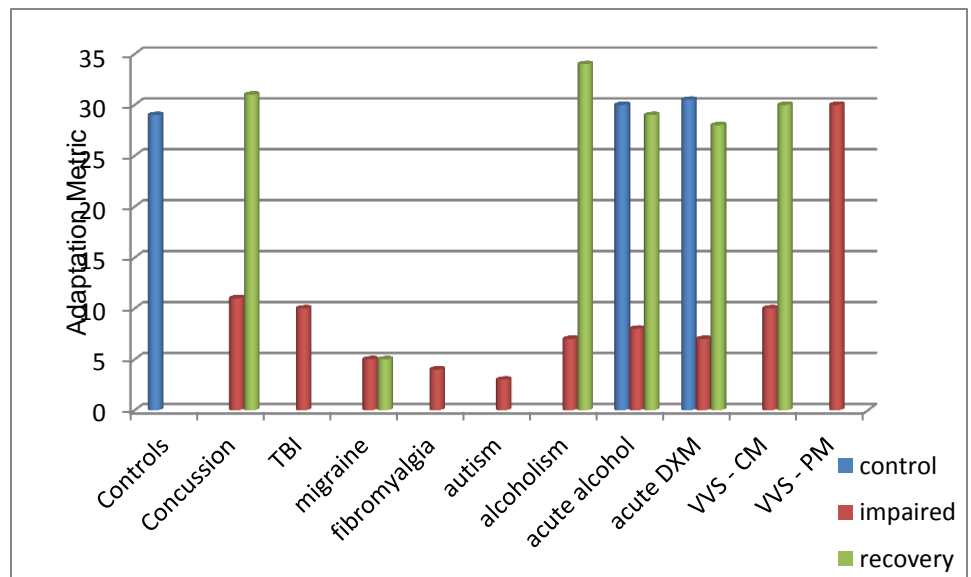
performing worse on the task because it is now more difficult to perceive that the larger stimulus is greater in intensity than the smaller stimulus (compare baseline to confound for healthy controls in the graph at right). This metric is robustly consistent across the age spectrum for healthy controls (Zhang et al, 2011b). How much a subject, or subject population adapts to the conditioning stimulus, is determined by the per cent difference between the baseline and confound measures, and is referred to as the adaptation metric.



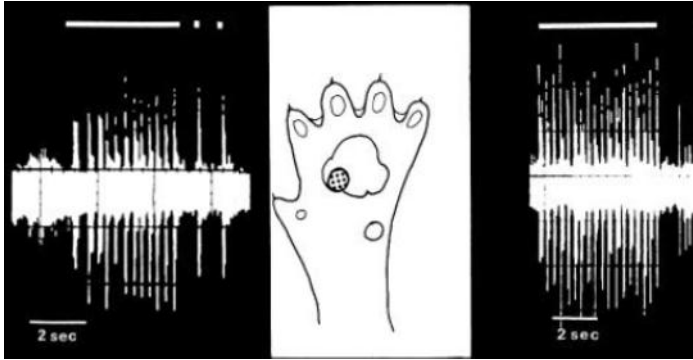
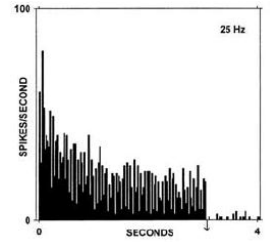
Note that in the migraine and concussion subject populations, the baseline metric is slightly elevated, but more significantly, the impact of the confound on the perceptual task is reduced. The larger values for the confound in the healthy controls indicate that those subjects adapted – or habituated – normally, while the other two subject populations did not.

**Outperformance with impairment.** The figure below demonstrates that the adaptation metric – derived as a

function of the per cent change from baseline to confound - deviates from the control values for a number of subject populations. Note that while performance of controls is impacted by the illusory confound by approximately 30%, a number of subject populations are not impacted as much, and thus, they *outperform* healthy controls on the discrimination task because they do not adapt as much to the conditioning stimulus. Recovery returns values to normative range for recovering from concussion, alcoholism, acute alcohol use, acute DXM ingestion and drug treatment for centrally mediated (CM) VVS. Peripherally mediated (PM) VVS is a pain condition not associated with centrally mediated mechanisms. Data is more fully described in Tannan et al., 2006, 2007a, 2008; Tommerdahl et al., 2007, 2010b; Folger et al., 2008; Francisco et al., 2008, 2011; Zhang et al., 2008, 2009, 2011a, 2011b and Nguyen, et al, 2012.



**Why it works.** Repetitive stimulation, such as occurs with the conditioning stimulus described above, normally results in an overall decrease in cortical response. In the figure at right, extracellular recordings that we recorded are plotted in spikes/second (how frequently neurons fired) in response to a 25Hz sinusoidal mechanical stimulus that is delivered to the skin for 3 seconds. Note that initially, the response is on the order of 60-75 spikes/second, but as the stimulus continues, the response rate of the neurons decreases – down to approximately 25 spikes/second (Whitsel et al, 2002).



Whenever there is a systemic cortical alteration, such as might occur with a neurological deficit, it usually results in an imbalance in excitation and inhibition, and the individual will not adapt normally. For this reason, they adapt less, and then *outperform* healthy controls because the conditioning stimulus does not alter their perception: thus, they perform better at the amplitude discrimination task post-illusory conditioning. A number of studies have been performed to examine changes in responsivity of SI cortex to altered excitation. One early example is demonstrated in the

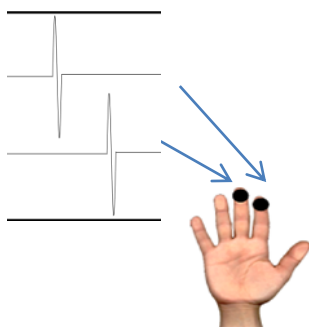
figure at the left (from our early work – see Juliano et al, 1989). Note the difference in the extracellularly recorded response in the pre-drug (left side) vs. the post-drug (right side) topical application of a very small amount of a GABA agonist, which produces hyper-excitability to stimulation. Solid white bar at the top of the figure indicates when a tactile stimulus was applied to the central pad of the cat’s forepaw. Increasing the excitation level of the cortex leads to less of a decrease in responsivity to repetitive stimulation (for review of dynamics involved, see Tommerdahl et al, 2010a) and will lead to changes in adaptation (for recent discussions, see Tommerdahl et al, 2010b; Zhang et al, 2011a).

**Hypothesis to be tested:** Adaptation metrics will quantitatively assess the degree to which an individual’s CNS is impacted by their condition, and the more that an individual is impacted by the neurological alteration, the less that performance will be impacted by adaptation. A degradation in performance will not be observed in healthy individuals.

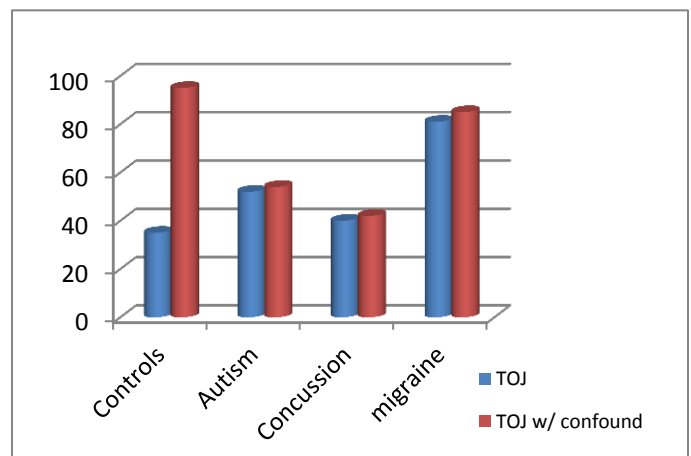
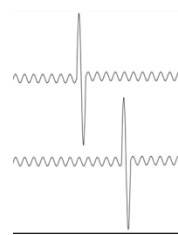
**Sensory metric category #2: Functional connectivity.**

**Baseline test: Which one came first?** Determination of which stimulus came first, as depicted at the left, describes an individual’s temporal order judgment (TOJ) capacity. Typically, healthy individuals have a TOJ capacity on the order of 30-40 msec. However, in the presence of an illusory conditioning stimulus, healthy controls perform significantly worse (Tommerdahl et al, 2007b).

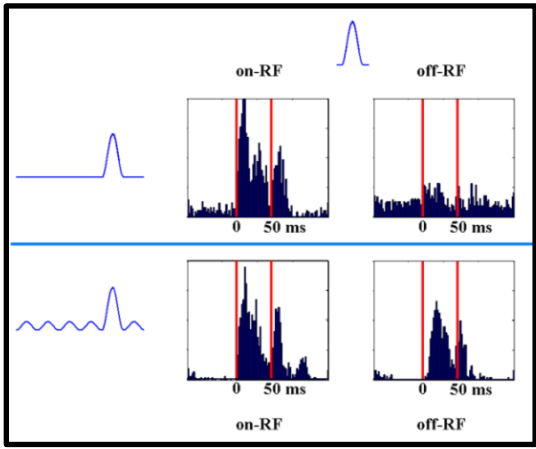
**Baseline**



**illusory condition**



**Outperformance with impairment.** Note that in each of the non-control subject populations in the graph above, individuals perform approximately the same in the presence of the confound (see Tommerdahl et al, 2007b and 2008 for more complete description). Functional connectivity between adjacent cortical regions normally leads to a reduction in TOJ performance in healthy controls with the introduction of the confound, but not in CNS impaired individuals.

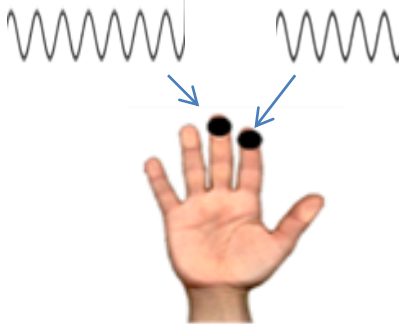


**Why it works.** consider the results displayed at left. Extracellular recordings were obtained from SI cortical regions corresponding to D2 (“on-RF”) and D3 (“off-RF”) in the squirrel monkey. When a vibrotactile pulse was delivered, a significant above background response was evoked at D2 (top left quadrant) but not at D3 representation (top right quadrant). When sub-threshold synchronized sinusoidal stimuli were delivered to both digits prior to the pulse (bottom half of figure), the pulse at D2 evokes a response at both the D2 and D3 representation (note absence of evoked activity before zero msec during subthreshold stimulation). From this type of data, we hypothesized that this response was the result of functional connectivity between adjacent and/or near adjacent cortical ensembles, and that delivery of synchronized conditioning stimuli would impact the topography of temporal perception, unless

there was a neurological deficit.

**Hypothesis to be tested:** The degree to which a subject is impaired will be negatively correlated to the degree with which TOJ is impacted by the illusory confound. Individuals who are not impaired will perform worse at the TOJ task in the presence of the confound. Individuals without a neurological impairment will not be able to perform as well as on the confounded task as on the simple TOJ task.

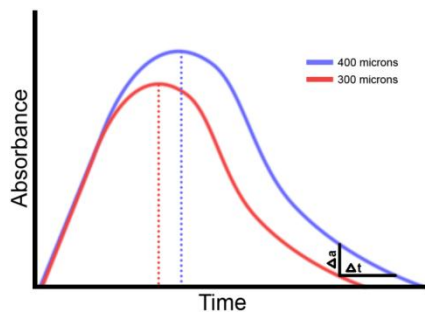
**Sensory metric category #3: Duration-intensity interactions.**



**Baseline test: Which one lasted longer?** Typically, healthy individuals can accurately discern which of two stimuli last longer (delivered sequentially) when there is approximately a 10% difference (e.g., 500 vs 550 msec). However, when the intensity of the shorter stimulus duration is increased, an illusion that the stimulus is longer is created, and duration discrimination capacity degrades (this is proportional to the increase in intensity; Francisco et al, 2012). Note that in the graph below, the difference limen (DL) gets worse with the introduction of the intensity confound for healthy controls. However, concussed and migraine subjects do not get worse with the introduction of the

intensity confound.

**Why it works.** We conducted several series of experiments in non-human primates using high resolution optical imaging methods (Simons et al, 2005, 2007).

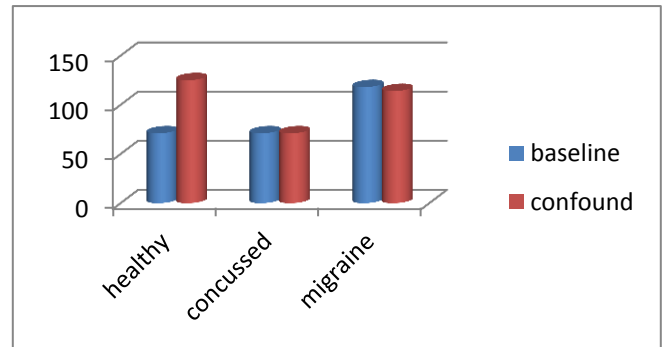


Observations from those experiments

demonstrated that increasing stimulus amplitude results in an

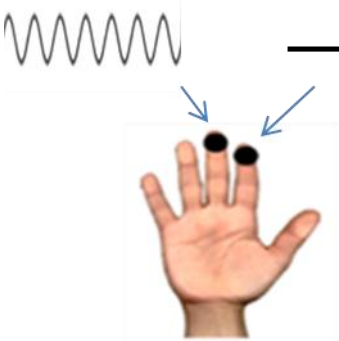
increasing duration of the optical response (see figure below; note the different durations of response for different magnitudes of stimulation). Additional experiments examining the source of the optical signal (Lee et al, 2005) led us to hypothesize that if neuron-glia interactions were significantly impacted, such as they would be with neuro-inflammation, the response duration of the optical signal would not be different with an increase in

amplitude. Thus, we hypothesized that neuro-inflammation would lead to a reduction in the impact of the intensity confound on an individual’s duration discrimination capacity.



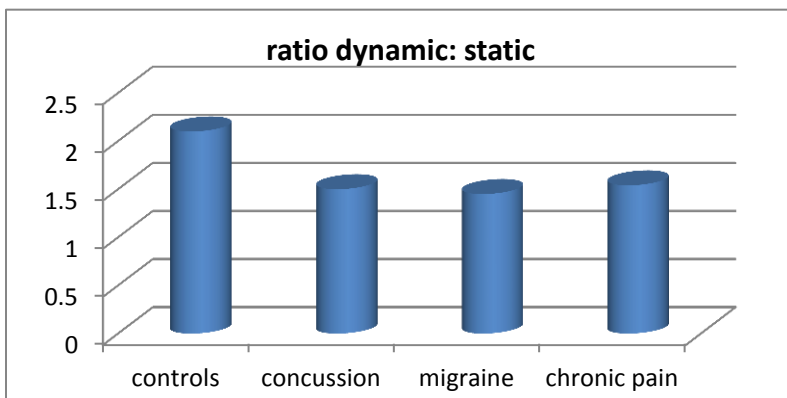
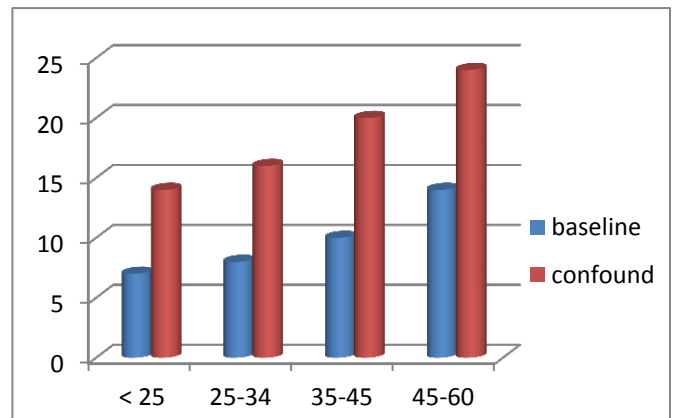
**Hypothesis to be tested:** The impact that an intensity confound has on duration discrimination capacity will be reduced with an increase in neurological impairment. Thus, subjects without impairment will not be able to outperform their baseline test.

## Sensory metric category #4: Feedforward inhibition.



**Baseline Test: Which one is larger?** The purpose of this metric is to determine the minimum stimulus that an individual can detect. This is accomplished by asking the subject to compare two stimuli – with one of them being zero – and tracking to their “static” detection threshold value. “Static” means that the stimulus (the larger one pictured to the left) does not change size during a test trial. The perceptual task that utilizes the illusory confound implements a “dynamic” or modulated stimulus. It starts out at a null value and increases in amplitude at a pre-determined rate (figurine at right). Even though the stimulus is not initially perceived by the subject, it has the effect of *raising* the subject’s threshold. In other words, sub-

threshold, or non-detectable, stimuli have an effect on perception, and the cortex tends to ignore the stimulus until it is much larger. Dynamic thresholds have been demonstrated to be significantly elevated in healthy controls across the age spectrum (Zhang et al, 2011b). The significance of this is that although static threshold changes with age (due to skin physiology), the ratio between the two measures remains relatively constant. However, individuals with some type of CNS impairment demonstrate a reduction in that ratio, even though static thresholds remain the same (Tommerdahl et al, 2010; Zhang et al, 2011a). Thus, these impaired individuals **outperform** healthy controls in terms of ratio of dynamic to static thresholds. The graph below summarizes the results that we have obtained from several such populations.



**Why it works, OR:** The role of sub-threshold stimulus-evoked inhibition – feed-forward inhibition and the role of within-column connectivity. A major well-documented feature of cortical functional organization is the presence of prominent feed-forward inhibition in the input layer 4, in which local layer 4 inhibitory cells receive direct thalamocortical input and in turn suppress responses of neighboring layer 4 excitatory cells to their thalamocortical drive,

thereby sharpening their RF properties (e.g., Douglas et al. 1995; Miller et al. 2001; Bruno and Simons 2002; Alonso and Swadlow 2005; Sun et al. 2006; Cruikshank et al. 2007). These inhibitory cells are more responsive to weak (near-threshold) afferent drive than are the excitatory layer 4 cells, and thus, sub-threshold or weak stimulus inputs will have the effect of raising the threshold at which excitatory layer 4 cells begin to respond to peripheral stimuli. In terms of sensory testing, this means that utilization of two different types of threshold tests – one with and one without subthreshold stimulus conditioning – should yield very different outcomes.

**Hypothesis to be tested:** Deficiencies in the underlying mechanisms that support feedforward inhibition will result in significant reductions in the differences observed between static and dynamic thresholds of neurologically impaired subjects.