SIGNIFICANCE: Electrical microstimulation is a powerful strategy for controlling neural dynamics of local populations mediating perception and action. Microstimulation is one of the oldest methods used to study biophysical properties of neurons[20], functional organization of brain areas[21,22], and the role of different brain regions in perception[23,24]. Intracortical microstimulation has also been used to provide sensory feedback to control robotic devices (e.g., prosthetics arms), a strategy referred to as the *write-in* neural stimulation method[1,3,4,25-29]. This strategy is different than the neural decoding or *read-out* approach, which is used to directly control movements of prosthetic devices using internally-generated brain signals[4,30-37].

The field of prosthetics has recently gone through a wave of innovation in developing dexterous robotic arms for neuroprosthetic applications. These robotic devices are equipped with miniaturized motors that provide multidimensional degrees of freedom in a light and compact design (e.g., DEKA arm and the Applied Physics Lab robotic arm). Studies show that these devices can be controlled using brain signals[38-41]. Yet, the vast majority of neuroprosthetic studies use visual inputs as a substitute for sensory signals that are normally conveyed by the tactile modality[34,35,40,42]. Although, vision provides accurate estimates of some physical properties of objects (e.g., location, and shape), dexterous grasping of objects with prostheses is best achieved by implementing algorithms that also leverage dynamics of functional tactile ensembles. In particular, real-time updates of physical signals occurring at the interface between the robotic device and the object (e.g., object's compliance, motion signals indicating slip) are critical for facilitating instantaneous adjustments of grasp. These sensory signals are best decoded by the somatosensory system, which has specialized circuits encoding these types of sensory features.

The volumetric electrical current spread evoked by microstimulation limits the control of functional ensembles that are not organized in a confined cluster. Calcium imaging studies show that microstimulation can drive distant cells (> 4 mm from the stimulation epicenter) by activating axons passing nearby the stimulating electrode[43,44]. Microstimulation effects are also more pronounced at the tip of the stimulating electrode[44], leading to heterogeneous activations of cells within the region of interest. The implication of these data is that electrical stimulation restricts the ability to selectively activate sparse and distributed neural representations encoding complex tactile sensations (e.g., size and shape of tactile objects, object slip). To the best of our knowledge, most microstimulation studies in somatosensory areas have only evoked tactile percepts related to rudimentary features such as location (e.g., *where on the hand the stimulus is presented)*[1], intensity (e.g., *weak `vs. strong stimuli*)[1], and vibrations (e.g., *fast vs. slow frequencies)*[24,45]. These studies take advantage of the topographical organization of neurons encoding these tactile features (e.g., somatotopy, or clustering of rapidly-adapting vs. slowly-adapting neurons). However, most elaborate tactile sensations are likely generated by sparse neural representations[5,46]. Certainly, the lack of evidence demonstrating that electrical stimulation can elicit more elaborate tactile sensations highlights the importance of incorporating novel strategies that enable more selective and uniform activation of cell circuits contributing to complex tactile percepts. An overarching goal of my research program is to develop neural stimulation strategies that elicit artificial tactile sensations mimicking those experienced through our hands in everyday life.

Refining neural activation patterns using combined microstimulation and optogenetics

Recent advances in molecular biology and optics have significantly improved the ability to selectively activate neural circuits with high spatial and temporal resolution[7,47-52]. One of the most widely used tools is optogenetics, a method that regulates activity of excitatory and/or inhibitory populations by controlling light-sensitive ion channels via light[8,17,53]. Optogenetic expression in neurons can also be restricted to the soma, avoiding activation of distant neurons by stimulating axons neighboring the optical fiber[54,55]. Further, rodent studies show that optogenetics can robustly drive sensory, motor, and cognitive-related neural circuits[15,56-70]. Importantly, recent studies have demonstrated similar successes in non-human primate models. Specifically, optogenetic stimulation in monkey brains has been shown to enhance stimulus processing and detection[71-74], to bias visual salience maps[75], to modulate motion discrimination[76], to promote reward signaling[77], and to drive saccades [78-82], among other functions[83,84]. Given the promising benefits of this genetic-based optical method, optogenetic stimulation has been proposed to be a viable method to use in brain-machine-interface (BMI) applications[43,85-90].

Optogenetic effects in monkeys are usually not as robust as those evoked by electrical stimulation. These sub-optimal effects are not caused by poor expression levels of the viral construct in primate brains[77,91-95]. One possible explanation is that, because of primates' large-scale brains, optical signals from fiber-optic cables are not strong enough to effectively activate a sufficient fraction of neurons that contribute to the percept. Potential solutions to

upregulate optogenetic effects in large brains are devices that illuminate larger volumes (e.g., light sheet optics) or optical sources that generate light within the cell itself (e.g., genetically-encoded bioluminescent light). Unfortunately, these techniques require further development in non-human primate brains. An alternate solution is to use optogenetics as a complement to electrical stimulation by inhibiting cell populations that are unintentionally activated by the electrical current. This combinatorial approach leverages the strong activation effects of microstimulation and the selective suppressive effects of optogenetics using inhibitory opsins. We contend that this multi-faceted strategy will lead to more refined stimulation patterns, enabling us to better study and manipulate functional circuits mediating haptic perception and control.

Benefits of computational modeling in neural stimulation

Determining the stimulation parameters that effectively drive neural ensembles typically requires an extensive search of electrical[28] and optical[15,17] parameters. This search is typically done *in vivo*, and, often times, stimulation parameters are non-stationary due to neural adaptation or other factors. Further, electrical stimulation patterns usually comprise monotonous stimulation trains such as biphasic square-wave pulses at a single frequency, emitted from a single electrode, and with uniform amplitude[1]. Similarly, optical light patterns that drive opsins tend to be composed of uniformly distributed train pulses with the same intensity and frequency emanating from a single optical source[15]. Yet, complex percepts typically emerge from neural dynamics among distributed neural ensembles, indicating that intracortical stimulation methods would benefit from using dynamic signals that vary in intensity, spatial, and temporal dimensions. However, searching through all these parameter spaces can be strenuous, especially when taking into account excitatory and inhibitory interactions between electrical and optogenetic stimulation. Thus, to reduce the search time *in vivo*, we will implement computational models to determine electrical and optical stimulation parameters that optimally drive somatosensory functional ensembles encoding tactile sensations.

Controlling the population's dynamics to study haptics and evoke enhanced touch sensations

Electrical and optogenetic effects can potentially be enhanced by applying stimulation during optimal brain states. Voigts and colleagues (2018) found that intracortical microstimulation effects in auditory cortex were greater at a particular phase of the broadband local field potential (LFP)[96]. In support of these findings, studies show that sensory processing and behavior are modulated during different epochs of the population's dynamics[97-117]. In particular, selective attention can increase gamma-band (30-100Hz) amplitude in neural areas encoding attended stimuli[110] that lead to enhanced detection rates[111]. These gamma-band attention effects are thought to sharpen neural encoding properties of local ensembles[118], and enhance signaling at the next stage of neural processing [14,119-122]. Gamma activity is also proposed to coordinate activity across widespread areas via phase-dependent mechanisms, whereby optimal neural signaling is achieved when cell ensembles spike at similar phases of a gamma rhythm[123]. These findings suggest that gamma oscillations may play a key role in haptic perception by coordinating activity between sensori-motor networks encoding relevant features of the tactile object.

Experimenter control of gamma-band activity can be a major asset in BMI applications by providing a mechanism that enables reliable intracortical stimulation during optimal brain states. A recent BMI showed that volitionally-generated gamma oscillations synchronized spiking between primary motor (M1) cortex cells, and improved grip and reach movements[124]. Unfortunately, a reliable method to systematically manipulate gamma activity in a non-human primate brain has not been developed, but studies in mice show that optogenetic stimulation of interneurons can induce gamma activity in sensory cortices[15,17,18]. These findings cemented the role of interneurons, particularly parvalbumin (PV) cells, as a major cell class generating gamma oscillations by imposing cyclical inhibition on neighboring ensembles every ~10-30 ms. (~30-100 Hz)[15,17,125]. The two major canonical models describing the network mechanisms that generate gamma-band oscillations are the *pyramidal interneuronal gamma (PING), and interneuronal gamma (ING)* (**Figure 1**)[126-137].

In PING, gamma oscillations emerge from reciprocal interactions between pyramidal and inhibitory neurons, whereby excitatory cells activate inhibitory interneurons that, in turn, suppress firing of local pyramidal networks. As inhibition wears off (~10-30ms), pyramidal cells renew firing and reactivate inhibitory interneurons, thus bringing the network into a gamma-band oscillatory state (~30-100Hz). Under the PING regime, the inhibition decay time constant determines the oscillation's frequency. Further, strong post-synaptic drive from excitatory

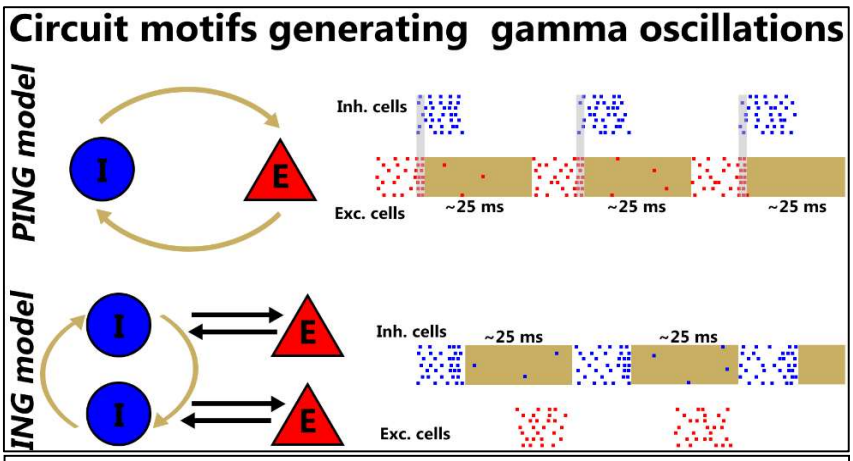

Figure 1. Canonical models generating gamma oscillations. The top and bottom diagrams show the neural circuit, and dynamics, underlying ***PING and ING models***, respectively. Under ***PING***, excitatory cells are necessary to drive inhibitory interneurons. Gamma oscillations emerge from the periodical interplay between excitatory and inhibitory cells (horizontal mustard-colored arrows). Under ***ING***, pyramidal cells are not required to drive interneurons. Gamma oscillations are generated by recurrent inhibition within an exclusive interneuron network (vertical mustard-colored arrows). Mustard-colored squares indicate the temporal window of inhibition imposed. Blue and red dots are spikes from inhibitory and excitatory cell populations, respectively. Gray vertical lines indicate the onset of the temporal window of inhibition imposed by pyramidal cells on interneurons.

cells (e.g., synchronized spiking between pyramidal cells[137]) is necessary to reactivate inhibitory interneurons. In ING, the interplay between excitatory and inhibitory cells is not the driver of gamma-band oscillations. Instead, gamma activity is generated by recurrent coupling between interneurons that leads to synchronized inhibition of the local network. The frequency of the oscillation depends on the inhibitory time constant of interneurons (~10-30ms), as well as the firing rate of the interneuron population, by increasing spike precision and, in turn, reducing the periodic variance[137].

It has been proposed that synchrony between interneurons, a driving mechanism of gamma activity, imposes a temporal window of activation for excitatory cells to transmit signals to the next stage of processing[138,139]. One hypothesis is that gamma-band activity, mediated by interneuronal synchronization, enhances the correlated spiking between tactile functional circuits to promote communication of object-related signals across the sensori-motor haptic network. Indeed, this is a major hypothesis I will test in my first R01 application.

The role of gamma-band oscillations in neural coding and behavior has been widely contested[140-148]. Studies have indicated that the power, stimulus dependency, and signal conduction properties of gamma oscillations are incompatible with effective neural coding and information transmission between neural areas[149]. Certainly, these are valid concerns that need further clarification. However, there is overwhelming *in vitro*, *in vivo*, and computational evidence demonstrating that gamma oscillations are not epiphenomenal[127,133], and play a role in neural processing and behavior[137,150-152]. Importantly, studies show that optogenetically-induced gamma activity can cause positive behavioral effects[15,125]. For the purpose of this application, determining whether optogenetic stimulation generates gamma activity in a primate brain is significant to 'causally' study how fast neural dynamics modulate encoding properties and signal transmission of sensori-motor neural ensembles in the haptic network. Further, experimenter control of gamma activity in primate brains will be a valuable feature for BMI applications by ensuring that neural stimulation is reliably applied during states of high gamma-band power.

INNOVATION: This application innovates on the use of neural stimulation strategies to study and manipulate neural dynamics that mediate haptic perception in monkeys. There are three specific innovative components.

(1) Tactile sensations elicited by electrical and optical neural stimulation: The plan is to leverage the benefits of electrical stimulation and optogenetics in a single setting. We will use electrical stimulation to robustly activate local populations encoding relevant tactile features, and pan-neuronal inhibitory optogenetic stimulation to suppress neural populations that are inadvertently activated by microstimulation. To the best of our knowledge, there has yet to be a study that utilizes both stimulation methods to manipulate tactile functional circuits.

(2) *Soma-targeted and cell-type specific opsin expression in non-human primate brains:* Most optogenetic monkey studies use viral promoters that express opsins ubiquitously across the population, preventing selective manipulation of cell classes. Studies in rodents usually circumvent this limitation by using transgenic animal models that express opsins in a specific cell type. Unfortunately, due to gestational periods in rhesus macaques and other factors, transgenic models in this species are unfeasible. To overcome this limitation, we will employ a set of viral strategies to selectively express opsins in interneurons, including PV cells of primate brains. Further, viral vectors will be restricted to the soma, limiting off-target effects of optical stimulation. Soma-targeted

expression of functional proteins (e.g., opsins and GCaMP) has been achieved in mice[54,55], but has yet to be implemented *in vivo* in non-human primate brains.

(3) *Closed-loop neural stimulation approach:* The goal is to evoke enhanced tactile sensations by applying intracortical stimulation during neural periods of high excitability states. We will implement an algorithm to monitor gamma-band dynamics, and apply electrical and optical stimulation during epochs of high gamma activity. We will also use optogenetics to evoke gamma oscillations. This stimulation strategy provides experimenter control of gamma activity, enabling reliable intracortical stimulation during 'optimal' brain states.

APPROACH: The general approach is to record single-unit (SU) and local field potential (LFP) activity, and apply electrical and/or optogenetic stimulation in area 1 of somatosensory cortex using an upgraded version of a transparent microelectrode array (**Figure 2**)[19]. We will implement a novel BMI method[153] to avoid electrical artifacts created by microstimulation. Further, we will inject pan neuronal and Cre-dependent viral vectors containing inhibitory or excitatory opsins in the neural area covered by the array in animals performing tactile motion discrimination tasks. Area 1 is the tactile homologue of the visual motion area, middle temporal (MT) cortex[154]. Area 1 contains neurons tuned to tactile motion direction and speed[155,156], with component and pattern response properties[156].

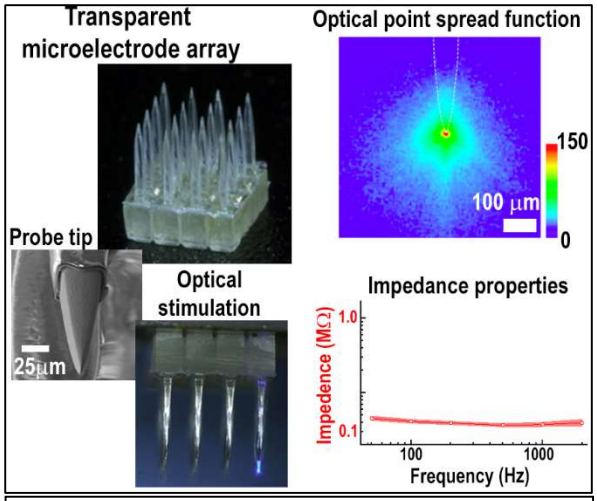

Figure 2. This figure shows a 16-channel transparent microelectrode array developed by our collaborators. This array provides both electrical and optical stimulation, and enables neural recordings at each individual probe site. The right hand side shows the array's optical point spread function (Top image), and electrical impedance properties derived from spectroscopy tests (bottom graph). The impedance trace shows uniform impedance values, indicating that electrical currents are not distorted across stimulation frequency.

Tactile stimuli will be delivered to the glabrous skin of monkeys, with the hand supinated and comfortably restrained to minimize movements (**Figure 3a**). Stimuli will be delivered using a motorized device that presents tactile stimuli moving in different directions (0º to 345º, in steps of 15º), and with speed of 40mm/s. Tactile stimuli will be presented within a sound isolated box to ensure that animals do not use visual or auditory cues to perform the task. Monkeys will be trained to respond via eye saccades on a visual monitor placed in front. **Table 2** in the Career Development, Training, and Objectives section shows the timeline of experiments.

Reducing learned stimulus/response associations: Type IV errors (correct rejection of the null hypothesis, but for the wrong reasons) are a major concern in neural stimulation studies. There are certain situations where monkeys make correct behavioral choices in response to a neural stimulation pattern that lead experimenters to conclude that the electrical or optogenetic stimulation activated the correct neural circuit(s). In many instances this inference is correct[1,24,45,157-159], but other times the behavioral response may occur from learned associations between the ambiguous/unnatural percept and the reward, which are established through many trial repetitions. Several studies avoid this issue by implementing clever experimental paradigms, and leveraging the topographical representation of the stimulated area (e.g., somatopy for area 1[1], motion direction columns for visual MT[157,158]). Unfortunately, area 1 motion-tuned cells are not organized in a topographical cluster. Thus, we will design experimental paradigms with large stimulus and response parameter sets to increase stimulus/response contingencies, and minimize (or even discourage) the likelihood that animals rely on learned strategies to obtain rewards. **First**, we will present a low number of neural stimulation trials (10% of each stimulating condition), and interleave them with mechanical trials to decrease the likelihood that animals learn to associate a particular artificially-induced sensation with a response. **Second**, we will implement a 3-alternative-forced-choice (3-AFC) task, vs. a 2-AFC, to increase contingencies between behavioral responses and neural (or mechanical) stimulation conditions, and decrease the probability that animals learn an arbitrary stimulus-response map. **Third**, we will randomly present a wide range of motion direction stimuli to derive psychometric functions, and quantify changes in threshold and response biases evoked by neural stimulation.

Aim 1 – Evoking tactile motion sensations using optogenetics and microstimulation

Hypotheses

- We will test the hypothesis that combined electrical and optogenetic stimulation synchronizes activity between motion-tuned somatosensory ensembles, which lead to enhanced discrimination of tactile motion stimuli. The mechanism of action is that microstimulation increases synchrony across the population, including motion-tuned cells, while a localized spatio-temporal pattern of inhibitory optogenetic stimulation desynchronizes and suppresses firing of non-motion tuned cells inadvertently activated by microstimulation.
- We will further test that neural stimulation effects are enhanced during epochs of high gamma-band amplitude, by further increasing synchronized spiking between motion-tuned neural ensembles.

Behavioral task design and experimental methods: Animals will be trained on a 3-AFC tactile motion direction discrimination task (see **Figure 3b**). A trial will commence when the animal fixates on a centrally presented cross for at least 500ms. Afterwards, two sequentially-presented moving tactile stimuli will be indented on the monkey's hand with an inter-stimulus interval (ISI) of 500 ms. The second stimulus will move to the left (1/3 of trials), right (1/3 of trials) or in the same direction (1/3 of trials) as the first stimulus. Animals will indicate whether the second stimulus moved to the left, right, or in the same direction as the first stimulus by saccading to a left, right, or central circle presented above the fixation cross, respectively. The motion direction of the second stimulus, in relation to the first, will be randomly varied across trials.

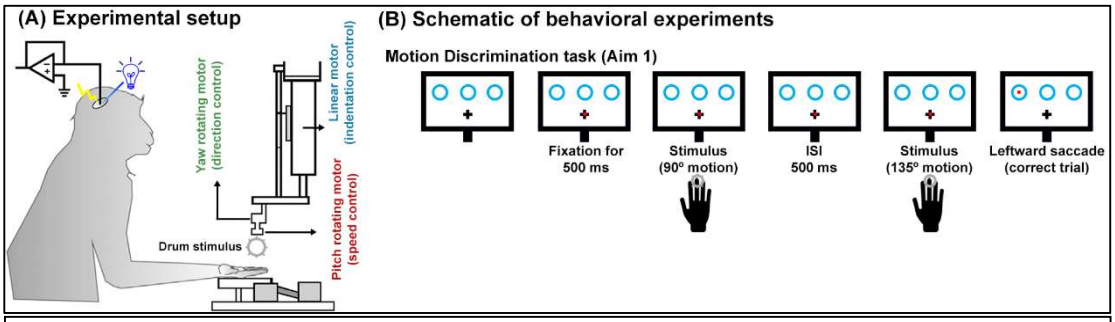

Figure 3. (A) Illustration of the experimental setup. Animals will sit comfortably on a chair with their hand supinated, while a drum stimulus (gray cylinder) is indented on their hand(s) with different direction. The indentation, motion direction, and speed of the drum will be controlled via linear (blue text), yaw (green text), and pitch (red text) motors, respectively. **(B) Typical sequence of events in experiments of aim 1 and 2**. The red dot shows the eye position of the monkey.

Mechanical stimuli will be presented either at threshold or supra-threshold amplitude levels. A liquid reward will be delivered if the animal makes a saccade to the correct circle within 1000ms after the second stimulus presentation. The location and motion directions of stimuli will be determined based on the somatotopic receptive field (RF) and direction tuning of neurons recorded by the array. Each block will contain 100 trials, and animals will perform at least 10 sessions per day, or until they are satiated of water. All trial conditions will be randomly permuted. On 30% of trials, we will present mechanical stimuli with threshold-level amplitude in combination with electrical-alone (10% of trials), optogenetic-alone (10% of trials), or combined electrical and optogenetic stimulation (10% of trials). On 10% of trials, we will provide combined electrical and optogenetic stimulation without mechanical stimuli to assay whether our combined stimulation strategy evokes tactile percepts in the absence of external sensory stimuli. The physical qualities (e.g., intensity, duration, frequency, etc.) and spatio-temporal patterns of electrical and optical stimulation will be determined *a priori* using computational models and machine learning decoders, and fine-tuned during *in vivo* experiments as needed (see General Methods).

Animals will be injected with a virus that expresses IC++ inhibitory opsins. Firing rate and LFP data will be analyzed for each stimulus condition. We will perform wavelet analyses in the 30-100Hz range *online* to obtain instantaneous readouts of gamma power, and apply neural stimulation during low, medium, and high epochs of gamma activity. Correlated activity will be assayed by computing spike-synchrony[160,161] and spike-field coherence[104]. A detailed description of data analysis strategies is provided in the General Methods section.

Predicted outcomes: Our hypotheses make clear predictions regarding the mechanism of action of combined electrical and inhibitory optogenetic stimulation, and their synergistic effects on the populations' dynamics and behavioral performance.

- We expect that strong microstimulation of area 1 neurons increases spike-synchrony between the entire population, relative to baseline, and that a localized spatio-temporal pattern of inhibitory optogenetic stimulation (derived from computational studies) will decrease spike-synchrony between neurons not tuned

for tactile motion direction. Importantly, we should expect that reductions in microstimulation amplitude will fail to strongly activate the population, and lead to decreases in spike-synchrony. Further, we predict that random patterns of inhibitory optogenetic stimulation will produce non-specific activations of tactile motion ensembles.

- We predict that combined electrical and optogenetic stimulation will enhance discrimination sensitivity (greater d-prime, and decreased perceptual thresholds) without affecting response-bias (no changes in β-criterion). We expect these behavioral effects to be greater in combined as compared to electrical-alone and optogenetic-alone stimulation conditions.
- We expect to find a systematic positive relationship between behavioral effects of neural stimulation and gamma amplitude. We further predict that neural stimulation trials with high discrimination performance will be associated with increases in gamma-band spike-field coherence in motion vs. non-motion tuned cells.

Alternate outcomes, pitfalls and solutions:

- Neural stimulation effects may not be enhanced during periods of high gamma amplitude. We will test for relationships in other frequency bands that are tightly linked to perception and discrimination (e.g., alpha: 8-14Hz[107,109,162,163]; and beta: 15-30Hz[117,164]).
- We designed the experiment such that the number of neural stimulation trials within a block is low. Given our design, we estimate that we will obtain about 50-100 trials per day for each neural stimulation condition. This may yield a low number of trials when parsing out data across gamma-band amplitude conditions, which may lead to concerns with statistical power. To avoid this limitation, we will pool data across multiple days to increase the number of trials in analyses that require binning of trials as a function of gamma amplitude.
- Stimulation parameters derived from the computational models may not be adequate to drive neurons during *in vivo* experiments. If that is the case, we will use traditional search algorithms to determine stimulation parameters that best drive neurons (e.g., Mazurek et al 2017). We will have sufficient time to explore all parameter spaces since we expect that the array will be chronically implanted for many months.

Aim 2 – Assay the role of interneurons in generating gamma oscillations in monkeys

Hypotheses

- We will test the hypothesis that gamma-band oscillations in primate brains are mediated by recurrent activation of inhibitory PV cells every ~10-30ms (30-100Hz). These PV inhibitory effects suppress firing in local pyramidal networks, that lead to a narrow window of pyramidal cell activation every ~10-30ms, thus bringing the entire local network into a gamma-band oscillatory state.
- We will also test the hypothesis that optogenetic drive of motion-tuned PV cells enhances motion discrimination by increasing gamma activity in the local population encoding tactile motion stimuli.

Behavioral task design and experimental methods: Animals will engage in the same behavioral task as in aim 1. However, monkeys will only receive optical stimulation to activate PV interneurons, which will be done on 30% of all trials. Further, on 50% of optogenetic stimulation trials, we will apply localized patterns of optical stimulation to activate PV cells tuned for motion stimuli. On the remaining optogenetic stimulation trials we will activate PV interneurons regardless of their tuning preference. On 10% of trials, we will apply optical stimulation during the baseline period (i.e., prior to a trial) to assay modulations in gamma power without contamination of sensory inputs. On 50% of trials, animals will not receive optogenetic stimulation.

Animals will be injected with a Cre-dependent virus under a PV promoter to express excitatory opsins (ChR2 or ChETA) in PV interneurons of area 1. Firing rate and LFP data will be analyzed for each stimulus condition. We will perform spike waveform shape analyses to functionally classify neurons into regular spiking (RS) and fast spiking (FS). FS cells are considered to be the electrophysiological correlate of PV interneurons. Gamma power will be characterized using wavelet analyses in the 30-100Hz range. Population correlated activity will be assayed by computing spike-synchrony and cross-correlograms[160,161]. A detailed description of data analysis strategies is provided in the General Methods section.

Predicted outcomes: Our hypotheses make clear predictions regarding effects of PV optogenetic stimulation on the local population neural dynamics, and pyramidal cell firing.

- We predict that 40Hz optogenetic stimulation of PV cells will drive FS spiking, and generate gamma activity in the LFP. These gamma-band optogenetic effects will also occur in the absence of mechanical stimuli.
- Optogenetic stimulation of motion tuned PV cells will increase gamma activity in local populations tuned for motion, that will lead to enhance discrimination of tactile motion stimuli. In contrast, optogenetic stimulation of non-motion tuned PV cells will decrease tactile motion discrimination by driving gamma in local populations that encode irrelevant features of the tactile stimulus
- We predict that optogenetic drive of PV interneurons will lead to an anti-phase relationship in the cross-correlograms of FS and RS firing. Specifically, we expect that increased spike-synchrony between FS cells will lead to decreased firing of RS neurons.

Alternate outcomes, pitfalls and solutions:

- It is possible that optogenetic activation of PV cells does not drive gamma activity. This leads to the hypothesis that gamma oscillations in non-human primates are generated by different mechanisms, a finding with cross-species implications. One possibility is that neocortical gamma oscillations in primates are generated by somatostatin/pyramidal interactions, as shown by a recent study in visual cortex of mice[165,166]. We can test this hypothesis using our same viral approach but with a somatostatin promoter.
- The transfection efficacy of the Cre-dependent double viral approach to target PV interneurons might be poor. An alternate solution to activate PV interneurons, is to inject viruses with DLX enhancers to express optogenetic elements in GABAergic cells, and optically stimulate at 40Hz to drive PV cells at their resonant frequency[15,17]. A second solution is to inject a non Cre- dependent virus with the PC-17 synthetic promoter. Both of these solutions have been shown to work in primate brains[167,168].

General Methods

Animals: All experimental and surgical procedures will conform to NIH guidelines, and be approved by the University of Rochester's Institutional Animal Care and Use Committee. We will use both mice and rhesus macaque monkey animal models. Experiments in mice will be performed during their dark (waking) cycle. Mice will be used to validate opsin expression in somas, to ensure that Cre-dependent PV viruses selectively express opsins in PV interneurons, and to debug the closed loop BMI system[153] before using it in monkeys. Mice will have free access to water, and we expect to run at least 10 mice per experiment.

Rhesus monkeys used in experiments will weigh ~3-6 kg, and will be purchased through the University of Rochester's Department of Laboratory Animal Medicine (see **Vertebrate Animals** section). During training and recording, animals will be under a water restricted diet, and brought to the laboratory 5 to 6 days a week for 4-6 hours each day. Monkeys will receive all of their water in lab during training and recording days. We expect that training on the tasks will take ~12 weeks for each monkey. Animals' weight and health will be carefully monitored throughout the training and experiment periods (see **Vertebrate Animals** section).

Tactile and visual devices: Tactile stimuli will be delivered using a 0.5-inch diameter cylinder with grooves (0.2 inches in height) that will be mounted on a platform with three motors that provide yaw, pitch, and linear control with high temporal and spatial precision. This enables us to present tactile stimuli moving in different directions (0º to 345º, 15º steps), speeds (1 mm/s to 80 mm/s, 10mm/s steps), and indented at different depths (0 to 2mm, .25mm steps). Tactile stimuli will be presented with 1 second duration. All visual stimuli will be presented on a gray background in a monitor place 2 feet in front of the monkey. The fixation cross will be colored in black with ~1º size. Response cue circles will be colored in blue with ~2.5º size, and presented 2º vertically above the fixation cross. Monkeys' eye movements will be monitored with a ViewPoint EyeTracker system from Arrington Research[161], and their hands comfortably restrained in a custom-made hand holder[161,169].

Neural recordings & optical and electrical stimulation: Neural recordings will be made in the left and right hemispheres of monkeys using a sub-durally implanted microelectrode array (**Figure 2**). We expect to implant up to three contiguous arrays to cover the entire hand representation of area 1. Placement of the arrays will be based on hand and tactile motion responses in fMRI experiments (see fMRI and DTI sections below). If development of the transparent array is delayed, we will use the Neural Probe with Monolithically Integrated micro-LEDs, which also affords neural recordings together with optical and electrical stimulation capacity[170].

We will implement a closed loop hardware/software integrated system[153] to provide continuous artifact-free neural recordings during electrical stimulation, enabling uninterrupted neural decoding and stimulation

functionality during experiments. The system provides artifact-free electrical recordings by implementing a series of signal blanking and digital filtering procedures in between the neural recording and stimulation stages.

Electrical and optical stimulation parameters will be determined using computational models, and fine-tuned during *in vivo* experiments (see Computational modeling section below). We will adhere to the stimulation safety guidelines determined by the Shannon equation to avoid tissue damage from electrical currents[171]. Similarly, we will apply safe levels of optical light stimulation to avoid tissue damage caused by thermal reactions. Optical light going into the array will be fully covered to ensure that animals do not use the optical stimulation as a visual cue.

Computational modeling: We will implement biophysical circuit-based models[172-175] and machine learning algorithms[176-180] to determine the physical qualities and spatio-temporal patterns of electrical and optical stimulation that best activate tactile circuits. The anatomical organization of the biophysical model will be based on the response properties, layer information, and axonal projections of neurons underneath the array derived from fMRI and DTI data. Further, the model will be composed of clusters of excitatory-inhibitory balanced networks that abide by the laminar neural distribution proposed by Ed Callaway's group[181,182].

Our strategy for determining the physical qualities and spatio-temporal patterns of electrical and optical stimulation is the following: **First,** use machine learning algorithms to decode the neural dynamics (e.g., population firing rate, neural synchrony, state-space models, etc.) that best predict the population's response of correct behavioral choices during mechanical stimulation trials. **Second,** run simulations with the biophysical circuit-based model to determine the combination of electrical and optical stimulation parameters (e.g., intensity, frequency, duration, and spatio-temporal patterns) that generates a population response that best match the population response activity decoded from the machine learning algorithm. **Third,** use the parameters derived from the biophysical models to stimulate area 1 cells. All models and parameters will be titrated to each monkey.

fMRI and DTI imaging: We will perform fMRI and DTI imaging to determine neuronal response properties and axonal projections of area 1 neurons in each animal using a Siemens 3T MRI with custom-made liquid nitrogen cooled phased array coil for rhesus macaque brains. These experiments will be conducted during anesthetized preparations using an anesthetic and dosage that minimally affect neural response properties in somatosensory cortices[183]. Conducting these imaging experiments during anesthesia provides us with enough time to present a variety of tactile stimuli so we can derive a comprehensive neural map of the response properties of a particular somatosensory area. Specifically, we will present tactile stimuli that vary in motion direction, motion speed, vibration, orientation, and texture. Stimuli will be scanned across different parts of the hand to derive a somatotopic map for each animal. Neural responses within each area will be co-registered with axonal projections to obtain a comprehensive functional anatomical map of the local ensemble. These data will be used in computational models to guide electrical and optical stimulation parameters. Dr. Gomez-Ramirez has experience performing functional imaging experiments in both humans and non-human primates (Gomez-Ramirez et al 2006 IMRF; 2007 Soc. Cog. Neurosci; Trzcinski et al 2012; 2013 SFN). We will build a plastic apparatus that delivers tactile stimuli via manual displacements by a human due to restrictions of electronic devices in the MRI room.

Viral surgery and development: We will develop a battery of viral vectors to express inhibitory and excitatory opsins in somatosensory neurons of non-human primates. Expression of all optogenetic elements will be restricted to the soma using a genetic 'tag' that imports proteins to the soma membrane[54,55]. We will use IC++, CHR2, and ChETA opsins because of their fast kinetic properties[8,184-186]. Aim 1 experiments will use the inhibitory opsin IC++, packaged in an AAV2/5 viral vector with the human synapsin promoter, and a red fluorescence protein (**AAV2/5-hSyn-iC++-tdTomato)**. For aim 2 experiments, we will develop a double Cre-dependent viral approach to express excitatory opsins (CHR2 or ChETA) in PV interneurons. First, we will create a PC17-Cre virus that expresses the Cre recombinase enzyme in PV cells under the synthetic promoter PC17. Secondly, we will develop a Cre-dependent-DLX-CHR2 (or ChETA) virus to express the excitatory opsin CHR2 (or ChETA) in interneurons that have the Cre recombinase enzyme. Using a Cre-dependent dlx enhancer further restricts the expression of opsins to PV interneurons. Viruses in aim 2 experiments will be packaged in an AAV2/9 serotype, which has high affinity for neocortical interneurons[187]. As a backup, we will develop viruses with DLX enhancers (mDLX or DLXi12b), which target interneurons in non-human primate brains with robust selectivity[167,168]. In total, the following viral constructs will potentially be developed for aim 2 experiments: **AAV2/9-PC17-Cre-tdTomato, AAV2/9-PC17-ChR2-eYFP, AAV2/9-PC17-ChETA-eYFP, AAV2/9-mDLX-Cre-tdTomato,**

AAV2/9-mDLX-DIO-ChR2-eYFP, AAV2/9-mDLX-DIO-ChETA-eYFP, AAV2/9-mDLXi12b-Cre-tdTomato, AAV2/9-mDLXi12b-DIO-ChR2-eYFP, AAV2/9-mDLXi12b-DIO-ChETA-eYFP.

Viral constructs will be injected in monkeys using MRI-guided convection-enhanced delivery (CED) methods, which lead to large-scale transduction of optogenetic elements in monkeys[94,95]. We will apply large volumes of the viral construct (40μl and 50μl) in at least 5 sites of area 1 cortex. We will inject at rate of 1 μl/min rate, increasing to 5 μl/min by 1 μl/min steps[94]. This viral delivery method increases the likelihood that viruses get effectively transfected in non-human primate brains[10,94,95].

Data analyses: We will derive psychophysical curves to estimate discrimination thresholds, and compute d-prime and β-criterion shifts. Analyses of spiking activity and LFPs will be time locked to the onset of mechanical, electrical, and optogenetic stimulation. LFP data will be analyzed in the frequency domain (1 to 100 Hz) using Fourier transforms, and in the time-frequency domain using wavelet analyses and band-pass filters with Hilbert transforms[108]. Correlated spiking activity between cells will be analyzed using spike-synchrony search methods[160,161], cross-correlograms, and spike-count correlations (Rsc)[188]. Relationships between spiking activity and the LFP will be assessed via spike-field coherence analyses[189]. Non-parametric bootstrapping tests will be used to statistically validate experimental results[108,161]. We will perform spike-waveform shape analyses to functionally classify neurons into RS and FS types[15].

Histology: We will perfuse and euthanize animals after completing experimental protocols, and perform histology on their brains to determine opsin expression levels and location with fluorescence microscopy methods.