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Using Optogenetics to Causally Test the Role of Local Gamma Synchrony in Downstream Sensory Responses

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Using Optogenetics to Causally Test the Role of Local Gamma Synchrony in Downstream Sensory Responses

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Submitted in Partial Fulfillment of the Prerequisite for Honors in Neuroscience

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-- K.S.
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Abstract

Conscious processes such as attention and sensory awareness have been associated with neural oscillations in the gamma frequency range (30-90 Hz). However, it remains unclear whether gamma rhythms play a functional role in sensory routing or are merely a byproduct of computation. Optogenetics has recently made it possible to manipulate synchrony exclusively in vivo. Therefore, to test the hypothesis that local gamma synchrony plays a functional role in routing sensory responses between brain regions, we launched an optogenetics protocol in mice to induce gamma synchrony in primary somatosensory cortex (S1) and measure somatosensory responses in frontal cortex (M2). To test the feasibility of our experimental setup we also carried out recordings in control mice and analyzed the relationship between spontaneous fluctuations in pre-stimulus S1 gamma synchronization and M2 response amplitudes. Our pilot experiments confirmed our ability to elicit and record sensory responses in anesthetized mice but initial analyses reveal no correlation between S1 synchrony and M2 response amplitude. Preliminary optogenetic experiments were unsuccessful in inducing synchrony but revealed the possible causes of the problem and the steps necessary to address them. Once we can successfully manipulate synchrony we can apply the analyses developed for control recordings to address the question of whether gamma synchrony modulates interarea signal transmission in the brain.
Attention is simultaneously one of the most ubiquitous and yet subtle processes of the human mind: we use it constantly, but rarely devote any conscious thought to it. It plays a central role in cognitive processing, and has been a primary focus of research in the fields of cognitive psychology and neuroscience since their births. Attention has been defined as a ‘bottleneck’ in the processing pathway of a given set of incoming stimuli – internal or external – that allows only their most relevant subset to undergo further processing and reach conscious awareness (Styles, 2006). Along the same lines, Broadbent (1958) described attention as a limited-capacity resource that can be distributed among a subset of inputs to make them the subject of conscious thought. In this view, attention is the limiting factor on our conscious cognitive capacities.

Evidence that such a processing bottleneck occurs at all is provided by the psychological refractory period, in which subjects respond more slowly to the second of two stimuli presented in quick succession (Welford, 1952). This suggests that, at some point in processing, a second input must await further processing until after the first is fully processed. In other words, there is a switch from parallel to serial processing sometime before the behavioral output. Because we consciously experience only the end result of attention, there has been controversy over where this bottleneck occurs along the processing pathway. Some researchers attempted to pinpoint its location using a dichotic listening task, in which separate audio recordings are streamed simultaneously to each ear and subjects are instructed to attend to just one. Upon questioning, subjects tend to remember only basic, physical properties of the unattended stream, such as the
timbre of the voice, but not the language or other semantic properties of the message (Broadbent, 1952, 1954; Cherry, 1953; Poulton, 1953, 1956). This suggested that the unattended messages were not processed thoroughly and that the attention bottleneck occurs early on in processing.

Other researchers challenged these findings, arguing that subjects may have fully processed the unattended stream but forgotten it by the time of questioning, or processed it unconsciously. Evidence of more extensive parallel processing and a later bottleneck came from findings that subjects recognize their own name in the unattended stream (Moray, 1959) and that when streams differ more in semantic content, subjects are better able to attend to one and ignore the other (Treisman, 1960). If the attended stream were selected for serial processing early on, these semantic parameters of the unattended stream would have gone unnoticed by listeners. Still, this breakthrough of the unattended tends to be the exception rather than the rule, and these conflicting findings framed the ‘early’-‘late’ debate in attention research, which continued for decades as each side expanded their findings and their corresponding theories.

In a review of visual and auditory selective attention literature, Lavie and Tsal (1994) noted that support for the early model came largely from complex tasks with many or ambiguous stimuli, termed high in perceptual load, while late selection was supported by studies with relatively simple paradigms. Lavie (1995) went on to show that, when perceptual load is high due to many distractor stimuli, subjects’ response times are not affected by response-incompatible distractors any more than neutral ones, implying that they are not processed fully. On the other hand, in simpler tasks these unattended stimuli have differential effects on response times because the lower perceptual load makes it possible to devote more processing capacity to the unattended stream. Thus, perceptual load theory proved the early-late dichotomy to be overly simplistic and provided a model that treats attention as a resource that always operates at
capacity, such that if target information is below that capacity it devotes the remainder to processing unattended information. Thus, the location of the bottleneck, and therefore the extent of information processing, varies with the complexity of the task.

While perceptual load theory is useful for a conceptual understanding of attention processing, a full account of attention must relate models in psychology to the physical workings of the brain in a way that makes it possible to test the empirical validity of theory and, conversely, to generate and test novel hypotheses from theory. Thus, a primary goal of research in neuroscience is to develop a rigorous understanding of the molecular and systems-level mechanisms underlying attention.

II. The neuroscience of attention

Much of what is known about the neural correlates of attention comes from studies that simultaneously measure attention via a behavioral task and neural activity in the form of electrophysiological recordings or functional imaging. Behavioral studies in humans and primates have shown that attention to target stimuli during behavioral tasks correlates with higher accuracy rates (Womelsdorf and Fries, 2006), faster reaction times (Womelsdorf et al., 2006), and lowered detection thresholds (Lee et al., 2005; White and Carrasco, 2011). Neurophysiological studies in primates have also found that neurons in areas corresponding to attended stimuli exhibit greater stimulus selectivity and sensitivity (Desimone and Duncan, 1995; Spitzer et al., 1988).
Frontal-Parietal Connectivity

Functional imaging, neurophysiology and lesion studies have consistently found that frontal and parietal cortex in particular are involved in attention in a number of species. Buschman and Miller (2007) reported that top-down, goal-directed attention activates monkeys’ frontal cortex earlier than parietal cortex, while the opposite is true for bottom-up, stimulus-driven attention. Increased interactions between these brain regions during both types of attention have also been reported in monkeys, as discussed further below (Buschman and Miller, 2007; Gregoriou et al., 2009). Positron emission tomography (PET) has revealed activation in these areas in human subjects performing an attention task (Corbetta et al., 1993; Nobre et al., 1997; Pardo et al., 1991), and human patients with parietal lesions display difficulty shifting attention (Posner, 1980).

While this evidence for a frontal-parietal attention network is focused on posterior parietal cortex (PPC), primary somatosensory regions may also project to frontal cortex either directly or after being integrated elsewhere such as in PPC. Golmayo et al. (2003) supplied electrical stimulation to the somatosensory hindlimb region of parietal neocortex in anesthetized rats and found responses in prefrontal cortex corresponding to M2. Response latencies of ~35 ms suggest these areas may be directly connected. Given that the topography of mouse prefrontal cortex closely resembles that of the rat (Guldin et al., 1981), it is possible that mice have analogous S1-M2 connections. Furthermore, anatomical homologies between mouse M2 and frontal cortex of higher order mammals (Ballesteros-Yáñez et al., 2010) provide a substrate for potential functional homologies between these areas in rodents and humans, and highlight the relevance of findings in mice to understanding cognitive processes in humans (Koch, 2012).
Neural synchronization

On the cellular level, one proposed mechanism for the behavioral and physiological changes observed during attention is neural synchronization, in which large numbers of neurons fire in unison such that their collective activation and suppression comes in carefully timed waves. When neural activity is assessed as a local field potential (LFP), which measures the electrical activity of a large local population of cells, synchronization can be reflected in the oscillatory patterns in the LFP. Synchronization in the gamma frequency range (30-90 Hz) (Freeman, 2006) in particular has been associated with attention. Womelsdorf et al. (2006) showed that local gamma synchrony in monkey visual cortex both before and after a stimulus predicts faster reaction time on a visual attention task. Gamma-band synchronization between two visual areas in monkeys was also found to vary with the behavioral relevance of the stimulus during an attention task (Grothe et al., 2012). Furthermore, Taylor et al. (2005) found that gamma synchrony in monkey visual cortex predicts successful allocation of attention, and that errors exclusively related to misdirected attention correlate with a shift in the site of elevated gamma synchrony from the neuronal population representing the target stimulus to that of the distracter stimulus.

Other research has implicated gamma synchrony in a host of other executive processes including perception, sensory integration, and memory, suggesting a role in neural computation beyond attention. In fact, gamma synchrony first caught the interest of the systems neuroscience community in the late 1980s and early 1990s with an investigation into its role in perceptual binding (Singer, 1999). This was prompted by observations in visual cortex of anesthetized cats that the extent of 30-50 Hz synchrony reflected global stimulus properties (Gray and Singer,
1987, *Soc. Neurosci.*, abstract; Gray and Singer, 1989). The binding-by-synchronization hypothesis that emerged from this research posits that different features of a stimulus, such as its shape and color, can be bound together to form an integrated percept through synchrony among the neural networks activated individually by each feature (Singer, 1999). Mice have also been found to exhibit greater gamma synchrony in visual cortex in response to coherent stimuli (e.g., gratings) than incoherent or degraded stimuli (e.g., random dot patterns) (Nase et al., 2003). Meador et al. (2002) demonstrated a link between conscious perception and gamma synchrony in humans, showing that coherent gamma oscillations in primary somatosensory cortex occurred 150-300 ms after perceived – but not unperceived – tactile stimuli. Carr et al. (2012) recorded from rat hippocampus and found that during sharp wave ripples associated with replay of stored memories, gamma synchrony correlated with the quality of memory replay. Gamma synchrony has even been implicated in sensory discrimination in insects, which lack cerebral cortex (Stopfer and Laurent, 1999; Stopfer et al., 1997).

Thus, gamma synchrony has been associated with various executive processes across many species and in brain regions ranging from all types of sensory cortex to subcortical nuclei (Fries, 2005). The common thread among these circumstances appears to be the need to coordinate disparate neuronal populations (Vidal et al., 2006). These observations have led to the hypothesis that gamma synchrony provides a dynamic mechanism for routing signals in the brain, making it a fundamental building block of cortical computation in general (Fries, 2005, 2009; Womelsdorf and Fries, 2006).

This hypothesis is controversial for several reasons. Many correlational studies that postulate synchrony as a functional mechanism do not account for alterations in other neural parameters (e.g., firing rates) that may accompany changes in synchrony (Tallon-Baudry, 2009).
Given its apparent ubiquity and the fact that it is often coincident with other potential mechanisms, synchrony is considered by some to be a byproduct of the brain’s functional connectivity that arises whenever a given region is active (Burns et al., 2011; Ray and Maunsell, 2010; Xing et al., 2012). In support of a more specialized role, studies of stimulus-evoked synchrony have shown that the probability of synchrony varies with the perceptual coherence of the stimulus rather than its mere presence or absence (Singer, 1999), and can even be selective for stimulus orientation (Fries, 2005). Synchrony at distinct and independent frequency bands within the gamma range associated with visual awareness and spatial attention also supports a functional specialization (Wyart and Tallon-Baudry, 2008).

Other challenges to the gamma hypothesis suggest that gamma synchrony is modulated not by attention but by normalization, in which a neuron’s response is suppressed by increased local activation. Ray et al. (2013) found that gamma synchrony in monkeys’ middle temporal cortex varied with the strength of normalization and independently of attentional load. At the same time, local gamma power (Reinhart et al., 2011) and coherence (Chalk et al., 2010) have been reported to decrease during attention in certain brain areas and experimental circumstances.

While debate is ongoing, it is evident that a functional role for gamma synchrony cannot be directly inferred from correlative evidence alone. Thus, a resolution to the controversy will rely on direct and independent manipulation of synchrony. The technology required to carry out such manipulation has recently been added to the repertoire of available scientific tools; with it comes the ability to rigorously address questions that were once only speculative, and whose answers may provide the key to understanding the relationship between synchrony and conscious thought.
**Functional Effects of Gamma Synchrony**

Two main physiological consequences of gamma synchrony provide insight into how it might play a functional role in processing. If neurons that converge onto a downstream target cell synchronize their firing, spatial summation of their action potentials can enhance their effect on the target (Hao et al., 2009; Xu et al., 2012). In this phenomenon, known as feed-forward coincidence detection, the excitatory post-synaptic potential (EPSP) evoked by simultaneous inputs could be sufficient to trigger an action potential in the target cell when a smaller EPSP from any individual input may not. This allows the downstream neuron to act as a “coincidence detector”, only firing in response to synchronous (i.e., coincident) upstream activity. Spatial summation requires spikes to arrive within a few milliseconds of one another, and a 40-80 Hz gamma oscillation corresponds to cycles of 12-25 ms, in which the majority of inputs would arrive during windows lasting approximately a half-cycle of 6-12 ms (Fries, 2005). Thus, inputs entrained to this frequency range would be sufficient for spatial summation, making gamma oscillations uniquely suited to enhance transmission of the information encoded in the circuits involved (Singer, 1999).

Viewing synchronized neurons as a target rather than an input group reveals another mechanism by which gamma synchrony could potentially modulate information processing. Gamma synchrony arises primarily from rhythmic inhibition of excitatory pyramidal cells by interneurons (see below), which creates brief windows for effective excitation by upstream excitatory inputs at the peak of the gamma cycle, when the network is disinhibited. EPSPs coming outside of this window are less effective at exciting their target cells because they coincide with the strong inhibition of the interneurons (Fries, 2005). In this way, the sensitivity
of the target network to the inputs – known as input gain – fluctuates with each gamma cycle, which is why this phenomenon is known as rhythmic input gain modulation. With no synchrony between the rhythms of inputs and cycles of input gain, an average number of inputs will arrive during the target network’s peak excitability. Models have shown that if input and input gain oscillate at the same frequency, however, input gain can be either maximized (inputs all arrive at peak excitability) or minimized (inputs all arrive at peak inhibition) (Tiesinga et al., 2004).

Womelsdorf et al. (2007) provided experimental support for this notion by demonstrating that the phase relation between two gamma-synchronized regions in cats and monkeys is correlated with the strength of their mutual influence.

This coordination not only within one area but also between two recording sites is termed neural coherence, and involves both coincidence detection to determine whether and which inputs achieve coherence with a downstream area, and gain modulation to lend a high gain to a coherent input and exclude (suppress the gain of) signals from incoherent areas (Fries, 2005).

Mechanisms of Gamma Synchrony

Given the apparent importance of the functional consequences of gamma synchrony, there has been interest in uncovering the cellular and synaptic mechanisms required to generate the oscillations themselves. While gamma rhythms have been observed in a huge variety of brain areas, one neuroanatomical feature these areas share is the presence of inhibitory interneurons acting at GABA\(_A\) synapses (Buzsáki and Wang, 2012). Evidence of the role of fast-spiking interneurons in synchrony first came from the observation that interneurons in rat hippocampus phase-locked their firing to gamma oscillations in the LFP (Buzsáki et al., 1983). Subsequent
studies in hippocampal areas CA1 and CA3 have found that interference with GABA\textsubscript{A} receptor-mediated inhibition, typically via the GABA\textsubscript{A} receptor antagonist bicuculline, consistently eliminates gamma oscillations; blockage of other receptor types has been observed to be only partially effective or region-specific (Bartos et al., 2007). This research supported the further investigation of inhibitory interneurons, which transmit GABA, as the driving force behind gamma oscillations.

Over a dozen types of interneurons have been identified, but those believed to be primarily responsible for generating gamma rhythms are a sub-type of basket cell interneurons, so named for their tendency to envelop target cell bodies in a “basket” of axonal projections (Bartos et al., 2007). The majority of basket cells exhibit a fast-spiking (FS) phenotype and are typically identified by their expression of parvalbumin (PV), a calcium-binding protein whose function is not well understood (Bartos et al., 2007). A number of features specific to PV+ FS basket cells make them particularly suited to drive gamma rhythms.

Perhaps most importantly, these interneurons inhibit target cells via shunting inhibition rather than hyperpolarization. In the case of hyperpolarization, the reversal potential for chloride is well below the cell’s resting membrane potential, so when GABA binds and opens chloride-conducting GABA\textsubscript{A} channels, the membrane potential falls further below threshold, reducing excitability. On the other hand, if chloride’s equilibrium potential is very close to the resting membrane potential, the cell exhibits shunting inhibition: when GABA\textsubscript{A} channels open, the cell’s potential remains largely unchanged, but its greatly increased membrane conductance effectively short-circuits concurrent excitatory inputs (Bartos et al., 2007). Thus rather than subtracting a fixed amount from the membrane potential, as in the case of hyperpolarization, shunting inhibition is thought to have a divisive effect on excitability (Prescott and De Koninck, 2003).
While Koch (1999) notes that this reverts to a subtractive effect on firing rate when applied to a spiking neuron model, the divisive effect has been found to emerge again at the network level. While the exact reasons for this are not well understood, experiments in vivo and in vitro have observed shunting inhibition to be perisomatic, fast and strong (Hasenstaub et al., 2005; Papp et al., 2001). The ability to innervate the soma directly is in keeping with basket cells’ unique architecture, and may further increase the strength and speed of shunting by avoiding the conduction delay and current decay potentially involved in passing the input along from a distal dendritic site before being integrated in the soma.

Basket cells’ ability to fire rapidly without fatigue further supports their ability to direct a high-frequency oscillation (Buzsáki and Wang, 2012). This function is substantiated by their resonance at (maximal responsiveness to) gamma-frequency inputs (Cardin et al., 2009). Each basket cell also tends to innervate large numbers of principal neurons (Bartos et al., 2007) as well as up to 60 other basket cells (Sik et al., 1995). These highly divergent connections, along with a low spiking threshold, allow basket cells to play a prominent role in network dynamics (Buzsáki and Wang, 2012).

Models of synchrony

While excitation seems a logical driver of rhythmic pyramidal cell firing, inhibition is in fact a more reliable timekeeper in the absence of perfect, instantaneous synaptic transmission (Bartos et al., 2002). Most initial models of synchrony in networks of inhibitory interneurons were based on weak, slow, hyperpolarizing inhibition, which was sufficient to generate rhythmic oscillations in response to tonic input but not to maintain synchrony in response to variable input.
drive (Wang and Buzsáki, 1996; White et al., 1998). However, subsequent experimental evidence revealed that the basket cells thought to be primarily responsible for generating such oscillations in vivo exhibit strong, fast, shunting inhibition. Models incorporating this type of inhibition, as well as the synaptic and conduction delays and network structure observed endogenously, are robust against variable drive and much more accurately reflect the properties of experimentally observed rhythms (Bartos et al., 2007).

There are two main network models of gamma synchrony generation that incorporate shunting inhibition. The first relies on mutual inhibition between highly connected interneurons. This inhibitory-inhibitory (I-I) network model generates a rhythmic output even in response to variable input because the recurrent connections make periodicity more stable than asynchrony. While some implementations have found the frequency of oscillation to be sensitive to the strength of input drive, modifications to include gap junctions between interneurons and resonant properties of basket cells can correct for this. Adding pyramidal cells to the I-I network causes them to become entrained to the gamma rhythm (Buzsáki and Wang, 2012).

An alternative paradigm relies on excitatory inputs to drive oscillations. In this model, pyramidal cells provide fast AMPA-mediated excitation to interneurons, which provide reciprocal inhibition via GABA_A receptors. Thus, the frequency is determined by synaptic and conduction delays amounting to a phase shift of ~5 ms between excitatory and inhibitory spikes, in keeping with the length of a gamma cycle. In contrast, in the I-I model frequency largely depends on the strength of input drive. The excitatory-inhibitory (E-I) model generally does not require I-I connections, which is consistent with observations in CA1 of mice (Wulff et al., 2009).
Both models provide a plausible explanation for how gamma rhythms arise in cortex, and they are not mutually exclusive. Several studies have found that in CA1 inhibitory firing occurs in advance and delay of excitatory firing phase, suggesting a potential role for both models (Buzsáki and Wang, 2012). It is also possible that the mechanism varies with the brain region in question or even with the particular state of the network at a given time.

**Optogenetics**

While synchronization is widely accepted as a neural correlate of attention, few studies have been able to test for a causal relationship. One of the only behavioral studies to successfully manipulate synchrony without altering the temporal firing patterns of individual neurons showed that disrupting local gamma oscillations impaired honeybees’ performance on an odor discrimination task (Fig. 1). However, the difficulty of manipulating synchrony exclusively during a behavioral paradigm has impeded the replication of this result in other animal systems. With the advent of optogenetics, it has become possible to target neural manipulation to specific cell sub-types without directly interfering with the processes of the surrounding tissue (Deisseroth et al., 2006). This technique employs genetic recombination to induce expression of a light-sensitive protein channel, which passes ions in response to photostimulation and thereby alters the cell’s membrane potential. Thus, when targeted to neuronal populations, optogenetics can be used to trigger or inhibit action potentials with light (Dugué et al., 2012).
Cardin et al. (2009) applied this technique to the study of neural synchronization and supplied some of the first causal evidence that gamma synchrony modulates sensory routing. The authors induced expression of Channelrhodopsin-2 (ChR2), a nonselective cation channel, in the fast-spiking inhibitory interneurons of barrel cortex (S1) in mice. They then showed that driving these cells at ~40 Hz is sufficient to induce local gamma synchrony, supporting the hypothesis that these cells underlie the generation of local gamma rhythms (Fig. 2A). Furthermore, they showed that the timing of a sensory input relative to the phase of a gamma cycle determined the amplitude and precision of evoked responses in S1, providing causal evidence that gamma rhythms gate local sensory responses (Fig. 2B).
Thus, using optogenetics to induce synchrony has lent unprecedented support to the hypothesis that gamma synchrony modulates sensory processing in local circuits. However, it remains unclear whether gamma rhythms route signals to target areas that may be responsible for higher-order processing. The mechanisms responsible for integrating information between one level of processing and the next, rather than merely within one level or brain region, are of critical importance in understanding how individual neural events give rise to conscious processes (Engel and Singer, 2001). The question of whether gamma synchrony plays a functional role in inter-area communication in the brain has long been a major component of such an understanding, but has only recently become tractable and remains to be addressed.

Figure 2. FS inhibitory interneurons generate local gamma oscillations, which gate local sensory responses. (A) Photostimulation of FS inhibitory interneurons at 40 Hz causes a specific increase in 40 Hz gamma power in the LFP. (B) Timing of whisker stimulation relative to phase of induced gamma oscillation determines amplitude of local sensory responses. Baseline activity is indicated in black. Reprinted from Cardin et al. (2009).
**Hypothesis**

To test whether local gamma synchrony in a primary sensory area modulates downstream sensory-evoked responses, we plan to induce gamma synchrony in mouse sensory cortex and measure evoked responses in frontal cortex to somatosensory, auditory and visual stimuli. To do this, we will use transgenic mice that express the light-sensitive protein Channelrhodopsin-2 (ChR2) in GABAergic inhibitory interneurons, including the specific sub-type of PV+ basket cells thought to drive gamma oscillations (Bartos et al., 2002). In anesthetized mice, we will induce gamma synchrony in the somatosensory hindlimb area (S1HL) while providing a tactile stimulus to the tail (Fig. 3). We will then measure the latency and amplitude of sensory-evoked responses in M2, a frontal area primarily devoted to processing motor inputs from M1 but also shown to be responsive to somatosensory information in rats and potentially involved in other higher cognitive functions (Golmayo et al., 2003).

To test whether gamma synchrony in a primary sensory area gates downstream processing, we will compare M2 responses to sensory stimulation delivered at various phases with respect to light-induced gamma oscillations. To test whether gamma synchrony amplifies inter-area communication, we will also draw comparisons between M2 responses to stimuli delivered during induced gamma and those delivered during baseline gamma synchrony in the absence of blue light. We will also compare these findings to M2 responses in control mice that do not express ChR2.

We hypothesize that stimuli arriving near the peak of a gamma cycle in S1 will elicit a larger response in M2 than those arriving near the trough, because inputs to S1 will be gated by gamma-frequency inhibition from interneurons such that those arriving during inhibition (e.g.,...
troughs) will be unable to trigger a response in S1 and therefore will not be passed to M2. In addition, we hypothesize that sensory responses to stimuli delivered in the presence or absence of gamma oscillations will evoke similar responses in frontal cortex, because without aligning to peaks of gamma synchrony, a sensory input has an equal chance of arriving during a peak or a trough of the gamma cycle, and thus an equal chance of getting through or getting blocked.

If successful, these experiments would be among the first to demonstrate a causal role for gamma synchronization in routing sensory signals between cortical areas. If we are unable to induce gamma synchrony without causing significant changes in local firing rates, the interpretation of our results will be less straightforward. However, even in this case we can test whether the phase of a sensory stimulation with respect to a gamma cycle gates sensory projections to higher-order areas responsible for generating behavioral responses. To our knowledge, this has not been tested by direct manipulation of gamma activity in any mammal.

Figure 3. Schematic of recording sites and optogenetic stimulation. (A) Electrode arrays will span S1 and M2 and will be accompanied by a cannula to hold the optic fiber for photostimulation. Cannulae will be fixed at the brain surface at an angle that restricts illumination as much as possible to S1HL. Image adapted from http://www.planitikos.gr.
Methods

Subjects. Data were collected from two male wild-type C57 mice and one male transgenic VGAT-mhChR2-EYFP (i.e., VGAT+) mouse (strain: B6.Cg-Tg(Slc32a1-COP4*H134R/EYFP)8Gfng/J) (The Jackson Laboratory; donating investigator: Guoping Feng, Massachusetts Institute of Technology) (Zhao et al., 2011). All animals were housed in group cages (prior to surgery) or individually (after surgery) at the Wellesley College Animal Care Facility on a 12:12 light/dark schedule (lights on at 11am/off at 11pm). Mice were 6-22 weeks of age at the time of surgery and weighed 23-26g. Mice were allowed free access to food and water and were weighed daily for one week following surgery and prior to each recording session. At the end of experiments mice were sacrificed by isoflurane overdose. All experiments were conducted in accordance with regulations set by the American Association for Accreditation of Laboratory Animal Care (AAALAC) International and approved by the Wellesley College Institutional Animal Care and Use Committee (IACUC).

Channelrhodopsin-2 expression. VGAT+ mice have been genetically modified to express the membrane protein channelrhodopsin (ChR2) in GABAergic interneurons. ChR2 consists of a G-protein coupled receptor, called an opsin, and a light-sensitive compound called a chromophore. The chromophore isomerizes in response to blue light (~473 nm) and causes the opsin pore to open and allow the passage of cations, including $H^+$, $K^+$, $Na^+$, and $Ca^{2+}$ (Dugué et al., 2012). In a neuron at resting potential, light-mediated opening of ChR2 results in an influx of positive charge and a subsequent depolarization. Thus, sufficient illumination of ChR2-expressing neurons triggers action potentials.
ChR2 has been targeted to this cell population using Cre-loxP recombination. Mice transfected with a bacterial artificial chromosome (BAC) transgene encode the Cre (“causes recombination”) enzyme after the promoter/enhancer region for the gene encoding vesicular GABA transporter (VGAT). In GABAergic interneurons, which express VGAT, this promoter is active and the Cre enzyme is expressed. In these cells, Cre deletes DNA regions between two lox-P sites, and in this case is used to delete the floxed (loxP-flanked) stop codon before the ChR2 gene. The result is that a ubiquitous promoter (expressed in all cell types) causes ChR2 to be expressed in VGAT+ cells but runs into the stop codon in all other cell types, allowing photostimulation to affect VGAT+ neurons exclusively (Zhao et al., 2011).

While ChR2 expression is targeted to all GABAergic interneurons, this includes the PV+ basket cells thought to underlie generation of gamma synchrony. Mice belonging to the more specific PV+ strain tend to show resonance at 40 Hz in response to photostimulation at different frequencies, whereas VGAT+ strains are more responsive to a wide range of stimulation frequencies (Jakob Voigts, personal communication). While VGAT+ mice may have a comparatively weaker 40 Hz response, the ability to activate a number of interneuron populations that may resonate at different frequencies would allow us to compare the effects of gamma synchrony with those of induced synchrony at other frequencies. In addition, PV+ mice available for purchase do not show expression in cortex, while VGAT+ mice display moderate cortical expression (Zhao et al., 2011).

Surgery. C57 or VGAT+ mice were anesthetized with isoflurane (induced at 2% in oxygen, maintained at 0.5-1.5%) and chronically implanted with 32 tungsten microelectrodes (Innovative Neurophysiology, Inc.) spanning S1HL and M2 in the right hemisphere (Fig. 4). In accordance
with stereotaxic coordinates set forth by Franklin (1997), craniotomies were centered at the following sites: S1HL, -0.1 AP, 1.8mm ML; M2, 1.5mm AP, 0.7mm ML. Electrodes were 35 µm in diameter and arranged in a 2x16 grid with 150-µm inter-electrode spacing and 300-µm row spacing (Fig. 5). In surgeries for optogenetic experiments, a fiber optic cannula was placed in the craniotomy in a position to illuminate S1HL. The cannula was held in place with clear-drying superglue to minimize light obstruction. Mice received 0.1 mg/kg Buprenex (sc, under the back skin) during surgery and every 6-12 hours for 3 days afterward. All surgeries were conducted according to standard techniques.
Figure 4. Locations of recording sites.
Craniotomies were centered at the midpoints of right hemisphere S1HL (A, C) and M2 (B, D). Both areas, as well as M1 in between (not shown), were spanned by a single microelectrode array. Electrodes were implanted at a depth of 800 μm. Grid indicates mm relative to bregma. Images adapted from Franklin (1997).
Recording. Mice were anesthetized with isoflurane (induced at 2% in oxygen, maintained at 0.5-1.5%) and placed in a stereotaxic apparatus inside a standard operant chamber (80003NS, Lafayette Instrument) (Fig. 6). Tactile, visual, or auditory stimuli were delivered via ABET-II software (Lafayette Instrument). Tactile stimulation consisted of providing pressure to the midpoint of the tail every 3-6 s either automatically via a 12 V tubular push solenoid (Jameco Electronics) or manually using a toothpick. During manual stimulation, the approximate time of stimulation was recorded by simultaneously lowering the toothpick with one hand and using the other to trigger the infrared sensor of a water dispenser in the operant chamber. Light stimulation consisted of a house light turning on for 3-6 s and then turning off for 3-6 s. Visual sessions were analyzed for responses to lights turning on. Auditory stimulation was in the form of 500-ms tones (2500 Hz, 75 dBA) delivered every 3-6 s through speakers in the chamber. Recording sessions typically lasted 15-30 minutes.
Local field potentials were sampled at 1 kHz using the Cerebus Data Acquisition System (Blackrock Microsystems) and highpass filtered offline at 250 Hz. Unit data was sampled at 30 kHz. During sessions in which spiking activity was discernible in the local field potential, units were sorted online by eye. This was done by setting a threshold amplitude above the noise; waveforms that crossed this threshold were then sorted into units by defining templates for consistent waveforms. All spikes that fit a given template were grouped together as a unit.
Without conducting offline sorting it is not possible to distinguish between single- and multi-unit activity but well-defined waveforms suggest the possibility of having captured single unit activity in some cases (Fig. 7). After recording sessions data were transferred to MATLAB using NeuroExplorer.

![Figure 7. Examples of online sorting of unit data.](image)

(A) Electrical activity crossing a threshold voltage in the LFP (plotted in left panel of A; threshold indicated by red line) was magnified (right panel of A) for further sorting into units. Signals passing through template voltage windows (indicated by arrows) determined by eye were stored separately as units. (B) Example electrode channel in which three separate units have been identified (pink, cyan, yellow).

**Optogenetic stimulation.** Optogenetic stimulation was provided in the form of blue light (470 nm) generated by a fiber-coupled light-emitting diode (LED) (Thorlabs). A 200-µm core diameter fiber optic delivered light to the brain surface via a 2.5 mm ceramic ferrule with 0.39 numerical aperture (NA). During recording sessions the ferrule was inserted into a fiber optic cannula (also 200 µm core, 0.39 NA) positioned at the brain surface. Light spreads from the fiber tip as a function of the NA such that the full angle divergence is given by $2 \cdot \sin^{-1}(NA)$ (by definition of NA). Thus for a 0.39 NA we have a full angle divergence of $2 \cdot \sin^{-1}(0.39) \approx 46^\circ$. Power at the fiber tip was measured at the beginning of each session by a power meter.
(PM100USB, Thorlabs) attached to a silicon power sensor head (S121C, 400-1100nm, 500mW, Thorlabs). Power averaged 2 mW over all sessions, which translates to a light intensity of

\[
\frac{2 \text{ mW}}{\pi (100 \mu \text{m})^2} = \frac{2 \text{ mW}}{\pi (0.01 \text{ mm})^2} = 64 \text{ mW mm}^{-2}
\]

Provided the beam is not excessively obstructed between the fiber tip and the target area (but still accounting for moderate effects of light diffusion through brain tissue), this is well within the range required for effective stimulation \textit{in vivo} in a strain of mice with robust cortical ChR2 expression induced by viral transduction (Cardin et al., 2010). The transgenic strain we are using (VGAT-mhChR2-EYFP) shows moderate ChR2 expression in cortex; while the range of light intensity for effective photostimulation \textit{in vivo} is not available for this strain, \textit{in vitro} recordings elicited robust firing of interneurons and silencing of layer V pyramidal cells in response to 2.1 mW mm\(^{-2}\) laser power (Zhao et al., 2011).

\textit{Data analysis}

LFPs and unit data were transferred from the Blackrock Neural Signal Processor via NeuroExplorer to MATLAB, where they were analyzed using custom routines. In MATLAB, all LFPs were segmented into 2-s trials surrounding each stimulus for a given tactile, visual or auditory stimulation session (.mouse_nseg.m).

\textit{Selecting data for analysis.} All sessions underwent cleaning to filter out 60 Hz noise and address artifacts or flat-line values in the LFP. Sessions showing 60 Hz line noise were filtered using a
500-ms sliding window prior to segmentation into trials (rmlinesc.m, Chronux Spectral Analysis Toolbox) (Bokil et al., 2010). For all sessions, trials during which the LFP in any electrode channel exceeded 500 µV were considered artifact trials and were discarded for all electrodes (clean3d.m). Flat-line trials, in which the voltage in any electrode remained constant for at least 5 ms at any point in the 2-s window surrounding the stimulus, were also discarded. If the proportion of artifact or flat-line trials for a particular channel exceeded 0.3 then this channel was discarded entirely. If the remaining data contained electrodes in both S1HL and M2 and more than 100 trials it was retained for further analysis. After discarding sessions with unacceptable LFP contamination, we retained three of four automatic and five of five manual tactile stimulation sessions, as well as three of four visual sessions and four of four auditory sessions.

*Sensory-evoked responses.* In sessions selected for further analysis, ERPs were graphed by averaging LFPs over electrodes and/or trials (mouse_nseg.m). For spatial plots, LFPs were classified as belonging to S1HL, M1 or M2 according to their positions relative to our coordinates of implantation and the coordinates of these areas in a stereotaxic atlas (chanlocs.m, eloc.m) (Franklin, 1997). A session was considered to exhibit a tactile response (1) if the 95% confidence intervals of the ERP surpassed the mean evoked potential in the 1-s pre-stimulus window for at least 50 ms and (2) if, contingent upon showing an ERP response, the response latencies varied across electrode positions such that a linear regression obtained an R² value of at least 0.5 and a slope significantly different from 0 (2-tailed t-test based on residuals of regression fit; p < 0.05). Depending on the most robust component of the ERP, response latencies were calculated as the time of the maximum or minimum evoked potential in the 1-s post-stimulus
window. Sessions in which response latencies appeared to fall into more than one temporal grouping were subdivided and examined separately based on time windows determined by eye.

*Spectral analysis.* In each sensory stimulation session, one electrode from S1HL and one from M2 were chosen randomly for spectral analysis. Spectral power at each recording site as well as coherence between them were calculated via the multi-taper method (time-bandwidth 3; 5 tapers) (*mouse_spectra.m; mtspectrume.m, mtspecgramc.m, coherencyc.m, cohgramc.m*, Chronux Spectral Analysis Toolbox). Comparisons of pre- and post-stimulus power and coherence were based on the average over trials in the 1-s window preceding and following each stimulus. For spectrograms and coheregrams, these calculations were carried out in a moving window of 500-ms duration in 50-ms steps. For sessions with LED stimulation, multi-taper power spectra (time-bandwidth 5; 9 tapers) were averaged over each 1-ms light pulse or each 1-s trial.

For analysis of the relationship between M2 amplitude and S1 power or S1-M2 phase coherence, the response amplitude on each trial for a randomly selected M2 electrode was calculated as the difference between the baseline maximum or minimum voltage in the 1-s post-stimulus window, depending on which component of the electrode-averaged ERP reached criterion. S1 gamma power was calculated at 40 Hz for a randomly selected S1 electrode during the 500-ms pre-stimulus window. The relative gamma phase between these electrodes was computed by taking the phase angle of the coherence calculation in the 500-ms pre-stimulus window. Trials with outlying values of pre-stimulus 40 Hz power were plotted individually for further analysis. In manual stimulation sessions, these trials tended to exhibit large pre-stimulus fluctuations attributable to jitter in stimulus delivery time (relative to the timestamp of the infrared trigger), such that a sensory-evoked response appeared in the pre-stimulus window (Fig.
8A). These trials were not included in power-amplitude and phase-amplitude correlations. In the automatic session that reached criterion for a significant response, there was much greater fluctuation in the LFP throughout the entire session and both pre- and post-stimulus (Fig. 8B). Trials with large pre-stimulus gamma power values were not readily distinguishable from other trials based on visual inspection of the raw LFP trace, so in this case all such trials were included in subsequent analysis. M2 amplitude was then plotted as a function of S1 gamma power and separately as a function of S1-M2 relative gamma phase (mouse_spectra.m).

![Figure 8. Examples of trials with high pre-stimulus gamma power.](image)

(A) In manual stimulation sessions, trials with high pre-stimulus S1 gamma power (e.g., 1.56 here) were almost exclusively those in which the stimulus was delivered prematurely relative to the timestamp and evoked a response in the pre-stimulus window. (B) On the other hand, the automatic stimulation session analyzed for trials with high gamma power had generally more fluctuation in the LFP throughout the session and especially for trials with outlying gamma power.

*Unit responses.* In sessions with defined multiunits, histograms were generated for each by calculating the number of spikes per second per trial in fifteen 0.13-s bins (mouse_units.m). Units’ responsivity to the stimulus was determined by 95% confidence intervals in NeuroExplorer.
Results

To begin addressing the hypothesis that gamma synchrony in a primary sensory area modulates sensory-evoked responses in a downstream area, local field potentials were recorded from three mice presented with tactile stimuli while under isoflurane anesthesia. Spectral analysis of these responses was then performed to look for a baseline relationship between features of the gamma spectrum and somatosensory responses. The specificity of somatosensory responses was tested by recording responses to visual or auditory stimulation as well. Finally, after confirming our ability to generate and record sensory responses in two wild-type mice (WT1 and WT2), we tested optogenetic stimulation in a transgenic VGAT+ mouse (VGAT1). The results of these experiments are presented below.

I. Tactile responses. Five manual and four automatic tactile stimulation sessions were carried out in three anesthetized mice. For sessions selected for further analysis (see Methods), event-related potentials (ERPs) were averaged over trials and electrodes and analyzed to determine the presence or absence of a response.
Two of five manual stimulation sessions met both criteria for classification as a tactile response, exhibiting (1) an ERP significantly exceeding the baseline mean for at least 50 ms (based on 95% confidence intervals) and (2) response latencies showing a linear variation with electrode position ($R^2 \geq 0.5$). In the first of these sessions (in WT1), the channel-averaged ERP passed outside the baseline mean for more than 50 ms at three points (Fig. 9). Latencies for the largest component of the ERP (positive deflection at 491 ms post-stimulus) showed a distinct effect of electrode position ($R^2 = 0.75$; slope = -0.068, t-test, $p < 0.001$; see Methods) (Fig 10). Based on the value of the regression fit at the midpoint of each area, S1 responses preceded M2 responses by 175 ms.

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**Figure 9. Event-related potentials in response to manual tactile stimulation.** (A) ERP response averaged over 10 electrode channels and 320 trials in one session (WT1). Blue shading indicates 95% confidence intervals based on variability across trials. (B) Spatial plot showing trial-averaged ERP for each electrode channel during the same session as in (A).
The second session (in VGAT1) meeting both response criteria showed a significant ERP (negative deflection at 383 ms post-stimulus). The overall spatial effect did not reach criterion because there appeared to be three distinct latency groupings. However, restricting analysis to each subset showed a spatial effect for those electrodes whose response latency occurred in the 250-500 ms post-stimulus window ($R^2 = 0.55$; slope = 0.15, t-test, $p < 0.001$). Based on the regression line, during this window responses in S1 preceded those in M2 by 105 ms. In two of the remaining sessions (in WT1 and WT2) the ERP did not reach significance, and in the third (in WT2) the ERP reached significance but no spatial effect was observed ($R^2 = 0.25$).

One automatic stimulation session was discarded on the basis of excessive artifacts (see Methods). Of the remaining three, one session (WT2) had a significant ERP (negative deflection at 279 ms post-stimulus) (Fig. 11) but no spatial pattern was apparent ($R^2 = 0.04$). In two other sessions (WT2, VGAT1) the ERP did not reach criterion. Of the four tactile stimulation sessions (manual and automatic, out of nine sessions total) with significant ERPs, three sessions (one in
each mouse) appeared to share certain components. The component reaching criterion in all of these was a negative deflection with a mean latency of 308 ± 38 ms (SE across sessions).

II. Visual and auditory responses. Visual and auditory stimulation did not show consistent responses in either S1HL or M2. The only session reaching criterion in each case showed activity of a similar magnitude before and after the stimulus. This was the case in one visual session (WT2) (Fig. 12); the spatial pattern of response latencies did not reach criterion (R² = .22). The other two visual sessions (WT1) showed no ERP response.
In all four auditory stimulation sessions (one in WT1, three in WT2) pre- and post-stimulus fluctuations were virtually indistinguishable. The single session (WT2) in which the ERP reached criterion (positive deflection at 147 ms) showed a pre-stimulus peak of similar magnitude (Fig. 13). This hinders interpretation of a spatial effect ($R^2 = 0.82$) for a subset of electrodes (9 of 11) that peaked in the 0-300 ms post-stimulus window, which showed M2 responses preceding those in S1.

Figure 12. Event-related potentials in response to visual stimulation. (A) ERP response averaged over 32 electrode channels and 110 trials in one session (WT2). Blue shading indicates 95% confidence intervals based on variability across trials. (B) Spatial plot showing trial-averaged ERP for each electrode channel during the same session.
III. Unit responses. In seven sessions showing discernible spiking activity, multiunits were sorted online by eye (see Methods). In WT1, 11 of 18 units showed robust spiking activity in response to manual tail stimulation (Fig. 14A). On the same day, these units showed no sensitivity to visual or auditory stimuli. Summed responses over all units for each of these sessions also showed a response to tactile, but not visual or auditory, stimulation (Fig. 14B). In WT2, 12 units were identified but none showed a response to tactile, visual or auditory stimuli (data collected for one session of each type). In another set of recording sessions (WT2), four of nine units showed a response to manual tail stimulation; unit responses to visual and auditory stimuli have not been analyzed yet. We have not observed any unit responses to tactile stimuli in VGAT1.
IV. Spectral analysis. For all tactile stimulation sessions the spectral power was calculated at randomly selected electrode pairs in S1 and M2. In all three mice and across all five manual stimulation sessions, including those that did not reach criterion for an ERP response, the spectral power in S1 and M2 increased across a wide range of frequencies following the stimulus (Fig. 15). Of three automatic stimulation sessions, only the one reaching criterion for an ERP response showed a similar increase in spectral power. Of all six tactile stimulation sessions (five manual, one automatic) showing a spectral response to stimulation, this response was very similar in S1 and M2 and across sessions.

Figure 14. Multiunit responses to manual tactile stimulation. (A) Histogram of example multiunit in S1HL during manual tactile stimulation (WT1). (B) Histogram of summed activity of all 18 defined units during the same session (displayed as spikes per unit). Data are averaged over 320 trials with 0.13-s bins.
Coherence between the same S1 and M2 electrode pairs used to calculate power spectra increased across a broad frequency range in four of five manual sessions in all three mice (Fig. 16). The single automatic session with an ERP response followed the same pattern, but the other two automatic sessions did not show any stimulus-dependent changes in coherence.

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**Figure 15. Power in S1HL in response to manual tactile stimulation.** (A) Evoked spectrogram in S1HL averaged over 328 trials in one session. The response may appear to precede the stimulus in part due to smoothing but also due to jitter in the timing of stimulus delivery. (B) Corresponding logarithmic power spectrum in S1HL calculated for the 500-ms windows before (blue) and after (red) stimulus onset.

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**Figure 16. Coherence in response to tactile stimulation.** Evoked cohoregram between randomly selected S1 and M2 electrodes, averaged over 328 trials in one manual stimulation session (WT1). Coherence was calculated in a moving window of 500-ms duration with 50-ms steps, which may be why coherence appears to change before stimulus onset.
V. Gamma rhythms and response amplitude. In tactile sessions with a significant ERP response we examined whether S1 gamma power or S1-M2 relative gamma phase predict the amplitude of responses in M2. M2 response amplitudes did not correlate with S1 gamma power or S1-M2 relative gamma phase (both 500 ms pre-stimulus) in any of these sessions (Fig. 17).

![Figure 17](image)

**Figure 17. Response amplitude in M2 as a function of S1HL-M2 gamma phase and S1HL gamma power.** (A) Example session showing no correlation between M2 response amplitude and pre-stimulus S1 40 Hz power ($R^2 < 0.01$). (B) Similarly, M2 amplitude and pre-stimulus S1-M2 relative gamma phase (40 Hz) show no linear correlation ($R^2 < 0.01$). Data are taken from a manual tactile stimulation session (WT1) with 320 trials.

VI. Optogenetic experiments. Given that low gamma power across trials could obscure a relationship between S1 gamma power and M2 response amplitudes, and that analysis of spontaneous gamma activity is only correlative, we also attempted to causally test whether pre-stimulus gamma synchrony modulates response amplitudes by optogenetically inducing gamma synchrony in S1. 1-s pulse trains of blue light (470 nm) delivered to S1 at 40 Hz (1-ms pulse width) did not produce any discernible elevation in 40 Hz power in S1 (Fig. 18).
Averaging the LFP and power spectrum over every individual 1-ms light pulse revealed a very low-amplitude but distinct 40 Hz oscillation (Fig. 19A, C). However, this oscillation was determined to be an artifact because it was also visible in a control mouse receiving light stimulation through a sham cannula (Fig. 19B, D). The effect was not as apparent in all electrodes or in the power spectrum in the control session; this may have been because the fiber optic cannula was inverted when implanted in the control animal, reducing light intensity at the recording site despite beginning with the same intensity at the fiber tip as the session in VGAT1 (~64 mW mm$^{-2}$).

Figure 18. LFP power in S1HL in response to 40 Hz optogenetic stimulation. (A) Spectrogram of randomly selected channel in S1HL with onset of 40 Hz light stimulation (~64 mW mm$^{-2}$) for 1s at time 0. (B) Corresponding power spectrum of the same channel in the 1-s window before (blue) and after (red) the stimulus.
VII. Technical results. The push solenoid worked relatively well to provide the desired amount of pressure to the tail in response to digital inputs. However, the surrounding setup, which involved batteries set up in series to supply the proper voltage, was unreliable when used in combination with the digital trigger. Furthermore, the solenoid consistently introduced electrical artifacts that could not be completely removed after cleaning. As a result, manual stimulation was favored.

Figure 19. Light stimulation induces 40 Hz artifact in pulse-averaged evoked potential. (A) LFP averaged over each 1-ms light pulse (~64 mW mm$^{-2}$, delivered every 25 ms for each 1-s trial, i.e. at 40 Hz) in a session shows 40 Hz oscillation. Responses in all channels (S1 and M2) were similar. (B) Control recording in WT2 shows similar 40 Hz oscillation in an M2 electrode in response to light stimulation. (C) Power spectrum (100 ms post-stimulus) corresponding to (A). (B) Comparison spectrum in control session (WT2) corresponding to (B) also showing a discernible peak at 40 Hz. Difference in peak size between (C) and (D) may be due to inverted cannula implantation in WT2 causing reduced light intensity at the recording site.
over automatic stimulation, but the lowered accuracy in stimulus timing points to the need to establish a more reliable setup for automatic stimulation.

Discussion

Observations of LFP and unit responses to tail stimulation in primary somatosensory hindlimb region (S1HL) and frontal cortex (M2) in three animals confirm our ability to elicit and record sensory responses in anesthetized mice. These data are a necessary prerequisite for testing the hypothesis that the synchronization state of the brain plays a functional role in inter-area communication. While we have not yet successfully implemented optogenetics to manipulate synchrony independently, we have begun to address this question by testing various aspects of our experimental design and performing a preliminary analysis of how spontaneous fluctuations in pre-stimulus synchronization state correlate with the strength of downstream responses.

I. Sensory responses. Manual tactile stimulation evoked a response in three of five sessions. Of the two remaining sessions, one was conducted 20 minutes after a session showing a robust response, at which point the animal may have become habituated. Although we made an effort to keep anesthesia just deep enough to prevent animals from waking up, it may have been too deep in some cases to allow a robust tactile response. Furthermore, jitter in the time of stimulus delivery could potentially have caused a response to become washed out after averaging over trials.

Our finding that response latencies varied with electrode position during one session evidences the propagation of stimulus information from S1 to M2. This is consistent with the
existence of projections from somatosensory hindlimb region to frontal cortex in rats (Golmayo et al., 2003). In the two tactile stimulation sessions (of nine total) exhibiting a latency effect, response latencies between S1 and M2 were 175 and 105 ms, respectively. We have not found comparable measurements of frontal-parietal latencies in mice in the literature, but these latencies contrast with the ~35-ms delay observed in rats (Golmayo et al., 2003). It is possible that latencies based on earlier peaks in the ERP (e.g., the first rather than the largest peak) would yield different frontal-parietal delays. If earlier peaks correspond more closely to the time required to relay signals between these areas, it could explain why defining response time based on later peaks yielded unexpectedly large delays. Alternatively, if these large delays are accurate, it could reflect the routing of sensory signals to other areas before they reach frontal cortex.

However, we need to collect more data and try different analyses to determine whether this large latency is typical and whether alternative interpretations of response time yield different latency results. In addition, we could use a histological tracing technique to directly measure the extent of S1-M2 connectivity.

Previous findings in mice (Nase et al., 2003) support the stimulus-induced increase in spectral power and S1-M2 coherence we observed in all four tactile stimulation sessions reaching criterion for a significant ERP response. Our finding that pre-stimulus synchrony in S1 did not correlate with M2 response amplitudes could be attributable to several factors. First of all, because the majority of our tactile data are responses to manual stimulation, there was some inherent trial-to-trial variability in the pressure and timing of the stimulus that we were not able to control for in our analysis. It could be that the main predictor of response amplitude on a given trial was the pressure of stimulation or even simply whether it fell within the predefined ‘post-stimulus’ time window at all. If this is the case, synchrony-amplitude correlations that might be
apparent under more precise stimulus conditions could have been obscured. In addition, the anesthesia may have been too deep for the LFP to reflect nuances in neural activity that are visible in awake animals. Isoflurane anesthesia has been found to differentially suppress feedback connections (Imas et al., 2005; Tononi and Koch, 2008); if these connections promote the inter-area coordination that would be required for a relationship between synchrony response amplitude, anesthesia may have prevented our observation of it. It is also possible that these correlations simply occur at another frequency band; while a cursory analysis of neighboring frequency bands in the gamma range yielded similar results, in the future when other potential confounds have been addressed it will be necessary to continue looking for these patterns at different frequencies. Finally, it is possible there is simply no relationship to be observed; however, to draw a conclusion one way or the other we must rule out alternative explanations, which we can begin to do by improving our automatic stimulation setup and working toward conducting experiments in awake animals (see below).

The lack of consistent responses in S1HL to visual or auditory stimuli is expected given its specialized function. While M2 is involved in processing inputs from other sensory modalities and may have been expected to show a response, Golmayo et al. (2003) report distinct regions of frontal cortex responsible for tactile versus visual processing in rats. Thus, it is possible we were in a region of M2 more exclusively devoted to processing tactile information. It is also possible the visual and auditory stimuli were generally less salient than in the case of tail stimulation; for example, if the mouse’s eyes were shadowed by the stereotax, the light may have been too weak to observe an effect. Thus, we may have seen a more significant response in M2 to louder tones or brighter lights.
II. Optogenetics. The lack of effect of photostimulation most likely indicates that the light intensity at the recording site was insufficient to evoke a response in the LFP. One possible reason for this is that the beam may have been occluded at the brain surface by blood, cerebrospinal fluid, or dental acrylic. This has been known to lead to excessive loss of light intensity (Cardin et al., 2010). To address this, we will implant another VGAT-mChR2-YFP transgenic mouse with a cannula that penetrates the brain surface to a depth of ~500 µm. In addition to avoiding beam obstruction by fluids at the brain surface, this will allow for greater light intensity at the recording site. While this raises the possibility of introducing a stronger light artifact, we will counteract this by placing our electrodes at such an angle to minimize this effect (see below).

Another possible reason we did not observe a response to light stimulation is that the mouse strain we are using may not have sufficient expression of ChR2 in cortex to allow for a significant effect at the light intensity we used (~64 mW mm\(^2\)). While this was well within the effective intensity range for comparable experiments in a different strain (Cardin et al., 2010), lower cortical ChR2 expression in VGAT-mChR2-EYFP mice might require compensation with higher light intensity or a light source closer to the recording site. We were operating our LED at maximum intensity, but if insufficient intensity proves to be the source of the problem we may replace our LED with a laser, which would allow for a much wider range of light intensities.

The 40 Hz artifact we observed is well-documented in the literature as the result of light coming into contact with metal electrodes (Ayling et al., 2009; Cardin et al., 2010; Han et al., 2009). Known as the photovoltaic effect, this occurs because light excites the electrons of the metal, which induces an electrical current (Schwoerer and Wolf, 2007). The effect can be
minimized by placing electrodes at such an angle that the insulated shaft blocks the exposed tip from direct contact with the light beam (Fig. 20) (Cardin et al., 2010). Alternatively, the persistence of the artifact we observed during the inter-trial interval suggests it could be the result of the external trigger to the LED, which checks for inputs at 40 Hz and could somehow affect the LFP on this timescale. To test this we will conduct recordings in which we pulse the LED for only 1 ms every 3-6 s (but the external trigger still checks for events every 25 ms). In addition to comparing such a session to our current data we can compare to a session in which we pulse the LED using its intrinsic trigger rather than a digital input from an external device.

The VGAT-mhChR2-YFP mouse strain used in this study has shown moderate cortical expression of ChR2 (Zhao et al., 2011). To confirm this is the case specifically for the mice used in this study we can use confocal microscopy to visualize yellow fluorescent protein (YFP), which has been fused to ChR2. Furthermore, to ensure that ChR2 expression was cell-type specific we can costain with and antibody for GAD67, a protein specific to GABAergic interneurons.
The ability to drive gamma activity in S1HL will allow us to extend our analysis of M2 response amplitudes as a function of gamma power and S1HL-M2 gamma phase coherence. If gamma phase gates responses we would expect M2 response amplitudes to be higher on trials with a particular S1-M2 relative phase. If gamma power amplifies responses we would expect M2 responses to be larger during optogenetic stimulation than at baseline. Other analyses of M2 response such as the timing and variability of peak latency, which have been found to vary with

Figure 20. Photostimulation induces electrical artifacts in metal electrodes. (A) Light delivered at 40 Hz to a cortical site expressing ChR-2 (red dots) generates an artifact in tungsten electrodes (top trace) that obscure endogenous gamma oscillations visible in a simultaneous recording via a glass electrode unaffected by the beam (middle trace; filtered in bottom trace). (B) Light artifacts can be avoided by placing metal electrodes at an angle that minimizes interaction between the beam and the electrode shaft. In this case the underlying gamma oscillations are comparable in metal (top traces) and glass (bottom traces) electrodes. Reprinted from Cardin et al. (2010).
the extent of local gamma synchrony (Cardin et al., 2009), will provide further insight into the potential functional consequences of gamma activity in sensory routing.

**III. Other future directions.** While manual stimulation was largely successful in eliciting a tactile response, to get more consistent data we need to improve the design of the automatic stimulation system. To address whether the solenoid or the circuit providing its digital inputs contribute artifacts to the evoked potential, we will run a control session in which the solenoid is not in direct contact with the mouse. If this still causes artifacts we can invest in a voltage converter to replace the circuit and/or wrap the solenoid with insulation. If the problem persists we can also look into alternative forms of tactile stimulation such as a clamp to provide pressure to the tail or hindlimb from both sides, or an air puff to stimulate the whiskers.

While we have not yet analyzed unit responses in detail, in the future we can use spiking data to address our main hypothesis by analyzing spike-field coherence, which has been found to predict the speed of change detection in monkeys (Womelsdorf et al., 2006). With our current experimental design we could test whether spike-field coherence predicts attributes of sensory responses on the neural level, such as ERP amplitude and response latency.

Once we have established a reliable setup for anesthetized recordings, we can also look to add a behavioral component to our experimental design. The optogenetics equipment used in this study can be adapted to work in freely behaving animals by adding a rotary joint between the fiber optic cable and the LED. This would not only avoid the problem of anesthesia potentially suppressing neural responses, but would allow us to directly test the effects of induced synchrony on behavior as measured by an attention or detection task.
IV. Conclusions. In this study we have demonstrated the feasibility of recording sensory-evoked responses in mice. We have identified the obstacles in supplying tactile stimuli and implementing optogenetics, and are currently taking steps to address them. The components of our experimental design and data analysis carried out successfully in control recordings can be applied to subsequent experiments and will inform our interpretation of their results. Thus, we have made critical steps toward addressing whether synchrony plays a functional role in inter-area communication in the brain.

This question is fundamental to our understanding of conscious processes including sensory integration and attention. The junction between one level of the processing hierarchy and the next, where often only a subset of information can be carried on, is arguably the most critical area of focus in the study of these processes. While our experiments did not address them directly because we conducted anesthetized recordings, the features of the neural responses we observed – specifically, ERP amplitudes and synchronization state – are known to vary with the behavioral significance of a stimulus (Broussard and Givens, 2010), which in turn involves feature integration and some kind of attentional mechanism (Engel and Singer, 2001). Thus, studying neurophysiological correlates of sensory awareness allows for a rigorous experimental design that is feasible with the currently available techniques, but still ultimately provides an avenue for addressing more abstract questions about conscious processes (Engel and Singer, 2001). By opening the door to causal manipulations of neural synchrony, our study has taken steps to further our understanding of one of the primary mechanisms hypothesized to underlie many executive processes and, ultimately, how these processes give rise to integrated conscious experience.
Literature Cited


