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Infrared neural stimulation of primary visual cortex in non-human primates

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Abstract

Infrared neural stimulation (INS) is an alternative neurostimulation modality that uses pulsed infrared light to evoke spatially precise neural activity that does not require direct contact with neural tissue. With these advantages INS has the potential to increase our understanding of specific neural pathways and impact current diagnostic and therapeutic clinical applications. In order to develop this technique, we investigate the feasibility of INS (λ = 1.875 μm, fiber diameter = 100–400 μm) to activate and modulate neural activity in primary visual cortex (V1) of Macaque monkeys. Infrared neural stimulation was found to evoke localized neural responses as evidenced by both electrophysiology and intrinsic signal optical imaging (OIS). Single unit recordings acquired during INS indicated statistically significant increases in neuron firing rates that demonstrate INS evoked excitatory neural activity. Consistent with this, INS stimulation led to focal intensity-dependent reflectance changes recorded with OIS. We also asked whether INS is capable of stimulating functionally specific domains in visual cortex and of modulating visually evoked activity in visual cortex. We found that application of INS via 100 μm or 200 μm fiber optics produced enhancement of visually evoked OIS response confined to the eye column where INS was applied and relative suppression of the other eye column. Stimulating the cortex with a 400 μm fiber, exceeding the ocular dominance width, led to relative suppression, consistent with involvement of inhibitory surrounds. This study is the first to demonstrate that INS can be used to either enhance or diminish visual cortical response and that this can be done in a functional domain specific manner. INS thus holds great potential for use as a safe, non-contact, focally specific brain stimulation technology in primate brains.

Introduction

In order to increase our understanding of neural circuitry of the brain, scientists continue to improve methods for assessing neural function through the development of new

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neurostimulation and detection techniques (Han et al., 2009; Heider et al., 2010; Jackson and Fetz, 2011). These new techniques with clinical potential are often first vetted in non-human primates before their clinical implementation in humans (Velliste et al., 2008). The non-human primate model permits a study of neural circuitry in ways that is not possible to do in humans. Such studies have led to increased understanding of neuronal basis underlying sensation, cognition, and behavior and have led to the development of treatments for neurological disorders (Capitanio and Emborg, 2008). In this study, we use the non-human primate model to evaluate a novel brain stimulation method, termed infrared neural stimulation (INS).

Infrared neural stimulation (INS) is a neurostimulation methodology that uses pulsed infrared light to excite neural tissue in a non-contact, artifact-free, spatially selective manner and without the need to genetically modify neural tissue (Wells et al., 2005, 2007b). The biophysical mechanism by which INS evokes neural activity is through absorption of infrared light by water that induces a spatially localized thermal gradient (Wells et al., 2007a). Several groups have reported findings that indicate multiple cellular mechanisms by which the thermal gradient is transduced into neural activity. Shapiro and colleagues recently have reported on a mechanism where the thermal gradient evokes a reversible change in the electrical capacitance of the cell membrane that leads to depolarization of the cell (Shapiro et al., 2012). A separate study implicates the heat sensitive TRPV4 calcium channel for generation of INS evoked neural activity in retinal ganglion cells (Albert et al., 2012); however, the distribution of TRPV4 channels is not uniform throughout the nervous system and is likely not the primary mechanism for INS evoked responses.

INS is a stimulation methodology that is broadly applicable for activating excitable tissue and has been shown to effectively evoke neural responses in peripheral nerves (Teudt et al., 2007; Tozburun et al., 2012; Wells et al., 2007b), in auditory and vestibular systems (Izzo et al., 2006; Rajguru et al., 2011), in dorsal ganglion cells (Katz et al., 2010), and in cardiac muscle (Dittami et al., 2011; Jenkins et al., 2010). While INS is clearly applicable for the stimulation of peripheral nerves, its use in the central nervous system has not been extensively explored. Initial feasibility and parametric studies have been conducted in rat thalamocortical brain slices where INS was found to evoke compound action potentials and initial laser stimulation parameters were characterized (Cayce et al., 2010). In a previous study, we demonstrated that INS can modulate cortical neural activity in rodent somatosensory cortex, as confirmed with optical intrinsic signal imaging and single unit electrophysiology, and characterized basic stimulation intensity dependence of this method (Cayce et al., 2011). However, a primary and important difference between rodent and nonhuman primates (NHPs) is the presence of functionally specific domains (e.g. ocular dominance columns, color blobs, orientation domains) in the NHP.

In this study, we investigate whether it is feasible to use INS to stimulate functionally specific cortical domains. As these domains are 200–300 μm in size INS stimulation must be restricted in size. We have thus examined the application of INS in nonhuman primate primary visual cortex (V1), an area that is characterized by well-defined functional modules (e.g. ocular dominance columns). We find that focal stimulation of ocular dominance columns with INS leads to eye-specific functional effects. This demonstrates that INS can be

used in a spatially selective and therefore functionally specific manner in primate cortex. This study represents the first application of INS to the cerebral cortex of non-human primates and provides the necessary feasibility data to move forward with studies in awake behaving animals and potential clinical application.

Methods

Experiments were conducted in two Macaque monkeys implanted with chronic imaging chambers (Chen et al., 2002; Roe, 2007; Tanigawa et al., 2010). All procedures were performed in accordance with protocols approved by the Vanderbilt University Institute for Animal Care and Use Committee and conformed to the guidelines of the US National Institutes of Health.

Surgical procedures

All surgeries were performed under aseptic conditions. Monkeys were initially anesthetized with ketamine HCL (10 mg/kg, I.M.) and maintained under isoflurane (1.5–2% in O_2) anesthesia throughout the duration of the craniotomy, duratomy, and implantation of chronic imaging chamber with artificial dura (Tecoflex or Silicone) (Chen et al., 2002). In all anesthetized procedures, vital signs (end-tidal CO_2, oximetry, body temperature, and heart rate) and anesthetic depth (via implanted wire electroencephalographic electrodes)were continuously monitored. Adequate depth was determined to be level 2, which is characterized by large-amplitude (up to 150 mV), low-frequency (one every 1–3 s) bursts. The implanted chamber was sealed with a nylon cap and opened under sterile conditions for intrinsic signal optical imaging and electrophysiology.

Animal preparation

For each optical imaging or electrophysiology session, animals were anesthetized and maintained with Brevital (1–2 mg/kg/h) and paralyzed with Norcuron (100 μg/kg/h). The animals' eyes were dilated with 1% atropine sulfate eye drops and fitted with contact lenses of appropriate curvature (Danker Laboratories Inc., Sarasota, FL) to focus on a visual stimulus computer monitor placed 57 cm in front of the animal. Foveal positions on the monitor were determined by plotting the optic disks using a reversible ophthalmoscope (Smith et al., 1990). The cortex was stabilized with warm 3% agarose (Type I, Sigma-Aldrich) and a glass coverslip for optical imaging. The stimulating fiber optic used to perform INS was placed in direct contact with artificial dura over targeted ocular dominance (OD) columns. Agar and a glass coverslip were carefully applied to stabilize the cortex for optical imaging and remained in place for the duration of imaging. To permit placement of electrodes and changing of optic fibers during optical imaging, a plastic brain stabilizer with a 5.5 mm diameter hole was used to stabilize the cortex in lieu of agar and the glass coverslip.

Optical imaging

Optical imaging of intrinsic signals was collected by an Imager 3001 with VDAQ/NT data acquisition software (Optical Imaging, Germantown, NY). The cortex was illuminated by 632 nm light band passed filtered from a halogen light source and focused onto the brain

using fiber optic light guides (Edmund Optics, Barrington, NJ). Light reflected from cortex was collected with a CCD camera (DALSA DS-21-01M60 Teledyne DALSA, Waterloo, ON, Canada) using a 85/50 mm lens in combination to yield an 8 × 8 mm field of view. Images were acquired at 5 Hz for 6–9 s (yielding 30–45 frames per condition) with an interstimulus interval of 5 s. This total period covers the 2–3 second time to peak of the intrinsic signal plus the ~8 second recovery to baseline. Signal to noise ratios were enhanced by syncing image acquisition to respiration and heart rate to reduce physiological noise and by trial averaging (25–40 per stimulus condition). Fig. 1A displays the experimental setup diagram and Fig. 1B outlines the imaging protocol used to collect images.

In experiments in which optical imaging was conducted in response to INS stimulation, images were acquired with agar and a half glass coverslip carefully placed over the agar next to the stimulating optical fiber. In animals that had an implanted optical chamber and clear artificial dura (Chen et al., 2002), we could not use this standard method. Instead, we developed a plastic brain stabilizer made of an optically clear stiff plastic material that, when fitted appropriately to the chamber, reduces the amplitude of brain pulsations. A hole in this stabilizer permits introduction of electrodes and placement of the fiber optic for light stimulation. The plastic brain stabilizer was used in electrophysiology experiments (Fig. 3) and experiments investigating the effects of spot/fiber diameter (Fig. 6).

Visual stimulation parameters

Full screen high contrast monochromatic drifting square-wave gratings (spatial frequency = 1 cycle/°, temporal frequency = 3 Hz), were created using custom software writing in Matlab (MathWorks, Natick, MA) controlling a ViSaGe Stimulus Generator (Cambridge Research Systems, Rochester, UK). Gratings were oriented at 0° or 90° on a 40 × 30 cm computer monitor and presented to each eye separately using electromechanical shutters in front of each eye. The duration of all visual stimulation was 3.5 s. Each block consisted of 6 conditions, presented in a randomly interleaved fashion. The visual stimulus was presented to each eye twice, once for visual stimulation and once for combined stimulation (simultaneous INS and visual stimulation) for a total of 4 visual stimulation conditions. Two additional conditions were an INS alone condition and a blank condition. During interstimulus intervals, blank condition, and INS condition, the shutters remained closed for both eyes (Fig. 1B).

Infrared neural stimulation parameters

Wavelength selection for infrared neural stimulation is based on the optical penetration depth of light in tissue; previous studies have identified an optimal penetration depth of 300–600 μm for evoking neural responses (Cayce et al., 2010, 2011; Wells et al., 2005). Anatomically, this penetration depth corresponds to the superficial layers of cortex (I–III). These are also the layers from which intrinsic optical signals are derived. Infrared neural stimulation was performed using a wavelength of 1.875 μm ± 0.02 μm (Capella Infrared Neuro-Stimulator, Lockheed Martin Aculight, Bothell WA) giving a penetration depth into tissue of approximately 465 μm. Light was delivered to the cortex through a 100, 200, or 400 μm diameter optical fiber (Ocean Optics, St. Petersburg, FL) with a numerical aperture (NA) of 0.22. The fiber was placed onto artificial dura using a hydraulic micromanipulator

(Narishige, Tokyo, Japan). A plastic brain stabilizer with a 5 mm diameter hole was used to stabilize the brain and allow for easy transition for each stimulating fiber during experiments where fiber size diameter was tested. Laser repetition rate ranged between 100 and 200 Hz, and pulse width ranged between 75 and 300 μs. The average power from the laser was measured at the fiber tip using a Power Max 500D laser power meter with a PM3detector head (Coherent, Santa Clara, CA). Radiant exposure was calculated assuming that the diameter of the fiber optic was equal to the spot size of the incident light onto the cortex. The radiant exposures were set between 0.5 and 1.3 J/cm^2 and were dependent on the stimulation parameters used for a given experiment. Pulse train duration for all INS experiments was 1000 or 500 ms. Laser triggering was controlled via LabVIEW software and National Instruments hardware interface that was synced with presentation of visual stimulation and/or optical imaging via digital inputs (Figs. 1A and B).

Optical imaging data analysis

Activation maps—Analysis of optical imaging data was performed with software written in Matlab (MathWorks, Natick, MA). For each condition, to examine change from baseline, the first baseline frame (taken prior to stimulation onset)was subtracted from each subsequent frame. For each condition, the first-frame subtracted values were then summed across trials to maximize signal to noise ratio. Trial by trial assessment of image quality was conducted to remove any bad trials due to lighting abnormalities, large physiological movement, or camera acquisition errors.

Both single-condition maps and difference maps were calculated (Lu and Roe, 2008). Single condition maps were generated by subtracting the blank condition from each experimental condition. Ocular dominance maps were generated by subtracting right eye stimulation conditions from left eye stimulation conditions, where darker pixels represent preference for left eye stimulation and lighter pixels represent preference for right eye stimulation. In this study, OD maps generated by visual stimulation alone are labeled as *vision* and OD maps generated by simultaneous visual and INS stimulation are labeled as *combined*. Display activation maps were clipped at a threshold to maximize contrast within image. In cases where multiple activation maps from different imaging runs are compared, the clipping threshold was determined by the activation map with strongest intrinsic signal. Large blood vessels were masked to remove from consideration pixels with signals related to surface vasculature (not to neural response). Standard Gaussian low-pass (3–8 pixels squared in size) and median high-pass filtering (100–200 pixels squared in size)were used to remove contamination from un-even illumination and from other physiological noise sources.

T-maps—A pixel by pixel Student's *t*-test was used to identify significant pixels activated for INS alone, visual stimulation, and combined stimulation (Wang et al., 1996). This analysis was used to identify locations of stimulus-related response, to identify regions of interest for time course analysis, and was useful for identifying the borders of OD columns. T-maps were generated in Matlab and were visualized by creating binary maps thresholded for a specific p-value (i.e. $p < 0.01$ or $p < 0.05$)where p-values falling below the set threshold were assigned a value of 1 and those above threshold assigned a value of zero.

Areas containing only a few significant pixels, along blood vessels, or along the edge of the imaging chamber were not included as the significance of these signals was ambiguous.

Time course analysis—The time course of the intrinsic signal was examined at selected sites by averaging the values of pixels within identified regions of interest (ROIs). For each condition, the summed pixel value from the first image frame was subtracted from each subsequent frame and then used as a divisor to measure the change in reflectance over background reflectance (dR/R). Imaging conditions were then subtracted from each other to determine the time course of the difference signal within the ROI. Laser stimulation alone was blank subtracted to view the direct intrinsic signal evoked by INS. To obtain eye preference within a specified ROI, right eye stimulation was subtracted from left eye stimulation time course; such subtractions were conducted for both visual alone and combined visual + laser stimulation conditions. A Welch's *t*-test, that assumes possible unequal variances, was performed on averaged data (typically frames 20–30 or 15–25) to determine statistical significance between INS alone, visual stimulation alone, and combined stimulation conditions.

Electrophysiology recordings

Single unit electrophysiology was used to assess the cortical neuronal responsiveness before, during, and after INS. Glass coated tungsten microelectrodes (1–3 MΩ, World Precision Instruments, Sarasota, FL) were inserted into cortex at depths of 50–500 μm in targeted OD columns identified with optical imaging. Single units were isolated that responded to brief INS (500 ms in duration). Signals were filtered and digitized using a 16 channel AM-Systems (Sequim, WA) differential amplifier using a 300–5 K bandpass filter. A LabVIEW interface was used to control presentation of laser stimulation, and Data Wave software (Loveland, CO) was used to collect single unit data. Collected single units were spike sorted and peristimulus time histograms (PSTH) were generated using DataView software. A paired Student's *t*-test analysis was used to determine the significance of changes observed in the PSTH related to laser stimulation.

Results

Efficacy of INS as a stimulation modality for primary visual cortex (V1) was assessed with optical imaging and electrophysiology techniques in two anesthetized Macaque monkeys. We examined the intensity dependency of the INS-induced optical signal, how spatially focal the effect would be, and whether it would produce functionally specific effects (i.e. confined to single functional compartments such as ocular dominance columns).

Radiant exposure of INS increases magnitude of OIS

Previous studies characterizing INS have indicated that radiant exposure is a primary parameter for determining strength of activation (Cayce et al., 2010, 2011; Feng et al., 2010; Richter et al., 2010; Tozburun et al., 2012;Wells et al., 2005). We first established the direct effects of radiant exposure on the intrinsic signal in V1 as assessed using optical imaging. We placed a 100 μm fiber over a location in V1 (Fig. 2A) and delivered different levels of INS stimulation; these values were guided by irradiance values determined to be safe and

effective in our previous study. The single condition maps in Figs. 2B–D represent the activation levels induced by increasing exposures of INS. The image maps show that activation was weakest for 0.5 J/cm^2 (Fig. 2B) and activation increased for 0.78 J/cm^2 (Fig. 2C) and 1.3 J/cm^2 radiant exposures (Fig. 2D). Significant pixels for the 1.3 J/cm^2 condition were identified with a pixel-by-pixel paired *t*-test (t-map, $p < 0.001$). The time course of response of these pixels to each of the INS stimulation levels (compared to a no stimulation control condition) is shown in Fig. 2E. This demonstrates that the peak intrinsic magnitude increases with radiant exposure, but a similar intrinsic signal time course is maintained (Fig. 2E). Statistical analysis (paired *t*-test) of the peak signal magnitude indicates a statistically significant increase in OIS as radiant exposure increases (Figs. 2G–F). These results demonstrate an intensity-dependent effect of INS on the imaged intrinsic signal, consistent with previous results obtained from rat cerebral cortex (Cayce et al., 2011).

Single unit recordings demonstrate neural excitability to INS

To examine whether the reflectance changes due to INS are based on underlying neural activity, we made a few sample recordings during the imaging sessions. Fig. 3 displays two post stimulus time histograms (PSTH) collected from single units recorded in V1. After determining locations of right and left eye columns with intrinsic signaling imaging, INS was delivered through a 200 μm fiber either to the same left eye column (PSTH in Fig. 3A) or a neighboring right eye column (PSTH in Fig. 3B) less than 1 mm away.

The PSTH shown in Fig. 3A illustrates neuronal response recorded from superficial layers (depth of 340 μm) and represents excitatory activity evoked by INS when the same (left) eye column was stimulated (fiber tip placed 500 μm away from recording electrode within the same eye column). The increased spike rate during INS was determined to be statistically significant when compared to spike rates 500 ms prior to stimulation ($p < 3.15E–08$, paired two-tailed *t* test) and 500 ms post stimulation ($p < 8.83E–09$, paired two-tailed *t* test). A second single unit, isolated at a depth of 650 μm, responded to infrared stimulation in the adjacent right eye column (500 μm away from recording electrode) (Fig. 2B). Paired *t*-tests indicated statistical significance when the spike rates acquired during stimulation were compared with spike rates 500 ms before stimulation ($p < 6.01E–6$) and spike rates 500 ms after stimulation ($p < 7.35E–6$). These single unit recordings demonstrate the capability of INS to evoke excitatory activity in superficial cortical neurons in V1 and support our optical imaging results that indicate that INS evokes optical signals driven by neural activity.

Infrared neural stimulation activates focal domains in V1

To evaluate whether the effects of INS can be specific to single functional domains, we examined effects of stimulating single ocular dominance columns which are 400 μm in width (Figs. 4A and B). We confined the stimulation to a single ocular dominance column by applying INS stimulation through a 100 μm diameter fiber optic, placed in direct contact with the optically clear artificial dura (Tecoflex or Silicone). In this case, after placement of the fiber, the imaging field of view was stabilized with 3% agarose and a glass coverslip (Fig. 4A). Starting with stimulation parameters we had previously identified as safe and effective in rat barrel cortex (Cayce et al., 2011), we stimulated primary visual cortex by

INS with three sets of laser parameters: 100 Hz for 500 ms (Fig. 4C), 200 Hz for 500 ms (Fig. 4D) and 200 Hz for 1000 ms (Fig. 4E).

Qualitatively, we found, consistent with Fig. 2 that INS produced a focal region of activation with the location of highest response intensity at the laser fiber tip, and that increasing INS intensity produced increased reflectance change (compare Figs. 4C, D, and E). This observation was quantified in Fig. 4F by comparing pixel time courses taken from region of interest 1 (ROI1). The weakest stimulation parameters (100 Hz, 500 ms) produced the smallest reflectance change (blue trace), the intermediate stimulation strength (200 Hz, 500 ms) produced intermediate levels of reflectance change (red trace), and strongest stimulation strength (200 Hz, 1000 ms) produced the largest reflectance change (green trace). Thus, as the stimulation strength increased (F: blue line to red line to green line), the peak signal and time to peak also increased; however the overall shape and duration of the time course remained relatively unchanged.

Interestingly, we observed that stimulation in the right eye column produced an accompanying change of opposite polarity in a neighboring left eye column (Fig. 4E). This effect also varied with INS stimulation intensity (compare C, D, E). As shown in Fig. 4G (ROI2), the effect is largely a positive reflectance change, with the weakest intensity level evoking the smallest reflectance change (blue trace), intermediate intensity producing a larger change (red trace), and the strongest intensity producing an initial brief negative deflection followed by a positive reflectance change. These different reflectance time courses are likely to result from an interplay of excitatory and inhibitory effects between ocular dominance columns (see the Discussion section).

To further examine the specificity of these effects, we surveyed reflectance change at other locations. As shown in Fig. 4H, the reflectance change at a distance of one ocular dominance cycle away, in a nearby right eye column (ROI3), produced a weaker response. These responses, although weaker, varied with intensity (compare blue: weakest, red: intermediate, green: strongest). This suggests that the effect of INS stimulation remains quite focal and is contained within one ocular dominance cycle. At distant locations, there are no significant responses to INS stimulation (Fig. 3I, ROI4), demonstrating that the effect of INS did not spread to distant sites.

Modulation of visually evoked OIS with INS

This study has shown that INS alone can evoke cortical response, as assessed with intrinsic signal optical imaging and with electrophysiological recordings and that INS can have specific and differential effects on ocular dominance columns. We now ask whether INS, coupled with visually evoked activity, can be used as a tool for modulating cortical responses. Such modulation would make this technique an important and useful tool for using INS under conditions of natural sensory and behavioral conditions.

We hypothesized that adding INS stimulation to, for example, a left eye ocular dominance column (Fig. 5A, schematic) would enhance visually evoked response to the left eye column (Fig. 5B) and diminish response to the right eye column (Fig. 5C). Thus, as in Fig. 3, a 100 μm fiber was placed in a single (left) eye column. Laser stimulation (100 Hz, 500 ms, 1.3

J/cm^2) was combined with visual stimulation (full screen containing square wave gratings of spatial frequency 1 c/°, drift velocity 3 Hz, 3.5 s, parameters typical of visual cortical stimulation in optical imaging experiments (Lu and Roe, 2007, 2008)) to test the ability of INS to modulate visually evoked cortical responses. Onset of the 500 ms INS stimulus was synchronized with 3.5 s of visual stimulation. Combined INS + visual stimulation was then compared to vision alone conditions. Cortical response was measured from left eye and right eye pixels within the field of view (roughly 4 mm, containing 3–4 ocular dominance cycles).

Our observations were consistent with our hypotheses. In comparison to ocular dominance maps produced by visual stimulation alone (Fig. 5A, middle panel), adding laser stimulation to a single (left eye) ocular dominance column resulted in an enhanced response. This observation is quantified in Figs. 5D and E. As shown in Fig. 5D, in the left eye columns, combined INS + vision (black trace) enhanced the response to vision alone (green trace). In contrast (Fig. 5E), in the right eye columns, INS + vision (black trace) diminished the effect of vision alone (green trace). This differential effect is quantified in Figs. 5F (left eye columns, $p < 0.03$) and G (right eye columns, $p < 0.26$). For both left and right OD columns, the comparison of combined versus vision alone identified a significant intrinsic component that can be related to neuromodulation evoked by INS. In the columns matching the ocular preference of the column being directly stimulated by INS, this effect is an enhancement; while in the opposing eye columns, it is a relative suppression.

The effect of INS spot size diameter

The organization of the brain into distinct modules suggests that the size of cortical area stimulated by INS will affect the nature of the evoked response. Based on previous electrophysiological studies (for review see Angelucci and Bressloff, 2006), stimulation beyond the extent of the functional domain involves inhibitory surrounds and can lead to relative suppression. In visual neurophysiology, small spot of light leads to neuronal activation; increasing the size of the light spot activates a larger proportion of the receptive field, leading to larger response. However, further increasing the spot size so that it impinges upon or covers the surround leads to relative suppression.

We predicted that the different fiber sizes would have different effects. As the diameter of orientation domains are ~200 μm and the ocular dominance column widths are ~400 μm in V1, we examined intrinsic signal response induced by three fiber optic sizes: 100, 200, and 400 μm fiber sizes (Fig. 6A). We hypothesized that, with INS alone, due to recruitment of greater cortical volume, the larger fiber size would produce the larger response (Fig. 6B). However, in combination with visual activation, we expected a facilitatory effect with smaller (sub-domain size, 100 μm or 200 μm diameter) fibers and a suppressive effect with larger (supra-domain size, 400 μm diameter) fibers (Fig. 6C).

The optical fibers were centered on the same cortical location with in a right eye ocular dominance column (green dot in Figs. 6D–L), and we then examined the effect of INS alone on the right eye column (ROI1) and the left eye column (ROI2) response. As shown in Fig. 6, the small 100 μm fiber evoked a small response (Figs. 6G and M blue), the intermediate 200 μm fiber produced an intermediate response (Figs. 6H and M red), and the largest 400 μm fiber produced the largest response (Figs. 6I and M green) for a radiant exposure of 0.64

J/cm^2. These effects were confined to the right eye column (Fig. 6M), as there was little effect on the left eye column (Fig. 6N).

We then examined how INS stimulation modulated intrinsic signal evoked by visual stimulation with respect to fiber size diameter. Infrared neural stimulation with a 100 μm fiber combined with visual stimulation resulted in an enhanced effect (Figs. 6J and O blue, ~0.03% change) compared to laser stimulation alone (compare with Fig. 6G, blue, ~0.02% change). The effect of a 200 μm fiber with visual stimulation slightly enhanced this effect further (Figs. 6K and O red, ~0.035% change) and resulted in a larger rebound, and the 400 μm fiber led to relatively suppressed response (Figs. 6L and O green, ~0.01% change). These effects were present only for the right eye column (Fig. 6O, ROI1) and not for the left eye column (Fig. 6P, ROI2).

Discussion

Summary

This paper demonstrates, for the first time, that pulsed infrared light can directly evoke and modulate functionally specific responses within the nonhuman primate brain in vivo. We demonstrate that: (1) INS can induce excitatory neural responses in vivo, (2) INS (introduced via a 100 μm optic fiber) can directly activate specific functional domains (ocular dominance columns), (3) INS can modulate effects of natural sensory (visual) stimulation, a finding with important implications for future applications under natural behavioral conditions, (4) the direction of modulation (enhancement vs suppression) depends on which ocular dominance column is stimulated, and (5) eye-specific modulation depends on size of activation area (by comparing 100, 200, and 400 μm fiber size), indicating the importance of choosing appropriate fiber size. Together, these data underscore the importance of precise targeting for selective activation of cortical domains and suggests that INS can be used for selective activation of functional brain circuits. This ability to stimulate brain circuits in a targeted manner will forward our understanding of cortical circuitry, and improve the ability to achieve targeted clinical intervention using light-based interfaces with the brain.

Relation to previous studies

Our group has established the ability to evoke neural activity in rodent models using pulsed infrared light (Cayce et al., 2010, 2011; Feng et al., 2010). In this paper, we have demonstrated the ability to evoke meaningful responses in anesthetized non-human primates. We demonstrated the ability of INS to evoke focal OIS in V1 with similarities in spatial and temporal features to OIS signals evoked by visual stimulation (Grinvald et al., 1994; Lu and Roe, 2007; Roe, 2007; Ts'o et al., 1990). The organization of activated areas and the time courses displayed in Fig. 4 suggest functional activation of underlying neural circuitry associated with ocular dominance, orientation domains, and/or color domains. This is an exciting finding suggesting that INS can be used to selectively activate neuronal circuitry if targeted properly (Lu and Roe, 2008), and this high degree of spatial precision is consistent with other applications of INS that have reported spatially precise evoked neural activity (Cayce et al., 2011; Duke et al., 2012a, 2012b; Feng et al., 2010; Jenkins et al.,

2010; Katz et al., 2010; Richter and Matic, 2012; Wells et al., 2007b). Furthermore, the time course of the INS evoked OIS in Fig. 4 combined with the data characterizing the effects of radiant exposure (Fig. 2) on intrinsic signal strength displays a strength response relationship with stimulation strength (increased repetition rate, increased radiant exposure, and increased stimulation duration) that corresponds to relationships identified in parametric studies of rodent somatosensory cortex characterizing OIS responses to INS (Cayce et al., 2010, 2011). In this study, the underlying neural response was confirmed to be excitatory as indicated by the single unit recordings summarized in Fig. 3. Demonstrating the ability to excite cortical neurons with pulsed infrared light establishes INS as a viable alternative stimulation modality for study of cortical microcircuitry in vivo.

The ability to modulate or perturb underlying cortical microcircuitry is an essential tool in determining the exact function of specific cortical structures (Stepniewska et al., 2011; Toth et al., 1996). Therefore, modulation of functionally relevant responses is an important feature to establish for INS to be accepted as a proven neurostimulation modality for studying cortical microcircuitry, in anesthetized and awake animals. We used brief periods of INS (500 ms) to modulate visually evoked OIS in eye specific OD columns. The results in Fig. 5 show left eye specific enhancement of OIS from combined high intensity (1.3 J/cm^2, fiber size = 100 μm) INS and visual stimulation when compared to the OIS evoked by visual stimulation alone. The slight decrease in right eye OD OIS evoked during combined stimulation suggests inhibition of OIS further supporting the ability of INS to modulate visual responses in an eye specific manner. We also determined how stimulating cortex with pulsed infrared light delivered through a 100, 200 and 400 μm fiber modulated visual evoked intrinsic optical signal. The 100 and 200 μm diameter fibers were hypothesized to be the most effective fiber size for modulation of eye specific OIS because the spot size of the laser light could be optimally targeted towards one OD column as the average diameter is 400 μm (Horton and Hocking, 1996; Lu and Roe, 2008). Combined stimulation with the 100 and 200 μm optical fibers was shown to enhance OIS signals in the targeted right eye column compared to OIS signals evoked by INS alone. Combined stimulation with the 400 μm optical fiber demonstrated relative local suppression of OIS signals (compared to the OIS signals evoked by combined stimulation with the 100 and 200 μm) suggesting that INS with the 400 μm fiber activates inhibitory surrounds and the 400 μm fiber may be too large to target a specific OD column. These findings support the importance of fiber size selection and positioning when performing INS for evoking excitatory neural activity.

The heat gradient responsible for evoking neural activity has consistently been identified as a concern for its potential in causing damage to neural tissue (Cayce et al., 2011; Duke et al., 2012b; Goyal et al., 2012; Teudt et al., 2007; Wells et al., 2007c). While a comprehensive damage study was not possible in these experiments, INS was performed in the same animal over the course of a year with no apparent signs of damage. Visible thermal damage was never observed in cortex following repetitive stimulation in the same location of cortex with these intensities. The animals never demonstrated loss of visual function in the awake state, and OIS and neural activity were consistent throughout the study. These observations lead to the conclusion that the parameters of INS used in this study were safe and did not cause acute or chronic damage to visual cortex.

INS evoked excitation versus inhibition

We demonstrate the ability of pulsed infrared light to evoke excitatory activity through single unit recordings in V1; however, we have previously shown INS to inhibit spontaneous neural activity in rat somatosensory cortex (Cayce et al., 2011). In this study, the use of fiber diameters of 100 and 200 μm used in this study allowed for more precise targeting of cortical modules than the 400 μm in diameter. The smaller stimulating fiber diameter allowed for focused INS of specific functional modules in V1, i.e. 400 μm in diameter is the size of primate OD columns (Horton and Hocking, 1996), that resulted in the observed INS evoked excitatory neural activity and apparent excitatory OIS activity. In the rodent study, where rat barrel columns are on the order of 400 (Derdikman et al., 2003), we exclusively used 400 μm stimulating fibers to activate somatosensory cortex (and a spot size between 400 and 800 μm in diameter (Cayce et al., 2011)). This larger spot size used in the rodent study was thought to be the primary reason why inhibition of neural activity was observed. There are many examples across species where large sized stimulation has frequently led to inhibition (Derdikman et al., 2003; Ghose, 2009; Levitt and Lund, 1997; Simons and Carvell, 1989). The observations made during the rodent study led us to hypothesize that the large spot size diameter used to stimulate rodent cortex led to INS evoking surround inhibition, and that INS with a small spot size diameter would evoke excitatory activity. In support of this hypothesis, we have demonstrated that targeted INS in primates evokes excitatory single unit activity indicating that spot size diameter plays a crucial role in determining INS evoked excitation or inhibition.

While the observed INS evoked inhibition in rodents was most likely due to activation of surround inhibition, the underlying cellular mechanisms for INS may still play a role in determining if INS evokes excitation or inhibition. The TRPV4 mechanisms (Albert et al., 2012) can help explain how INS may excite neural tissue; however, the capacitance mechanism (Shapiro et al., 2012) can explain both excitation and inhibition. The INS evoked change in capacitance is not cell specific meaning any cell membrane that absorbs incident light will be depolarized. This indicates that excitatory and inhibitory neurons and their processes will be depolarized in the presence of a thermal gradient evoked by INS. Activation of a higher number of inhibitory neurons could have led to inhibition of spontaneous neural activity as observed in the rodent study (Cayce et al., 2011). The ability of INS to activate inhibitory neural pathways has already been demonstrated through increasing GABA current in plated cultured neurons (Feng et al., 2010). While significant gains have been made in understanding the cellular mechanisms involved in INS evoked potentials, significant cellular research is needed to fully understand how these mechanisms interact in determining if a neuron will be excited or inhibited.

Conclusions and future directions

This feasibility study in NHPs represents a significant step in the development of INS as a neurostimulation modality for application in the CNS. Pulsed infrared light was shown to evoke and modulate OIS in V1 corresponding to eye specific ocular dominance columns. Additionally, electrophysiological recordings demonstrated that INS evoked excitatory neural responses suggesting that OIS evoked by INS was related to excitatory neural

activity. Together, these results demonstrate that INS can be used to evoke excitatory neural activity in V1 of NHPs providing a new tool for neuroscientist to study NHP neurocircuitry.

This study demonstrates the feasibility of INS as a neurostimulation modality applicable in non-human primates paving the way for advanced studies in primates. The high spatial precision of INS will allow researchers to probe neural circuitry using a different modality that may reveal a new understanding of cortical neural circuitry. Infrared neural stimulation has the advantage of being minimally invasive as pulsed infrared light was delivered to the cortex through artificial dura eliminating the risk of damage associated with direct contact with tissue which makes INS ideal for awake behavioral studies. Additionally, the optical fiber used to deliver infrared light to the brain is MRI compatible as glass is inert in a magnetic field highlighting the potential of INS to be applied in fMRI studies. The minimal invasiveness of INS also promotes the clinical applicability of the technique in humans. Intraoperative high spatial resolution cortical mapping and application towards deep brain stimulation are future possibilities. The work presented here represents the first building block towards more advanced non-human primate and human studies.

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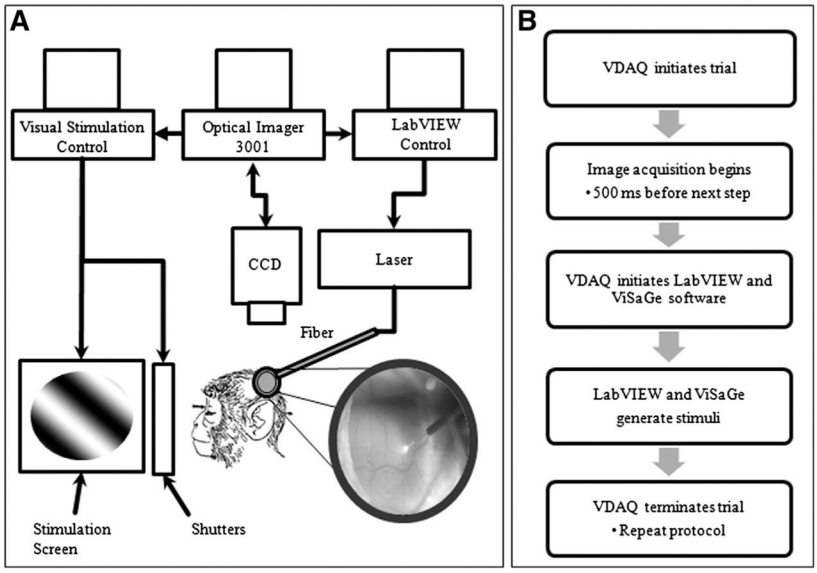

Fig. 1.

Methods for infrared neural stimulation and optical imaging. (A) Schematic diagram of the experimental setup. Experiment is controlled by VDAQ/NT imaging software that acquires images and determines when stimuli are presented through signaling separate computers running ViSaGe software (visual stimulus presentation) and LabVIEW software (INS presentation). ViSaGe software generates the monochromatic drifting square-wave gratings that are presented to the animal through electromagnetic shutters placed over the eyes. The LabVIEW control computer triggers laser stimuli by sending TTL pulses to the laser at the desired repetition rate. Intensity of INS was set manually by adjusting the diode current and pulse width of the laser. The optical fiber used to deliver infrared light is positioned on the artificial dura covering the cortex in the desired stimulation location. Image shows an example of a fiber positioned on the cortex through the imaging system. (B) Imaging protocol flow chart of one trial for a given condition.

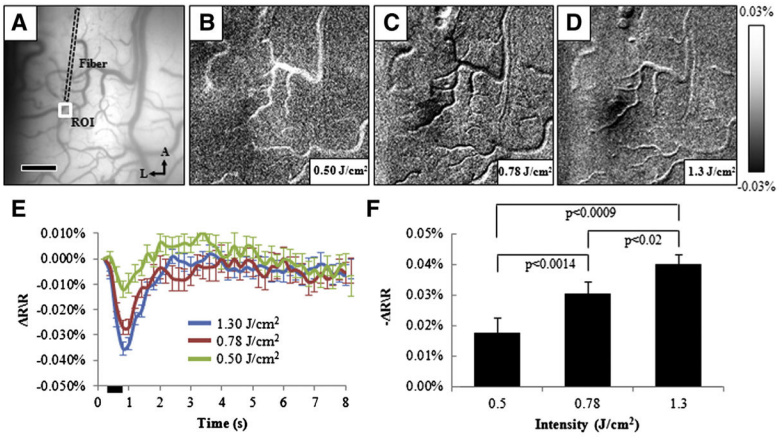

Fig. 2.

Increasing radiant exposure of INS increases intrinsic reflectance signal magnitude. (A.) Blood vessel map. Region of interest demarcated by white box. (B–D) Activation maps in response to INS demonstrate increases in increased intrinsic optical signal for (B) 0.5 J/cm^2, (C) 0.78 J/cm^2, and (D) 1.3 J/cm^2. (E) Time course of intrinsic signal in response to different radiant exposures. Black bar: INS stimulus on. Error bars: standard error of the mean. (F) Peak magnitude of intrinsic reflectance signal change. Statistical significance: p-Values obtained from paired two-tailed *t*-test. INS stimulation parameters: λ = 1.875 μm, pulse width = 250 μs, pulse train length = 500 ms, fiber size = 100 μm. Imaging parameters: 5 fps, ITI = 8 s, number of trials = 40 (1.3 J/cm^2), 22 (0.78 J/cm^2), and 20 (0.50 J/cm^2). Scale bar in A: 1 mm. A = anterior, L = lateral. Data from Monkey 1 left hemisphere.

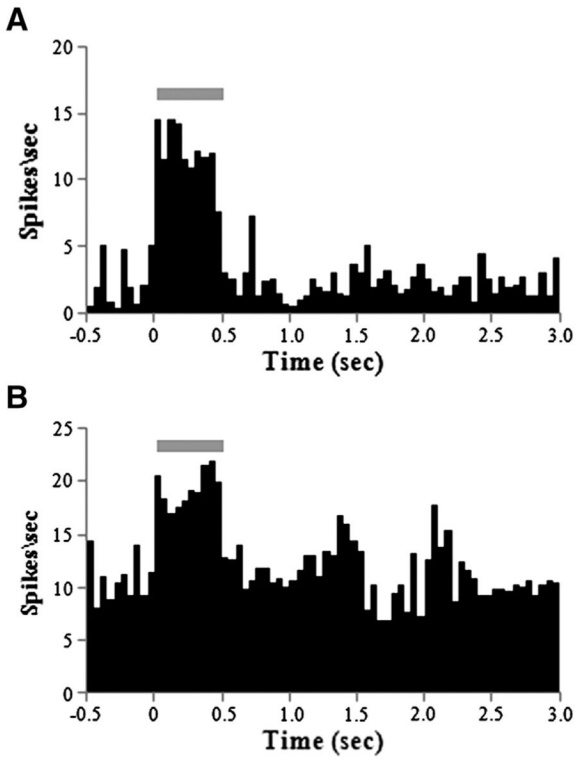

Fig. 3.

Excitatory single unit response evoked by INS of the primary visual cortex. (A) Single unit response near INS site located in left eye ocular dominance column. Electrode depth at 340 μm. (B) Single unit response in right eye ocular dominance column to INS located in left eye ocular dominance column near INS site. Electrode depth at 650 μm. Gray bars: INS stimulation on. INS stimulation parameters: λ = 1.875 μm, pulse width = 250 μs, radiant exposure = 0.57 J/cm^2, fiber size = 200 μm, repetition rate = 200 Hz, stimulation duration = 500 ms. Data from Monkey 2.

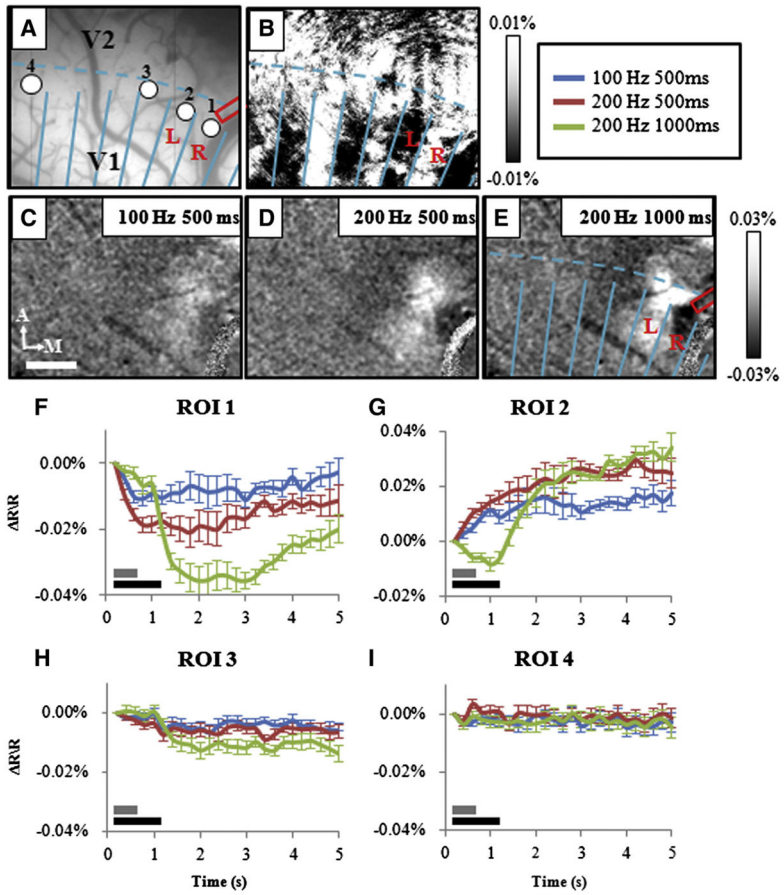

Fig. 4.

Infrared neural stimulation generates focal responses in primary visual cortex. (A.) Blood vessel map. Dashed blue line: V1/V2 border. Solid blue lines: Ocular dominance borders based on B. White dots: Regions of interest from which time courses in F–I were taken. Red outline: fiber optic tip targeted in the right eye column. (B) Ocular dominance map (dark pixels: left eye, light pixels: right eye). (C–E) Activation maps in response to (C) 100 Hz pulse train, 500 ms, (D) 200 Hz pulse train, 500 ms, and (E) 200 Hz pulse train, 1000 ms. Overlay of ocular dominance borders in E shows specificity of activation of the right eye column (dark pixels) and adjacent left eye column (light pixels). (F–I) Time course of intrinsic signals taken from regions of interest 1–4 in A. Traces in blue, red, and green: time course of response to INS in D, E, and F, respectively. (F) ROI1: response at fiber tip location in the right eye column reveals a strong intensity dependent negative reflectance change. (G) ROI2: response to location in adjacent left eye column reveals an opposite (positive) reflectance change. (H) ROI3: smaller response at location further away from fiber tip. (I) ROI4: null response at control location distant from fiber tip. INS parameters: λ = 1.875 μm, pulse width = 250 μs, radiant exposure = 0.6 J/cm^2, fiber size = 100 μm. Imaging parameters: 5 fps, ITI = 8 s, trials = 40. A = anterior, L = lateral. Stimulation begins at 0.4 s following imaging onset and ends at 0.9 s (500 ms train) or 1.4 s (1 s train). Scale bar in B: 500 μm, applies to A–E. Data from Monkey 1 left hemisphere.

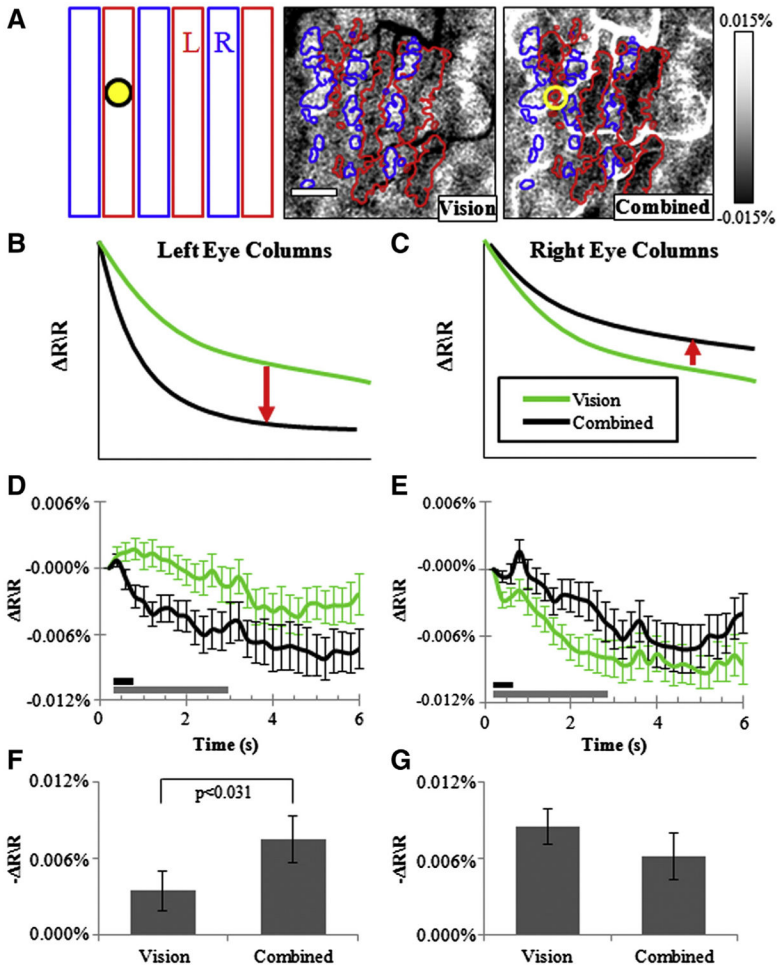

Fig. 5.

Intrinsic optical response to visual stimulation potentiated by INS. (A) Blood vessel map. Left eye OD columns demarcated by red outline and right eye OD columns demarcated by blue outline. Location of INS demarcated by yellow circle. Scale bar = 1 mm. Middle panel: ocular dominance column activation maps generated by visual stimulation of both eyes (vision, Le–Re). Right panel: ocular dominance columns generated by INS during visual stimulation of both eyes (Combined, LeC–ReC). (B & C) Hypothesized potentiation (B) and attenuation (C) of intrinsic signal from INS targeted in the left eye column. (D & E) Time course of intrinsic signal for visual and combined stimulation for (D) left and (E) right eye OD columns demarcated in activation maps in A. (F & G) Averaged reflectance change corresponding to 4–6 s of time courses in D & E indicates that INS potentiates visually evoked intrinsic signals for left eye OD column but attenuates signal in right eye OD columns. p value comparing visual to combined stimulation obtained from paired two-tailed *t*-test. Error bars represent standard error of the mean. INS parameters: λ = 1.875 μm, radiant exposure: 1.3 J/cm^2, repetition rate = 200 Hz, pulse width = 250 μs, pulse train length = 500 ms, fiber size = 100 μm. Visual stimulation parameters: Prestim gray = 1 s, stimulation duration = 3.5 s, spatial frequency = 1 Hz, temporal frequency 3 Hz, square wave grating. Imaging parameters: 5 fps, ITI = 8 s, trials = 40. Subtraction maps in B and C correspond to

frames 20 to 30 (4 to 6 s). Scale bar in A: 1 mm. (A = anterior, L = lateral). Data from Monkey 1 left hemisphere.

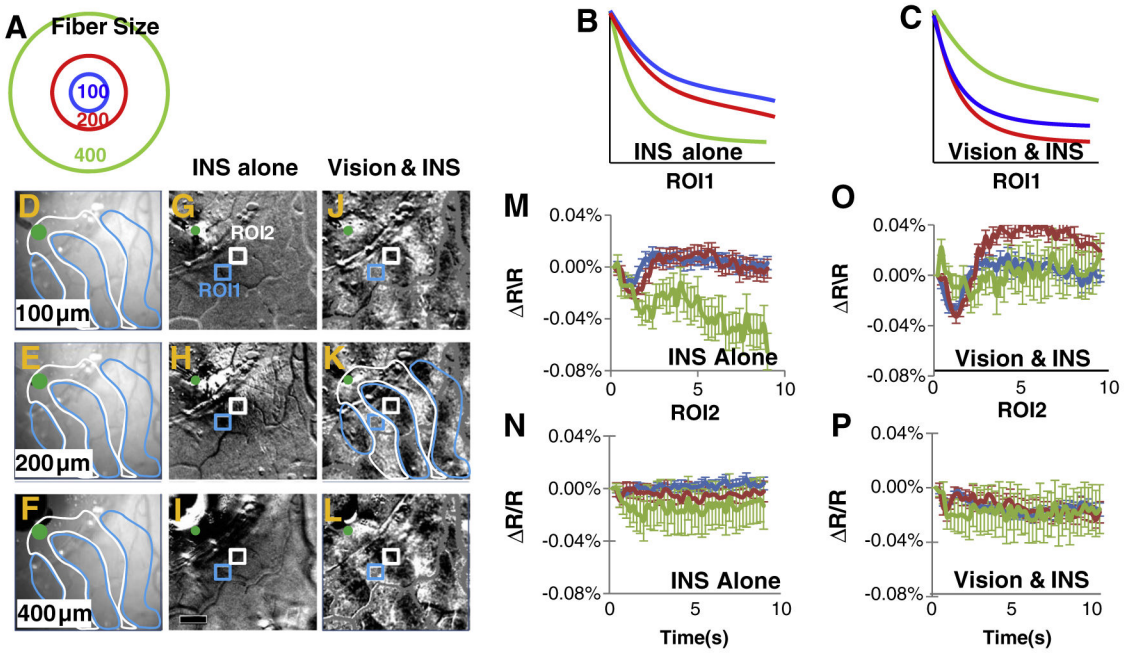

Fig. 6.

Modulation of visual signal by INS dependent on fiber size. (A) Schematic displaying diameter difference of 100, 200, and 400 μm fibers. (B & C) Hypothesized intrinsic responses in right and left OD columns in V1 for INS alone in a right eye OD column and in combination with visual stimulation for each optical fiber diameter tested. (D–F) Blood vessel maps displaying placement of 100 (D), 200 (E), and 400 (F) μm fibers in relation to ocular dominance columns (white outlines: right eye; green outlines: left eye). Green dot: location of fiber tip. Error of fiber placement < 100 μm. Visual stimulation and combined stimulation were performed as previously described. Laser parameters for combined stimulation consisted of a radiant exposure = 0.64 J/cm^2, repetition rate = 200 Hz, pulse width = 250 μs, and pulse train length of 500 ms. Black scale bar = 500 μm. (G–I) Activation maps generated by INS alone with 100, 200, and 400 μm diameter fibers. Intrinsic signal magnitude increases with increased fiber diameter. (J–L) Ocular dominance maps generated by visual stimulation combined with INS. (M & N) Intrinsic signal time course for INS alone for an ROI positioned in a right (ROI1 (M)) and left ROI2 (N) OD column for each fiber diameter. (O & P) Intrinsic signal time course evoked by combined stimulation during left eye stimulation (Le + laser-blank). Time course magnitude is enhanced in the right OD column with the 200 μm diameter optical fiber. INS parameters: λ = 1.875 μm, radiant exposure = 0.64 J/cm^2, repetition rate = 200 Hz, pulse width = 75–300 μs, pulse train length = 500 ms. Trials = 38 for 100 and 200 μm fiber, trials = 25 for 400 μm fiber. Subtraction maps in (J–L) correspond to frames 15 to 25 (3 to 5 s). (A = anterior, L = lateral). Data from Monkey 1 right hemisphere.