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Imaging separation of neuronal from vascular effects of cocaine on rat cortical brain *in vivo*

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Abstract

MRI techniques to study brain function assume coupling between neuronal activity, metabolism and flow. However, recent evidence of physiological uncoupling between neuronal and cerebrovascular events highlights the need for methods to simultaneously measure these three properties. We report a multimodality optical approach that integrates dual-wavelength laser speckle imaging (measures changes in blood flow, blood volume and hemoglobin oxygenation), digital-frequency-ramping optical coherence tomography (images quantitative 3D vascular network) and Rhod2 fluorescence (images intracellular calcium for measure of neuronal activity) at high spatiotemporal resolutions (30 μ m, 10Hz) and over a large field of view (3 $\times$ 5mm²). We apply it to assess cocaine's effects in rat cortical brain and show an immediate decrease (3.5 $\pm$ 0.9min, phase 1) in the oxygen content of hemoglobin and the cerebral blood flow followed by an overshoot (7.1 $\pm$ 0.2min, phase 2) lasting over 20min whereas Ca²⁺ increased immediately (peaked at t=4.1 $\pm$ 0.4min) and remained elevated. This enabled us to identify a delay (2.9 $\pm$ 0.5min) between peak neuronal and vascular responses in phase 2. The ability of this multimodality optical approach for simultaneous imaging at high spatiotemporal resolutions permits us to distinguish the vascular versus cellular changes of the brain, thus complimenting other neuroimaging modalities for brain functional studies (e. g., PET, fMRI).

Keywords

cocaine; cerebral blood flow; calcium [Ca]; optical imaging

1. Introduction

Functional magnetic resonance imaging (fMRI) has transformed the way we investigate brain function (Hanlon et al., 2010; Sokoloff, 2008). fMRI is based on the measurement of blood-oxygenation-level-dependent (BOLD) contrast signals (Ogawa et al., 1990; Raichle, 2001) that are complex and reflect the interplay between neuronal activation and the

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metabolic (oxygen metabolism or CMRO₂) and hemodynamic (cerebral blood flow and volume changes) responses of the brain (Donahue et al., 2009; Logothetis et al., 1999; Raichle, 2003). A comprehensive understanding of the mechanisms underlying the relationship between hemodynamic and metabolic responses to neuronal activation is thus critical for brain function studies. However, currently available imaging modalities are constrained by their limited spatiotemporal resolution, their small field of view (FOV) or their lack of absolute quantification capabilities. For example, PET (Fox and Raichle, 1986; Petersen et al., 1988; Volkow et al., 1996), fMRI (Kwong et al., 1992; Ogawa et al., 1990), and diffuse optical imaging (e.g., NIR spectroscopy and imaging) (Boas et al., 2001; Gratton and Fabiani, 2001; Villringer and Chance, 1997)) are macroscopic imaging methods whose spatial resolutions are over 1mm and thus insufficient to resolve individual vascular compartments or cells. Advanced light microscopy (e.g., confocal and multi-photon microscopy) (Dirnagl et al., 1991; Hudetz, 1997; Kleinfeld et al., 1998; Villringer et al., 1994) can provide with superior spatial resolution for visualizing capillary vasculature and cellular details of animal brain *in vivo*; however, their FOV and imaging depth are limited (e.g., ~300µm) (Dirnagl et al., 1991) restricting its potential for measurements that require imaging of neurovascular networks. Other recent optical methods include laminar optical tomography (Hillman et al., 2004) and laser speckle imaging (LSI) (Dunn et al., 2001) to improve the spatial resolution for mapping brain hemodynamic activities by deploying intrinsic hemoglobin absorption contrast. Unfortunately, laminar optical tomography is unable to identify individual vessels. Although LSI can resolve individual blood vessels, it only provides “relative” measures of cerebral blood flow (CBF).

Despite numerous neuroimaging studies on cocaine’s neurobiological effects, it is still not clear the extent of cocaine’s effects on vascular versus its neuronal effects in the brain. This is because cocaine not only exerts direct effects on neuronal tissue but also directly affects cerebral blood vessels (He et al., 1994). For example, human MRI and PET studies have shown reduced cerebral blood flow (CBF) in cocaine abusers (Hanlon et al., 2010; Johnson et al., 2001; Lu et al., 2007; Volkow et al., 1996). However, the mechanisms underlying cocaine-induced CBF reduction is not well understood, which may result from 1) direct vasoconstrictive properties of cocaine and/or indirect vasoconstriction secondary to release of sympathomimetic amines; or 2) indirect consequence of reduced neural activity and metabolic demand. This knowledge gap is due in part to limitation in our ability to concurrently assess the vascular and the neuronal effects of cocaine and to limited spatiotemporal resolutions of currently available imaging methodologies.

In this study, a newly developed optical/fluorescence imaging technique (OFI) is presented to permit simultaneous assessment of cerebral hemodynamic and neuronal activities in cortical brain *in vivo*. OFI integrates 1) dual-wavelength laser speckle imaging (DW-LSI) for concurrent detection of changes in CBF, blood volume (CBV) and hemoglobin oxygenation at high spatiotemporal resolutions across a large field of view; (2) digital-frequency-ramping Doppler optical coherence tomography (DFR-OCT) to permit quantitative 3D imaging of the neurovascular network; (3) Rhod₂ fluorescence imaging to measure the intracellular calcium ([Ca²⁺]_i) changes of the brain, which serves as an indicator of neuronal activation. To validate the efficacy of OFI, a rat model is used to assess the effects on the vascular and neuronal activities of brain in response to a cocaine challenge.

2. Methods

2.1. Animal model

Cocaine-naïve female Sprague–Dawley rats (250–300g/each, n=12 total) were anesthetized and ventilated with 2% isoflurane mixed in pure oxygen during the surgical procedures. The right carotid artery was catheterized for continuous arterial blood pressure monitoring and

the left carotid artery was set apart by a 3.0 suture. A ~ ϕ 6mm cranial window was created on one lateral side of the parietal bone that exposed the somatosensory and motor cortex area. After the dura was carefully removed, the exposed brain surface was immediately submerged in saline to avoid dehydration which might otherwise result in increased surface scattering and specular reflection. After the surgery, the anesthesia was then switched to α -chloralose using an initial bolus of 50mg/kg followed by continuous infusion of 25mg/kg/hr through the femoral vein during the experiment. For *in vivo* fluorescence imaging of $[Ca^{2+}]_i$ shown in Figs. 5(B₀–B₄), Rhod₂-AM (Molecular Probes, Eugene) which labels intracellular calcium was slowly infused (100 μ M, 3 μ l/min) into a rat cortical brain using a micro injector and a waiting period of ~60min was needed to allow for intracellular Rhod2 dye uptake for fluorescence imaging studies (Du et al., 2009; Du et al., 2006). The cellular uptake of Rhod2 was visualized from the cryostatic section of the brain after the *in vivo* experiments. The procedures of the cryo-sectioning and immunostaining have been described previously (Du et al., 2009). To do the drug challenges in the brain, a bolus of cocaine (1mg/kg, i.v.) was administrated through the tail vein followed by a 0.5cc saline. The vascular types (i.e., venous vs. arterial vessels) were characterized by using a transient ischemic insult after the cocaine experiment was completed, as has been described previously (Du et al., 2005; Luo et al., 2009a). During the experiment, the physiological parameters of the animal were continuously monitored, including the mean arterial blood pressure (MABP), respiration rate and body temperature (PC-SAM monitor, SA Inc.). In addition, blood gases were periodically measured (ABL 700, Radiometer Medical) to ensure that the rat remained under normal physiological condition. For example, the typical physiological parameters in the baseline were: pH=7.35–7.40, pCO₂=35–45mmHg, sO₂=97.5–100%, MABP=80–100mmHg, and T=36.5–37.5°C.

2.2. Instrumentation and Image acquisition

Fig. 1 illustrates the multimodal OFI system that integrates DW-LSI and fluorescence imaging (upper dashed box) with 3D DFR-OCT (lower dashed box). OFI is a custom-built imaging platform whose major subsystems and modules are summarized as follows.

DW-LSI & fluorescence imaging—A custom lighting module which comprised 2 single-mode laser diodes at the wavelengths of 785nm (50mW, HL7851G, Hitachi) and 830nm (30mW, DL5032, Sanyo) symmetric to hemoglobin isobetic point of 805nm for DWLSI and 1 diode laser at 532nm (50mW, G30/R100, Optlaser) for Rhod₂-Ca²⁺ excitation was employed to sequentially illuminate the cortical window (> ϕ 5mm) via 3 optical fibers. A cooled 12-bit CCD camera (pixel size: 6.45 μ m; Retiga Ex, QImaging) synchronized with the laser pulses acquired 2 diffuse reflectance images (exposure: T_{1,2}=10ms) for DW-LSI and 1 Rhod₂-Ca²⁺ fluorescence image peaked around 570–589nm (exposure: T/3 $\approx$ 30ms). The pulse sequences for laser and camera exposures were generated by a Time Base (PCI-6221, NI) to enable sequential DW-LSI and fluorescence imaging acquisitions. The OFI was based on a modified fluorescence zoom microscope (AZ100, Nikon) to take advantage of its long working distance (WD=45mm), large FOV (e.g., ~ ϕ 5mm) and high NA (NA=0.22) optics, e.g., 2 $\times$ Plan Apo objective (L1). A custom epi-illumination cube (C1) mounted in front of L1 integrated the 1.3 μ m DFR-OCT system with a dichroic mirror (DM1, cut off at $\lambda \approx 1\mu$ m). The 785nm and 830nm laser beams were delivered by 2 pigtailed SM fibers (NA $\approx$ 0.1) to obliquely (24°) illuminate the cranial window (over ϕ 6mm) to be overlapped with the 532nm laser beam introduced by the epi-illumination cube 2 (C2). The dichroic mirror (CM2, cut off at $\lambda \approx 550$ nm) reflected the 532nm to cortical brain for fluorescence excitation and transmitted the light beams at all 3 wavelengths (i.e., 785nm, 830nm, and fluorescence emission around 570–589nm) together with a barrier filter (BP2: $\lambda > 570$ nm) to be detected by the CCD camera operated in a time-sharing mode. The captured image sequences were streamed into workstation 2 via an IEEE-1394 interface for image

processing to extract 2D images of CBF, and changes in oxygenated (HbO₂), deoxygenated-hemoglobin HbR (thus, total hemoglobin HbT) and [Ca²⁺]_I fluorescence.

3D DFR-OCT—A SDOCT system was used to perform Doppler flow imaging in which a pigtailed broadband source (BBS: 12mW, $\lambda=1.3\mu\text{m}$, $\Delta\lambda=90\text{nm}$, coherence length $L_C\approx 8\mu\text{m}$; Inphenix) was employed to illuminate a fiberoptic Michelson interferometer. The reference beam was collimated to transmit through a pair of variable-thickness wedge prisms and reflected by a stationary mirror to minimize the dispersion mismatch between the two arms and match the pathlength of the sample beam. The sample arm was connected to C1 in which light exiting the SM fiber was collimated ($\phi 5\text{mm}$), transversely scanned by a pair of servo mirrors (x-y scanner), focused by an achromate (f/40mm), and reflected by dichroic mirror DM1 onto the cortical surface; while the light backscattered from the cortical brain was coupled back into the sample fiber through the same optical pathway. Then, the recombined sample and the reference beams in the detection fiber were connected to a custom spectrometer in which light was collimated ($\phi 10\text{mm}$), linearly diffracted by a grating ($d^{-1}=1200/\text{mm}$), and focused by a lens group (f/140mm) to be detected by a fast InGaAs line camera to enable 2D OCT (xz cross-section) at 47fps. A 3D OCT image cube (e.g., $x/1\text{k} \times z/1\text{k} \times y/400$ voxels) could be acquired in approximately 8s to cover a volume of $2.5\times 2\times 2.5\text{mm}^3$ on cortical brain. Post image processing with digital-frequency ramping (i.e., DFR-OCT) was applied to retrieve Doppler flow signals and reconstruct the 3D CBF network of the brain cortex quantitatively. The transverse and axial resolutions of the SDOCT system were $12\mu\text{m}$ and $8\mu\text{m}$, respectively.

2.3. Image processing

For DW-LSI, a 2D flow index map was retrieved from the speckle patterns in an acquired raw photographic image based on the dynamic features resulting from local CBFs (Dunn et al., 2003; Luo et al., 2008). To extract the flow contrast embedded in the speckle patterns of a raw reflectance image, 5×5 binning was performed to compute the speckle contrast map $K=\sigma/\langle I \rangle$, where $\langle I \rangle$ and σ were the mean intensity and standard deviation of each binning window (i.e., $6 \times 5 \approx 30\mu\text{m}$). The relation between K and the dynamic features of speckles is highly complex, which can be approximated as (Bandyopadhyay et al., 2005)

$$K = \left\{ \frac{\tau_c}{T} + \frac{\tau_c^2}{2T^2} \left[\exp\left(-\frac{2T}{\tau_c}\right) - 1 \right] \right\}^{1/2} \quad (1)$$

where $\tau_c = [ka\langle v^2 \rangle^{1/2}]^{-1}$ is the autocorrelation time of the speckle intensity fluctuation, $k=2\pi/\lambda$ is the wave number of light, and $\langle v^2 \rangle^{1/2}$ represents the rooted-mean-square speed of moving scattering particles. As a is an unknown factor associated with v distribution and tissue scattering characteristics, the combined product $a\langle v^2 \rangle^{1/2}$ is defined as the speed index which was obtained from a pre-calculated look-up table between K and $a\langle v^2 \rangle^{1/2}$ to minimize computation.

The changes in blood volume (CBV) and hemoglobin oxygenation can be derived from DW-LSI images under the assumption that $\Delta[\text{HbO}_2]$ and $\Delta[\text{HbR}]$ dominate the dynamic changes of light absorption and thus the measured diffuse reflectance (Dunn et al., 2003; Zhang et al., 2007), which can be given by

$$\begin{bmatrix} \Delta[HbO_2(t)] \\ \Delta[HbR(t)] \end{bmatrix} = \begin{bmatrix} \varepsilon_{HbO_2}^{\lambda_1} & \varepsilon_{HbR}^{\lambda_1} \\ \varepsilon_{HbO_2}^{\lambda_2} & \varepsilon_{HbR}^{\lambda_2} \end{bmatrix}^{-1} \cdot \begin{bmatrix} \frac{\ln(R_{\lambda_1}(0)/R_{\lambda_1}(t))}{L_{\lambda_1}(t)} \\ \frac{\ln(R_{\lambda_2}(0)/R_{\lambda_2}(t))}{L_{\lambda_2}(t)} \end{bmatrix} \quad (2)$$

where ε refers to the molar spectral absorptivity of the chromophore. $R_{\lambda 1}(t)$, $R_{\lambda 2}(t)$ are the diffuse reflectance images measured at these two wavelengths after being averaged over 5 consecutive frames for speckle noise reduction at time t , and $R_{\lambda 1}(0)$, $R_{\lambda 2}(0)$ are their baseline values prior to a stimulation (e.g., cocaine challenge). $L_{\lambda 1}(t) \approx L_{\lambda 2}(t)$ where $L_{\lambda 1}(t)$, $L_{\lambda 2}(t)$ are their pathlengths. The total hemoglobin concentration change can be obtained by $\Delta[HbT] = \Delta[HbO_2] + \Delta[HbR]$, assuming that it is linearly proportional to local CBV (Dunn et al., 2003; Zhang et al., 2007). According to Eq.(1) and Eq.(2), the changes in CBF, CBV and the hemoglobin oxygenation of cortical brain can be simultaneously imaged by DW-LSI at high spatiotemporal resolutions. For $[Ca^{2+}]_i$ fluorescence imaging, a cross-correlation test between baseline ($t \leq 0$) and post cocaine injection ($t > 0$) periods was used to analyze $[Ca^{2+}]_i$ fluorescence change in response to cocaine administration. Specifically, for each pixel (x , y), cross correlation between measured fluorescence $I_{xy}(t)$ and a step function $u(t)$ ($u(t)=0$, $t \leq 0$; $u(t)=1$, $t > 0$) was calculated to statistically determine a significant increase in the $[Ca^{2+}]_i$ fluorescence ($p < 0.01$), which was used to mask the active regions in the $[Ca^{2+}]_i$ fluorescence image (e.g., the overlapped $[Ca^{2+}]_i$ clouds in Fig. 5(B0–B4)). The averaged $[Ca^{2+}]_i$ fluorescence change within the mask at each time point was calculated to present the time course of $[Ca^{2+}]_i$ -fluorescence change in response to cocaine administration (e.g., Fig. 5(B5)). The influence of the hemoglobin absorption on the fluorescence emission can be corrected empirically through the CBF changes measured simultaneously (Supplement 1).

For quantitative Doppler OCT, the detected spectral interference can be derived as

$$I_{OCT}(k; \Delta L, x) = S(k) \left[(I_s + I_r) + 2 \sqrt{I_s I_r} \cdot \cos(k \Delta L + \varphi_r(\Delta L, x)) \right] \quad (3)$$

where I_s and I_r are the intensities in the sample and reference arms, $S(k)$ is the source spectrum ($k = 2\pi/\lambda$ is the wave number of light), and $\Delta L = L_s - L_r$ is the round-trip pathlength difference between the sample and the reference arms. The interferometric term spectrally modulating $S(k)$ at a frequency of $\Delta k = \pi/\Delta L$ can be decoded via an inverse FFT to restore the A-scan (Dorrer et al., 2000). It is noteworthy that as the detected spectral interferogram at a transverse position x , $I_{OCT}(k; \Delta L, x)$, preserves the relative phase $\varphi_r(\Delta L, x)$, SDOCT allows for detection of Doppler flow velocity along with tissue morphology (i.e., amplitude). By detecting the phase shift $\Delta \varphi_r(\Delta L, x)$ between two consecutive A-scans with τ interval, the Doppler flow velocity can be derived as $v_f(z, x) = [\lambda \Delta \varphi_r(\Delta L, x) / (4\pi n \cos \alpha)]$ (Leitgeb et al., 2003; Luo et al., 2008; Zhenguo Wang 2007), where n is refractive index of tissue and α is the angle between flow and incident light. However, this method for Doppler flow imaging is prone to phase noise induced by various effects (e.g., multiple scattering, tissue heterogeneity and motion, and electronic noise), which leads to severe under detection of subsurface blood flows. Recent advances in OCT angiography (Wang et al., 2007) dramatically improved the sensitivity for flow detection by applying Hilbert transform to the detected $I_{OCT}(k; \Delta L, x)$ along the transverse x -axis, i.e., frequency offsetting to reduce background phase noise. We further developed DFR-OCT (Yuan et al., 2009), a simple digital-frequency-ramping method to enhance flow detection which can be applied to conventional SDOCT with no hardware modification. Importantly, DFR-OCT enables quantitative 2D and 3D Doppler flow imaging by digitally ramping threshold frequency ν_R in Hilbert transforms, i.e., $\varphi_r(\Delta L, x)$ in Eq.(2) can be expressed as (Yuan et al., 2009)

$$\varphi_{\tau}(\Delta L, x) = \varphi_0 + (2\pi/\lambda) \cdot (v_f + v_b + v_R) \cdot \tau \cdot n_x \quad (4)$$

where φ_{τ} is the initial phase, $n_x = 1, 2, \dots, N_x$ is A-scan index (N_x is the number of scans along x-axis), v_f and v_b are the velocities of Doppler flow and background noise flow. If the digitally imposed frequency-ramping offset v_R is chosen to ensure,

$$(v_f + v_b + v_R) \cdot (v_b + v_R) < 0 \quad (5)$$

Hilbert transform (sensitive to frequency thresholding, i.e., flipping sign) along x-axis can easily separate these two types of flow signals, i.e., to differentiate all Doppler flows with v_f above the noise ground v_b for non-quantitative angiographic detection (Yuan et al., 2009). To further quantify Doppler flow rate v_f which can be normalized to the range of $(-1, 1]$, v_R can be similarly ramped over N steps with a step size of $\Delta v = 1/N$ or $v_R(i) = v_R + i \cdot \Delta v$ (where $i = -N/2, \dots, N/2$) to search for points at which the transverse modulation frequency of Hilbert transform flips sign. Here, because of phase wrapping effect, the ramping range can be further reduced to half to save computation efforts in our new algorithm. Then, based on Eq. (4) and Eq.(5), the flow rate for DFR-OCT can be derived as

$$|v_f| = \Delta v \left\{ \sum_{i=-N/2}^{-1} [(v_f - i\Delta v) < 0] + \sum_{i=1}^{N/2} [(v_f - i\Delta v) > 0] \right\} \quad (6)$$

Compared with the previous DFR algorithm (Yuan et al., 2009), the two sets of summation operations in Eq.(6) accumulate the results from each step and further reduce the noise level because of the averaging effect. Here, $(v_f - i \cdot \Delta v) > 0$ and $(v_f - i \cdot \Delta v) < 0$ are both Boolean functions (i.e., 1 for 'true', 0 for 'false'), and the sign of v_f can be determined by

$$\text{sign}(v_f) = \begin{cases} (+) & \text{if } \sum_{i=1}^{N/2} [(v_f - i\Delta v) > 0] > \sum_{i=-N/2}^{-1} [(v_f - i\Delta v) < 0] \\ (-) & \text{if } \sum_{i=1}^{N/2} [(v_f - i\Delta v) > 0] < \sum_{i=-N/2}^{-1} [(v_f - i\Delta v) < 0] \end{cases} \quad (7)$$

The step size was optimized and set to $\Delta v = 0.02$, which is a compromise of extensive computation and the discernable Doppler flow rate resolution.

3. Results

3.1. Quantitative imaging of cocaine-induced CBF changes

Fig. 2 shows the results of simultaneous CBF images by LSI and 3D DFR-OCT before and after cocaine administration (1mg/kg, i.v., which is a clinically relevant dose). Fig. 2(a) shows that LSI provides *en face* CBF images over a large FOV of $5 \times 3 \text{ mm}^2$ at a high frame rate of $\sim 10 \text{ fps}$, thus permitting continuous monitoring of time-varying CBF changes in the full field which includes large and small vessels (e.g., 1, 2) and CBF perfusion in the surrounding cortical tissue (e.g., 3, resulting from micro flows irresolvable by LSI, i.e., $\phi 30 \mu\text{m}$ or less). The CBF flow indices ($v_1 \approx 7.8 \text{ k}$, $v_2 \approx 3.5 \text{ k}$) in different-size vessels can be readily differentiated by LSI despite its reliance on relative measurements. Unlike LSI, DFR-OCT enables quantitative 3D imaging of the local CBF network at $\sim 10 \mu\text{m}$ spatial

resolution. Fig. 2(c) shows a projected 3D DFR-OCT image of the vascular CBF network ($2.5 \times 2.5 \times 2\text{mm}^3$) whose *en face* FOV corresponds to the cortical area landmarked by the dashed rectangle in Fig. 2(a). Fig. 2(e) reveals that the relative CBF indices change as a function of time in response to cocaine. The flow indices of the three flow traces (1, 2, and 3) measured by LSI (e.g., left axis) can be calibrated by DFR-OCT to convert to absolute flow rates (e.g., right axis) (Luo et al., 2008; Yuan et al., 2009), where the vertical lines at t_b and t_p represent the time points for concurrent LSI and DFR-OCT acquisitions at the baseline, e.g., $t_b = -2.5\text{min}$ as illustrated in Fig. 2(a) and Fig. 2(c) and at the elevated response to cocaine (phase 2), e.g., $t_p \approx 9\text{min}$ as illustrated in Fig. 2(b) and Fig. 2(d), respectively. Fig. 2(g) summarized the mean CBF changes measured by LSI and DFR-OCT in different vascular compartments before (t_b) and after (t_p) a cocaine challenge from different animals ($n=4$). The results indicate that the CBF rates by LSI increased $28\% \pm 7\%$, $26\% \pm 8\%$, and $32\% \pm 3\%$ in arterial (A), venous (V), and tissue perfusion (T), respectively. The corresponding CBF increases (A, V) measured by DFR-OCT, i.e., $22\% \pm 8\%$ and $23\% \pm 7\%$ correlated with the LSI measurements.

A comparison between the upper and lower panels indicates that both LSI and DFR-OCT imaging modalities were able to detect the CBF increase in response to a cocaine challenge as indicated by flow rates (i.e., in pseudo color) in both larger and smaller vessels. Specifically, the flow profiles for vessel 1 ($\sim \phi 170\mu\text{m}$, vein) and vessel 2 ($\sim \phi 40\mu\text{m}$, arteriole) before and after cocaine injection were plotted, indicating that the averaged flow rates increased from $v_1(t_b) \approx 3.1\text{mm/s}$, $v_2(t_b) \approx 1.4\text{mm/s}$ to $v_1(t_p) \approx 4.1\text{mm/s}$, $v_2(t_p) \approx 2.0\text{mm/s}$. Despite the flow rate increase, no significant vasodilation (i.e., increase in vessel size) was observed in response to cocaine challenge. The normalized CBF changes in Fig. 2(f) demonstrate the high spatiotemporal resolution of LSI to distinguish the time-lapse CBF responses to cocaine (green curve) in the different-size vessels (e.g., curves 1, 2) and within the brain tissue (e.g., curve 3, perfused micro flows). The transient decrease in CBF (phase 1, $t = 3.5 \pm 0.9\text{min}$, $n=4$) following cocaine administration was consistently observed in all experiments (e.g., Fig. 4, Fig. 5A₅). This early dynamic change in CBF (e.g., the ‘dipping’ effect) did not appear in the vehicle animals challenged by saline, suggesting that it was a pharmacological effect from acute cocaine administration rather than measurement artifacts, e.g., induced by scattering decrease. Similar effects on CBV and BOLD were observed in previous MRI studies (Luo et al., 2003; Schwarz et al., 2003). In contrast to the “dip”, which was not seen with saline administration, the immediate transient increase in CBF (peaked within 1min) upon cocaine administration was also observed after saline administration, indicating that it is most likely caused by the bolus flush of solution.

3.2. Mapping cocaine-induced changes in 3D CBF network

In addition to quantitative flow measurement, 3D DFR-OCT can provide the depth profile of the CBF measurements, which 2D LSI is unable to determine. This technique effectively enhances the sensitivity and spatial resolution of OCT for Doppler flow imaging by employing image processing technique (i.e., digital-frequency ramping), it enables fast 3D CBF imaging suitable for brain functional studies on a conventional SDOCT with no need of additional hardware modifications or compromise in the imaging rate. Fig. 3(b) is an example of 3D DFR-OCT of the vascular CBF network in the cerebral cortex of a rat brain where Fig. 3(a) is the corresponding maximum-intensity-projection image similar to those shown in Figs. 2(c, d). Full-size 2D DFR-OCT images (i.e., $1\text{k} \times 1\text{k}$ pixels in x-z cross section) were acquired at up to 47fps; therefore, a 3D volume consisting of 400 slices in y-axis (i.e., $1\text{k} \times 1\text{k} \times 400$ voxels) could be obtained in merely 8s to image the CBF network over $2.5 \times 2 \times 2.5\text{mm}^3$ on the cortical brain at $10\mu\text{m}$ resolution. Fig. 3(c) shows that owing to 3D imaging capability, a minute CBF ($\sim \phi 13\mu\text{m}$) in Fig. 3(a, b), descending from $150\mu\text{m}$ to $750\mu\text{m}$ under the cortical surface, could be traced and quantitatively measured in near real

time, which shows the capabilities of 3D DFR-OCT for high-resolution imaging of detailed CBF changes in different layers of the cerebral cortex in a rat cortical brain. For instance, the pie-cut graph of 3D DFR-OCT in Fig. 3(d) illustrates the vascular CBF network of the rat cortical brain (α -chloralose anesthesia), and the 4 panels show the time course of flow changes induced by a cocaine challenge (1mg/kg, i.v.) in 4 typical CBFs: e) large ($\sim\phi 80\mu\text{m}$) and deep ($\sim 430\mu\text{m}$), f) small ($\sim\phi 20\mu\text{m}$) and deep ($\sim 670\mu\text{m}$), g) large ($\sim\phi 70\mu\text{m}$) and superficial ($\sim 130\mu\text{m}$), and h) small ($\sim\phi 25\mu\text{m}$) and superficial ($\sim 50\mu\text{m}$). A detailed comparison (normalized to baseline) in Fig. 3(i) indicate that CBF in all 4 vessels exhibited a cocaine-induced transient decrease (phase 1) and a following overshoot (phase 2) similar to those in Fig. 2 and Figs. 4–5. It also shows a slight delay between large flows and small, deep flows as highlighted by the dashed green line ($\Delta t = 0.8 \pm 0.4\text{min}$, $n=4$). More interestingly, small flows, particularly deep small flows tended to show more vibrant pulsive changes (e.g., dashed purple curve) in response to cocaine challenge.

3.3. Simultaneous imaging of cerebral hemodynamic and oxygenation changes

DW-LSI allows for simultaneous detection of the changes in CBF (i.e., ΔCBF), and oxygenated and deoxygenated hemoglobin concentrations (i.e., $\Delta [\text{HbO}_2]$, $\Delta [\text{HbR}]$). The changes in total hemoglobin ($\Delta [\text{HbT}]$), i.e., the changes in cerebral blood volume (ΔCBV) can be determined accordingly by $\Delta [\text{HbT}] = \Delta [\text{HbO}_2] + \Delta [\text{HbR}]$ (Luo et al., 2009). Fig. 4 shows the time courses of ΔCBF , $\Delta [\text{HbO}_2]$, $\Delta [\text{HbR}]$ and $\Delta [\text{HbT}]$ in a cortical brain (α -chloralose anesthesia). Figs. 4(a,b,c) compare the temporal responses to a cocaine challenge (1mg/kg, i.v.) at $t=0\text{s}$ of an arteriolar flow, tissue perfusion (i.e., from micro flows irresolvable by DW-LSI), and a venous flow, as indicated by 3 arrows in the lower middle panel. The time traces of ΔCBF , $\Delta [\text{HbO}_2]$, $\Delta [\text{HbR}]$, and ΔCBV characterized by dotted, red, blue, and green curves show that after an initial dip (phase 1), CBF, $[\text{HbO}_2]$, and CBV increased following cocaine injection whereas $[\text{HbR}]$ decreased (phase 2). Here, t_b , t_p , and t_r represent 3 typical time points (i.e., baseline, $\sim 20\text{min}$ and $\sim 40\text{min}$ after cocaine challenge) at which the corresponding full-field CBF images are shown in Figs. 4(d,e,f) in which the quantitative flow rates in the segmented arteriolar, tissue perfusion and venous flow compartments are presented by red, green and blue colors, respectively.

3.4. Simultaneous imaging of cocaine-induced CBF and intracellular $[\text{Ca}^{2+}]$ fluorescence

Fig. 5 shows time-lapse simultaneous images of the CBF network (A0–A4) and the cortical brain $[\text{Ca}^{2+}]_i$ fluorescence (B0–B4) labeled with Rhod2 in response to a cocaine challenge (1mg/kg, i.v.) at $t=0\text{s}$ in a drug-naïve rat (α -chloralose anesthesia). The detailed time-course characteristics of ΔCBF and $\Delta [\text{Ca}^{2+}]_i$ are plotted in A5 and B5, respectively. The results indicate that cocaine induced a transient decrease (phase 1) in CBF within $t = 3.3 \pm 0.8\text{min}$ ($n=4$) after cocaine injection, followed by an overshoot (phase 2) that reached plateau around $t = 7.1 \pm 0.2\text{min}$ ($n=4$). In contrast, $[\text{Ca}^{2+}]_i$ fluorescence increased immediately in response to cocaine challenge (peaked at $t = 4.1 \pm 0.4\text{min}$, $n=4$) and persisted over 20min followed by a gradual decay. A comparison between panels A3 and B2 reveals a time lapse ($\Delta t = 2.9 \pm 0.5\text{min}$, $n=4$) between the peak cellular (e.g., $\Delta [\text{Ca}^{2+}]_i$) and phase 2 vascular (e.g., ΔCBF) responses to cocaine. Additionally, it is noteworthy that cocaine-induced blood absorption increase (e.g., ΔCBF) might affect the measured Rhod2-fluorescence emission. An empirical model was derived to correct the artifact (see Supplement 1 for details) and the result (solid curve) in Fig. 5(B5) indicate that although the time profile between the two curves did not differ significantly, especially in the early phase ($t < 4\text{min}$, phase 1), the measured $\Delta [\text{Ca}^{2+}]_i$ curve (dashed curve) could be 25% underestimated in later stage ($t > 4\text{min}$, phase 2) due to increased CBF and should thus be corrected. Fig. 5(C) is the raw baseline fluorescence image to illustrate the loading spot and the Rhod2 distribution on the cortical brain *in vivo* and Fig. 5(D–F) are the corresponding cryosection fluorescence

microscopic images where the bright spots in (D, E) indicate intracellular Rhod2 uptake of Ca^{2+} and the bright area in (F) shows their intracellular localizations.

4. Discussions

Noninvasive and high spatiotemporal resolution imaging of cerebral hemodynamic and neuronal effects in response to various types of stimulations (e.g., electrical stimulations, drug challenges) remains a major challenge in neuroimaging. While LSI permits 2D imaging of CBF at high spatiotemporal resolutions (e.g., $30\mu\text{m}$, 10Hz), it is based on *en face* imaging and only measures the relative flow indices rather than the absolute flow rates. Doppler OCT is an emerging optical technique that enables quantitative 3D imaging of the vascular CBF network (absolute flow rate detection) at high spatial resolution ($\sim 10\mu\text{m}$) over a large FOV in the cerebral cortex of the rodent's brain. Recent advances in DFR-OCT have dramatically improved the sensitivity of Doppler OCT for detection of cerebral capillary flow (e.g., $\sim 10\mu\text{m}$, 0.16mm/s) and the frame rate needed to render 3D imaging of the vascular CBF network within 8s/volume (e.g., Fig. 3); however, post image processing is needed because of the intensive computation required to reconstruct quantitative 3D DFR-OCT flow images. By co-registering with DFR-OCT, it is found that LSI can be calibrated (e.g., Fig. 2) to allow for high-resolution absolute quantitative imaging of transient CBF changes in real time (e.g., 10–29Hz). Moreover, DW-LSI can measure changes in both $[\text{HbO}_2]$ and $[\text{HbR}]$, thus enabling determination of the change in total hemoglobin concentration (i.e., $\Delta [\text{HbT}]$ or ΔCBV) in both CBFs and tissue perfusion (i.e., irresolvable capillary flows). Therefore, a multimodality neuroimaging platform that combines DFR-OCT, DW-LSI and fluorescence can allow for simultaneous characterization of the local changes in cerebrovascular hemodynamics (CBF, CBV), hemoglobin oxygenation (HbO_2) and intracellular calcium ($[\text{Ca}^{2+}]_i$ fluorescence) as shown here for monitoring the effects of cocaine. Such a multimodality imaging technique (OFI) provides several uniquely important merits, including: 1) large FOV ($\sim 3 \times 5\text{mm}^2$), 2) high spatiotemporal resolutions ($\sim 30\mu\text{m}$, $\sim 10\text{Hz}$), 3) quantitative 3D imaging of the CBF network by co-registering with DFR-OCT, 4) label-free imaging of hemodynamic changes, 5) separation of vascular compartments between arterial and venous vessels and monitoring of cortical brain metabolic changes, 6) simultaneous imaging and thus separation of cellular (neuronal) from vascular responses, and 7) the ability to separately measure CBF in the layers of the cerebral cortex in the rodent brain.

The animal study presented here validates the technological feasibility of this multi-modal approach for simultaneous imaging. Experimental results presented in Fig. 2 – Fig. 5 demonstrate the utility of this new technique to enable *in vivo* imaging of rat cortical brain functional changes in response to cocaine administration at high spatiotemporal resolutions over a large of field of view. The critical significance of such imaging modalities on neuroimaging and the drug-induced pharmacologic effects on the brain function can be summarized as follows:

1. Full-Field and high spatial resolution: DW-LSI enables imaging of the vascular CBF network at high spatial resolution ($\sim 30\mu\text{m}$) and over a large FOV ($4 \times 5\text{mm}^2$). By co-registering with DFR-OCT, quantitative 3D imaging of the CBF network is permitted over a cubic cortical volume of $2.5 \times 2 \times 2.5\text{mm}^3$ at $\sim 10\mu\text{m}$ resolution. Such high spatial resolution and large FOV will bridge the gap between conventional mesoscopic (e.g., fMRI, PET) and microscopic (e.g., confocal, 2-photon) imaging modalities to study cerebral neurovascular coupling effects which require both high resolution and large field of view.
2. High temporal resolution: The frame rates of our current DW-LSI and 2D DFR-OCT are 10fps and 47fps and can be easily upgraded to 30fps and 94fps (i.e.,

0.25Hz for 3D DFR-OCT). Such high temporal-resolution 2D and 3D CBF imaging enables to capture transient neurovascular events (as shown in this study for the case of cocaine) to provide new insights to advance our understanding of the coupling between neuronal activity, metabolism and hemodynamic changes and the perturbation by pharmacological agents and animal models of brain diseases. Additionally, it will likely allow us to monitor the treatment or therapeutic effects aimed at vascular as well as neuronal repair.

3. Multimodality simultaneous imaging: The optical/fluorescence techniques that combine DW-LSI, 3D DFR-OCT and $[Ca^{2+}]_i$ fluorescence enable concurrent imaging of changes in hemodynamics (CBF, CBV), hemoglobin oxygenation (HbO_2) and $[Ca^{2+}]_i$ from cortical brain *in vivo*. The value for simultaneous imaging of neurovascular and cellular functions is illustrated by our results with cocaine that clearly differentiated cocaine-induced cellular (neuronal) effects from its neurovascular effects.

The findings we report here with cocaine corroborate and advance our prior studies that measured the effects of cocaine in local cerebral hemodynamics (CBV), oxygenation and $[Ca^{2+}]_i$ (Rhod₂ fluorescence). In our prior optical spectroscopy study we showed that acute cocaine (e.g., 1mg/kg, i.v.) induced a local increase in CBV and hemoglobin oxygenation in rats (α -chloralose anesthesia)(Du et al., 2006). The current study advances our findings of cocaine's effects on the brain in the following 2 important aspects. First, we advance from point measurement (acquiring averaged signal across a brain area defined by fiberoptic probe, e.g., $\phi=5$ mm of our prior spectroscopy) to high-resolution imaging. With DW-LSI, we achieve high spatiotemporal resolutions (e.g., $\sim 30\mu m$ and ~ 10 Hz) which allow us to resolve individual vascular compartments and distinguish the vascular effects (i.e., changes in CBF, $[HbR]$, $[HbO_2]$ and CBV) from the cellular effects (i.e., changes in $[Ca^{2+}]_i$ fluorescence) induced by cocaine in the cortical brain; Secondly, we integrate three imaging techniques (DW-LSI, DFR-OCT, and $[Ca^{2+}]_i$ fluorescence) into a multimodal imaging platform (OFI) to enable tri-modal simultaneous imaging. By co-registering with DFR-OCT, the 3D CBF network can be quantitatively 'visualized' across various depths of a rodent cortical brain (e.g., up to $z=1$ mm in a rat brain) and image their changes in response to cocaine administration. The image results using DW-LSI and DFR-OCT show more detailed cocaine-induced effects. For instance, we observed the late phase (phase 2) of cocaine's neurovascular effect, e.g., increases in CBF (Figs. 2–5), CBV or $[HbT]$ and $[HbO_2]$ along with a decrease of $[HbR]$ (Fig. 4) after 4–5min of cocaine injection with single-vessel resolution. During this time period, DW-LSI showed an increase in $[Ca^{2+}]_i$ fluorescence with concurrent CBF elevation induced by cocaine, in agreement with prior studies(Du et al., 2006; Hu, 2007; Lu et al., 2007; Nasif et al., 2005). Importantly, the high temporal resolution and simultaneous imaging capability of our OFI allowed us to visualize the early phase (phase 1) of cocaine's neurovascular effect, e.g., an immediate early increase in $[Ca^{2+}]_i$ along with an immediate transient decrease in CBF during the first 3.5 ± 0.9 min ($n=4$) following cocaine administration (e.g., Fig. 5). Indeed, the early transient CBF 'dip' observed in this study is consistent with the report of sharp negative BOLD signal measured with fMRI at up to 120s after cocaine administration in rat cortical brain (urethane anesthesia)(Luo et al., 2003), which was interpreted to reflect neuronal-based vascular constriction induced by cocaine. Our findings of an immediate increase in $[Ca^{2+}]_i$ (indicator of neuronal activation) with a concomitant decrease in CBF and in $[HbO_2]$ and increase in $[HbR]$ and CBV in arteriole (Fig. 4, upper left panel) suggest that the transient negative BOLD signal reported with fMRI shortly after cocaine reflects a decrease in hemoglobin oxygenation secondary to the temporally lagging in CBF response to the increases in neuronal activation elicited by cocaine. Cocaine's immediate increases in neuronal activity and abrupt decrease (dip) in CBF and $[HbO_2]$ could underlie the cerebrovascular complications associated with cocaine abuse, e.g., ischemic stroke(Johnson et al., 2001).

The findings of cocaine-induced Ca^{2+} increases could also underlie the reported enhanced hemodynamic and field potential responses to sensory stimulation after acute cocaine administration (Devonshire et al., 2004). It is noteworthy that a sampling rate of 10Hz in this study was not fast enough to capture the calcium transients of individual neuron firings. However, unlike somatic electrical stimulation, cocaine directly stimulates neuronal activity and neuron firings might not necessarily synchronize, which would make it difficult to distinguish even at a higher frame rate (e.g., 30fps). The cocaine-induced mean $[\text{Ca}^{2+}]_i$ increase measured here (Fig. 5B5) could reflect the amplitude increase in neuronal Ca transients or the increase in neuronal firing rate or a combination of both.

Recent technological advances in OCT angiography have dramatically improved the sensitivity for Doppler flow imaging so that subsurface minute blood flows, including capillary flows that can be uncovered (Mariampillai et al., 2010; Srinivasan et al., 2010; Wang et al., 2007). For instance, Fig. 6 shows a maximum-intensity projection of a full-field 3D OCT angiography of a mouse cortex which resolves far more detailed cerebral microvasculature than white-light surface imaging. Noteworthy, despite more microvasculature seen in Fig. 6 than in DFR-OCT (e.g., Figs. 2–3), OCT angiography is unable to provide quantitative flow changes crucial to brain functional studies such as cocaine effects presented in this work. Therefore, our future work will combine quantitative DFR-OCT with OCT angiography to lift the limitation. Additionally, two wavelengths (785nm, 830nm) close to the isosbestic point of hemoglobin absorption (805nm) were chosen for DW-LSI, which might benefit to increase the flow imaging depth (due to reduced tissue scattering with wavelength) and alleviate complications in $\Delta[\text{HbO}_2]$ and $\Delta[\text{HbR}]$ calculation in Eq.(2) induced by pathlength difference between these two wavelengths. However, the low hemoglobin absorption may lead to reduced sensitivity in detecting HbO_2 changes. Alternative approaches include using 830nm for LSI and decoupled reflectance images at other wavelengths (Kawauchi et al., 2009; Okui and Okada, 2005; Strangman et al., 2003; Uludag et al., 2004) with a stronger HbO_2 absorbance (e.g., 690nm) for $\Delta[\text{HbO}_2]$ detection, which in return might encounter potential problems such as reduced depth for flow imaging and complications in $\Delta[\text{HbO}_2]$ computation and image registration with LSI. Nevertheless, it will be interesting to compare the advantages and limitations between these approaches.

In summary, we present a multimodality optical imaging technique which combines DW-LSI, DFR-OCT and Rhod2-labeled $[\text{Ca}^{2+}]_i$ fluorescence. Results of *in vivo* animal studies demonstrate the potential of such imaging platform for simultaneous imaging and absolute quantification of changes in neuronal, metabolic and hemodynamic parameters of the cortical brain. More specifically, it allows us: 1) to analyze the temporal effects of cocaine on neurovascular network and oxygenation; 2) to delineate the dynamic processes that occur between the vascular CBF network and the surrounding neuronal tissue and to separately evaluate cellular from vascular effects by comparing the transient changes between $[\text{Ca}^{2+}]_i$ fluorescence from $[\text{HbO}_2]$ and CBF; and 3) to do in quantitative CBF measures at different depths in the cortex. Using this multimodality imaging platform we show a transient response to cocaine that revealed an immediate and transient decrease (phase 1) in local oxygen content (Fig. 4) and CBF ($t < 4\text{min}$) followed by a longer lasting overshoot (up to 40min, phase 2) whereas cocaine induced an immediate Ca^{2+} increase (peaked at $4.1 \pm 0.4\text{min}$) that remained elevated over 20min of the measurements (Fig. 5). This identifies a $2.9 \pm 0.5\text{min}$ lagging time between the vascular and the neuronal responses to cocaine. This method complements other existing neuroimaging approaches for use in neuroscience research including that of the investigation of the coupling between neuronal activation and hemodynamic and metabolic responses of cerebral tissue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Bandyopadhyay R, Gittings AS, Suh SS, Dixon PK, Durian DJ. Speckle-visibility spectroscopy: A tool to study time-varying dynamics. *Review of Scientific Instruments*. 2005; 76:093110.
- Boas DA, Gaudette T, Strangman G, Cheng X, Marota JJ, Mandeville JB. The accuracy of near infrared spectroscopy and imaging during focal changes in cerebral hemodynamics. *Neuroimage*. 2001; 13:76–90. [PubMed: 11133311]
- Devonshire IM, Berwick J, Jones M, Martindale J, Johnston D, Overton PG, Mayhew JEW. Haemodynamic responses to sensory stimulation are enhanced following acute cocaine administration. *Neuroimage*. 2004; 22:1744–1753. [PubMed: 15275930]
- Dirnagl U, Villringer A, Gebhardt R, Haberl RL, Schmiedek P, Einhaupl KM. Three-dimensional reconstruction of the rat brain cortical microcirculation in vivo. *J Cereb Blood Flow Metab*. 1991; 11:353–360. [PubMed: 2016343]
- Donahue MJ, Stevens RD, de Boorder M, Pekar JJ, Hendrikse J, van Zijl PCM. Hemodynamic changes after visual stimulation and breath holding provide evidence for an uncoupling of cerebral blood flow and volume from oxygen metabolism. *Journal of Cerebral Blood Flow and Metabolism*. 2009; 29:176–185. [PubMed: 18797471]
- Dorrer C, Belabas N, Likforman JP, Joffre M. Spectral resolution and sampling issues in Fourier-transform spectral interferometry. *Journal of the Optical Society of America B-Optical Physics*. 2000; 17:1795–1802.
- Du C, Luo Z, Yu M, Benveniste H, Tully M, Pan R, Chance B. Detection of Ca²⁺-dependent neuronal activity simultaneously with dynamic changes in CBV and tissue oxygenation from the live rat brain. *Journal of Innovative Optical Health Sciences*. 2009; 2:189–200.
- Du CW, Yu M, Volkow ND, Koretsky AP, Fowler JS, Benveniste H. Cocaine increases the intracellular calcium concentration in brain independently of its cerebrovascular effects. *Journal of Neuroscience*. 2006; 26:11522–11531. [PubMed: 17093073]
- Dunn AK, Bolay T, Moskowitz MA, Boas DA. Dynamic imaging of cerebral blood flow using laser speckle. *Journal of Cerebral Blood Flow and Metabolism*. 2001; 21:195–201. [PubMed: 11295873]
- Dunn AK, Devor A, Bolay H, Andermann ML, Moskowitz MA, Dale AM, Boas DA. Simultaneous imaging of total cerebral hemoglobin concentration, oxygenation, and blood flow during functional activation. *Optics Letters*. 2003; 28:28–30. [PubMed: 12656525]
- Fox PT, Raichle ME. Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc Natl Acad Sci U S A*. 1986; 83:1140–1144. [PubMed: 3485282]
- Frahm J, Baudewig J, Kallenberg K, Kastrup A, Merboldt KD, Dechent P. The post-stimulation undershoot in BOLD fMRI of human brain is not caused by elevated cerebral blood volume. *Neuroimage*. 2008; 40:473–481. [PubMed: 18201912]
- Gratton G, Fabiani M. The event-related optical signal: a new tool for studying brain function. *International Journal of Psychophysiology*. 2001; 42:109–121. [PubMed: 11587771]
- Hanlon CA, Wesley MJ, Roth AJ, Miller MD, Porrino LJ. Loss of laterality in chronic cocaine users: An fMRI investigation of sensorimotor control. *Psychiatry Research-Neuroimaging*. 2010; 181:15–23.
- He GQ, Zhang A, Altura BT, Altura BM. Cocaine-induced cerebrovasospasm and its possible mechanism of action. *J Pharmacol Exp Ther*. 1994; 268:1532–1539. [PubMed: 8138965]

- Hillman EM, Boas DA, Dale AM, Dunn AK. Laminar optical tomography: demonstration of millimeter-scale depth-resolved imaging in turbid media. *Opt Lett*. 2004; 29:1650–1652. [PubMed: 15309848]
- Hu XT. Cocaine withdrawal and neuro-adaptations in ion channel function. *Molecular Neurobiology*. 2007; 35:95–112. [PubMed: 17519508]
- Hudetz AG. Blood flow in the cerebral capillary network: a review emphasizing observations with intravital microscopy. *Microcirculation*. 1997; 4:233–252. [PubMed: 9219216]
- Johnson BA, Devous MD, Ruiz P, Ait-Daoud N. Treatment advances for cocaine-induced ischemic stroke: Focus on dihydropyridine-class calcium channel antagonists. *American Journal of Psychiatry*. 2001; 158:1191–1198. [PubMed: 11481148]
- Kawauchi S, Sato S, Ooigawa H, Nawashiro H, Ishihara M, Kikuchi M. Light scattering change precedes loss of cerebral adenosine triphosphate in a rat global ischemic brain model. *Neuroscience Letters*. 2009; 459:152–156. [PubMed: 19446006]
- Kleinfeld D, Mitra PP, Helmchen F, Denk W. Fluctuations and stimulus-induced changes in blood flow observed in individual capillaries in layers 2 through 4 of rat neocortex. *Proc Natl Acad Sci U S A*. 1998; 95:15741–15746. [PubMed: 9861040]
- Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, Kennedy DN, Hoppel BE, Cohen MS, Turner R, et al. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci U S A*. 1992; 89:5675–5679. [PubMed: 1608978]
- Leitgeb RA, Schmetterer L, Drexler W, Fercher AF, Zawadzki RJ, Bajraszewski T. Real-time assessment of retinal blood flow with ultrafast acquisition by color Doppler Fourier domain optical coherence tomography. *Optics Express*. 2003; 11:3116–3121. [PubMed: 19471434]
- Logothetis NK, Guggenberger H, Peled S, Pauls J. Functional imaging of the monkey brain. *Nature Neuroscience*. 1999; 2:555–562.
- Lu HB, Xi ZX, Gitajn L, Rea W, Yang YH, Stein EA. Cocaine-induced brain activation detected by dynamic manganese-enhanced magnetic resonance imaging (MEMRI). *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:2489–2494. [PubMed: 17287361]
- Luo F, Wu GH, Li Z, Li SJ. Characterization of effects of mean arterial blood pressure induced by cocaine and cocaine methiodide on BOLD signals in rat brain. *Magnetic Resonance in Medicine*. 2003; 49:264–270. [PubMed: 12541246]
- Luo Z, Wang Z, Yuan Z, Du C, Pan Y. Optical coherence Doppler tomography quantifies laser speckle contrast imaging for blood flow imaging in the rat cerebral cortex. *Opt Lett*. 2008; 33:1156–1158. [PubMed: 18483544]
- Luo ZC, Yuan ZJ, Pan YT, Du CW. Simultaneous imaging of cortical hemodynamics and blood oxygenation change during cerebral ischemia using dual-wavelength laser speckle contrast imaging. *Optics Letters*. 2009; 34:1480–1482. [PubMed: 19412312]
- Mariampillai A, Leung MKK, Jarvi M, Standish BA, Lee K, Wilson BC, Vitkin A, Yang VXD. Optimized speckle variance OCT imaging of microvasculature. *Optics Letters*. 2010; 35:1257–1259. [PubMed: 20410985]
- Nasif FJ, Hu XT, White FJ. Repeated cocaine administration increases voltage-sensitive calcium currents in response to membrane depolarization in medial prefrontal cortex pyramidal neurons. *Journal of Neuroscience*. 2005; 25:3674–3679. [PubMed: 15814798]
- Ogawa S, Lee TM, Kay AR, Tank DW. Brain Magnetic-Resonance-Imaging with Contrast Dependent on Blood Oxygenation. *Proceedings of the National Academy of Sciences of the United States of America*. 1990; 87:9868–9872. [PubMed: 2124706]
- Okui N, Okada E. Wavelength dependence of crosstalk in dual-wavelength measurement of oxy- and deoxy-hemoglobin. *Journal of Biomedical Optics*. 2005; 10:011015.
- Petersen SE, Fox PT, Posner MI, Mintun M, Raichle ME. Positron Emission Tomographic Studies of the Cortical Anatomy of Single-Word Processing. *Nature*. 1988; 331:585–589. [PubMed: 3277066]
- Raichle ME. Cognitive neuroscience - Bold insights. *Nature*. 2001; 412:128–130. [PubMed: 11449247]

- Raichle ME. Functional brain Imaging and human brain function. *Journal of Neuroscience*. 2003; 23:3959–3962. [PubMed: 12764079]
- Schwarz AJ, Reese T, Gozzi A, Bifone A. Functional MRI using intravascular contrast agents: detrending of the relative cerebrovascular (rCBV) time course. *Magnetic Resonance Imaging*. 2003; 21:1191–1200. [PubMed: 14725926]
- Sokoloff L. The physiological and biochemical bases of functional brain imaging. *Cognitive Neurodynamics*. 2008; 2:1–5. [PubMed: 19003468]
- Srinivasan VJ, Jiang JY, Yaseen MA, Radhakrishnan H, Wu WC, Barry S, Cable AE, Boas DA. Rapid volumetric angiography of cortical microvasculature with optical coherence tomography. *Optics Letters*. 2010; 35:43–45. [PubMed: 20664667]
- Strangman G, Franceschini MA, Boas DA. Factors affecting the accuracy of near-infrared spectroscopy concentration calculations for focal changes in oxygenation parameters. *Neuroimage*. 2003; 18:865–879. [PubMed: 12725763]
- Uludag K, Steinbrink J, Villringer A, Obrig H. Separability and cross talk: optimizing dual wavelength combinations for near-infrared spectroscopy of the adult head. *Neuroimage*. 2004; 22:583–589. [PubMed: 15193586]
- Villringer A, Chance B. Non-invasive optical spectroscopy and imaging of human brain function. *Trends Neurosci*. 1997; 20:435–442. [PubMed: 9347608]
- Villringer A, Them A, Lindauer U, Einhaupl K, Dirnagl U. Capillary perfusion of the rat brain cortex. An in vivo confocal microscopy study. *Circ Res*. 1994; 75:55–62. [PubMed: 8013082]
- Volkow ND, Ding YS, Fowler JS, Wang GJ. Cocaine addiction: hypothesis derived from imaging studies with PET. *J Addict Dis*. 1996; 15:55–71. [PubMed: 8943582]
- Wang RK, Jacques SL, Ma Z, Hurst S, Hanson SR, Gruber A. Three dimensional optical angiography. *Optics Express*. 2007; 15:4083–4097. [PubMed: 19532651]
- Yuan ZJ, Luo ZC, Ren HG, Du CW, Pan YT. A digital frequency ramping method for enhancing Doppler flow imaging in Fourier-domain optical coherence tomography. *Optics Express*. 2009; 17:3951–3963. [PubMed: 19259236]
- Zhang HF, Maslov K, Sivaramakrishnan M, Stoica G, Wang LHV. Imaging of hemoglobin oxygen saturation variations in single vessels in vivo using photoacoustic microscopy. *Applied Physics Letters*. 2007; 90:053901.
- Wang, Zhenguo; CSDL; Waltzer, Wayne; Liu, Jingxuan; Xie, Huikai; Yuan, Zhijia. In vivo Bladder Imaging with MEMS-based Endoscopic Spectral Domain Optical Coherence Tomography. *Journal of Biomedical Optics*. 2007:034009. [PubMed: 17614717]

Fig. 1.

A schematic illustrating the principle of a multimodality optical and fluorescence imaging platform (OFI) that combines DW-LSI, DFR-OCT for fluorescence imaging used in the study. Upper dashed box: DW-LSI and fluorescence imager. LD_{1,2}: laser diodes at $\lambda_{1,2}=785, 830\text{nm}$ for CBF and metabolic imaging; λ_{ex} : 532nm for excitation of Rhod₂-[Ca²⁺]_i fluorescence imaging (λ_{em} : $570\text{--}589\text{nm}$), SM: single mode. Lower dashed box: 3D DFR-OCT. CM: collimator; BBS: broadband source ($\lambda=1.3\mu\text{m}$), LD: aiming laser ($\lambda=670\text{nm}$), FPC: fiberoptic polarization controller. Left dash box: modified zoom microscope. C1: epi-illumination cube 1. DM1: dichroic beam splitter ($\lambda_D=1\mu\text{m}$); L1: $2\times$ APO ($f=45\text{mm}$, $\text{NA}=0.22$). C2: epi-illumination cube 2. DM2: dichroic beam splitter ($\lambda_D=550\text{nm}$); BP2: barrier filter ($\lambda_B \geq 570\text{nm}$).

Fig. 2.

DW-LSI and 3D DFR-OCT of vascular CBF network in a rat cortical brain (α -chloralose anesthesia) prior (a, c) and post (b, d) cocaine injection (1mg/kg, i.v.). Relative 1D and quantitative 2D flow profiles of vessel 1 ($\sim\phi 170\mu\text{m}$, vein) and vessel 2 ($\sim\phi 40\mu\text{m}$, arteriole) are shown in the left and right small panels. Flow depths of $z_{1,2}=240, 330\mu\text{m}$ are given by 3D OCT. The cocaine-evoked CBF changes from the selected ROIs of 1 (large), 2 (small) and 3 (tissue perfusion by irresolvable capillary flows, e.g., $<\phi 30\mu\text{m}$) are shown in panels e)–f). t_b , t_p : baseline and elevated CBF periods. G) Statistical analysis of cocaine-induced CBF increase (%) in arteriolar (A), venous (V) and perfusion (T) flows.

Fig. 3.

3D DFR-OCT images of local CBF network in a rat cortical brain and its response to cocaine (1mg/kg, i.v.). a), b) Maximal intensity projection and 3D CBF images, respectively; c) Magnified view of a minute CBF marked by the blue box in the panels a) and b), indicating that the 3D DFR-OCT can detect subcortical vascular flow at 0.7–0.9mm under the cortical surface depending on flow size, flow rate and the projection angle with the incident light. d) Pie-cut graph of 3D DRF-OCT, e)–h) time course of flow changes in response to cocaine (1mg/kg, i.v.) in 4 typical CBFs of large deep ($\sim\phi 80\mu\text{m}$, $z\approx 430\mu\text{m}$, solid red), small deep ($\sim\phi 20\mu\text{m}$, $z\approx 670\mu\text{m}$, dashed pink), large superficial ($\sim\phi 70\mu\text{m}$, $z\approx 130\mu\text{m}$, solid blue), and small superficial ($\sim\phi 25\mu\text{m}$, $z\approx 50\mu\text{m}$, dashed blue). i) Normalized time course of cocaine-induced CBF changes in all 4 vessels. Small, deep flows tended to exhibit more pulsive variations in response to cocaine challenge.

Fig. 4.

DW-LSI of rat cortical arteriolar flow, venous flow, tissue perfusion changes in response to cocaine injection (1mg/kg, i.v.). Upper panels show the traces of the relative changes in CBF, [HbO₂], [HbR] and CBV (i.e., [HbT]) in selected ROIs marked by 3 arrows. t_b , t_p , t_r : time points at baseline, 20min and 40min after cocaine challenge corresponding to the 3 bottom panels, respectively. The rat was under α -chloralose anesthesia. H: head, T: tail, L: lateral.

Fig. 5. Cocaine-induced CBF (A₀–A₅) and $[Ca^{2+}]_i$ fluorescence (B₀–B₅) changes in a rat cortical brain. A₅, B₅) Time courses of CBF and Rhod2- $[Ca^{2+}]_i$ changes (masked Ca^{2+} fluorescence clouds were overlapped on LSI images) in response to cocaine. C) Raw baseline Ca^{2+} fluorescence image *in vivo* to indicate injection spot and Rhod2 distribution. D)–F) *Ex-vivo* cryosection fluorescence microscopic images ($4 \times$, $40 \times$) of the brain specimen after *in vivo* imaging to indicate the distribution of Rhod2 uptake of Ca^{2+} in the cortical brain. F) An immunostained fluorescence microscopic image to indicate intracellular Rhod2 localization in a neuronal cell *ex vivo*.

Fig. 6. Enhanced cerebral microvasculature with 3D OCT angiography. A) Surface image of a mouse cranial window. B) Maximum intensity projection of a full-field 3D OCT angiography within the cranial window. Although not quantitative, OCT angiography was able to visualize more detailed microvasculature than DFR-OCT. OCT Image size: $2.4 \times 2.4 \text{ mm}^2$