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Cocaine-Induced Abnormal Cerebral Hemodynamic Responses to Forepaw Stimulation Assessed by Integrated Multi-wavelength Spectroimaging and Laser Speckle Contrast Imaging

Wei Chen,

Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794, USA

Kicheon Park,

Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794, USA

Nora Volkow,

National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH), Bethesda, MD 20892, USA

Yingtian Pan, and

Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794, USA

Congwu Du

Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11794, USA

Abstract

Simultaneous imaging of cerebral hemodynamic changes in response to functional activation during drug intoxication provides a valuable strategy to assess cocaine induced neurovascular dysfunction. However, this requires tools with sufficient spatiotemporal resolution and adequate signal to noise ratio (SNR). Though several technologies have been developed to address this demand during functional brain activation, their spatiotemporal resolution has been compromised to preserve SNR. In this study, we combine spatiotemporal-domain laser speckle contrast analysis and image correlation techniques to integrate multi-wavelength spectroimaging and laser speckle contrast imaging (MW-LSCI). Experimental results show that optimized spatiotemporal resolution with enhanced SNR were achieved that enabled simultaneous measurement of multiple hemodynamic responses (i.e., HbO_2 , HbR , HbT and CBF) during cocaine administration. Specifically, cocaine-induced functional cerebral hemodynamic changes were accessed by measuring the activation responses to forepaw electrical stimulation at different times after cocaine administration. With improved spatiotemporal resolution and SNR, the system was able to differentiate the heterogeneity of cocaine's effects on the cerebral vasculature and on tissue metabolism, demonstrating the unique capability of MW-LSCI for various brain functional and pharmacological studies.

Index Terms

Biomedical optical imaging; blood flow measurement; brain stimulation; drug delivery

I. Introduction

Impaired neuronal activity is widely reported in cocaine abusers and many researches have shown that abnormal cerebral hemodynamic change may be the underlying cause [1, 2]. Simultaneous measurement of the hemodynamic changes after cocaine administration would provide valuable information in evaluating cocaine-elicited neurovascular defects. Various imaging modalities, for example, functional magnetic resonance imaging (fMRI) [3], and single photon emission computed tomography (SPECT) [4], have been applied to study this complicated problem. Constrained by their spatiotemporal resolutions, these technologies failed to resolve the rapid hemodynamic changes in small brain compartments such as individual blood vessels and perfusion flows. Recent advances in light sheet scanning multiphoton microscopy may significantly enhance the imaging rate without compromising spatial resolution [5]. However, the relatively small field of view (e.g., $1 \times 0.5 \text{ mm}^2$) restricts its applications in monitoring cerebral neurovascular network dynamics. Other new imaging methods such as optical coherence Doppler tomography [6] and photoacoustic microscopy [7] hold the potential to quantify cocaine induced neurovascular abnormalities, yet optical studies on the hemodynamic responses to brain activation after cocaine administration have not yet been reported.

Laser speckle contrast imaging (LSCI) is an optical imaging approach for blood flow estimation based on the analysis of dynamic speckle patterns induced by moving particles such as red blood cells in blood vessels [8]. Owing to its high spatiotemporal resolutions, LSCI is widely used in functional imaging studies, including mapping cerebral blood flow (CBF) in the cortex and monitoring the CBF changes during functional stimulations [9–11]. Furthermore, combining LSCI with spectroimaging – termed as integrated multi-wavelength spectroimaging and laser speckle contrast imaging (MW-LSCI) – provides the capability for simultaneously quantifying hemodynamics, e.g., changes in oxygenated- and deoxygenated hemoglobin (HbO_2 and HbR) and CBF [12, 13]. We recently reported the use of MW-LSCI for simultaneous measurement of the CBF, HbO_2 and HbR (and thus HbT) and their changes in response to acute cocaine. However, to our knowledge, the interaction between the activation responses (i.e., forepaw electrical stimulation) and the cocaine effects on hemodynamic and metabolic characteristics of brain have not yet been well studied [6]. Separation of cerebrovascular effects reflecting cocaine-induced vasoconstriction from those reflecting flow and metabolic changes due to activation requires both high signal to noise ratio (SNR) and sufficient spatiotemporal resolutions. Several LSCI techniques have been reported attempting to improve the SNR for flow detection, but they were at the risk of compromising either the spatial resolution or the temporal resolution [9, 14–15]. Therefore, a good strategy can be derived by combining both spatial and temporal domain LSCI to achieve a balance between the SNR enhancement and the preservation of the spatiotemporal resolutions, both of which are key to studying cocaine-elicited brain dysfunction.

In this animal study, we implemented the combination of spatiotemporal-domain LSCI reconstruction algorithms with image correlation in MW-LSCI to optimize SNR enhancement and high spatiotemporal resolutions tailored to assess cocaine effects on cortical brain function. Both the CBF responses and HbO_2 and HbR dynamics to forepaw

electrical stimulation before and after acute cocaine administration were recorded simultaneously, which allowed us to characterize cocaine's effects on brain function based on the hemodynamic responses. In addition, results also showed that the high spatiotemporal resolutions of MW-LSCI enabled us to differentiate the heterogeneity of cocaine's effects on cerebral vasculature and on tissue metabolism.

II. Method

A. Animal model

Male Sprague–Dawley rats (250–300 g/each, $n=12$ total) were used in the experiments. As shown in the Table I, 4 rats received 2-week cocaine (20mg/kg/day, *i.p.*) pretreatment (chronic cocaine group), 4 rats received 2-week saline (0.1cc/100g/day, *i.p.*) pretreatment (saline group) and 4 rats were cocaine naïve rats which received no pretreatment of either cocaine or saline except 1 dose of cocaine (1mg/kg, *i.v.*) challenge during the experiment (acute cocaine group).

During the surgical procedure, all animals were anesthetized and ventilated with 2% isoflurane mixed in pure oxygen. The left femoral artery was catheterized for blood pressure and blood gas monitoring and a femoral vein was catheterized for α -chloralose infusion or saline/cocaine administration during experiment. The stimulator (A-M System 2100) was connected to the animal through stimulation electrodes. A $5 \times 4 \text{ mm}^2$ cranial window was created on the contralateral side to expose the somatosensory motor cortex area (e.g., i.e., AP: +2mm to –3mm; LR: +2mm to +6mm), and agarose was immediately applied onto the cortex after dura was removed to fill the gap between brain and cover glass. 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/h through the femoral vein during the experiment. To investigate cocaine's effects on cortical brain function, a bolus of cocaine was administrated (1mg/kg, *i.v.*) to both the naïve cocaine group and the chronic cocaine group through the femoral vein. Similarly, a bolus of saline was administrated (0.1cc/100g, *i.v.*) for comparison. The physiological condition of the animal including mean arterial blood pressure (MABP), respiration rate and body temperature (PC-SAM monitor, SA Inc.) were continuously monitored and recorded in the image workstation. In addition, blood gases were regularly monitored to ensure the animal was within normal physiological condition. The experimental procedures were approved by the Institutional Animal Care and Use Committees of Stony Brook University.

B. MW-LSCI setup and image acquisition protocol

The schematic diagram of our MW-LSCI used to acquire all the image data presented in this study is illustrated in Fig. 1, in which both the optical setup (a) modified based on a zoom fluorescence microscope (AZ100, Nikon) with a $2 \times$ Plan APO objective to allow for cranial imaging over a large field of view (e.g., $\text{FOV} \sim 4 \times 5 \text{ mm}^2$), long working distance ($\text{WD} = 45 \text{ mm}$) and a relatively high NA ($\text{NA} = 0.22$). The schematics for sequential multi-wavelength LEDs/LD illumination and sCMOS camera synchronization (b), and the image processing approaches (c) are also demonstrated in Fig. 1.

Specifically, a custom multi-channel light engine (Fig. 1a) consisting of two LEDs at 568nm and 630nm (Spectra Light Engine, Lumencor) coupled in a fiber bundle and a laser diode at 830nm (DL8142-201-830, Thorlabs) coupled in a single-mode fiber were employed to sequentially illuminate the cranial window on the rat's somatosensory motor cortex. A custom TTL-pulse sequence was programmed to synchronize the 14-bit sCMOS camera (pixel size: 6.5 μ m; Zyla4.2, Andor) with the time-sharing multi-wavelength illumination at an imaging rate of up to 12.5fps per channel by the image workstation via a time base (PCI-6221, NI). The exposure time for HbO₂ and HbR acquisitions was set to be 10ms, and the same exposure was set for LSCI to gain sufficient sensitivity for detecting slow perfusion flows.

All of the acquired multi-channel images were then streamed to the image workstation for image processing to extract 2D images of CBF, HbO₂ and HbR in response to forepaw stimulation. For each animal, a total of 17 forepaw stimulation trials with a 2-min separation were conducted over a period of 32min (i.e., from -4min before to 28min after a bolus cocaine injection at t=0min) as illustrated in Fig. 1b. For each trial, multiple-channel images were acquired for 60s shown as the shaded gray periods in Fig. 1b, during which forepaw electrical stimulation started at t=35s and lasted t=5s as shown by the shaded yellow periods in Figs. 1(b-d). The paradigm of the electrical forepaw stimulation was: 0.3ms pulse width, 2mA peak-to-peak amplitude, 3Hz repetition rate over 5s duration. Three trials of forepaw stimulation (e.g., at t=-4, -2, 0 min) before a bolus *i.v.* injection of either cocaine or saline (the shaded red period in Fig. 1b) were included as baseline data. During post-image processing, the hemodynamic responses for each trial were quantified as the relative changes (e.g., HbO₂, HbR and CBF) in percentage against their mean basal or resting values (e.g., HbO_{2b} and CBF_b) at t=20s before the forepaw stimulation (Fig. 1c).

C. Spatiotemporal-domain LSCI analysis to optimize spatiotemporal resolutions and SNR

LSCI is a full-field photographic imaging approach for blood flow imaging, which is based on extracting the dynamic laser speckle contrast. As has been previously outlined [8], if a Lorentzian velocity profile is assumed, the typical mathematic relationship between the blood flow rate and the dynamic speckle contrast constant K can be derived as,

$$K = \frac{\sigma}{\langle I \rangle} = \left[\frac{\tau_c}{2T} (1 - \exp(-\frac{2T\tau_c}{\tau_c})) \right]^2 \quad (1)$$

where $\tau_c = 1/(ak_0v)$ is the correlation time, T is the camera exposure time, v is the flow rate or speed, k_0 is the wavenumber, and a is an unknown factor that relates to the optical properties and the speed distribution of moving scatterers. However, due to residual laser speckles, multiple scattering, and tissue optical heterogeneity, the reconstructed CBF image is usually very noisy and requires additional image processing to enhance the detection SNR.

For detection of weak CBF response to forepaw stimulation that requires both high sensitivity and spatiotemporal resolutions, we have found that a spatiotemporal-domain

LSCI reconstruction algorithm (st-LSCI) can be well suited for the study. Figs. 2(a–c) compare 3 different algorithms, i.e., spatial-domain LSCI (s-LSCI), temporal-domain LSCI (t-LSCI), and st-LSCI for flow imaging reconstruction. Instead of calculating the laser speckle contrast solely from adjacent pixel binning ($N_s \times N_s$) within each frame or one pixel across adjacent frames ($1 \times N_t$), st-LSCI utilizes pixels directly from an $N_s \times N_s \times N_t$ cube to calculate the speckle contrast and can thus be expressed as

$$K_{i,j,t}^2 = \frac{\delta^2}{\langle I \rangle^2}$$

$$\delta^2 = \frac{1}{(1+n)^2} \sum_{x=i-N_s/2}^{i+N_s/2} \sum_{y=i-N_s/2}^{i+N_s/2} \sum_{t=i-N_t/2}^{i+N_t/2} I_{x,y,t}^2 - \left(\frac{1}{(1+n)^2} \sum_{x=i-N_s/2}^{i+N_s/2} \sum_{y=i-N_s/2}^{i+N_s/2} \sum_{t=i-N_t/2}^{i+N_t/2} I_{x,y,t} \right)^2$$

$$\langle I \rangle = \frac{1}{(1+n)^2} \sum_{x=i-N_s/2}^{i+N_s/2} \sum_{y=i-N_s/2}^{i+N_s/2} \sum_{t=i-N_t/2}^{i+N_t/2} I_{x,y,t}$$

(2)

where $I_{x,y,t}$ is the intensity of a pixel (x, y) within the t-th frame and $n = N_s \times N_s \times N_t$ is the total pixel number contained in the cube.

Figs. 2(a', b', c') are the LSCI maps acquired from a rat and reconstructed using s-, t-, and st-LSCI algorithms, respectively. The effective spatial resolution is 45.5 μ m ($N_s=7$) in s-LSCI image, 6.5 μ m in t-LSCI image, and 19.5 μ m in st-LSCI image, respectively; therefore, st-LSCI provides a good balance between high SNR and high spatial resolution for CBF imaging. Fig. 2d compares the 3 time traces of CBF within the ROI (white circle) reconstructed by the 3 algorithms, respectively. The effective temporal resolution for s-, t-, and st-LSCI are 80ms, 560ms, and 160ms; thus, st-LSCI better balances between high SNR and high temporal resolution to detect CBF dynamics.

D. HbO₂/HbR quantification with cross correlation to improve the measurement of the hemodynamic responses

The dynamics in HbO₂ and HbR can be determined by the measurements at $\lambda_1=560$ nm and $\lambda_2=630$ nm under the assumption that HbO₂ and HbR dominate the changes of light attenuation (e.g., absorption) within these two wavelength ranges in the measured diffuse reflectance, which can be derived as [16]

$$\begin{bmatrix} \Delta HbO_2 \\ \Delta HbR \end{bmatrix} = \begin{bmatrix} \varepsilon_{HbO_2}^{\lambda_1} & \varepsilon_{HbR}^{\lambda_1} \\ \varepsilon_{HbO_2}^{\lambda_2} & \varepsilon_{HbR}^{\lambda_2} \end{bmatrix}^{-1} \begin{bmatrix} \ln(R_{\lambda_1}(0)/R_{\lambda_1}(t))/L_{\lambda_1}(t) \\ \ln(R_{\lambda_2}(0)/R_{\lambda_2}(t))/L_{\lambda_2}(t) \end{bmatrix} \quad (3)$$

where $\varepsilon_{HbO_2}^{\lambda_1}$, $\varepsilon_{HbR}^{\lambda_1}$, $\varepsilon_{HbO_2}^{\lambda_2}$ and $\varepsilon_{HbR}^{\lambda_2}$ are the molar extinction coefficients for HbO₂ and HbR at the two wavelengths, $R_{\lambda_1}(t)$ and $R_{\lambda_2}(t)$ are the measured diffuse reflectance matrices (2D images) at these two wavelengths, and $L_{\lambda_1}(t)$ and $L_{\lambda_2}(t)$ are the pathlengths of light propagation, which is assumed to be approximately equivalent because the wavelengths of λ_1 and λ_2 are close (i.e., $\lambda_1 - \lambda_2 < 100$ nm).

Similarly, because of high tissue heterogeneity in cortical brain and speckle noise (mostly in CBF channel), the measured hemodynamic responses often suffer low SNR (e.g., Fig. 3c) and poorly defined boundary to identify the forepaw-response brain region. To solve this problem, we implemented a masking approach derived from cross-correlation analysis. For each pixel (x, y) in the time-lapse image stacks, a threshold (e.g. Pearson correlation coefficient =0.8) was set to calculate the cross correlation $C(x, y)$ between the signal sequence and an activation function, which can be given as:

$$C_i(x, y) = \text{corr}[I(x, y, t), f(t)] \quad (4)$$

where $I(x, y, t)$ is the intensity profile for pixel (x, y) , $f(t)$ is a reference vector or activation function (e.g., $f(t)=0.1$, $t<35$ and $t>45$; $f(t)=1$, $35<t<45$: activation period). If $C(x, y) \geq 0.8$, the pixel was regarded as an effectively activated spot during stimulation to be included into the response mask. For CBF images or animals with weak responses or poor SNR which resulted in scattered or nonunified masks, additional t-test between baseline and peak-activation periods was performed to derive the activation mask [17] (e.g., pixels with $p<0.05$).

For each hemodynamic response, a cross-correlation mask was defined from the stimulation trials during the baseline period (i.e., baseline trials). Once it was generated, the same mask was applied to the following stimulation trials (e.g., after cocaine administration) for simplicity and consistency.

Figs. 3(a–c) show the peak hemodynamic responses in HbO_2 , HbR , and CBF evoked by forepaw stimulation. Although HbO_2 (Fig. 3a) in the middle is evident (e.g., up to 5% increase), it is difficult to objectively draw the boundary of the response region without masking. For weaker change in the HbR (Fig. 3b) or noisy CBF image (Fig. 3c), detection of the forepaw response margin is even more difficult, which can be well apprehended by the low SNR (e.g., black trace for CBF) in Fig. 3d. By applying the correlation mask, the response regions can be well defined and affirmatively overlaid on the HbO_2 , HbR , and CBF images as shown Figs. 3(a'–c'). As a result of increased SNR and more effective averaging over the well-defined ROIs, the temporal response profiles in Fig. 3d' show better SNR, especially for HbR and CBF channels.

E. Statistical Analysis

Results were reported as mean $\pm$ standard error (SEM). Comparison of the forepaw responses in baseline between different groups was conducted by using one-tailed student's t-test. Differences of a hemodynamic response to forepaw from the baseline level within the same group were evaluated by the repeated measurement one-way analysis of variance (RM ANOVA). Paired t-test was used to compare two different time periods (e.g., baseline vs. 10–14 min) within the same group. Significance levels were set at $p < 0.05$ for all analyses.

III. Result

As outlined in Table 1, 3 groups of animals (control, naïve, and chronic cocaine, $n=4$ per group), totaling 12 rats, were used in the experiment to simultaneously track forepaw-evoked hemodynamic changes (HbO₂, HbR, HbT, and CBF) before and after a saline (control) or cocaine challenge (naïve and chronic). Fig. 4–Fig. 7 summarize the results of our *in vivo* image studies of the cocaine effects on forepaw stimulation.

Fig. 4 compares the differences in forepaw stimulation evoked HbO₂ responses before and after administration of saline or acute cocaine (1mg/kg, *i.v.*) among these 3 groups. Each row (top: control, middle: naïve, bottom: chronic) in Fig. 4a represents the time-lapse

HbO₂ responses to forepaw stimulations, from time periods of baseline (−4 to 0 min) to 2–6 min, 10–14 min, and 20–24 min after a bolus injection of saline or cocaine. All images are presented in percentage (dynamic range: from −5% to 5%) and overlaid with the correlation mask as described in the Method. The results in Fig. 4b, i.e., HbO₂ averaged within the correlation mask, indicate that the baseline forepaw evoked HbO₂ ($t<0$ min) in the chronic cocaine group ($3.3\pm0.6\%$) was $28\pm3.2\%$ ($p<0.001$) lower than that of the control ($4.6\pm0.24\%$) and the naïve ($4.3\pm0.2\%$) groups. Saline injection did not change the HbO₂ response ($p=0.71$) as expected. However, acute cocaine resulted in a dramatic decrease in the

HbO₂ response within 4–8 min after injection, e.g., a $-65\pm12.3\%$ reduction to $1.5\pm0.5\%$ for naïve cocaine group ($p<0.001$) and a $-62\pm16.6\%$ reduction to $1.26\pm0.45\%$ for the chronic cocaine group. Cocaine induced a persistent disturbance to the HbO₂ responses to forepaw stimulation which did not recover within the entire observation window (>28 min). Fig. 4c summarizes the statistical comparisons.

Fig. 5 compares the differences in forepaw stimulation evoked HbR responses before and after administration of saline or acute cocaine (1mg/kg, *i.v.*) among these 3 groups. The results in Fig. 5b indicate that the baseline forepaw evoked HbR decrease ($t<0$ min) in the chronic cocaine group ($-1.4\pm0.25\%$) was $22\pm2.3\%$ ($p<0.001$) lower than that of the control ($-1.8\pm0.06\%$) and naïve ($-2.0\pm0.13\%$) groups. Saline injection did not change the HbR response ($p=0.46$), but acute cocaine resulted in a remarkable decrease in the HbR response, e.g., a $-83\pm12.4\%$ reduction to $-0.34\pm0.08\%$ within ~4min after the injection for naïve cocaine group ($p<0.001$), and a $-43\pm6.3\%$ reduction to $-0.79\pm0.09\%$ within ~6min after the injection for chronic cocaine group. These decreases did not recover for at least 28min after the cocaine injection.

Fig. 6 compares the differences in forepaw stimulation evoked HbT responses before and after administration of saline or acute cocaine (1mg/kg, *i.v.*) among these 3 groups. The results in Fig. 6b indicate that the baseline forepaw evoked HbT ($t<0$ min) in the chronic cocaine group ($2\pm0.36\%$) was $28\pm3.7\%$ ($p<0.001$) lower than that of the control ($2.8\pm0.22\%$) and naïve ($2.7\pm0.13\%$) groups. Saline injection did not change the HbT response ($p=0.82$), but acute cocaine resulted in a marked decrease in the HbT response within ~4min after the injection, e.g., a $-52\pm5.2\%$ reduction to $1.3\pm0.2\%$ for naïve cocaine group ($p<0.001$), and a $-65\pm15\%$ reduction to $0.7\pm0.2\%$ for chronic cocaine group. These decreases did not recover after 28min of the cocaine injection.

In addition, Fig. 7 compares the differences in forepaw stimulation evoked CBF responses before and after administration of saline or acute cocaine (1mg/kg, *i.v.*) among these 3 groups. All images in Fig. 7a are presented in percentage with a dynamic range from 0% to 30%, in which the forepaw evoked CBF increases were observed in both major vessels and the avascular brain tissue (tissue perfusion) within the cross correlation mask. Fig. 7b shows that the chronic group exhibited a significantly ($28\pm3.7\%$, $p<0.001$) lower baseline forepaw evoked CBF ($16.5\pm1.3\%$) than that of the control ($24.7\pm1.4\%$) and the naïve ($26.7\pm1.0\%$) groups (no significant difference between these two groups, $p=0.22$). Again, saline injection did not change the CBF response ($p=0.812$). However, acute cocaine induced a $-70\pm8\%$ reduction in the CBF response down to $7.9\pm1.5\%$ within ~ 4 min after injection for the naïve group ($p<0.001$), which followed a slow recovery to $19.1\pm6.1\%$ at $t=28$ min that was still lower than baseline level ($p<0.05$). For the chronic cocaine group, the already dramatically low baseline CBF response ($16.5\pm1.3\%$) was further decreased $-41\pm9.8\%$ to $10.2\pm2.6\%$ ($p=0.03$) with 4–6min after the injection, which started to recover to $14.1\pm 1.3\%$ ($p=0.18$) at $t>10$ min after cocaine injection.

IV. Discussion

The simultaneous evaluation of cocaine's effects on the dynamic responses to functional activation on CBF and on oxygen hemoglobin concentration during forepaw stimulation has been challenging. This is relevant in order to study how the direct effects of cocaine on blood vessels influence the brain activation responses measured with CBF. Here we used multi-wavelength spectroimaging integrated with laser speckle contrast imaging to simultaneously measure metabolic and hemodynamic changes. Several techniques had previously been developed with different emphasis on improving either effective spatial resolution [14], temporal resolution, or SNR [18–20]. While these technologies indeed demonstrated their advantage in one of those aspects, they had trade-off between high spatiotemporal resolutions and detection sensitivity. In functional brain activation studies the hemodynamic responses have often been averaged across several stimulation trials in order to improve SNR without sacrificing temporal resolution with the assumption that animal hemodynamic condition (e.g., CBF) did not change [13, 21]. This strategy does not apply to studies that investigate the effects of drug (e.g., cocaine) challenges as CBF changes (due to the vasoconstriction effect of cocaine) with time after drug administration. In this study, we combined spatiotemporal-domain laser speckle contrast imaging reconstruction with image cross correlation to further improve the system's SNR and spatiotemporal resolutions.

As shown in Figs. 2(a'–c'), the effective spatial resolution in the s-LSCI method is only $45.5\mu\text{m}$ ($N_s=7$, Fig. 2a') while the effective spatial resolution for t-LSCI and st-LSCI is $6.5\mu\text{m}$ and $19.5\mu\text{m}$, as shown in Fig. 2b' and Fig. 2c', respectively. These improvements in spatial resolution are evident compared to s-LSCI method whereas the difference in spatial resolution between t-LSCI and st-LSCI is insignificant as the evident microcirculations can be resolved with both methods (Fig. 2b' and Fig. 2c'). In order to maintain the accuracy of LSCI analysis for flow quantification based on a fully developed speckle pattern, sufficient pixel numbers across frames should be included, which compromises the temporal resolution in the t-LSCI method [22]. For a similar size of pixels ($N_f=7$) used for the s-LSCI method, the temporal resolution was 560ms for t-LSCI; this was improved to 160ms ($N_s=3$,

$N_f=2$) for st-LSCI. The SNR is significantly improved in both t-LSCI and st-LSCI methods compared to the s-LSCI method (Fig. 2d). However, more sampling points used in t-LSCI method scarify its time resolution in reflecting the CBF dynamics (temporal profile) after stimulation. Thus, a time retardant in CBF (blue line in Fig. 2d) was observed with t-LSCI. In contrast, st-LSCI is capable of revealing the rapid CBF increase after forepaw stimulation with improved SNR, which demonstrates its advantage for enhancing the temporal resolution.

Generally, a difference/ratio method would be used to identify the hemodynamic response regions on the cortex and a random but constant ROI would be used to quantify the changes from forepaw stimulation. Though this technique can provide a general idea about the activated cortical regions, it usually fails to define the boundaries where the responses occur. Some artifacts or errors may also be included in the quantification due to substantial spontaneous hemodynamic oscillations and instrument noise. The obvious advantage of the correlation-based ROIs is an unbiased selection to represent the whole evoked cortical brain region from forepaw stimulation. As compared in the Fig. 3d and Fig. 3d', without the correlation mask, it would be difficult to tell the rising point of forepaw evoked oxygenation hemoglobin concentration changes (e.g., HbO_2 , the red curve in Fig. 3d or CBF, the black curve in Fig. 3d). As a result, the dynamical range of the hemodynamic responses to forepaw stimulation, were more clearly identified after applying the correlation mask. The SNR also increased benefitting from excluding irrelevant pixels from a 27.2% increase without correlation mask to 36.1% with the correlation mask in CBF. From Fig. 3d', with the correlation mask we can also tell that after forepaw stimulation, CBF increased first followed by the increase in HbO_2 and the decrease in HbR . These advances improve the accuracy of functional brain imaging studies, such as forepaw stimulations in animals subjected to various pharmacological exposures as shown here for acute and chronic cocaine.

The animal study here validates those improvements in the system and its feasibility to conduct accurate quantification of cocaine induced abnormal functional cerebral hemodynamic responses. Constant decreases in the oxygenation hemoglobin concentration in response to forepaw stimulation were observed after cocaine administration in both the naïve and chronic groups. While the chronic cocaine group went through a relatively slow decrease to the minimum value ~16 min after cocaine administration, the naïve cocaine group showed a rapid decrease to its minimum value at 4–8 min. The drop in oxygenation hemoglobin responses were not recovery till 28 min after cocaine injection ($p<0.001$). A similar decrease was also observed in the CBF responses whereas the CBF responses partially recovered for the naïve group at 28 min and fully recovered for the chronic group at around 20 min after cocaine administration ($p=0.18$). However, since the CBF activation prior to cocaine was already lower in the chronic group the recovery in the chronic group after acute cocaine returned to levels that were still lower than the baseline CBF activation responses in the naïve or controls rats. Similarly, hemoglobin responses were weaker in the chronic cocaine group compared to those in the control group during the baseline period. Using our system, we successfully differentiated the heterogeneity of cocaine's effects on vasculature and tissue metabolism enabling us to compare the effects of acute cocaine in naïve versus chronically cocaine exposed rats.

It is noteworthy that for each hemodynamic change, a mask for activation area generated at baseline was used post cocaine periods for simplicity. Although cocaine reduced the activation area, this method did not result in missing assessment of cocaine efforts. Nevertheless, cocaine-induced area change of forepaw stimulation evoked responses may also be of interest for studying cocaine-induced brain dysfunction. This requires calculation of activation masks at each time point [23] and will be implemented in our future studies.

V. Conclusion

Combining laser speckle contrast imaging technique and multi-wavelength spectroimaging, we performed simultaneous measurement of cerebral hemodynamic changes in response to forepaw stimulation prior to and during cocaine intoxication in naïve rats versus rats chronically exposed to cocaine. Using spatiotemporal-domain LSCI image reconstruction algorithm and cross correlation masking, we improved the spatiotemporal resolutions and the SNR. Our experiment results obtained from *in vivo* animal imaging studies demonstrated the advantages of our methods in resolving the rapid changes in hemodynamics during forepaw stimulation along with an acute cocaine challenge. Using this system we were able to uncover that cocaine induced abnormal functional cerebral hemodynamic responses to forepaw stimulation in both naïve and chronic cocaine exposed rats.

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Biographies

Wei Chen received his B.S. degree in optical information of science and technology from Huazhong University of Science and Technology, Wuhan, China, in 2010 and M.S. degree in optical engineering from Wuhan National Lab for Optoelectronics, Wuhan, China, in 2012. He is currently pursuing his Ph.D. degree in Biomedical Engineering at Stony Brook University, New York, USA. Currently, he focuses on optimization of laser speckle imaging technology and optical coherence tomography for brain functional imaging of living animals.

Yingtian Pan, PhD, is professor of the biomedical engineering at Stony Brook University, New York. He received his PhD in 1992 from the National Lab of Laser Technologies, Huazhong University of Science and Technology, China. Since then, he has been conducting research and development in advanced biophotonic imaging techniques, as well as their applications for biological tissues *in vivo*. His current research focuses on optical coherence tomography, laser-scanning endoscopy, and 3D Doppler tomography. Preclinical and clinical applications in his lab include early diagnoses of bladder cancer, interstitial cystitis, tissue engineering growth and high-resolution 3D imaging of cerebral hemodynamics.

Congwu Du, Ph.D., is professor in the Department of Biomedical engineering, Stony Brook University, New York. She was trained as a biomedical engineer at Univ. of Luebeck,

Germany, and has spent 20 years in the application of optical spectroscopy and imaging for characterization and detection of physiological processes in the brain and heart. Her current projects include the simultaneous detection of the cerebral blood flow, blood volume and oxygenation as well as intracellular calcium in vivo using fluorescence and photon migration techniques. Her long-term goal is to develop optical imaging tools and to combine with other imaging modalities such as OCT, MRI and PET for brain functional studies.

Kicheon Park is a Research Support Specialist in Stony Brook University. He received his B.S and M.S. in Biomedical Engineering from Stony Brook University, New York in 2009 and 2011. Since graduation, he has been applying various imaging techniques for brain studies including laser speckle and optical coherence tomography. Currently his research focuses on drug-addicted brain function through in vivo fluorescence or hemodynamics imaging.

Nora D. Volkow, M.D., is Director of the National Institute on Drug Abuse (NIDA) at the National Institutes of Health. NIDA supports most of the world's research on the health aspects of drug abuse and addiction.

Dr. Volkow's work has been instrumental in demonstrating that drug addiction is a disease of the human brain. Dr. Volkow pioneered the use of brain imaging to investigate the toxic effects and addictive properties of abusable drugs. Her studies have documented changes in the dopamine system affecting, among others, the functions of frontal brain regions involved with motivation, drive, and pleasure in addiction. She has also made important contributions to the neurobiology of obesity, ADHD, and aging.

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Fig. 1.

A schematic illustration of the MW-LSCI setup and image acquisition protocol. (a) MW-LSCI setup with a laser diode at 830nm for imaging the CBF change (ΔCBF) and two LEDs at 560nm and 630nm for imaging HbO_2 and HbR dynamics (i.e., HbO_2 and HbR); (b) The pulse sequence protocol to illustrate the acquisition of trials at baseline (from -4min to 0min) and after cocaine administration (from 0min to 28min), where the yellow shaded periods are for forepaw stimulations and grey shaded periods are for image acquisitions; (c) The relative changes (in unit of %) in ΔCBF and in ΔHbO_2 per trial, i.e., the responses to a brain stimulation against their resting-state values illustrated in both the images at a specific time point and as a function of time from a specific ROI (white boxes as pointed by arrows).

Fig. 2.

LSCI image reconstruction. (a, b, c): schematic illustration of image reconstruction algorithms for spatial-domain LSCI (s-LSCI), temporal-domain LSCI (t-LSCI), and spatiotemporal-domain LSCI (st-LSCI) analyses; (a', b', c'): a comparison to show the advantage of st-LSCI vs s-LSCI to preserve high spatial resolution for CBF imaging; (d) a comparison to show st-LSCI (red trace) vs t-LSCI (blue trace) and s-LSCI (black trace) to enhance the temporal resolution and the SNR for CBF imaging (perfusion flow changes).

Fig. 3.

Forepaw stimulation evoked hemodynamic responses (HbO_2 , HbR , CBF) reconstructed without and with cross-correlation masking. (a–c) peak hemodynamic responses calculated without cross-correlation masking; (a'–c') peak hemodynamic responses with cross-correlation masking; (d, d') time courses of hemodynamic responses to the forepaw stimulation without and with cross-correlation masking, respectively. Apparently, masked analysis yields improved SNR.

Fig. 4.

HbO₂ responses to forepaw stimulation before and after saline/cocaine challenge. (a) Time-lapse images of masked HbO₂ response maps overlaid with LSCI images for 3 different groups. (b) The averaged HbO₂ responses with time. (c) Statistical comparisons of the average HbO₂ response levels before and after a saline/cocaine challenge. p*, p**<0.001. Cocaine significantly reduced the HbO₂ increases evoked by forepaw stimulation.

Fig. 5.

HbR responses to forepaw stimulation before and after saline/cocaine challenge. (a) Time-lapse images of masked HbR response maps overlaid with LSCI images for 3 different groups. (b) The averaged HbR responses with time. (c) Statistical comparisons of the average HbR response levels before and after a saline/cocaine challenge. p^* , $p^{**}<0.001$. Cocaine significantly reduced the HbR decreases evoked by forepaw stimulation.

Fig. 6.

HbT responses to forepaw stimulation before and after saline/cocaine challenge. (a) Time-lapse images of masked HbT response maps overlaid with LSCI images for 3 different groups. (b) The averaged HbT responses with time. (c) Statistical comparison of the average HbT response levels before and after a saline/cocaine challenge. p^* , $p^{**} < 0.001$. Cocaine significantly reduced the HbT increases evoked by forepaw stimulation.

Fig. 7.

CBF responses to forepaw stimulation before and after saline/cocaine challenge. (a) Time-lapse images of masked CBF response maps overlaid with LSCI images for 3 different groups. (b) The averaged CBF responses with time. (c) Statistical comparison of the average CBF response levels before and after a saline/cocaine challenge. $p^* < 0.001$, $p^{**} = 0.05$. Cocaine significantly reduced the CBF increases evoked by forepaw stimulation for naïve rats only. A less significant effect in chronic rats could reflect their very low CBF reactivity to forepaw stimulation even prior to acute cocaine.

TABLE I

Animal groups and experiment paradigms

Group#	Pretreatment (2 weeks daily)	Drug challenge (during experiment)
Control (n=4)	Saline (0.9% NaCl, 0.1cc/100g, <i>i.p.</i>)	Saline (0.9% NaCl, 0.1 cc/100g, <i>i.v.</i>)
<i>Naive (n=4)</i>	None	Cocaine hydrochloride (1mg/kg, <i>i.v.</i>)
<i>Chronic (n=4)</i>	Cocaine (20mg/kg, <i>i.p.</i>)	Cocaine hydrochloride (1mg/kg, <i>i.v.</i>)