Cocaine-induced ischemia in prefrontal cortex is associated with escalation of cocaine intake in rodents

Congwu Du1 · Nora D. Volkow2 · Jiang You1 · Kicheon Park1 · Craig P. Allen1 · George F. Koob3 · Yingtian Pan1

Received: 6 April 2018 / Revised: 13 August 2018 / Accepted: 6 September 2018 / Published online: 3 October 2018
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Abstract
Cocaine-induced vasoconstriction reduces blood flow, which can jeopardize neuronal function and in the prefrontal cortex (PFC) it may contribute to compulsive cocaine intake. Here, we used integrated optical imaging in a rat self-administration and a mouse noncontingent model, to investigate whether changes in the cerebrovascular system in the PFC contribute to cocaine self-administration, and whether they recover with detoxification. In both animal models, cocaine induced severe vasoconstriction and marked reductions in cerebral blood flow (CBF) in the PFC, which were exacerbated with chronic exposure and with escalation of cocaine intake. Though there was a significant proliferation of blood vessels in areas of vasoconstriction (angiogenesis), CBF remained reduced even after 1 month of detoxification. Treatment with Nifedipine (Ca2+ antagonist and vasodilator) prevented cocaine-induced CBF decreases and neuronal Ca2+ changes in the PFC, and decreased cocaine intake and blocked reinstatement of drug seeking. These findings provide support for the hypothesis that cocaine-induced CBF reductions lead to neuronal deficits that contribute to hypofrontality and to compulsive-like cocaine intake in addiction, and document that these deficits persist at least one month after detoxification. Our preliminary data showed that nifedipine might be beneficial in preventing cocaine-induced vascular toxicity and in reducing cocaine intake and preventing relapse.

Introduction
Deficits in the function of the prefrontal cortex (PFC) play a crucial role in promoting compulsive cocaine use in humans [1]. Prefrontal dysfunction is in part attributable to cocaine-induced disruption of dopamine striato-cortical circuits [2]. However, cocaine’s direct disruption of cerebral blood vessels and cerebral blood flow (CBF) is also likely to contribute to and exacerbate hypofrontality. Indeed, cocaine abusers are at higher risk of ischemic and hemorrhagic strokes in the brain than non-abusers [3–5] and imaging studies in cocaine abusers have documented marked decreases in CBF, which are most prominent in the PFC [6]. Similarly, studies in rodents have shown that chronic-cocaine triggers vasoconstriction, reduces CBF and results in cerebral ischemia [7]. However, the extent to which cocaine-induced CBF decreases affect neuronal activity in the PFC and contribute to compulsive-like cocaine taking and the extent to which the CBF decreases recover with detoxification have been minimally investigated [7].

To investigate the changes in the cerebrovascular system with cocaine exposure and their rate of recovery with detoxification along with their relationship to compulsive cocaine intake we used an integrated optical imaging tool in rodents. We previously reported, using ultrahigh-resolution optical coherence Doppler tomography (µODT) for 3D imaging of CBF, that acute cocaine reduced cortical CBF and that repeated injections within the same session (two to
three doses) exacerbated these deficits triggering cortical microischemia in the mouse brain [8, 9]. However, such studies were done in the somatosensory cortex (not the PFC), cocaine was noncontingently administered (rather than self-administered) and it was given acutely (3 doses within 3 h). To address these limitations, here we performed two parallel studies that assessed the effects of chronic cocaine in neurovascular networks in the PFC: one in a rat model that allowed us to assess the effects of compulsive-like cocaine self-administration (long access, LgA) [10], and the other in a longitudinal mouse model that allowed us to monitor changes as a function of chronicity and withdrawal. The LgA model emulates the compulsive-like pattern of cocaine intake characteristic of cocaine addiction [11, 12]. In this model rats are allowed to intravenously self-administer cocaine with extended (long) access (LgA, 6 h/day, 0.5 mg/kg/injection), which increases their motivation to take more cocaine leading to escalation in intake. The longitudinal mouse model uses an implanted cranial window on the PFC to serve as comparison, which allowed us to assess cocaine-induced changes as a function of chronicity and to assess if the changes recovered following withdrawal. Finally, we examined whether pretreatment with nifedipine, a Ca$^{2+}$ antagonist and vasodilator, could prevent cocaine-induced changes in CBF and reduce cocaine-self-administration and prevent reinstatement of cocaine seeking.

We hypothesized that both cocaine self-administration and noncontingent cocaine administration would result in vasoconstriction, reductions in CBF, and ischemia in the PFC and that prevention of vasoconstriction and ischemia by nifedipine would reduce escalation of cocaine self-administration and prevent reinstatement of cocaine seeking. We also hypothesized that without treatment cocaine-induced reductions in CBF and neuronal activity would persist after detoxification.

Fig. 1 Self-administration of cocaine decreased CBF, but increased vascular density in prefrontal cortex (PFC). Rat model for assessing the effects of long access (LgA) cocaine self-administration on the prefrontal cortex in j. Rats self-administered cocaine (Groups 2 and 3) under a fixed ratio 1 schedule (0.5 mg/kg/injection) to compare with the controls (Group 1). a, e Representative images of quantitative cerebral blood flow velocity (CBFv) in the PFC in control and LgA rats, respectively, illustrating the global CBFv decrease in the LgA animal. b, f Representative images of vasculature angiographies of the PFC in control and LgA rats, and their skeletonized vasculature maps are presented in c and g illustrating the vascular density increase in the PFC of the LgA rat. d Increased drug intake in LgA during the escalation period (red curve, n = 6) versus a stable drug intakes in ShA (blue curve, n = 7). Left axis: number of injection per session, right axis: mg/kg/session. h Significant CBFv decreases in CBFv in medium vessels (ϕ = 124.27 ± 16.09 µm) between control (n = 6) and LgA (n = 6) rats. i Statistical comparisons of the fill factor (FF) of vasculature indicating that the vascular density was significantly increased in LgA compared to controls. k Correlation of the mean CBFv with the total dose of cocaine intake (in log10 of mg/kg), indicating a moderately strong association (correlation coefficient r = −0.6, p = 0.008) between the CBFv and the amount of cocaine administered to the animals.
Materials and methods

Animal preparation and physiology measurement

All procedures were carried out in accordance with animal protocols approved by the Institutional Animal Care and Use Committees of Stony Brook University. A total of 83 animals were used, including 53 rats and 30 mice, for the various experiments in this study (for detail, see Supplemental 1, Table S1). For all animals (rats or mice), cocaine pretreatment was conducted while animals were awake. Imaging was conducted while animals were anesthetized with 1.5–2.5% isoflurane in a 60–70% O₂/air mixture either by intubation (rats) or face mask (mice).

For the rat study, we continuously monitored physiological measures during the imaging procedure using a Small Animal Instrument (Module 224002) to record heartbeat, mean arterial blood pressure (MABP), respiration rate, and body temperature. The end-tidal CO₂ was monitored continuously using a Poet IQ2, (Criticare Technologies). Rats were kept under stable conditions, including MABP, body temperature, respiration, and heart rate. The end-tidal CO₂ was kept in the normocapnic range (~35–45 mmHg, Supplementary 6, Table S2).

For the mouse study, heartbeat, respiration rate, and body temperature were measured noninvasively with a Small Animal Instrument (Module 224002) during imaging. However, the small blood volume of mice precluded measurements of blood gases during the imaging experiments since frequent bleeding could result in hypovolemia. Instead, to evaluate for potential effects of chronic cocaine on blood gases we assessed the effects in a separate group of control mice (saline, ~0.1 cc/100 g/day, i.p. n = 3) and chronic cocaine exposed mice (cocaine, 30 mg/kg/day, i.p. n = 3) pretreated for ~4 weeks (for detail procedures, see Supplementary 6). In these mice, pH, pCO₂ and pO₂ did not differ between groups and remained normocapnic for both control and cocaine-exposed mice (Table S3).

Studies in the rat model

The experiments were performed in Wistar male rats, and the self-administration protocols and timelines for imaging are illustrated in Fig. 1j. The animals were implanted with chronic intravenous (i.v.) catheters and chronically exposed to either saline (control group) or cocaine (ShA, LgA groups) for 26 consecutive days via intravenous self-administration. After first 7–9 days of cocaine self-administration, ShA rats received a daily 1 h session under a fixed-ratio schedule (FR1 with a 20 s timeout) for the duration of ~17 days pretreatment period, whereas the LgA group received a daily 6 h session also under FR1, during this period. Groups were assigned to balance responding during first 7–9 days. For this purpose, the rats were trained to self-administer i.v. saline or cocaine at a volume of 0.1 cc/injection (saline) or at a dose of 0.5 mg/kg/injection (cocaine). The details for surgery, training, and self-administration procedures were as described previously [13].

Animals were imaged 7–8 days after their last self-administration session. During imaging, animals were anesthetized and ventilated with 1.5–2% isoflurane mixed in a 60–70% O₂/air mixture. During the surgery, a femoral artery was catheterized for continuous arterial blood pressure monitoring and a femoral vein was catheterized for drug administration. The animal was then positioned in a stereotaxic frame (KOPF 900) to minimize brain motion. A cranial window (~6 × 4 mm²) was created above the PFC (anterior: +1 to +5 mm; lateral: ±3 mm from the bregma, (33)). The dura was carefully removed, and the exposed brain surface was immediately covered with 1.25% agarose gel and affixed with a 100 μm-thick glass coverslip to maintain normal cranial pressure. After the surgery, the animal and the stereotaxic frame were transferred to the image suite for experiments. The animal’s physiology was continuously monitored during the surgery and imaging, including electrocardiography, MABP, respiration rate, and body temperature (Module 224002, Small Animal Instruments). For the first experiment only, rats underwent self-administration in Dr. Koob’s lab before transportation to Dr. Du’s lab for imaging.

A home-built multimodal imaging platform was used to assess the effect of cocaine on cerebral blood flow velocity (CBFv) networks and on oxygenated hemoglobin. 3D ODT was used to visualize angiography and quantify CBF in cortical neurovascular networks [8]. It was integrated with a dual-wavelength imaging system (DWI) into a modified zoom fluorescence microscope (AZ100, Nikon) to allow for simultaneous imaging of CBFv and the oxygenated- and deoxygenated-hemoglobin concentrations within the surrounding tissue. For 3D ODT, a fast spectral-domain OCT system illuminated with a broadband source (λ₀ = 1.3 μm, Δλ ≥ 90 nm; Inphenix) was coupled to the zoom microscope via a custom dichroic mirror (DM1) to provide an axial resolution of 8 μm [14]. The collimated light beam (φ5 mm) exiting the sample arm of an optical coherence tomograph (OCT) was transversely scanned by a pair of servo mirrors (VM500, Cambridge Tech), focused with an achromatic lens (f40 mm/0.1NA), which allows for a lateral resolution of ~φ12 μm. The backscattered light from the brain was recombined with the reference light and detected by a high-speed linear spectrograph (a 1024-pixel InGaAs array; Goodrich, 47 kHz). For the DWI system, two light emitting diodes (150 mW/each) at wavelengths of λ₁ = 570 nm and λ₂ = 630 nm were coupled into a φ3 mm fiber bundle (NA/0.25) for illuminating the exposed cortex. The back-
reflected light from the cortex encoded with the dynamic changes of total blood volume ($\Delta[HbT]$) and deoxygenated hemoglobin ($\Delta[HbR]$) was collected via the microscope optics (2×0.22NA) and imaged by a 16-bit sCMOS camera (Zyla 5.5, Andor). Time-sharing illumination scheme at $\lambda_1$ (=570 nm) and $\lambda_2$ (=630 nm) allowed for imaging at a frame rate of up to 16 Hz/channel and both measures were then used to compute $[\Delta[HbO_2]]$, i.e.,

$$\frac{[\Delta[HbO_2](t)]}{[\Delta[HbR](t)]} = \left[ \frac{\varepsilon_{\lambda_1}^t \varepsilon_{\lambda_1}^0}{\varepsilon_{\lambda_2}^t \varepsilon_{\lambda_2}^0} \right]^{-1} \cdot \left[ \frac{\ln(\frac{R_{\lambda_1}(0)/R_{\lambda_2}(0)}{L_{\lambda_1}(t)/L_{\lambda_2}(t)})}{\ln(\frac{R_{\lambda_1}(0)/R_{\lambda_2}(0)}{L_{\lambda_1}(t)/L_{\lambda_2}(t)})} \right],$$

where $\varepsilon$ refers to the molar spectral absorptivity of a chromophore. $R_{\lambda_1}(t), R_{\lambda_2}(t)$ are the diffuse reflectance images measured at these two wavelengths, and $R_{\lambda_1}(0), R_{\lambda_2}(0)$ are their baseline values prior to the cocaine challenge. $L_{\lambda_1}(t), L_{\lambda_2}(t)$ are their pathlengths. The dynamic changes in $[\Delta[HbO_2]]$ and in $[\Delta[HbT]]$ were captured in response to a drug (e.g., cocaine challenge of 1 mg/kg, i.v.) from both control and cocaine-exposed animals. Region of interests (ROIs) in cortical areas where there were no visually distinguishable vessels were selected to compute $[\Delta[HbO_2]]$ in cortical tissue at each time point for a total of 40 min, including a 10 min baseline measure before cocaine challenge and a 30 min cocaine measure following its injection. For the comparisons between control and LgA animals, we integrated the measure following its injection. For the comparisons between control and LgA animals, we integrated the measure before cocaine challenge and a 30 min cocaine challenge and compared the means of $[\Delta[HbO_2]]$ and $[\Delta[HbT]]$ between the two groups. To analyze whether the “low” $[\Delta[HbO_2]]$ area in the PFC was correlated to the dose of cocaine consumed during self-administration, the mean area of $[\Delta[HbO_2]]$ was calculated from the $[\Delta[HbO_2]]$ image for each LgA animal, which was used to compute the correlation with the total escalation doses of cocaine consumed by each animal.

To assess if cocaine-induced CBF decreases in the PFC in LgA rats was causally related to their compulsive-like cocaine intake, we evaluated whether the vasodilating effects of the L-type Ca$^{2+}$ antagonist drug nifedipine in the PFC were associated with reduced cocaine intake and with changes in neuronal activity. Self-administration procedures were the same as above, including one saline group ($n = 6$), one LgA group ($n = 5$) and a second LgA group that received intraperitoneal (i.p.) nifedipine ($20 \text{ mg/kg}$, $n = 5$; [15]) 20 min prior to the initiation of the self-administration sessions. Groups were assigned based on responding during acquisition. After 14 days of self-administration rats were prepared for imaging 24 h after the last session as described above. $\Delta[HbT]$ was measured as above, while CBFv was calculated by reconstructing laser speckle flow image series by computing the speckle variances in both the spatial and temporal domains [14, 16]. To assess the changes in neuronal activity, the [Ca$^{2+}$] indicator GCaMP6f was used. Three weeks prior to the commencement of experiments the rats were injected with AAV1.Syn.GCaMP6f.WPRE.SV40 virus (Penn Vector Core) into the right PFC (A/P: +3; M/L: 0.8) to induce GCaMP expression using procedures previously described [17]. Two infusions of 0.5 μl were made (D/V: −1.4 and −1 mm from skull) at a rate of 0.2 μl/min, and the injector was left in place for 20 min following each infusion to allow for diffusion. Additionally, to test whether nifedipine could prevent reinstatement of cocaine seeking in rats, a second experiment was performed. Following LgA self-administration, as described above, rats underwent extinction training (lever presses resulted in saline infusion, $n = 8$) until they reached a criterion of less than ten infusions in a session. Animals were tested for cocaine-induced reinstatement 24 h after they reached extinction criteria. For reinstatement, animals were injected with 10 mg/kg (i.p.) cocaine immediately before they were placed in the self-administration chambers. Prior to reinstatement animals were randomly selected to be pretreated 20 min prior to cocaine either with saline or with 20 mg/kg nifedipine, which was followed by an additional extinction session. The saline or nifedipine reinstatement sessions were counterbalanced and were 48 h apart separated by an extinction session.

For the noncontingent cocaine administration studies in rats, we used two groups of animals: one received daily i.p. injections of saline (0.7 cc/100 g/day) and another group received the cocaine (30 mg/kg/day) for four consecutive weeks (Supplemental 3 Fig. S2O). Afterward, in vivo imaging was conducted for each animal to record the CBFv change in the somatosensory cortex in response to an acute intravenous saline (0.1 cc/100 g, i.v., for control animals) or cocaine injection (1 mg/kg, i.v., for cocaine animals). The somatosensory cortex was used as a control region to determine if the vasoactive effects of cocaine were specific to PFC or occurred in other cortical regions.

For the assessment of microvascular density and vascular endothelial growth factor (VEGF) in PFC and somatosensory cortex, we used ex vivo fluorescence measurement to quantify microvascular density in the brain of rats with or without chronic-cocaine pretreatment (Supplemental 3, Fig. S2). Animals were divided into 2 groups: in the control group, the animals were treated with saline (0.7 cc/100 g/day, i.p., $n = 4$) for 28 days (4 weeks), whereas the animals in the cocaine group were treated with cocaine (30 mg/kg/day, i.p., $n = 6$) for 4 weeks. FITC-Dextran (mol wt 2 × 10$^6$, 50 mg/ml), a fluorescence dye, was intracardially infused (500 μl) 1 min before the animal was sacrificed to label the microvessels, and the whole brain of the rat was removed from the skull, then incubated in cold 4% formaldehyde solution (Thermo Fisher Scientific, Waltham, MA)
overnight. The brain was then immersed in sucrose (≥99.5%, Sigma-Aldrich St. Louis, MO) solution with increasing concentrations from 10% to 30%. The brain was cryosectioned, and the brain regions of the PFC and somatosensory cortex were imaged by fluorescence microscope (E80i, Nikon Instruments).

To visualize the VEGF expression, brain slices were bathed with 5% goat serum (ab 7481, Abcam) for 30 min. The samples were then incubated with anti-VEGF antibody (ab 52917, Abcam) at 1:200 dilution for 1 h, followed by a further 1 h incubation with an Alexa Fluor® 488-conjugated Goat Anti-Rabbit IgG H&L (1:1000, ab150077, Abcam). The slices were finally mounted with Dapi Fluoromount-G® (SouthernBiotech, Birmingham, Alabama, USA) for imaging. ImageJ software was used to count microvascular density and to assess VEGF fluorescence density.

### Studies in the mouse model

For the longitudinal imaging studies of the vascular tree and CBF we used C57BL mice. For studies of the PFC (Naïve $n = 5$; cocaine, $n = 7$, Fig. 2j), a cranial window was implanted on the frontal cortex (lateral: 0.1–0.31 mm; anterior: 1.5–4.5 mm from the bregma [18]), and for studies of the somatosensory cortex (Naïve $n = 6$; cocaine $n = 6$, Fig. 3i), the cranial window was implanted on the
sensorimotor cortex (lateral: 0.25–2.75 mm; anterior: −0.25 to −2.75 mm from the bregma [18]). Each mouse was anesthetized with a mixture of ~2.5% isoflurane in a 60–70% O2/air mixture by a face mask, and its head was then mounted on a customized stereotaxic frame, shaved, and cleaned with alcohol and iodine. For cranial window implantation, all surgical tools and supplements, operation area and cranial window cover glasses were sterilized by

### Fig. 3 Longitudinal ultrahigh resolution μOCA and μODT to identify chronic-cocaine elicited vasoconstriction and angiogenesis in somatosensory cortex.

- **a, c, e, g** μOCA images of mouse cortex before cocaine (i.e., Day 0) and after cocaine exposures of 12 and 27 days (30 mg/kg/day i.p) and 22 days after withdraw from the last dose of cocaine exposure; **b, d, f, h** Corresponding μODT images simultaneously recorded with μOCA to monitor CBFv changes in the neurovascular network, indicating the chronic cocaine elicited vasoconstriction (e.g., pink arrows in c, e, g) and the corresponding microvascular adaptation (CBF reduction illustrated in d, f, h) to compare with baseline in b, and microneoangiogenesis, green circles).

Interestingly, although neoangiogenesis occurred on Day 12 as shown in (c), little detectable flows were observed in these vessels as shown in (d) (e.g., neovessel pointed by green circles in c and green arrows in d). Image size: 2.0 × 1.5 × 1.0 mm3.

I Longitudinal imaging of cocaine-induced changes in neurovascular networks in the mouse somatosensory cortex and effects of 35 days of withdrawal. Mice were pretreated with saline (~0.1 cc/10 g/day, i.p.) or cocaine (~30 mg/kg/day, i.p.) for 30 days followed by 30 days of withdrawal, μOCA and μODT imaging were done once every 3 days until 28 days into the pretreatment period, and then reduced to once every 7 days (i.e., each week) during the withdrawal period until the end of the experiment (i.e., ~60 days). **j** Time course of blood flow (CBFv) changes in individual arteries (red dashed lines) and veins (blue dashed lines) and CBFv development in angiogenesis (green dashed lines). Their mean changes are presented as solid lines correspondingly. **k** Statistical results of CBFv changes in arteries and veins after 21 days of cocaine treatment with respect to the baseline (day 0). It shows the CBFv decreased from 13.07 ± 0.92 to 9.40 ± 1.95 mm/s in the arteries (n = 6) and from 10.32 ± 0.56 to 6.36 ± 0.94 mm/s in the veins (n = 6), corresponding to mean CBFv reductions of 28.08% in arteries and 38.42% in veins. From last day of cocaine treatment (Day 27) to the withdrawal of 25 ± 5.4 days, the CBFv in arteries were 8.08 ± 1.29 and 9.01 ± 2.09 mm/s, respectively (p = 0.879 > 0.05), and in veins 6.02 ± 0.63 and 7.74 ± 1.52 mm/s, respectively (p = 0.983 > 0.05). **l** Changes in diameters and blood flow in new microvessels. It shows that the vessel diameter peaked prior to their CBFv peak, which indicates angiogenesis development.

**Statistical results of vascular density changes in capillary bed and middle size vessels after 21 days of chronic cocaine compared with their baseline (0 day). It shows the vascular density increased from 0.12 ± 0.02 to 0.20 ± 0.02 in capillaries (fill factor) (p = 0.029) and from 0.02 ± 0.002 to 0.03 ± 0.01 (p = 0.026) in middle vessels.**
autoclaving and 70% alcohol. The cortical skin was removed and the fascia and connective tissue on the skull were cleaned with a hydrogen dioxide solution with q-tips. Once dried, the skull was thinned with a dental drill (Ideal Micro Drill; Roboz) and the bone was carefully removed over the ROI. A drop of dexamethasone sodium phosphate (2 mg/ml) was applied to the brain to prevent cerebral edema and the exposed brain surface was covered using a glass coverslip (4 × 3 mm², 100 µm thick). The edge of the coverslip was sealed with glue and dental cement, which was spread around the coverslip to further secure its attachment to the skull. For post-surgery treatment, an anti-inflammatory drug was injected (Flunixin 2.5 mg/kg, subcutaneous injection) every 12 h for 2 days. The cranial window was implanted over the area of the PFC or the somatosensory cortex and was used to image the animals repeatedly during cocaine exposure and after cocaine discontinuation. After 3 days to allow for recovery from the surgery, saline was administered (~0.1 cc/100 g/day, i.p.) in the control animals and cocaine was administered (30 mg/kg/day, i.p.) in the chronic-cocaine animals for ~28 days (Fig. 2j). The animals used to study the sensorimotor cortex were continuously imaged for an additional one month during withdrawal until day 60 (Fig. 3i).

We used a newly developed μOCA/μODT for 3D imaging of CBF networks in the mouse cortex. Similar to OCA/ODT, it can simultaneously image the cerebral microvasculature (μOCA) and quantify CBF without the need for extrinsic labeling. In addition, it has dramatically enhanced blood flow detection sensitivity to facilitate fast, quantitative 3D capillary CBF imaging (e.g., resolving ϕ < 5 µm capillaries with a minute flow rate of <20 µm/s) over a larger field of view (FOV; e.g., 3 × 3 × 1.5 mm³). Our custom μOCA/μODT system is powered by an ultrabroad band light source (λ = 1310 nm, ΔλFWHM = 220 nm) to allow for a high-axial resolution (2.5 µm), which is defined by the transform-limited coherence length, \( L_c = 2(ln2)/πλ_0^2/Δλ_{cs} \). In the sample arm the collimated light was transversely scanned by a fast servo mirror and focused onto the mouse cortex through a cranial window with a f/16 mm/NA0.25 NIR objective yielding a lateral resolution of 3.2 µm. The backscattered interference fringes spectrally encoding the depth profile were detected by a high-speed linescan InGaAs camera (2048-pixels, 145k-lines/s; GL2048, Sensors Unlimited) synchronized with sequential transverse scans for 2D/3D μOCT acquisition. The Doppler flow (phase subtraction) image and maximum intensity projection image were instantaneously processed by a graphic processing unit, permitting reconstruction of μODT images as fast as 473 fps for a B-scan containing 1 k × 2 k pixels [9, 19].

### Quantification of CBFv and angiogenesis

The CBFv was determined from the ODT image based on Doppler flow reconstruction algorithms including the phase subtraction method (PSM) and phase intensity method (PIM) to enhance the blood flow detection [8, 20]. To assess the vascular density, 3D OCA images were used. A binary operation was then applied to the OCA images to skeletonize the cerebral vascular networks using MATLAB software [21]. Additionally, an adaptive rolling window method (window size: 50 pixels × 50 pixels) was utilized to provide a spatially resolved measure of vessel density from the skeleton of cortical vessels within the FOV. The local vascular density fraction was quantified using vascular fill factor (\( FF \)), which is defined as the ratio of the pixels occupied by vessels to the total pixel numbers within the rolling window, i.e.,

\[
FF = \frac{\text{Total vessel pixel number}}{\text{Total pixel number of ROI}}
\]  

(2)

The spatial resolved density map can be obtained by rolling the adaptive window in both the vertical and horizontal directions with an increment step, e.g., a fraction of the window size \( w \) such as 0.1 \( w \) in order to attain sufficient lateral resolution. Using this approach, the localization of the angiogenesis within the OCA image can be determined. In addition, the mean \( FF \) of the vessels over the FOV of the images can be calculated,

\[
\overline{FF} = \frac{1}{M} \sum_{i=1}^{M} FF_i
\]

(3)

where \( M \) represents the total number of ROIs selected in each image.

To assess vasoconstriction as a function of time during chronic-cocaine exposure, the diameters of arteries and veins in the PFC (\( n = 7 \)) and in the somatosensory cortex (\( n = 6 \)) were tracked from μOCA images. For comparison with controls (\( n = 5 \) for PFC and \( n = 6 \) for somatosensory cortex) similar vessels were measured. The ROIs were selected randomly for each vessel type and the diameters of these selected vessels, i.e., \( \phi(t_0) \) were traced every 7 days until the end of treatment with saline or cocaine (e.g., \( t_d = 0, 7, 14, 21, 28 \) for PFC studies, and \( t_d = 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, \) and additionally at 34, 41, 49 and 62 during cocaine withdrawal for the somatosensory cortex studies). The diameter change of the specific vessel was compared with its baseline, i.e., \( \phi(t_0) \) before the saline/cocaine pretreatment, thus presenting as,

\[
\Delta \phi(\%) = \{\phi(t_d) - \phi(t_0)\}/\phi(t_0) \times 100
\]

(4)
To analyze the CBF in constricted vessels and in the surrounding new vessels (angiogenesis), we measured their CBFv from the repeated µODT images obtained in the cocaine or saline exposed mice (controls) as a function of time, which were conducted for 28 days for PFC and for ~60 days for sensorimotor cortex studies, respectively.

**Statistical analysis**

Statistical tests were performed with SYSTAT Software (Chicago, IL, USA). The differences in CBFv and the vascular density between the control, ShA and LgA animals were tested by one-way ANOVA followed by post hoc tests (Student–Newman–Keuls) for intergroup comparison. Two-way repeated measures ANOVA was used to test the hypothesis of CBFv increases in newly grown vessels and the hypothesis of density increases, and the intergroup comparisons were performed using the Student–Newman–Keuls method. Pearson product moment correlation analyses were used to assess the correlation between CBFv reduction and the cocaine consumed by the animals. The correlation with cocaine intake was done both for the untransformed and also for the log transformed data, which was applied to show the increase in cocaine intake between control, ShA and LgA groups. In the mouse model, time courses for the CBFv changes were quantified and the comparisons with respect to the vessel’s baseline measure (pretreatment) were assessed for within and between groups (controls vs cocaine groups) using a factorial repeated measures ANOVA. Significance was set at $p < 0.05$. All data are presented as mean ± s.e.m.
Results

We used a multimodal imaging platform to image vascular morphology (Fig. 4), CBF and oxygenated hemoglobin in the PFC and somatosensory cortex (used as comparison). µOCA was used to assess vascular morphology in vivo (Fig. 4b) and simultaneously µODT was used for quantitative three-dimensional (3D) imaging of CBFv networks (Fig. 4a, c), e.g., in arteries (red dashed-lines), veins (blue dashed lines) and capillaries (as illustrated in Fig. 4b). We used DWI to capture dynamic changes in oxygenated (\(\Delta [\text{HbO}_2]\)) and deoxygenated hemoglobin (\(\Delta [\text{HbR}]\)) concentrations in response to cocaine administration (see Eq. (1)). This was effected by switching between two wavelengths, \(\lambda_1 = 570\ \text{nm}\) (brown, equally absorbed in oxygenated, and deoxygenated hemoglobin; Fig. 4d, e) and \(\lambda_2 = 630\ \text{nm}\) (red, absorbed more strongly in deoxygenated hemoglobin; Fig. 4f).

Studies in the rat model

Self-administration model

Both ShA (\(n = 7\)) and LgA (\(n = 6\)) rats were initially habituated to one hour daily of cocaine exposure for self-administration and compared to control animals (\(n = 6\); Fig. 1j). Controls animals were handled identically to the cocaine exposed rats (see Methods). After training, ShA rats were allowed to self-administer cocaine for 1 h/day (0.5 mg/kg i.v., 7–8 mg/kg average total daily dose) and LgA rats for 6 h/day (50–60 mg/kg average total daily dose; Fig. 1d). LgA rats have been shown to escalate cocaine intake with time, to develop hedonic tolerance and withdrawal, and show compulsive-like responding for cocaine [12]. ShA rats do not show any of these changes but keep a stable day-to-day self-administration pattern with no escalation [12]. Consistent with the literature, LgA rats, in the present study, escalated their cocaine self-administration (Fig. 1d).

Fig. 5 Decreases in oxygenated hemoglobin (\(\Delta [\text{HbO}_2]\)) in the PFC with acute cocaine challenge are greater in LgA than in Control animals. a Representative full field [HbO_2] map of the PFC before and after acute cocaine (1 mg/kg, i.v.) in a cocaine-naïve (i.e., control) and in an LgA rat in b. c Time course of mean [HbO_2] changes in response to cocaine (1 mg/kg) in control (\(n = 6\)) and LgA (\(n = 6\)). d Total decrease of [HbO_2] over the recording period (\(t = 30\ \text{min}\)) induced by cocaine in control and LgA rats (\(p = 0.024\)). Cocaine induced a transient [HbO_2] change (i.e., \(t < 5\ \text{min}\)) in controls whereas the [HbO_2] decrease in LgA animals was persistent (i.e., \(t \geq 27\ \text{min}\)) and observed across the whole FOV. The HbO2 reductions are consistent with ‘ischemic’ effects of cocaine in the LgA animals. e Correlation of the “ischemic” area (i.e., total pixels of \(\Delta [\text{HbO}_2] \leq -10\%\)) with the total escalation dose of cocaine in the LgA animals, indicating a strong association between the severity of the ischemic area and the doses of cocaine administered by the animals.
Figure 1 shows images of quantitative CBFv and of angiography in the PFC of a LgA animal (Fig. 1e, f) and of a yoked control (i.e., saline infusion instead of cocaine; Fig. 1a, b). The CBFv in the PFC of the LgA animal (Fig. 1e) was much lower than that of the control (Fig. 1a) throughout the cerebrovascular tree. Three group comparisons (one-way ANOVA) of CBFv in middle-size vessels (i.e., diameter $\phi = 124.27 \pm 16.09 \mu m$) showed a significant difference between groups $[F(2,35) = 3.33, p = 0.047]$. Post hoc $t$ tests revealed that controls ($8.99 \pm 1.02 \text{mm/s}$) had significantly faster CBFv than LgA rats ($6.39 \pm 0.48 \text{mm/s}; p = 0.043$; Fig. 1h), whereas CBFv in ShA rats ($n = 7$) did not differ from controls ($p = 0.18; \text{ShA}: 7.18 \pm 0.56 \text{mm/s}$) nor from LgA rats ($p = 0.43$) suggesting an intermediate state between the two groups (Supplemental 2, Fig. S1). Correlation analyses between CBFv in the PFC and the doses of cocaine self-administered by the animals (in log$_{10}$ scale) revealed a significant inverse association (Fig. 1k), such that rats with the greater reductions in CBFv in the PFC consumed the largest doses of cocaine ($r = -0.6, p = 0.008$). The correlation with the untransformed values (raw cocaine intake) with CBFv changes showed a similar association ($r = -0.45, p = 0.054$). The correlation with the raw data was significant when we controlled for the non-normal distribution using Spearman rank order correlation ($rs = -0.48, p = 0.037$).

To evaluate whether CBFv reductions triggered changes in vascular density, we quantified the vasculature present over the FOV in the μOCA images. We computed vascular density as the mean vascular fill factor (i.e., $FF$ as described in Eq. (3)), which was defined as the ratio of the number of pixels occupied by vessels to the total number of pixels in the FOV (see Eq. (2) for detail). To do this, the OCA images were skeletonized where each vessel and its branches were simplified into lines (Palagyi and Kuba, 1998). Figure 1c, g exemplify the skeletonized vasculature maps of a control (2c) and a LgA (2g) animal. $FF$ values (one-way ANOVA) differed significantly between groups $[F(2, 35) = 5.56, p = 0.008]$. LgA rats ($0.035 \pm 0.0015, n = 6, \text{ROIs} = 12$) had a significantly greater density than controls ($0.029 \pm 0.0008; n = 6, \text{ROIs} = 12; p = 0.011$, Fig. 1i) and ShA rats ($FF = 0.030 \pm 0.0015; n = 7, \text{ROIs} = 14; p = 0.011$). There was no difference between ShA rats and controls ($p = 0.96$; Fig. S1d).

To assess whether cocaine-induced disruptions of vascular networks in LgA animals were exacerbated during cocaine intoxication, we measured the hemodynamic response in the PFC to an acute cocaine challenge (1 mg/kg, i.v.). Spectral images at $\lambda_1$, and $\lambda_2$ were acquired using a time-sharing approach (i.e., 12.5 Hz sampling rate for each channel) continuously during the baseline period and up to 30 min after cocaine administration. Changes in $[\text{HbO}_2]$ and $[\text{HbR}]$ in cortical tissue were calculated from the time-lapse images at $\lambda_1$, and $\lambda_2$. (Eq. (1); [14]). Figure 5a, b illustrates the spatiotemporal changes of $\Delta[\text{HbO}_2]$ in the PFC in response to acute cocaine in a control and an LgA animal, respectively. These images show that in the control, $\Delta [\text{HbO}_2]$ decreased within 3 min after cocaine injection, predominantly around the central vein above the middle PFC, and this decrease was short lasting and recovered within a few minutes of cocaine injection (Fig. 5a). In contrast, the $\Delta [\text{HbO}_2]$ decrease in the LgA animal (Fig. 5b) was longer lasting (persisted $> 20$ min postcocaine injection) and widespread (observed over the full FOV). Group comparisons of the time courses for cocaine-induced decreases in $\Delta [\text{HbO}_2]$ showed that for controls (green curve, $n = 6$), acute cocaine abruptly decreased $\Delta [\text{HbO}_2]$ within $t = 2$ min of injection with peak decreases occurring at $\sim 3$ min postinjection ($-10.7\% \pm 1.2; p = 0.001$), and with $60-70\%$ recovery by $t = 6-9$ min and complete recovery by $t = 18$ min; whereas for LgA animals (red curve, $n = 6$), peak decreases occurred at $\sim 4$ min postcocaine injection, were larger ($-20.7\% \pm 3.8; p = 0.002$), and did not recover until $t = 29$ min postcocaine (Fig. 5c). The peak decrease in $\Delta [\text{HbO}_2]$ triggered by cocaine was significantly stronger ($p = 0.024$), and the duration longer for LgA than for controls ($p = 0.02$).

We also compared the magnitude of $\Delta [\text{HbO}_2]$ in response to acute cocaine by integrating $\Delta [\text{HbO}_2]$ over $t = 30$ min after cocaine challenge. Decreases in $\Delta [\text{HbO}_2]$ in LgA animals were $\sim 1.4$ times greater than in controls ($p = 0.02$, Fig. 5d). To assess the correlation between the doses of cocaine self-administered by LgA animals and the magnitude of the $\Delta [\text{HbO}_2]$ decreases triggered by acute cocaine, we computed the percentage of the area with “low” $\text{HbO}_2$ (i.e., percentage of pixels with $\Delta [\text{HbO}_2] \leq -10.0\%$ with respect to the total number of pixels in the FOV) and showed that it was significantly correlated with the cocaine doses administered ($r = 0.98; p < 0.001$; Fig. 5e).

**Noncontingent administration model**

To assess if cocaine’s effects in CBFv and $\text{HbO}_2$ were specific to the self-administration model and/or the PFC we evaluated the effects of an acute cocaine injection (1 mg/kg, i.v.) in the somatosensory cortex in rats exposed chronically (4 weeks) to noncontingent cocaine administration (30 mg/kg/day, i.p.). Responses were compared to those after an acute i.v. saline injection in animals exposed chronically (4 weeks) to saline (0.7 cc/100 g/day, i.p, Fig. S2o, Suplemental 3). The effects of noncontingent cocaine administration in the somatosensory cortex were similar to those in the PFC obtained with the self-administration model. Fig. S2B0-B3 illustrated the CBFv changes in response to acute cocaine (1 mg/kg, i.v.) in the chronically cocaine exposed rats, Fig. S2B4 showed a decrease within $t = 2-6$
min postcocaine injection to ΔCBFv = −18.8 ± 6.2% from baseline (i.e., t ≤ 0 min, p = 0.039,), which was long lasting and did not fully recover by 28 min postinjection. Acute saline injection in the control animals did not change CBFv (Fig. S2A–A4).

Ex vivo histochemistry analyses showed that the microvascular density, using fluorescence labeling (FITC-Dextran fluorescence indicator), was significantly increased in the cocaine treated rats (4 weeks, noncontingent i.p cocaine 30 mg/kg/day) both in somatosensory cortex (p = 0.007; controls: 287.3 ± 24.5/mm²; chronic cocaine: 379.4 ± 23.2/mm²; Fig. S2c–e), and in PFC (p = 0.009; controls: 246.4 ± 17.7/mm²; chronic cocaine: 298.4 ± 7.5/mm²; Fig. S2i–k). We also showed that the VEGF, a potent angiogenic factor involved in blood vessel growth, was increased both in the somatosensory cortex (p = 0.006; controls: 0.45 ± 0.13; chronic cocaine: 1.32 ± 0.3; Fig. S2f–h) and in the PFC (p = 0.006; controls: 0.38 ± 0.07; chronic cocaine: 1.06 ± 0.2; Fig. S2l–n). These results indicate that angiogenesis occurred in the brains of animals exposed to chronic cocaine as shown by the increase in vessel density and VEGF levels when compared to controls.

L-Type Ca²⁺ blockade improved cocaine-induced [Ca²⁺] and CBF changes in PFC and reduced cocaine intake

In a separate self-administration experiment, we injected rats with a virus expressing GCaMP6f (a calcium indicator) to measure neuronal activity concurrent to CBFv changes (Fig. 6). To assess if preventing the hemodynamic disruption triggered by cocaine would prevent escalation of cocaine intake we evaluated the effect of nifedipine (L-type Ca²⁺ antagonist and vasodilator) pretreatment in LgA animals. We hypothesized that by dilating vessels and ensuring

![Image](https://example.com/image.png)

**Fig. 6** Treatment with calcium antagonist during self-administration reduces drug taking and prevents reduction in CBFv. a A representative image of laser speckle (830 nm) used to calculate CBFv. b A representative Ca²⁺ fluorescence image. c A cross-sectional representation of fluorescence. d Time course of calcium change following a cocaine (1 mg/kg) injection at time 0, in animals with either history of cocaine or saline SA. e The timeline for the nifedipine (NIF) SA experiment. f Total cocaine intake by LgA and LgA + NIF rats over 14 days of self-administration; 20 mg/kg NIF (i.p.) reduced cocaine intake supporting the hypothesis that reduction in CBFv contributes to escalation of intake. g Mean cocaine intake across sessions. h Peak fluorescence amplitude. LgA causes increased response to cocaine challenge, which was blocked by nifedipine treatment. i CBFv in rats with a history of saline, LgA or LgA + NIF; treatment with NIF blocked the reduction of CBFv caused by cocaine self-administration. j Test of reinstatement (n = 8) showing the number of cocaine infusions in the last day of self-administration (SA), following extinction (EXT), reinstatement following vehicle (RI + V), following 20 mg/kg nifedipine (RI + N). * Significant difference (p < 0.05).
adequate blood supply, PFC dysfunction would be diminished, which in turn would protect against escalation of cocaine intake. Figure 6e illustrates the study protocol. Briefly, nifedipine (20 mg/kg, i.p.) was given 30 min prior to each self-administration session to LgA rats (LgA-NIF) and cocaine intake, CBF, and [Ca\textsuperscript{2+}] reactivity in PFC, were compared to that in LgA animals not given nifedipine (LgA). Nifedipine pretreatment significantly reduced cocaine intake over 14 days of self-administration ($F(1, 8) = 10.81, p = 0.011$); total intake over self-administration LgA = 1074.4 ± 46.65, $n = 5$; LgA-NIF = 779 ± 76.8, $n = 5$; Fig. 6f). And the reduction in cocaine intake was observed starting from the first pretreatment session and was consistently reduced throughout all of the sessions (Fig. 6g). When imaged after self-administration, LgA animals showed significantly greater changes in neuronal intracellular calcium ([Ca\textsuperscript{2+}]) in response to cocaine than controls or LgA-NIF rats ($F(2,13) = 4.53, p = 0.032$, Fig. 6d). This group difference was due to an increase in peak calcium in LgA rats compared to controls and LgA-NIF rats ($F(2,13) = 7.49, p = 0.007$); control = 4.3 ± 0.22, $n = 5$; LgA = 6.35 ± 0.71, $n = 5$; LgA-NIF = 3.24 ± 0.64, $n = 5$; Fig. 6h). Finally, nifedipine also prevented the reductions in CBF following chronic-cocaine administration (Fig. 6i). Specifically whereas LgA rats showed a significant reduction in CBFv in the PFC following self-administration, LgA-NIF rats did not ($F(2,13) = 4.83, p = 0.027$); control = 2288.9 ± 749.3, $n = 6$; LgA = 1683.19 ± 1831.4, $n = 5$; LgA-NIF = 24734.56 ± 2726.6, $n = 5$). It is important to note that animals were imaged 24 h after the last dose of nifedipine, which has a 2 h elimination half-life, and therefore the differences compared to the non-nifedipine group are due to a blockade of cocaine-induced adaptations rather than a lingering effect of the medication. Furthermore, as both changes in CBF and neuronal activity were prevented by nifedipine, one could speculate that reductions in CBF contributed to neuronal dysfunction.

Disruption of the PFC is known to contribute to relapse in humans and thus we hypothesized that nifedipine by counteracting the vascular and neuronal effects of cocaine in the PFC would prevent reinstatement in the rats. To test this hypothesis, we assessed the effects of nifedipine in the cocaine priming model of reinstatement [22]. After extinction rats were treated either with saline or with 20 mg/kg of nifedipine prior to priming them with cocaine (10 mg/kg ip) for reinstatement. A repeated measures ANOVA found a significant effect of session type on number of infusions [$F (3,19) = 33.76, p < 0.001$] (Fig. 6j). Post hoc analysis showed a significant decrease in the number of infusions from the end of self-administration (78.25 ± 5.08) to the end of extinction (7.13 ± 1.76, $p < 0.001$). When pretreated with saline, priming with cocaine significantly increased infusions (RI + N: 41.29 ± 10.63, $p = 0.001$, Fig. 6j) relative to the end of extinction, consistent with cocaine-induced reinstatement. In contrast when pretreated with nifedipine, priming with cocaine did not significantly increase infusions (RI + N: 18.71 ± 5.19, $p = 0.19$, Fig. 6j) relative to extinction. Infusions after cocaine priming were significantly lower when rats were pretreated with nifedipine than when they were not ($p = 0.01$). These results suggest that even when administered after chronic-cocaine use, nifedipine may have beneficial effects at preventing cocaine-induced reinstatement of drug seeking.

### Studies in the mouse model

The mouse model allowed us to perform longitudinal imaging for 30–60 days [23], and hence measure the changes in vascular density and CBFv as a function of chronicity and detoxification. For this purpose, we implanted a cranial window over the PFC (or the somatosensory cortex; see below) of the mouse’s brain and concurrently acquired μOCA for 3D angiography and μODT for quantitative 3D CBFv networks at high spatial resolution (~3 μm) over a relatively large FOV (2.5 × 2.5 × 1.2 mm\textsuperscript{3}).

The control group ($n = 5$) received a daily injection of saline (~0.1 cc/10 g/day i.p.); the cocaine group ($n = 7$), received a daily injection of cocaine (~30 mg/kg/day i.p.) for 28 consecutive days. Animals were periodically scanned with μOCA/μODT (Fig. 2j). Figure 2a-c shows the time-lapse 3D μOCA images of vascular networks in PFC from a control animal on Days 0, 14, and 28, and Fig. 2d-f shows the images from a cocaine animal. For illustration purposes, “large” (ϕ ~150 μm, “1”) and “middle” (ϕ ~100 μm, “2”) sized vessels were selected in the control and the cocaine mice (Fig. 2a, d) for comparison. In the control animal neither vessel “1” nor “2” changed between day 0 and day 28 (Fig. 2a vs. Fig. 2c), whereas in the cocaine animal the vessels’ diameters decreased from ϕ ~150 μm to ϕ ~80 μm for vessel “1”, and from ϕ ~100 to ϕ ~40 μm for vessel “2” (Fig. 2d-f). Figure 2k shows the time courses for the diameter changes of arteries and veins in controls ($n = 5$, total ROIs = 40) and in cocaine animals ($n = 7$, total ROIs = 56) during saline or cocaine exposure over day 0 to day 28. The diameters of both arteries and veins decreased significantly as a function of cocaine chronicity (Fig. 2k).

In cocaine treated animals, the mean diameters of arteries and veins decreased from baseline (prior to cocaine) to the end of treatment (28 days). For the arteries, the diameter was reduced 21.1 ± 9% (from 57.8 ± 37.8 to 42.6 ± 25.0 μm) and two-way repeated measure ANOVA test showed a statistically significant difference between cocaine-treated animals and controls as a function of time (i.e., $F(4,40) = 7.467, p = 0.0001$; Fig. 2k). For the veins, the diameter was reduced 30.3 ± 17% (from 71.5 ± 44.2 to 48.0 ± 29.3 μm; $F(4,40) = 5.822, p = 0.0008$).
differed significantly from those in controls \( (p < 0.05) \). Figure 21 summarizes the comparison of the cocaine-treated animals to the controls (saline-treated animals) after 28 days of treatment. A separate one-way ANOVA shows that the diameter reductions induced by chronic-cocaine exposures were significantly different from control, both for arteries (CocA versus SalineA, \( [F(1,40) = 9.982, \ p = 0.011] \) and veins (CocV versus SalineV) \( [F(1,40) = 12.452, \ p = 0.005] \).

To determine the relationship between cocaine-induced vasoconstriction and the formation of new vessels, we assessed the temporal sequence between these two events. Figure 2g–i shows the “zoom-in” ROIs within Fig. 2d–f (marked as yellow boxes) to emphasize the progression of angiogenesis around the area of vasoconstriction (e.g., vessel “2”, pink tracks). Angiogenesis appears as an extension of branches from preexisting vessels (e.g., vessels “3” and “4”, green tracks shown in Fig. 2g) that surround the constricted vessel from day 0 to day 28 of cocaine infusions. It shows that, these two events were co-localized. To characterize the CBFv in the constricted vessels and in the surrounding area of angiogenesis, we selected 4–5 ROIs along the length (axis) of vessels “2” and “3” to track the CBFv changes as a function of time (for details, see Fig. S3). As illustrated in Fig. 2m, the diameter of vessel “2” decreased as a function of cocaine treatment days (dashed line, C2 in Fig. 2m), as did CBFv (pink line, C2 in Fig. 2m), from 9.28 ± 1.12 mm/s at baseline (day 0) and 9.95 ± 2.27 mm/s on day 7, to 6.35 ± 1.34 mm/s on day 14, 3.35 ± 0.53 mm/s on day 21, and 2.20 ± 0.22 mm/s on day 28. Note that in the “growing” vessel branch “4”, CBFv gradually increased from 4.50 ± 0.96 mm/s (green line, A1 in Fig. 2m) on day 0 to 7.43 ± 2.23 mm/s on day 7 and 7.98 ± 2.18 mm/s on day 14, and then plateaued at 8.70 ± 0.93 mm/s on day 21 and 8.77 ± 1.39 mm/s on day 28. These results show that, while cocaine-induced vasoconstriction decreased CBFv, it stimulated angiogenesis, which helped compensate for some of the CBFv decreases. However, the slope of the CBFv decrease \( (k_v) \) in the constricted vessel (e.g., “2”) from day 7 to day 28 is greater than that of the CBFv increase \( (k_v) \) in the angiogenetic vessel (e.g., “4”), i.e., \( |k_v| = 0.381 > |k_v| = 0.07 \) (Fig. S3B, Supplemental 4). This suggests that, although angiogenesis might ameliorate to a certain extent cocaine-induced CBFv reductions in the PFC, this compensation is insufficient to return CBFv to baseline values.

To assess if cocaine-induced changes in the vasculature and its hemodynamics were specific to the PFC or occurred in other cortical regions, and to assess if it recovered during withdrawal, we evaluated cocaine’s effects on the somatosensory cortex longitudinally over a 2-month period, during which time mice were noncontingently exposed to cocaine for 4 weeks (i.e., 28 days) and then withdrawn for the following 4–5 weeks (i.e., 30–32 days). A cranial window was implanted over the sensorimotor cortex in controls (saline, −0.1 cc/10 g/day, i.p., \( n = 6 \)) and in chronic-cocaine exposed animals (cocaine, 30 mg/kg/day, i.p., \( n = 6 \)). Using the same protocol as for the PFC (described above) we obtained µOCA (angiographic) and µODT (quantitative CBFv) images periodically over 60 days to track their changes with time (Fig. 3i). Repeated µOCA and µODT images obtained before cocaine (i.e., 0 day) and after 12 and 27 days of cocaine exposures followed by withdrawal for 22 days are shown in Fig. 3a–h. “Pink” arrows highlight some of the constricted vessels, and green circles mark the surrounding angiogenetic vessels growing with time. It shows that chronic cocaine also induced vasoconstriction (e.g., pink arrows in Fig. 3a–h) in the somatosensory cortex. Similar to what was observed in the PFC, angiogenesis (including angiogenetic capillary nets) developed in the areas surrounding constricted vessels (green circles Fig. 3c).

Note that, although angiogenesis occurred on Day 12 (as shown in Fig. 3c), there was little detectable blood flow in these vessels (green arrows pointed in Fig. 3d). This is likely the result of these new vessels being nonfunctional, e.g., unable to properly recruit blood flow. After 12 days of cocaine-induced vasoconstriction the new microangiogenic vessels started to show perfusion and microvessel collateralization was observed (as illustrated in Fig. 3e–f). Quantitative analysis of the diameters of neovessels is provided in Supplemental material (Fig. S4I, Supplemental 5). The analysis indicates that angiogenesis in cortex developed in the capillary bed early on (e.g., on Day 12) and was followed by microvessel collateralization later on (e.g., after 27 days of cocaine exposure). These neovessels were located within brain tissue (Fig. S4II). Both the reduced vessel diameter and increased density were long lasting and persisted for at least 22 days after cocaine treatment discontinuation (i.e., from Day 27 to Day 49 as shown in Fig. 3e, g, pink arrows).

Figure 3j shows cocaine-induced diameter changes in individual arteries and veins, as well as the hemodynamic (i.e., CBFv) changes in neovessels. It indicates that the response of arteries (red dashed lines) to cocaine varied from vessel-to-vessel, with some decreasing their diameter by 50–70%, whereas others did not change. In contrast, cocaine consistently decreased the diameter of veins (blue dashed lines). The mean diameter changes in both arteries (red solid line) and veins decreased as a function of time. The angiogenic vessel started to recruit flow starting on Day 10–12 and CBFv increased as a function of time and plateaued after Day 25 (green curve).

The CBFv changes in arteries and veins across different animals \( (n = 6) \) are summarized in Fig. 3k. Figure 3k shows that after 21 days of cocaine treatment, CBFv decreased −28.08% in arteries (from 13.07 ± 0.92 to 9.40 ± 1.95 mm/s).
and ∼38.42% in veins (from 10.32 ± 0.56 to 6.36 ± 0.94 mm/s) with respect to baseline (day 0). The local CBFv in these vessels did not change from the last day of cocaine exposure (i.e., day 27) to after 25 ± 5.4 days of cocaine abstinence (arteries: 8.08 ± 1.3 versus 9.0 ± 2.1 mm/s, \( p = 0.942 \); veins: 6.02 ± 0.64 m/s versus 7.73 ± 1.53 mm/s, \( p = 0.127 \)). This indicates that the hemodynamic dysfunction did not recover following 4 weeks of cocaine abstinence.

The development of angiogenesis, reflected by neovessel ΔD/D (black traces) and their flow recruitment reflected by CBFv (red traces) as a function of time is shown in Fig. 3l. It indicates that neovessels grew rapidly after day 7–8 and reached a peak about day 12, after which they plateaued (ROIs = 15). CBFv in these neovessels appeared with a 1–2 day delay from the time they were formed and then increased up to 3.46 ± 0.74 mm/s around day 27. However, the CBFv in these neovessels did not change further after cocaine withdrawal (from day 28 to day 62), indicating that the neovessels’ capability for flow recruitment was limited and unable to fully compensate for the local CBFv decrease. Comparison of vascular density (assessed by \( FF \)) between baseline (day 0) and after 21 days of cocaine in Fig. 3m showed that \( FF \) of medium vessels (i.e., MID) increased from 0.02 ± 0.002 to 0.03 ± 0.006 (\( p = 0.026 \)) and \( FF \) of small vessels including capillaries (CAP) increased from 0.12 ± 0.021 to 0.20 ± 0.019 (\( p = 0.035 \)).

**Discussion**

Here, we document significant vasoconstriction in arterial and venous blood vessels along with CBF reductions in the PFC, both in rats that showed compulsive-like self-administration of cocaine and in mice noncontingently exposed to cocaine chronically. The vascular and hemodynamic changes occurred early in the history of cocaine administration (within 1 week of cocaine exposure) and persisted after cocaine discontinuation. The longitudinal assessment allowed us to map the dynamic nature of the changes triggered by cocaine self-administration and revealed that cocaine induced significant vasoconstriction, which triggered proliferation of local blood vessels. However, despite the angiogenesis, CBF remained significantly reduced even after ~1 month of detoxification. Finally, we found that the CBF reductions in the PFC co-occurred with increases in neuronal reactivity to cocaine (as assessed with neuronal intracellular calcium ([Ca**2+**])), and that pretreatment with the calcium channel blocker nifedipine prevented both these changes in the PFC and reduced cocaine intake and blocked cocaine-primed reinstatement.

Our findings of reduced CBF in the PFC of LgA rats, and passively treated mice, are consistent with clinical findings of reduced CBF in the cortex (most prominently in the PFC) of cocaine abusers [6]. Our results illuminate the clinical findings by demonstrating that cocaine-induced reductions in CBF are due to cocaine-induced vasoconstriction of both arterial and venous blood vessels. Studies on isolated cerebral arterioles have shown that application of cocaine or its metabolites induced vasoconstriction, documenting a direct effect of cocaine on blood vessels [24]. Most studies of vasoconstriction of cerebral vessels report on diameter reductions of arterial vessels, but previous studies have also shown that venous vessels undergo vasoconstriction [25]. In the present study, the cocaine-induced diameter decreases were observed across different types and sizes of vessels, including arteries, veins, arterioles and venules. Our studies also reveal that the vasoconstricting effects of cocaine in cerebral vessels are sensitized with repeated administration. It is possible that the mechanisms underlying the changes in vessel diameters differ for arteries and veins, as well as a function of vessels’ size. For arteries that are large enough to have sympathetic innervation, the decreases might reflect vasoconstriction, whereas the diameter decreases in veins are likely to reflect both adaptations to accommodate for reduced flow to the tissue, as well as direct vasoconstricting effects of cocaine [26, 27]. Though mechanism(s) are unclear, there is evidence that norepinephrine (NE) and L-type Ca**2+** channels may play a role in the sensitization of cocaine-induced vasoconstriction [28, 29].

Cocaine’s effects in cerebral blood vessels might also be due in part to its dopaminergic effects as demonstrated by the fact that dopamine D2 receptor blockade with haloperidol blocked cocaine-induced vasoconstriction [24]. Dopamine transporters (pharmacological targets of cocaine) are expressed in cerebral blood vessels [30], and cocaine could increase dopamine from dopamine terminals in close contact with arterioles and capillaries, resulting in vasoconstriction [31]. Cocaine’s noradrenergic enhancing actions are also likely to contribute, for NE also has vasoconstricting effects [32]. While vasoconstriction would reduce CBF, further reduction could be produced by the neuronal effects of cocaine [8] through neurovascular coupling. Indeed, we previously reported that acute cocaine (1 mg/kg, i.v.), depressed the resting-state field potentials of neurons, which correlated with decreases in CBF [16]. This suggests that cocaine-induced reduction in synchronized spontaneous neuronal activity might also contribute to CBF reductions. However, in the LgA animals we observed that acute cocaine accentuated the CBFv decreases despite it enhancing the neuronal intracellular calcium ([Ca**2+**]) increases, consistent with the disruption of neurovascular coupling by chronic-cocaine exposure [33].

Our findings also revealed sensitization to cocaine-induced CBF reductions, such that an acute cocaine challenge triggered significantly larger and longer-lasting CBF reductions and Δ[HbO2] decreases in LgA than in drug
naïve or in ShA animals. Indeed, in LgA animals, cocaine-induced decreases in Δ[HbO₂] were double the magnitude of those in controls (20.7% versus 10.8%). In humans, studies using infrared optical imaging revealed that decreases in brain oxygen saturation of 13% were associated with EEG changes characteristic of cerebral ischemia in 97% of cases [34]. Considering that the chronically exposed animals had a 20.7% reduction in Δ[HbO₂] when exposed to an acute cocaine dose, this indicates that these reductions are well within values associated with ischemia. Occurrence of ischemia with chronic-cocaine exposure is consistent with clinical reports of transient ischemic attacks (TIA) and cerebral strokes in cocaine abusers [35–37] and with preclinical findings of TIA in mice exposed chronically to cocaine [38].

We also documented angiogenesis with chronic cocaine exposure in both the rat and the mouse, in both self-administration and noncontingent administration models, indicating that this is not a unique phenomenon limited to a specific animal model or species. Furthermore, using the longitudinal mouse model we show that cocaine-induced vasoconstriction and the associated reductions in CBF in the PFC were regionally and temporarily associated with the formation of new vessels. Similar findings were obtained in the somatosensory cortex, indicating that cocaine-induced vascular and hemodynamic changes are not specific to the PFC and occur in other cortical regions. Cortical CBFv remained significantly reduced even after 25–30 days of cocaine withdrawal and despite the occurrence of angiogenesis.

Ischemia is known to trigger angiogenesis [39], so it is likely that cocaine-induced ischemia underlies the angiogenesis we observed with chronic cocaine in both the rat and the mouse models and in the PFC and somatosensory cortex. Moreover, the regional and temporal correspondence between cocaine-induced vasoconstriction and angiogenesis in the surrounding area is consistent with the hypothesis that cocaine-induced ischemia triggers angiogenesis.

Though the mechanisms by which hypoxia triggers angiogenesis with chronic cocaine exposure are unclear, we showed that in rats chronic cocaine increased the levels of VEGF in somatosensory cortex after 2 and 4 weeks of cocaine exposure [7]. In the current study, we also show that microvascular density and VEGF are increased in the PFC after 4 weeks of cocaine exposure (Fig. S2(I–K) and Fig. S2(L–N)). Consistent with these results, clinical studies have reported increases in VEGF in the pleura of cocaine abusers [40]. Since VEGF is a potent angiogenic factor involved in blood vessel growth in ischemic diseases [41], increases in VEGF could underlie the angiogenesis we observed in the PFC and somatosensory cortex of animals chronically exposed to cocaine. Though VEGF triggers endothelial cell proliferation and enhances vascular permeability in nascent vessels, newly formed vessels are immature and leaky [42], which could explain why CBF remained decreased in the cocaine-exposed animals despite the increases in vessel numbers.

The longitudinal mouse model and our recently developed µOCA/µODT imaging tools enabled us to image vascular networks during 28 days of cocaine exposure and following 1 month of abstinence (Fig. 3). The longitudinal analyses revealed that angiogenesis occurred around day 10 of cocaine exposure but then, along with CBFv, stabilized after ~25 days of cocaine administration. Noteworthy was the fact that CBFv remained unchanged following 30 days of cocaine abstinence and remained significantly reduced relative to the baseline, indicating that after the initial burst of angiogenesis there was little further recovery. Indeed, even after ~30 days of cocaine withdrawal and concomitant with an overall increase in vascular density, CBF remained significantly reduced. Such a time course implies that angiogenesis was insufficient to compensate for cocaine-induced disruption of cerebrovascular function and that cocaine-induced vascular and hemodynamic changes are long lasting and persist even after cocaine discontinuation. The lack of recovery in CBF despite the presence of angiogenesis could indicate that these new vessels are not functioning optimally. Clinically, it has been reported that in cerebral ischemic stroke there is active angiogenesis, which is most prominent in the penumbra [43, 44], and greater neovascularization was associated with longer survival [43]. However, the number of microvessels filled with blood cells was significantly lower in the infarcted hemispheres, and the functionality of the new cerebral vessels following an ischemic event is unclear [39]. Nevertheless, more attention is needed to investigate the role of angiogenesis, which could determine neuronal survival after cerebral ischemia, in the recovery of cocaine abusers.

In LgA rats we observed significant changes in vasoconstriction, CBFv and angiogenesis, and although a similar direction of effects was observed in the ShA rats, they were not significant. However, they were in the same direction and intermediate between values in LgA and controls. This indicates that there is a dose effect for cocaine-induced vascular toxicity. The fact that these same responses were observed in the mouse model, where animals were exposed to chronic cocaine noncontingently, indicates that these are direct pharmacological effects of cocaine and not dependent on the animal model used for exposure. These results also show that they occur both in rats and mice (and in humans as show in clinical studies); and are not specific to PFC since they occurred also in somatosensory cortex (and presumably other cortical areas).

By measuring neuronal Ca²⁺ (marker of neuronal function) in the present study we were able to demonstrate that
the PFC in LgA animals was hyper-responsive to an acute cocaine challenge. In our study this enhanced reactivity (reflected by an increase in neuronal Ca\(^{2+}\)) occurred concurrently with the reduction in CBF. In fact, when the rats were treated with nifedipine, a vasodilator and l-type Ca\(^{2+}\) channel antagonist, prior to self-administration sessions, both the reduction in CBF and neuronal hyper-responsiveness were blocked. Prevention of these adaptations was associated with reduced cocaine intake, suggesting that CBF hypofrontality and neuronal sensitivity contribute to compulsive-like drug taking in the LgA model. Indeed, it has been reported that escalation of drug intake is associated with loss of PFC neurons [45], which could potentially result from the ischemic effects of cocaine. Similarly, hypoactivity of the prelimbic cortex was associated with increased cocaine seeking, which was reduced by optogenetic stimulation of the PFC [46]. Thus, one hypothesis is that by preventing the ischemic consequences of cocaine-induced vasoconstriction in the PFC its dysfunction can be minimized, and escalation of cocaine intake can be diminished. Similarly strengthening PFC function might provide resistance to relapse and account for the reduced cocaine-primed reinstatement we observed with nifedipine pretreatment. A similar disruption of cocaine-induced reinstatement (also cue induced reinstatement) was reported with fendiline pretreatment, which is another l-type channel blocker [47]. Note, nifedipine could be altering cocaine intake by mechanisms other than vasodilation and recovery of PFC function. In cocaine users nifedipine attenuated the subjective effects of cocaine [48], and in rodents it blocked the rewarding effects of cocaine as measured by conditioned place preference [49]. Therefore, it is possible that nifedipine reduced cocaine intake in part by interfering with its rewarding effects. Alternatively, Ca\(^{2+}\) antagonism may interfere with cocaine-induced synaptic plasticity and contextual memory [50], which could also affect cocaine intake and reinstatement. Together these findings indicated that nifedipine may be beneficial both in preventing cortical damage and developing addiction when administered early, as well as aiding in the maintenance of recovery in those with a history of abuse. Because the noradrenergic enhancing effects of cocaine contribute to its vasoactive effects it is possible that combining a drug that attenuates NE signaling (i.e., \(\alpha_1\) antagonist or \(\alpha_2\) agonist) with an l-type calcium channel antagonist might enhance its beneficial hemodynamic and functional effects in the PFC and merits further investigation.

Our findings are clinically relevant, as they indicate that the sensitized reactivity of cerebral blood vessels to chronic cocaine will place cocaine abusers at increased risk of cerebral ischemia, particularly during cocaine intoxication. The increase in vascular density observed in our study may reflect an attempt to compensate for cocaine-induced hypoxia. Inasmuch as the PFC is a main target for cocaine-induced neurovascular toxicity, improving CBF may be particularly beneficial in helping recover executive function and self-regulation. Clinical and preclinical studies of chronic-cocaine exposure have corroborated a decrease in PFC function, which has been associated in animals with compulsive-like cocaine intake and in humans with relapse and impairments in cognitive performance [51]. The mechanisms underlying hypofrontality are poorly understood, although there is evidence that adaptations in striatocortical dopamine neurotransmission are involved [52]. However, the present study suggests a contribution of cocaine-induced cerebrovascular pathology to hypofrontality. Our finding that the \([\text{HbO}_2]\) decreases in the PFC triggered by cocaine were associated with the amount of cocaine self-administered by animals suggests that hemodynamic deficits are involved in hypofrontality and thus likely to contribute to the escalation of cocaine intake in addiction.

One of the limitations of this study is that the measures of vasoconstriction were recorded while imaging the animals under isoflurane anesthesia. Isoflurane could have counteracted the vasoconstriction from cocaine since it produces cerebral vasodilation [53], so it is possible that cocaine-induced vasoconstriction might have been attenuated by the anesthesia. Another limitation is that optical imaging has limited penetration so we could not access subcortical brain regions. It would have also been desirable to obtain additional measures to characterize the impact of the hemodynamic changes triggered by cocaine such as disruption of blood–brain barrier permeability, neuroinflammation or white matter changes.

In summary, here we document significant changes in perfusion in the PFC associated with chronic cocaine exposure that persist after cocaine discontinuation and are associated with altered neuronal activity. These findings show that cocaine-induced vasoconstriction, and the resulting ischemia, together with abnormal neuronal reactivity induced by cocaine contribute to PFC dysregulation that is associated with compulsive cocaine intake. We also show that nifedipine pretreatment attenuated the hemodynamic and neuronal changes in PFC from chronic cocaine and reduced cocaine intake and that it prevented cocaine-induced reinstatement following extinction. This highlights the potential therapeutic benefits of l-type Ca channel blockers in the management of cocaine use disorders [54].

Acknowledgments We specially thank to Sunmee Wee for conducting the initial self-administration experiment and analyzing that behavior data. Also to J. Li for partially assisting with figure illustration (Fig. 5) and Q.J. Zhang for assisting with noncontingent cocaine administration of rats and their VEGF studies on PFC and the somatosensory cortex. This work was supported in part by National Institutes of
Health (NIH) grants 1R01DA029718 (C.D. and Y.P.), R21DA042597 (Y.P. and C.D.), R01DA04398 (G.F.K. when he was at The Scripps Research Institute), and NIH’s Intramural Program of NIAAA (NDV). The authors would also like to thank the NIDA drug supply program for providing the cocaine used in the calcium antagonist and mouse model experiments.

**Author contributions** C.D. and Y.P. designed and built the optical setups. N.D.V., C.D., and Y.P. designed the experiments. G.F.K. designed the self-administration model and provided self-administering animals and helped with analysis and interpretation of the results. C.P.A. conducted self-administration animals for Ca²⁺ antagonist studies. C.P.A., J.Y., and K.P. carried out the imaging experiments and data analysis. All authors proofread the manuscript.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**References**

1. Rapinesi C, et al. Add-on high frequency deep transcranial magnetic stimulation (dTMS) to bilateral prefrontal cortex reduces cocaine craving in patients with cocaine use disorder. Neurosci Lett. 2016 Aug 26;629:43–47.
2. Volkow ND, Morales M. The Brain on Drugs: From Reward to Addiction. Cell. 2015 Aug 13; 162(4):712–725.
3. Levine SR, et al. “Crack” cocaine-associated stroke. Neurology. 1987;37:1849–53.
4. Levine SR, et al. A comparative study of the cerebrovascular complications of cocaine: alkaloidal versus hydrochloride—a review. Neurology. 1991;41:1173–7.
5. Tuchman AJ, Daras M, Zalpal P, Mangiardi J. Intracranial hemorrhage after cocaine abuse. J Am Med Assoc. 1987;257:1175.
6. Volkow ND, Mullani N, Gould KL, Adler S, Krajewski K. Cerebral blood flow in chronic-cocaine users: a study with positron emission tomography. Br J Psychiatry. 1988;152:641–8.
7. Zhang Q, et al. Chronic-cocaine disrupts neurovascular networks and cerebral function: optical imaging studies in rodents. J Biomed Opt. 2016;21:26006.
8. Ren H, et al. Cocaine-induced cortical microischemia in the rodent brain: clinical implications. Mol Psychiatry. 2012;17:1017–25.
9. You J, Du C, Volkow ND, Pan Y. Optical coherence Doppler tomography for quantitative cerebral blood flow imaging. Biomed Opt Express. 2014;5:3217–30.
10. Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. Science. 1998;282:298–300.
11. Koob GF, in Addiction Medicine: Science and Practice, AB Johnson, editor. New York, NY: Springer; 2011. p. 333–57.
12. Koob GF. Neurobiological substrates for the dark side of compulsivity in addiction. Neuropharmacology. 2009;56(Suppl. 1): S18–S31.
13. Wee S, Orto L, Ghirrai S, Cashman JR, Koob GF. Inhibition of kappa opioid receptors attenuated increased cocaine intake in rats with extended access to cocaine. Psychopharmacology. 2009;205:565–75.
14. Yuan Z, Luo Z, Volkow ND, Pan Y, Du C. Imaging separation of neuronal from vascular effects of cocaine on rat cortical brain in vivo. Neuroimage. 2011;54:1130–9.
15. Araki H, Hino N, Karasawa Y, Kawasaki H, Gomita Y. Effect of calcium channel blockers on cerebral ischemia-induced hyperactivity in Mongolian gerbils. Physiol Behav. 1999;67:573–7.
16. Chen W, Liu P, Volkow ND, Pan Y, Du C. Cocaine attenuates blood flow but not neuronal responses to stimulation while preserving neurovascular coupling for resting brain activity. Mol Psychiatry. 2016;21:1408–16.
17. Gu X, Chen W, You J, Koretsky AP, Volkow ND, Pan Y, et al. Long-term optical imaging of neurovascular coupling in mouse cortex using GCaMP6f and intrinsic hemodynamic signals. Neuroimage. 2018;165:251–64.
18. Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. Elsevier Academic Press; 2004.
19. You J, Zhang Q, Park K, Du C, Pan Y. Quantitative imaging of microvascular blood flow networks in deep cortical layers by 1310 nm μODT. Opt Lett. 2015;40:4293–6.
20. Zhao Y, et al. Phase-resolved optical coherence tomography and optical Doppler tomography for imaging blood flow in human skin with fast scanning speed and high velocity sensitivity. Opt Lett. 2000;25:114–6.
21. Palagyi K, Kuba A. A 3D 6-subiteration thinning algorithm for extracting medial lines. Pattern Recognition Letters, 1998;19:627.
22. McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug seeking behavior. J Neurosci. 2001;21:8655–63.
23. Park K, You J, Du C, Pan Y. Cranial window implantation on mouse cortex to study microvascular change induced by cocaine. Quant Imaging Med Surg. 2015;5:97–107.
24. He GQ, Zhang A, Altura BT, Altura BM. Cocaine-induced cerebrovasospasm and its possible mechanism of action. J Pharmacol Exp Ther. 1994;268:1532.
25. Shen Y, Pu IM, Ahearn T, Clemence M, Schwarzbauer C. Quantification of venous vessel size in human brain in response to hypercapnia and hyperoxia using magnetic resonance imaging. Magn Reson Med. 2013;69:1541–52.
26. Abboud FM, Eckstein JW, Zimmerman BG, Graham MH. Sensitization of arteries, veins, and small vessels to nor-epinephrine after cocaine. Circ Res. 1964;15:247–57.
27. Sofuoglu M, Nelson D, Babb DA, Hatsuuki DM. Intravenous cocaine increases plasma epinephrine and norepinephrine in humans. Pharmacol Biochem Behav. 2001;68:455–9.
28. Kalsner S. Cocaine sensitization of coronary artery contractions: mechanism of drug-induced spasm. J Pharmacol Exp Ther. 1993;264:1132–40.
29. Laporte R, DeRoth L. Modulation of the effects of norepinephrine uptake inhibitors on the norepinephrine-induced contractile response of the porcine uterine artery during early pregnancy. Can J Vet Res. 1997;61:214–20.
30. Ohtsuki S, Yamaguchi H, Kang YS, Hori S, Terasaki T. Reduction of L-type amino acid transporter 1 mRNA expression in brain capillaries in a mouse model of Parkinson’s disease. Biol Pharm Bull. 2010;33:1250–2.
31. Krimer LS, Muly EC, Williams GV, Goldman-Rakic PS. Dopaminergic regulation of cerebral cortical microcirculation. Nat Neurosci. 1998;1:286–9.
32. Giessler C, Wamemmam T, Silber RE, Dhein S, Brodde OE. Noradrenaline-induced contraction of human saphenous vein and human internal mammary artery: involvement of different alpha-adrenoceptor subtypes. Naunyn Schmiede Arch Pharmacol. 2002;366:104–9. Epub 2002 Jun 14.
33. Chen W, Volkow ND, Li J, Pan Y, Du C. Cocaine decreases spontaneous neuronal activity and increases low-frequency neuronal and hemodynamic cortical oscillations. Cereb Cortex. 2018. https://doi.org/10.1093/cercor/bhy057
34. Al-Rawi PG, Kirkpatrick PJ. Tissue oxygen index: thresholds for cerebral ischemia using near-infrared spectroscopy. Stroke. 2006;37:2720–5.
35. Sordo L, et al. Cocaine use and risk of stroke: a systematic review. Drug Alcohol Depend. 2014;142:1–13.
36. Toossi S, Hess CP, Hills NK, Josephson SA. Neurovascular complications of cocaine use at a tertiary stroke center. J Stroke Cerebrovasc Dis. 2010;19:273–8.

37. Treadwell SD, Robinson TG. Cocaine use and stroke. Postgrad Med J. 2007;83:389–94.

38. You J, Volkow ND, Park K, Zhang Q, Clare K, Du C, et al. Cerebrovascular adaptations to cocaine-induced transient ischemic attacks in the rodent brain. JCI Insight. 2017;2:e90809. https://doi.org/10.1172/jci.insight.90809

39. Hayashi T, Noshita N, Sugawara T, Chan PH. Temporal profile of angiogenesis and expression of related genes in the brain after ischemia. J Cereb Blood Flow Metab. 2003;23:166–80.

40. Strong DH, et al. Eosinophilic “empyema” associated with crack cocaine use. Thorax. 2003;58:823–4.

41. Marti HJ, et al. Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. Am J Pathol. 2000;156:965–76.

42. Zhang ZG, et al. Correlation of VEGF and angiopoietin expression with disruption of blood–brain barrier and angiogenesis after focal cerebral ischemia. J Cereb Blood Flow Metab. 2002;22:379–92.

43. Krupinski J, Kaluz J, Kumar P, Kumar S, Wang JM. Role of angiogenesis in patients with cerebral ischemic stroke. Stroke. 1994;25:1794–8.

44. Wei L, Erinjeri JP, Rovainen CM, Woolsey TA. Collateral growth and angiogenesis around cortical stroke. Stroke. 2001;32:2179–84.

45. George O, Mandyam CD, Wee S, Koob GF. Extended access to cocaine self-administration produces long-lasting prefrontal cortex-dependent working memory impairments. Neuropsychopharmacology. 2008;33:2474–82. Epub 2007 Nov 21.

46. Chen BT, Yau HJ, Hatch C, Kusumoto-Yoshida I, Cho SL, Hopf FW, et al. Rescuing cocaine-induced prefrontal cortex hypovascularity prevents compulsive cocaine seeking. Nature. 2013;496:359–62.

47. Cunningham JJ, Orr E, Lothian BC, Morgen J, Brebner K. Effects of fendiline on cocaine-seeking behavior in the rat. Psychopharmacology (Berl). 2015;232:4401–10.

48. Muntaner C, Kumon KM, Nagoshi C, Jaffe JH. Effects of nifedipine pretreatment on subjective and cardiovascular responses to intravenous cocaine in humans. Psychopharmacology (Berl). 1991;105:37–41.

49. Calcagnetti DJ, Keck BJ, Quattrella LA, Schechter MD. Blockade of cocaine-induced conditioned place preference: relevance to cocaine abuse therapeutics. Life Sci. 1995;56:475–83.

50. Degoulet M, Stelly CE, Ahn KC, Morikawa H. L-type Ca²⁺ channel blockade with antihypertensive medication disrupts VTA synaptic plasticity and drug-associated contextual memory. Mol Psychiatry. 2016;21:394–402.

51. Buttner A. Review: the neuropathology of drug abuse. Neuropathol Appl Neurobiol. 2011;37:118–34.

52. Volkow ND, Morales M. The brain on drugs: from reward to addiction. Cell. 2015;162:712–25.

53. Maekawa T, Tommasino C, Shapiro HM, Keifer-Goodman J, Kohlenberger RW. Local cerebral blood flow and glucose utilization during isoflurane anesthesia in the rat. Anesthesiology. 1986;65:144–51.

54. Edvinsson L, Johansson BB, Larsson B, MacKenzie ET, Skarby T, Young AR. Calcium antagonists: effects on cerebral blood flow and blood–brain barrier permeability in the rat. Br J Pharmacol. 1985;79:141–8.