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Cocaine attenuates blood flow but not neuronal responses to stimulation while preserving neurovascular coupling for resting brain activity

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Abstract

Cocaine affects neuronal activity and constricts cerebral blood vessels, making it difficult to determine whether cocaine-induced changes in cerebral blood flow (CBF) reflect neuronal activation or its vasoactive effects. Here we assessed the effects of acute cocaine on both resting-state and stimulation responses to investigate cocaine's effects on neurovascular coupling and to differentiate its effects on neuronal activity from its vasoactive actions. We concurrently measured cortical field potentials via thinned skull EEG recordings and CBF with laser Doppler flowmetry in the rat's somatosensory cortex for both resting state and forepaw stimulation prior to and following cocaine administration (1mg/kg, *i.v.*). Results show both resting-state field potentials and CBF were depressed after cocaine administration (19.8±4.7% and 52.1±13.4%, respectively) and these changes were strongly correlated with each other ($r=0.81$, $p<0.001$) indicating that cocaine did not affect neurovascular coupling at rest and that the reduction in resting CBF reflected reduction in synchronized spontaneous neuronal activity rather than vasoconstriction. In contrast, the forepaw-stimulation-evoked neuronal activity was not changed by cocaine ($p=0.244$) whereas the CBF to the stimulation was reduced 49.9±2.6% ($p=0.028$) gradually recovering ~20min post cocaine injection, indicating that neurovascular coupling during stimulation was temporarily disrupted by cocaine. Neurovascular uncoupling by cocaine during stimulation but not during rest indicates that distinct processes might underlie regulation of neurovascular coupling for spontaneous than for stimulation-induced activity. The greater reductions by cocaine to the stimulation-induced CBF increases than to the background CBF should be considered when interpreting fMRI studies comparing activation responses between controls and cocaine abusers. Neurovascular uncoupling could contribute to cocaine's neurotoxicity particularly for stimulation conditions when CBF might be insufficient to cover for the energetic demands of neuronal tissue.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Supplementary Information is available at ___ website

INTRODUCTION

Cocaine is one of the most commonly abused psychoactive substances in the United States and its abuse is associated with significant morbidity and mortality. Among the most serious adverse events from cocaine abuse are cerebrovascular accidents¹, which reflect in part cocaine-induced vasoconstriction². Indeed, brain imaging studies have consistently demonstrated widespread reductions in cerebral blood flow (CBF) in cocaine abusers³⁻⁷. However, from the imaging studies it is not possible to differentiate if the CBF defects reflect cocaine's vasoconstricting properties⁸ or are secondary to cocaine-induced decreases in neuronal activity and associated decreases in flow⁹.

Imaging technologies have profoundly advanced our understanding of cocaine's effects on the brain's vasculature. For example, cocaine, at doses typically used by drug abusers, decreases blood velocity, increases pulsatility¹⁰⁻¹², and decreases cerebral blood volume (CBV) in the human¹³ and laboratory animals' brain¹⁴, which are effects that most likely reflect vasoconstriction. Indeed, reports of cerebral hemorrhages and ischemic strokes in cocaine abusers indicate that cocaine's vasoconstricting effects are clinically relevant¹⁵⁻¹⁹. Recently, using ultrahigh-resolution optical coherence Doppler tomography we showed that acute cocaine induced cortical microischemia in the rodent brain²⁰ - an effect that was exacerbated with repeated cocaine exposures^{20, 21}.

Functional MRI (fMRI) is used to study the regional pattern of brain activation triggered by tasks, stimuli and drugs such as cocaine. Activation and deactivation responses from fMRI are based on hemodynamic changes measured using blood-oxygen-level dependent (BOLD) signals that assume a tight neurovascular coupling between neuronal activity and vascular responses²²⁻²⁴. However, the relationship between brain activation or deactivation and the dynamic changes in the vascular responses could be disrupted by pharmacological agents that have vasoactive effects, such as cocaine²⁵, complicating the interpretation of fMRI findings. For example, an fMRI study that assessed visual cortical activation before and after acute cocaine reported no differences in BOLD responses but reported decreases in CBF as assessed with the FAIR (flow sensitive alternating inversion recovery) method²⁶. However, others reported that acute cocaine markedly reduced the connectivity of the visual cortex, which was interpreted to reflect a reduction in neuronal activity²⁷. Thus, acute and chronic effects of cocaine on cerebrovascular reactivity may complicate the interpretation of BOLD-contrast fMRI as well as results from other imaging studies that rely on CBF measurements. To distinguish cocaine-induced vascular effect from its neuronal actions on the brain, here we assessed in parallel cerebrovascular function and neuronal activity from the anaesthetized animals both in the non-stimulation state, which we refer to as "resting-state activity" and during the electrical forepaw stimulation, which we refer to as "stimulation-induced activity".

For this purpose, we combined laser Doppler flowmetry (LDF) with electroencephalography (EEG) to simultaneously measure CBF (reflecting neurovascular hemodynamics) and field potential (reflecting neuronal activities) in the somatosensory cortex of the rodent brain during resting-state and during stimulation before and after acute cocaine. Specifically,

electrical forepaw stimulation was performed periodically throughout the experiment before and after cocaine while the vascular and the neuronal responses to the stimuli were monitored. We hypothesized that cocaine would reduce CBF and result in an attenuated CBF response to the stimulation, meanwhile cocaine through its catecholaminergic enhancing effects²⁸ would reduce basal neuronal activity without affecting stimuli-elicited neuronal activation^{27, 29, 30}.

MATERIALS AND METHODS

Animal preparation

Male Sprague-Dawley (SD) rats (300~350g/ea, n=10) were used for the experiments as illustrated in Fig.1; 5 rats received an acute cocaine challenge and the other 5 control rats received a saline injection. During the experiments, each rat was intubated and anesthetized using 2–3% isoflurane in 60–70% O₂/air mixture in order to perform the surgical procedure. Catheterization of the femoral artery and vein was done for continuous arterial blood pressure monitoring and for intravenous (*i.v.*) drug (e.g., cocaine) administration. Afterwards the rat was placed on a stereotaxic frame (Kopf 900) to fix the head. The skin of ~5×4mm² on the somatosensory cortex (i.e., AP: +2mm to –3mm; LR: +2mm to +6mm) was removed and the skull was carefully thinned to ~90–100μm using a micro-dental drill (0.8mm drill bit, Ideal micro drill; Roboz). During the drilling procedure, the drill bit was gently touched to the skull and the cold saline was applied on the operating area each 10s to prevent heating (Suppl. Fig.s1A). A drop of mineral oil was immediately applied to avoid surface dehydration and anesthesia was switched to α-chloralose using an initial bolus of 50mg/kg followed by continuous infusion of 25mg/kg/hr through the femoral vein^{31–33}.

During experiments cocaine or saline (placebo) was administered intravenously (1mg/kg for cocaine, 1ml/kg for saline). The experimental procedures were approved by the Institutional Animal Care and Use Committees of Stony Brook University and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The physiological condition of the rat, e.g., electrocardiogram (ECG) or heart rate, mean arterial blood pressure (MABP), respiratory rate and body temperature, was continuously monitored and recorded (Module 224002, Small Animal Instr.), and blood gases were sampled periodically (Model 700, Radiometer) to ensure PCO₂ in the range of 30–45mmHg.

CBF and field potential recording

CBF and field potential were continuously recorded on the thinned skull above the somatosensory cortex (AP –0.25; LR +3.0) during the experiment, including 10min baseline before cocaine administration till 30–40min post cocaine administration. Both resting-state (i.e., non-stimulation) signals and the signals during forepaw electrical stimulations were recorded (Fig.1c). For CBF detection, a φ0.8mm fiberoptic LDF probe (Moor Instr., VP3/moorVMS) was gently touched to the thinned-skull and a droplet of mineral oil was applied to optimize light coupling between the skull and the LDF probe. The LDF probe measured the changes in CBF of the cortex (sampled at 20Hz). The difference in CBF, CBF, (i.e., between activation vs. resting state) was used to quantify the relative flow change to brain activation. For field potential detection, a pair of φ0.3mm EEG electrodes (EL450, Biopac) -

with one affixed on the thinned skull in the cranial window, one on the thinned skull on the contralateral side of the brain, and a third ground electrode (Biopac, EL452) inserted under the neck skin (Fig.1a), were connected to a multi-channel EEG amplifier (Biopac, MP150/EEG100C). The signal was digitized at 2kHz, bandpass filtered (0.1–35 Hz), amplified, and then interfaced to PC for recording and real-time display (Fig.2a). To optimize the signal recordings, the LDF probe was slightly adjusted in the cortical area until the maximal response was reached (i.e., the location at peak CBF_p shown in Fig.1b). Then the signal electrode was positioned on the skull next to the LDF probe for EEG recording above the somatosensory cortex (AP -0.25 ; LR $+3.0$). Measurement on the thinned skull maintains the integrity of the cortex environment.

Electrical forepaw stimulation

Two needle electrodes inserted under the skin of contralateral forepaws of the rat were connected to an electrical stimulator (A–M System 2100) for forepaw stimulation. Synchronized with PC, each forepaw stimulation epoch lasted 10s during which 30 bipolar rectangular electrical pulses (0.3ms pulse width, 2mA peak-to-peak amplitude) were delivered at 3Hz (Fig.1c and Suppl. Fig.s1B₀). Prior to forepaw stimulation and drug administration, the rat was kept in the resting state for >15 min to minimize physiological fluctuations. Moreover, rat was in the resting state for 3min between 2 adjacent forepaw stimulations to reduce baseline drift. Briefly, the whole experiment procedure included 3 forepaw stimulation epochs during the baseline period (e.g., 9min) followed by 10 forepaw stimulation epochs following cocaine or saline administration (30min), thus totaling 13 stimulation epochs (i.e., 40min including 1min for cocaine administration) for each rat (Figs. 1b & 1c).

Data analysis for field potential and CBF

As shown in Suppl. Fig.s1B₁, the electrical forepaw stimulation evoked field potential, referred to as stimulation evoked potentials (SEP) was quantified by the average peak-to-peak intensity V_{SEP} over all of the spikes within a pulse epoch, i.e.,

$$V_{SEP} = \frac{1}{N_{SEP}} \sum_{i=1}^{N_{SEP}} V_i \quad (1)$$

where V_i ($i=1, 2, \dots, N_{SEP}$) denotes the amplitudes of forepaw stimulation evoked SEP spikes and N_{SEP} is the total number of spikes within the pulse epoch. Meanwhile, spontaneous field potential spikes between two adjacent forepaw stimulations that reflect resting-state neuronal activity were evaluated by the field potential spike counts per minute,

$$n_{rest} = \frac{1}{\Delta t} \sum_{\Delta t} i \quad (2)$$

where t is the time duration to count field potential spikes. $t=1$ min before each forepaw stimulation was used in the study. For simplicity, resting-state spontaneous neuronal activity, which is quantified by n_{rest} , refers to the spontaneous synchronized neuronal activity.

Similarly, CBF_0 , an average of the CBF level over 20s prior to each forepaw stimulation epoch was used to evaluate the resting-state CBF between forepaw stimulation epochs. The forepaw stimulation evoked CBF change was quantified by 1) the maximum CBF change ($\Delta CBF_p = CBF_p - CBF_0$), and 2) the total CBF change (ΔCBF_t) over the response period t_{FP} , (Suppl. Fig.s1B2),

$$\Delta CBF_t = \int_{\Delta t_{FP}} (CBF - CBF_0) dt \quad (3)$$

which describes the integrated CBF change in response to forepaw stimulation.

Statistics

All data are presented as means and s.e.m. Comparison made between two different time points or two different time periods was analyzed using one-tailed Student's t-test. Comparison made across multiple time points within a group (control/cocaine) was analyzed using one-way analysis of variance (ANOVA). In all cases, a $p < 0.05$ was considered statistically significant.

RESULTS

As summarized in Suppl. II Table s1, the physiology of the animals was stable during the experiments. The mean artery blood pressure (MABP) values were within the range of physiological autoregulation and the pCO_2 level was maintained within the normal range of 35–45mmHg.

Time-dependent CBF and field potential at rest and in response to forepaw stimulation

Fig.2 shows typical dynamic traces for CBF and field potential changes from one of the rats, which were recorded simultaneously and continuously before and after cocaine administration. During the experiment, forepaw stimulations (2mA, 3Hz, 10s) were conducted periodically (at 3min interval); the CBF and field potential responses to forepaw stimulation are indicated by the red shadowed areas (Fig.2), between which the signals reflect spontaneous neuronal activity at rest (i.e., without forepaw stimulations). Figs.2(c, d) are 'zoom-in' views of the signals corresponding to the time period from -4min prior to cocaine to 3min post cocaine challenge.

Field potential signals represent neuronal activity with or without forepaw stimulation and the CBF measurements represent both the mean basal blood flow (CBF_0) during the resting state (i.e., at non-stimulation time period) and the CBF change (i.e., ΔCBF) in response to forepaw stimulation. As shown in Fig.2c, the cortical neurons' activity was spontaneous and random during the resting state; whereas during forepaw stimulation epochs, the field potential spikes were synchronized to the stimuli, i.e., a total of 30 spikes during the 10s

stimulation epoch (3Hz, see Suppl. Fig.s1B₁). In addition, there was a transient CBF increase in response to forepaw stimulation (Fig.s1B₂), indicating the regional hemodynamic increase in response to the local changes in neuronal activation and thus demonstrating the neurovascular coupling in the somatosensory cortex of the rat's brain.

Stimulation-evoked CBF and SEP changes before and after cocaine administration

Fig.3 shows typical CBF (a1–a4) and stimulation-evoked potential (SEP) responses (b1–b4) to the forepaw stimulation at different time points before and after cocaine administration (1mg/kg, *i.v.*). As shown in Fig.3b(1–4), after acute cocaine administration the SEP spikes (blue traces) did not differ from the SEP spikes recorded prior to cocaine administration (black trace); whereas the corresponding CBF changes differed dramatically ($p < 0.001$) as shown in Fig.3a(1–4). Both the resting-state CBF₀ and the forepaw stimulation evoked CBF increase (e.g., CBF_p, CBF_t) were reduced by acute cocaine: CBF quickly dropped to a minimum (e.g., $t = 3\text{min}$) followed by a gradual recovery till 21–24mins after cocaine administration. Interestingly, the resting-state CBF₀ remained decreased even after 24min post cocaine administration.

Table 1 summarizes the peak CBF change (CBF_p) to forepaw stimulation. Specifically, it shows that the forepaw stimulation elicited CBF_p dropped significantly ($-50.43\% \pm 2.6\%$) from 49.5 ± 1.5 in the baseline to 24.5 ± 1.3 after cocaine ($t = 3\text{min}$); it then gradually recovered to 33.5 ± 3.0 (i.e., $-32.34\% \pm 6.0\%$, $p < 0.001$) at $t = 9\text{min}$ and fully recovered at $t = 21\text{min}$ post cocaine ($p = 0.433 > 0.05$). The lowering of CBF_p after cocaine indicates that cocaine temporarily modified the hemodynamic response of the brain to forepaw stimulation.

Forepaw stimulation: Cocaine reduces CBF response but not field potential activation response

Fig.4 shows the quantitative analyses of the forepaw stimulation induced CBF and field potential changes before and after cocaine across the animals ($n = 5$) compared to the control rats ($n = 5$). As shown by the blue curves in panels (a), the total CBF change (i.e., CBF_t) in response to forepaw stimulation was reduced from its baseline value of 210.4 ± 8.6 ($0.0\% \pm 4.1\%$) to 45.1 ± 9.1 ($-78.6\% \pm 4.3\%$) at $t = 3\text{min}$ after cocaine and did not fully recover till $t = 21\text{--}24\text{min}$ post cocaine injection ($p < 0.05$). In contrast, there were no changes in CBF_t in the control rats after saline injection (red curve), indicating that the decrease in CBF_t to forepaw stimulation observed after cocaine administration reflects cocaine's disruption of the hemodynamic response to stimulation. Similarly, Fig.4b shows the field potential responses (i.e., V_{SEP} signals) to forepaw stimulation before and after saline (in control rats) or cocaine (in cocaine rats). Forepaw stimulation evoked V_{SEP} was around $0.278 \pm 0.006\text{V}$ and remained unchanged before and after cocaine (blue curve). Although it fluctuated during the recording period (e.g., $t = 15\text{min}$), this effect was not significant ($p = 0.147$, $n = 5$). A comparison of V_{SEP} signals between control (red curve) and cocaine (blue) rats showed no significant difference ($p = 0.64$, $n = 5$). This indicates that acute cocaine did not significantly affect the amplitude of field potential in response to forepaw stimulation.

Resting activity: Cocaine reduces resting CBF and spontaneous field potential activity

Fig.5 shows the quantitative changes of the resting-state CBF (CBF_0) and spontaneous field potential activities (n_{rest}) with time before and after cocaine. As shown in Fig.5a cocaine resulted in $-19.8 \pm 4.7\%$ CBF_0 decrease from 151.5 ± 1.57 at baseline (e.g., $t=-3$ min) to a minimum of 121.8 ± 7.13 at $t=9$ min ($p=0.001$) followed by a slight recovery to 132.9 ± 9.6 at $t=30$ min post cocaine (blue trace, $p<0.05$). In contrast, CBF_0 in control rats remained unchanged in response to saline administration ($p=0.433$, red trace in Fig.5a) thus indicating that the CBF_0 decrease observed in the cocaine rats resulted from cocaine's effects.

In parallel, a depression in resting spontaneous activity was observed after cocaine administration. Fig.5b shows that the field potential counts (n_{rest}) in cocaine rats (blue trace) continued to decrease $-52.1 \pm 13.4\%$ from 101.3 ± 14.3 /min in the baseline period ($t=-3$ min; pre-cocaine) to the minimal 45.6 ± 12.8 /min at $t=12$ min ($p=0.004$) after cocaine injection. Interestingly, similar to CBF_0 the decrease in n_{rest} was also long lasting and had not recovered at 30min post cocaine administration (time of last measurement). In contrast, no decreases in n_{rest} were observed in the control group (red trace, Fig.5b). Figs.5(c, d) present the mean changes in field potential spikes and CBF_0 for individual animals from baseline ($t=-6$ ~ 0 min) to ($t=9$ ~ 15 min) after saline/cocaine injection. While the resting neuronal activity (n_{rest}) was not changed by saline injection in control animals (red markers), both n_{rest} and CBF_0 were significantly decreased in response to cocaine and this change was consistent for all tested animals as shown in Figs.5(c, d) and Suppl. IV. Fig.5e shows the correlation analysis between CBF_0 and n_{rest} that includes both control (red markers) and cocaine (blue markers) animals. It indicates that CBF_0 was highly correlated with neuronal activity ($r=0.81$, $p<0.001$) in both control and cocaine groups. Most of the data from the control group were located in the 'Baseline region', which is associated with high resting neuronal activity and high resting CBF. In contrast, the data from the cocaine group (blue markers in the figure) showed two distinct patterns: before cocaine administration the points locate in the 'Baseline region' (as expected) but after cocaine administration, the points shift to the labeled 'Cocaine region', which is associated with low resting neuronal activity and low resting CBF. The strong correlation observed suggests that the decreases in resting CBF after cocaine are likely due to decreases in resting spontaneous neuronal activity rather than just reflecting cocaine induced vasoconstriction. This also indicates that neurovascular coupling during rest is not affected by cocaine.

Cocaine increased the ratio of stimulation-elicited vs spontaneous neuronal activity but temporarily decreased the ratio of stimulation-evoked CBF response vs resting CBF

Since cocaine depressed both resting-state neuronal activity and resting CBF as well as the forepaw stimulation induced CBF, we wanted to assess how cocaine would affect the ratio of stimulation-evoked neuronal response (N_{SEP}) to that of spontaneous neuronal activity (n_{rest}), and how it would affect the ratio of stimulation-evoked CBF to that of resting-state CBF (i.e., CBF_0). Fig.6 shows the time courses of N_{SEP}/n_{rest} and CBF_t/CBF_0 (i.e., normalized CBF) before and after cocaine injection. As shown in Fig.6a, N_{SEP}/n_{rest} was ~ 0.35 during the baseline period ($t<0$ min) but increased after cocaine injection to a peak value of 0.72 ± 0.18 at $t=12$ min ($p=0.027$) and a value of 0.43 ± 0.07 at $t=24$ min ($p=0.042$) after cocaine administration. In contrast, as shown in Fig.6b, cocaine decreased CBF_t/CBF_0

CBF₀ from 1.39±0.06 during the baseline period (t<0min) to 0.34±0.06 at t=3min (p<0.001), and to 1.12±0.07 at t=9–15min (p<0.001, lower than baseline) recovering to 1.52±0.11 at t=21min with a slight overshoot to 1.68±0.22 at 30min post cocaine (p=0.01). The results reveal that acute cocaine depressed background neuronal activity, resulting in an enhancement of the signal-to-noise ratio to the activation (e.g., increased stimulation-induced neuronal activation vs background spontaneous neuronal activity), as reflected by N_{SEP}/n_{rest} . However, CBF_t/CBF₀ was temporarily decreased (i.e., t<9min), thus demonstrating the decoupling of the CBF response to stimulation-induced neuronal activation.

Confounds from anesthesia

Since the study was performed on anesthetized animals and stimulant drugs increase arousal from anesthesia³⁴ we assessed the effects of depth of anesthesia on spontaneous neuronal activity. For this purpose we compared the field potential signal for different anesthetic depths: i.e., increase α-chloralose dose from 25-, 32.5- to 50-mg/kg/h. We found no significant changes in neuronal activity as a function of depth of anesthesia (p=0.413) (Suppl. Fig.s4).

Physiological changes with cocaine

To ensure that the decreases in CBF in the resting state and that the attenuation of the forepaw stimulation response were not due to cocaine-induced systemic physiological changes we monitored mean artery blood pressure (MABP). Cocaine administration induced a brief increase in MABP from 108±2.8 mmHg to 123±3.7 mmHg within 3–6min after cocaine injection that was sustained at 124–125 mmHg till 30min post cocaine (Suppl. Table s1). The mild increase in MABP was within the range where cerebral autoregulation is maintained³⁵.

DISCUSSION

Here we show that acute cocaine during the resting state decreased spontaneous neuronal activity and this was associated with a parallel decrease in CBF indicating preservation of neurovascular coupling during rest. In contrast acute cocaine did not affect forepaw stimulation-induced neuronal activation but it significantly decreased stimulation-induced increases in CBF indicating that cocaine dissociated the neuronal and vascular responses to stimulation. Additionally, since cocaine reduced spontaneous neuronal activity but did not affect stimulation-induced neuronal activation, the neuronal responses to the stimulation over the spontaneous neuronal activity was increased (Fig.6a). In contrast, cocaine reduced the stimulation-induced CBF increases to a greater extent than its reduction of background CBF, which decreased the ratio of the CBF response to forepaw activation over the background CBF (Fig.6b).

Cocaine-induced decreases in basal CBF₀ (i.e., CBF₀) and in CBF differed in their magnitude and duration, indicative of distinct processes regulating stimulation driven increases in CBF from those sustaining resting CBF. Specifically, cocaine injection gradually decreased cortical basal CBF₀ with a maximal decrease of ~19.8% around 9min

post cocaine followed by a slow recovery that was still ~10% below baseline 30min post cocaine. In contrast, the attenuation of stimulation-evoked CBF_t was faster, larger and of shorter duration, e.g., about 78.5% at 3min followed by full recovery 20min post cocaine. Importantly, despite a global CBF_0 decrease in the cortex, the CBF increase in response to forepaw stimulation though attenuated was still detectable. This implies that imaging studies measuring activation responses to stimulation during cocaine intoxication using BOLD or CBF measurements will be reduced (even when neuronal activation responses are not affected) and the magnitude of the changes will vary as a function of the time post cocaine administration at which the measurements are made.

The decreases in basal CBF_0 observed following cocaine administration are consistent with our prior findings showing decreases in basal CBF_0 and in oxygen content of hemoglobin in the cortex of the rodent's brain following an acute cocaine challenge³⁶. They are also consistent with findings in cocaine abusers given cocaine in experimental settings. Specifically, a SPECT study reported that acute cocaine infusion to cocaine abusers resulted in a ~30% decrease in absolute CBF at the time of peak brain cocaine uptake³⁷. Using MRI, Gollub and colleagues²⁶ observed a ~14% decrease in cortical CBF over 15–30min after cocaine (0.6 mg/kg, *i.v.*) in human subjects. In addition, acute methylphenidate, which has similar pharmacological effects as cocaine including vasoconstriction, decreased CBF globally for at least 30min post injection as assessed by PET and [¹⁵O]H₂O³⁸, which seems consistent with our current findings of a long-lasting decrease in baseline CBF_0 by cocaine. Indeed long-lasting reduction in CBF from cocaine could account for the reductions in CBF reported in cocaine abusers when tested within the early stages of detoxification³.

Along with the basal CBF_0 decrease, the spontaneous neuronal activity at rest (i.e., n_{rest}) was decreased about 30–40% over 10–30min following cocaine injection, consistent with the long lasting reductions in basal CBF_0 (Figs.5(a, b)). Previous human studies have reported decreases in baseline brain glucose metabolism after intravenous cocaine³⁹. Thus it is possible that the decrease in brain glucose metabolism reported after acute cocaine in cocaine abusers might reflect reduced baseline neuronal activity.

The neuronal activity reflected by the field potential signals was random during the resting-state but synchronized to the stimuli during forepaw stimulation (Fig.s1B₁, Fig.s2c). Our results showed that forepaw stimulation resulted in comparable field potential signals in the somatosensory cortex before and after cocaine that did not differ from those obtained after saline injection (Fig.4b). In other words, despite cocaine inducing a significant decrease in spontaneous neuronal activity (i.e., n_{rest}), the stimulation-evoked increase in field potential was not disrupted. As a result, the contrast of neuronal activation induced by forepaw stimulation relative to that of the neuronal background activity was enhanced post cocaine administration (Fig.6a). This contrasted with the larger (i.e., >50%) decrease in CBF response to forepaw stimulation compared to the smaller decrease (i.e., <19.8%) in basal CBF_0 . As a result, the contrast of forepaw stimulation induced increases in CBF (i.e., CBF_t) relative to the basal CBF_0 was attenuated by cocaine till 20min post cocaine injection (Fig. 6b). Therefore, these results demonstrate that acute cocaine transiently interrupts neurovascular coupling in the somatosensory cortex of the rodent brain. Uncoupling of neurovascular responses will confound interpretation of stimulation-driven BOLD signals or

of CBF measurements when performed during the state of cocaine intoxication. Thus a reduced BOLD signal following stimulation during cocaine intoxication might not necessarily reflect decreased neuronal activity but instead cocaine-induced hemodynamic changes interfering with the delivery of the oxygenated blood to the area of activation that generates the BOLD signal. To the extent that chronic cocaine exposure might result in long lasting changes in blood flow dysfunction this could also contribute to the stimulation-induced BOLD signals being attenuated in cocaine abusers⁴⁰ that might not necessarily reflect disruptions in neuronal activity.

The neurovascular uncoupling by cocaine during stimulation contrasts with the preservation of the neurovascular coupling during the resting state. Specifically, cocaine reduced spontaneous neuronal activity, which was associated with parallel decreases in basal CBF₀. This indicates that during cocaine intoxication measures of resting functional connectivity (assessed as spontaneous BOLD oscillations with fMRI) will still reflect cocaine-induced changes in spontaneous neuronal activity. Cocaine's disruption of neurovascular coupling during stimulation but not for spontaneous activity indicates that they are affected differently by changes in vascular tone. Cocaine induces vasoconstriction abruptly in arterioles triggering a gradual decrease in CBF in veins²¹ and thus the differential selectivity to stimulation-induced neurovascular uncoupling would be consistent with activation-elicited CBF responses predominantly reflecting an excess delivery of oxygenated blood by arterioles to the capillaries and veins in the stimulated area⁴¹. Vasoconstriction of arterioles during cocaine intoxication would interfere with this process. However, the LDF probe applied for CBF detection is a non-imaging approach that did not allow us to measure blood vessel diameters and thus we could not assess whether cocaine induced vasoconstriction. The maintenance of neurovascular coupling during resting activity even when the overall CBF is decreased would be consistent with preservation of the reactivity of microvessels (including capillaries and veins) to changes in spontaneous neuronal activity⁴², which at rest might not be driven by abrupt vasodilation of arterial vessels. However, without a proper understanding of the mechanisms underlying neurovascular coupling it is not possible to assess if preservation of neurovascular coupling for rest but not for stimulation during cocaine intoxication could also reflect distinct regulation processes.

Though cocaine triggered a mild increase in MABP it was within the range where cerebral autoregulation is maintained³⁵. Based on the fact that autoregulatory mechanisms maintain CBF constant, this implies that the transient decrease in CBF in response to the forepaw stimulation after cocaine and the cocaine-induced decrease in basal CBF₀ were not due to changes in MABP. Intubation of the animals enabled us to maintain pCO₂ constant prior to and post cocaine ensuring that the drop in CBF and CBF after cocaine was independent of respiratory-driven changes in pCO₂ as well.

The findings of a reduction in CBF but not in neuronal activity in response to stimulation (neurovascular uncoupling) is clinically relevant for it could contribute to cocaine's neurotoxicity, including cocaine associated stroke⁴³. Since the vasoconstricting effects from cocaine are accentuated with repeated administration⁴⁴, neurovascular uncoupling could render neuronal tissue vulnerable to hypoxia particularly during states of enhanced stimulation (as might be observed during cocaine intoxication and bingeing).

A limitation for this study is that our experiments were done in anesthetized animals. We selected α -chloralose as the anesthetic agent since when compared with other anesthetics (1) it has minimal effects on depressing neuronal activity⁴⁵, (2) it preserves metabolic coupling for somatosensory stimulation⁴⁵, (3) it provides a normal CBF baseline close to that measured in the awake state compared with other anesthetic agents such as isoflurane³², and (4) it preserves cerebrovascular reactivity⁴⁶. In addition, we showed no significant change in neuronal activity as a function of depth of anesthesia (Suppl. V), which is consistent with findings from prior study in rats that showed no difference in EEG power spectrum for different α -chloralose doses⁴⁷. Therefore, the decreases in resting state neuronal activity (n_{rest}) observed with cocaine are unlikely to reflect its interactions with the anesthetic agent. Another limitation has to do with the inability of our methodology to assess the contribution of individual neurons to LFP events. However, the responses we obtained to forepaw stimulation were robust and comparable whether we used LFP, electrical recording from the exposed cortex (ECoG) or from EEG signals to record them (shown in Suppl. III). The comparisons showed that: (1) the thinned-bone EEG recording employed in this study enabled us to detect all 30 pluses in response to 30 forepaw electrical stimulations during the 10s stimulation paradigm (i.e., 3Hz); (2) both EEG and ECoG showed similar pulse transients to the pulse transient obtained with LFP recorded from within layers III-IV of the cortex, although their amplitudes were different depending on the signal amplifiers employed in the electrical circuits; (3) the pulse durations of measured by EEG and ECoG were ~80ms and ~60ms, respectively, which were broader than the pulse duration of 30–40ms measured by LFP. Overall, these results indicate that our EEG measurement observed from the thinned skull reflects local field potential changes resulting from neuronal activity in response to forepaw electrical stimulation. Moreover our findings are also consistent with prior electrophysiological and optical Ca^{2+} imaging studies showing that forepaw stimulation is associated with synchronized activation of multiple neurons in the somatosensory cortex^{48–50}.

Here we interpret the correlation between the decreases in resting CBF and those in resting local potential after cocaine to indicate that cocaine induced reduction in spontaneous neuronal activity underlie the reductions in resting CBF. However correlations do not connote causality and future studies that measure dynamic changes in cerebral blood vessel diameters after cocaine is needed to rule out the possibility that reductions in CBF might drive the decreases in spontaneous neuronal activity. Finally it would have been desirable to assess the mechanisms that underlie the neurovascular uncoupling and the reduction of resting activity by cocaine, which is a drug that not only enhances dopamine and norepinephrine but that also has local anesthetic effects.

In summary, acute cocaine temporarily interrupted neurovascular coupling for stimulation driven responses, though it did not modify neuronal responses to stimulation; whereas it did not uncouple neurovascular responses during rest, even though it reduced spontaneous neuronal activity. Cocaine-induced decrease in background neuronal activity but its lack of an effect in forepaw stimulation evoked neuronal activation, resulted in an enhanced stimulation-induced to background neuronal signal ratio. In contrast cocaine reduced stimulation-induced increases in CBF to a greater extent that it decreased baseline CBF_0 attenuating the task to resting CBF signal ratio. Thus these findings provide evidence that

cocaine uncouples hemodynamic responses in the brain, which is an effect that will confound BOLD and CBF findings with activation but not the measures of resting connectivity when performed during cocaine intoxication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

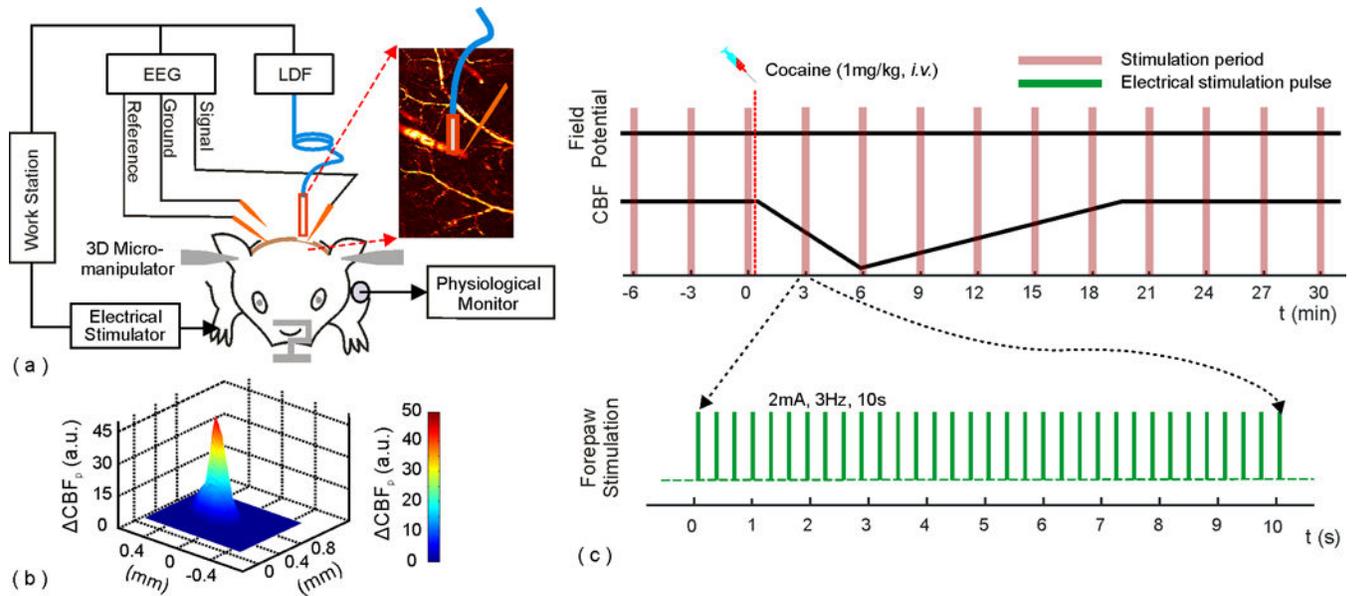
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References

1. Sordo L, Indave BI, Barrio G, Degenhardt L, de la Fuente L, Bravo MJ. Cocaine use and risk of stroke: a systematic review. *Drug Alcohol Depend.* 2014; 142:1–13. [PubMed: 25066468]
2. De Giorgi A, Fabbian F, Pala M, Bonetti F, Babini I, Bagnaresi I, et al. Cocaine and acute vascular diseases. *Curr Drug Abuse Rev.* 2012; 5(2):129–134. [PubMed: 22455504]
3. 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; 152:641–648. [PubMed: 3262397]
4. Tumeh SS, Nagel JS, English RJ, Moore M, Holman BL. Cerebral abnormalities in cocaine abusers: demonstration by SPECT perfusion brain scintigraphy. Work in progress. *Radiology.* 1990; 176(3): 821–824. [PubMed: 2389042]
5. Mena I, Giombetti RJ, Miller BL, Garrett K, Villanueva-Meyer J, Mody C, et al. Cerebral blood flow changes with acute cocaine intoxication: clinical correlations with SPECT, CT, and MRI. *NIDA Res Monogr.* 1994; 138:161–173. [PubMed: 7603541]
6. Kosten TR, Cheeves C, Palumbo J, Seibyl JP, Price LH, Woods SW. Regional cerebral blood flow during acute and chronic abstinence from combined cocaine-alcohol abuse. *Drug Alcohol Depend.* 1998; 50(3):187–195. [PubMed: 9649971]
7. Ernst T, Chang L, Oropilla G, Gustavson A, Speck O. Cerebral perfusion abnormalities in abstinent cocaine abusers: a perfusion MRI and SPECT study. *Psychiatry Res.* 2000; 99(2):63–74. [PubMed: 10963982]
8. Kaufman MJ, Levin JM, Ross MH, Lange N, Rose SL, Kukes TJ, et al. Cocaine-induced cerebral vasoconstriction detected in humans with magnetic resonance angiography. *JAMA.* 1998; 279(5): 376–380. [PubMed: 9459471]
9. Volkow ND, Ding YS, Fowler JS, Wang GJ. Cocaine addiction: hypothesis derived from imaging studies with PET. *J Addict Dis.* 1996; 15(4):55–71. [PubMed: 8943582]
10. Martin PJ, Evans DH, Naylor AR. Transcranial color-coded sonography of the basal cerebral circulation. Reference data from 115 volunteers. *Stroke.* 1994; 25(2):390–396. [PubMed: 7905680]
11. Cho SJ, Sohn YH, Kim GW, Kim JS. Blood flow velocity changes in the middle cerebral artery as an index of the chronicity of hypertension. *Journal of the Neurological Sciences.* 1997; 150(1):77–80. [PubMed: 9260861]
12. Schondorf R, Benoit J, Wein T. Cerebrovascular and cardiovascular measurements during neurally mediated syncope induced by head-up tilt. *Stroke.* 1997; 28(8):1564–1568. [PubMed: 9259749]
13. Kaufman MJ, Levin JM, Maas LC, Rose SL, Lukas SE, Mendelson JH, et al. Cocaine decreases relative cerebral blood volume in humans: a dynamic susceptibility contrast magnetic resonance imaging study. *Psychopharmacology (Berl).* 1998; 138(1):76–81. [PubMed: 9694529]
14. Du C, Pan Y. Optical detection of brain function: simultaneous imaging of cerebral vascular response, tissue metabolism, and cellular activity in vivo. *Rev Neurosci.* 2011; 22(6):695–709. [PubMed: 22098474]

15. Mody CK, Miller BL, McIntyre HB, Cobb SK, Goldberg MA. Neurologic complications of cocaine abuse. *Neurology*. 1988; 38(8):1189–1193. [PubMed: 3399066]
16. Peterson PK, Gekker G, Chao CC, Schut R, Molitor TW, Balfour HH Jr. Cocaine potentiates HIV-1 replication in human peripheral blood mononuclear cell cocultures. Involvement of transforming growth factor-beta. *J Immunol*. 1991; 146(1):81–84. [PubMed: 1984454]
17. Neiman J, Haapaniemi HM, Hillbom M. Neurological complications of drug abuse: pathophysiological mechanisms. *Eur J Neurol*. 2000; 7(6):595–606. [PubMed: 11136345]
18. Levine SR, Welch KM. Cocaine and stroke. *Stroke*. 1988; 19(6):779–783. [PubMed: 3076698]
19. Klonoff DC, Andrews BT, Obana WG. Stroke associated with cocaine use. *Archives of neurology*. 1989; 46(9):989. [PubMed: 2673163]
20. Ren H, Du C, Pan Y. Cerebral blood flow imaged with ultrahigh-resolution optical coherence angiography and Doppler tomography. *Opt Lett*. 2012; 37(8):1388–1390. [PubMed: 22513695]
21. Pan Y, You J, Volkow ND, Park K, Du C. Ultrasensitive detection of 3D cerebral microvascular network dynamics in vivo. *NeuroImage*. 2014; 103:492–501. [PubMed: 25192654]
22. Li B, Freeman RD. High-resolution neurometabolic coupling in the lateral geniculate nucleus. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 2007; 27(38):10223–10229. [PubMed: 17881528]
23. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. *Nature*. 2001; 412(6843):150–157. [PubMed: 11449264]
24. Roy CS, Sherrington CS. On the Regulation of the Blood-supply of the Brain. *J Physiol*. 1890; 11(1–2):85–158. 117.
25. Iannetti G, Wise RG. BOLD functional MRI in disease and pharmacological studies: room for improvement? *Magnetic resonance imaging*. 2007; 25(6):978–988. [PubMed: 17499469]
26. Gollub RL, Breiter HC, Kantor H, Kennedy D, Gastfriend D, Mathew RT, et al. Cocaine decreases cortical cerebral blood flow but does not obscure regional activation in functional magnetic resonance imaging in human subjects. *Journal of Cerebral Blood Flow & Metabolism*. 1998; 18(7):724–734. [PubMed: 9663502]
27. Li SJ, Biswal B, Li Z, Risinger R, Rainey C, Cho JK, et al. Cocaine administration decreases functional connectivity in human primary visual and motor cortex as detected by functional MRI. *Magnetic Resonance in Medicine*. 2000; 43(1):45–51. [PubMed: 10642730]
28. Ritz MC, Cone EJ, Kuhar MJ. Cocaine Inhibition of Ligand-Binding at Dopamine, Norepinephrine and Serotonin Transporters - a Structure-Activity Study. *Life sciences*. 1990; 46(9):635–645. [PubMed: 2308472]
29. Rebec GV. Behavioral electrophysiology of psychostimulants. *Neuropsychopharmacology*. 2006; 31(11):2341–2348. [PubMed: 16855534]
30. Jimenez-Rivera CA, Segarra O, Jimenez Z, Waterhouse BD. Effects of intravenous cocaine administration on cerebellar Purkinje cell activity. *Eur J Pharmacol*. 2000; 407(1–2):91–100. [PubMed: 11050295]
31. Ueki M, Mies G, Hossmann KA. Effect of alpha-chloralose, halothane, pentobarbital and nitrous oxide anesthesia on metabolic coupling in somatosensory cortex of rat. *Acta Anaesthesiol Scand*. 1992; 36(4):318–322. [PubMed: 1595336]
32. Masamoto K, Kim T, Fukuda M, Wang P, Kim SG. Relationship between neural, vascular, and BOLD signals in isoflurane-anesthetized rat somatosensory cortex. *Cereb Cortex*. 2007; 17(4): 942–950. [PubMed: 16731882]
33. Bonvento G, Charbonné R, Corrèze J-L, Borredon J, Seylaz J, Lacombe P. Is α -chloralose plus halothane induction a suitable anesthetic regimen for cerebrovascular research? *Brain research*. 1994; 665(2):213–221. [PubMed: 7895056]
34. Benveniste H, Volkow ND. Dopamine-enhancing medications to accelerate emergence from general anesthesia. *Anesthesiology*. 2013; 118(1):5–6. [PubMed: 23208519]
35. Gozzi A, Ceolin L, Schwarz A, Reese T, Bertani S, Crestan V, et al. A multimodality investigation of cerebral hemodynamics and autoregulation in pharmacological MRI. *Magnetic resonance imaging*. 2007; 25(6):826–833. [PubMed: 17451905]
36. 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(2):1130–1139. [PubMed: 20804849]

37. Wallace DR, Mactutus CF, Booze RM. Repeated intravenous cocaine administration: locomotor activity and dopamine D2/D3 receptors. *Synapse*. 1996; 23(3):152–163. [PubMed: 8807743]
38. Wang G-J, Volkow ND, Fowler JS, Ferrieri R, Schlyer DJ, Alexoff D, et al. Methylphenidate decreases regional cerebral blood flow in normal human subjects. *Life sciences*. 1994; 54(9):PL143–PL146. [PubMed: 8114609]
39. London ED, Cascella NG, Wong DF, Phillips RL, Dannals RF, Links JM, et al. Cocaine-induced reduction of glucose utilization in human brain: A study using positron emission tomography and [Fluorine 18]-fluorodeoxyglucose. *Archives of General Psychiatry*. 1990; 47(6):567–574. [PubMed: 2350209]
40. Lee J-H, Telang FW, Springer CS Jr, Volkow ND. Abnormal brain activation to visual stimulation in cocaine abusers. *Life sciences*. 2003; 73(15):1953–1961. [PubMed: 12899920]
41. Hillman EM. Coupling Mechanism and Significance of the BOLD Signal: A Status Report. *Annual Review of Neuroscience*. 2014; 37(1)
42. Du C, Volkow ND, Koretsky AP, Pan Y. Low-frequency calcium oscillations accompany deoxyhemoglobin oscillations in rat somatosensory cortex. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111(43):E4677–4686. [PubMed: 25313035]
43. Sordo L, Indave B, Barrio G, Degenhardt L, de la Fuente L, Bravo M. Cocaine use and risk of stroke: A systematic review. *Drug and alcohol dependence*. 2014; 142:1–13. [PubMed: 25066468]
44. Ren H, Du C, Yuan Z, Park K, Volkow ND, Pan Y. Cocaine-induced cortical microischemia in the rodent brain: clinical implications. *Mol Psychiatry*. 2012; 17(10):1017–1025. [PubMed: 22124273]
45. Ueki M, Mies G, Hossmann KA. Effect of alpha-chloralose, halothane, pentobarbital and nitrous oxide anesthesia on metabolic coupling in somatosensory cortex of rat. *Acta anaesthesiologica scandinavica*. 1992; 36(4):318–322. [PubMed: 1595336]
46. Bonvento G, Charbonne R, Correze JL, Borredon J, Seylaz J, Lacombe P. Is Alpha-Chloralose Plus Halothane Induction a Suitable Anesthetic Regimen for Cerebrovascular Research. *Brain Research*. 1994; 665(2):213–221. [PubMed: 7895056]
47. Lu H, Zuo Y, Gu H, Waltz JA, Zhan W, Scholl CA, et al. Synchronized delta oscillations correlate with the resting-state functional MRI signal. *Proceedings of the National Academy of Sciences*. 2007; 104(46):18265–18269.
48. Schulz K, Sydekum E, Krueppel R, Engelbrecht CJ, Schlegel F, Schröter A, et al. Simultaneous BOLD fMRI and fiber-optic calcium recording in rat neocortex. *Nature methods*. 2012; 9(6):597–602. [PubMed: 22561989]
49. Ma H, Harris S, Rahmani R, Lacefield CO, Zhao M, Daniel AG, et al. Wide-field in vivo neocortical calcium dye imaging using a convection-enhanced loading technique combined with simultaneous multiwavelength imaging of voltage-sensitive dyes and hemodynamic signals. *Neurophotonics*. 2014; 1(1):015003–015003. [PubMed: 25525611]
50. Moxon K, Hale L, Aguilar J, Foffani G. Responses of infragranular neurons in the rat primary somatosensory cortex to forepaw and hindpaw tactile stimuli. *Neuroscience*. 2008; 156(4):1083–1092. [PubMed: 18775766]

**Fig.1.**

A sketch to illustrate the experimental setup and the measurement protocol. (a) Experimental setup for simultaneous recording of field potential (using EEG) and CBF (using LDF) in the somatosensory cortex of the rat's brain *in vivo*; (b) Localization of the somatosensory cortex for maximal CBF response to forepaw stimulation; (c) Protocol for simultaneous recording of field potential and CBF changes elicited by acute cocaine administration (1mg/kg, *i.v.*, at $t=0$ min) and repeated forepaw stimulations (paradigm: 2mA, 3Hz, 10s, 3min/repetition).

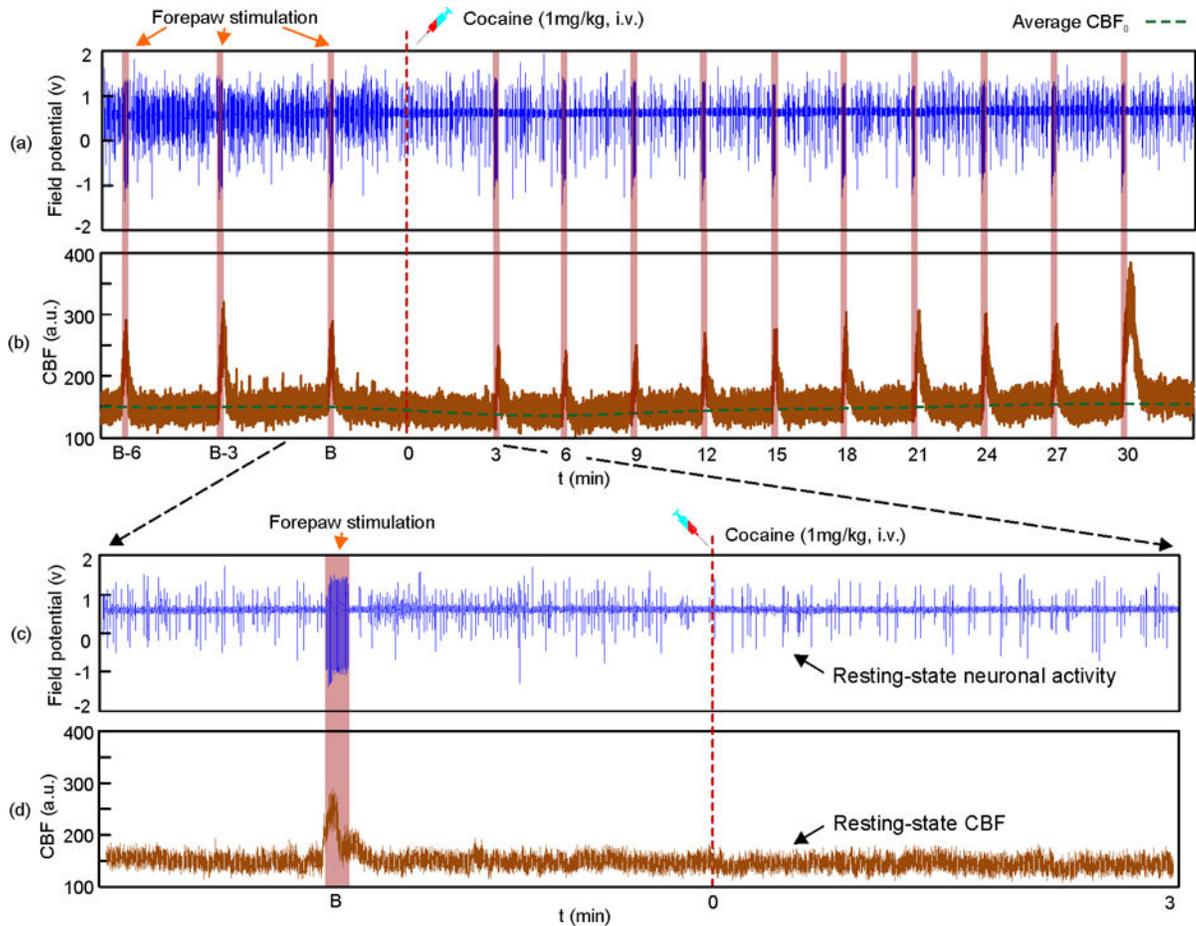
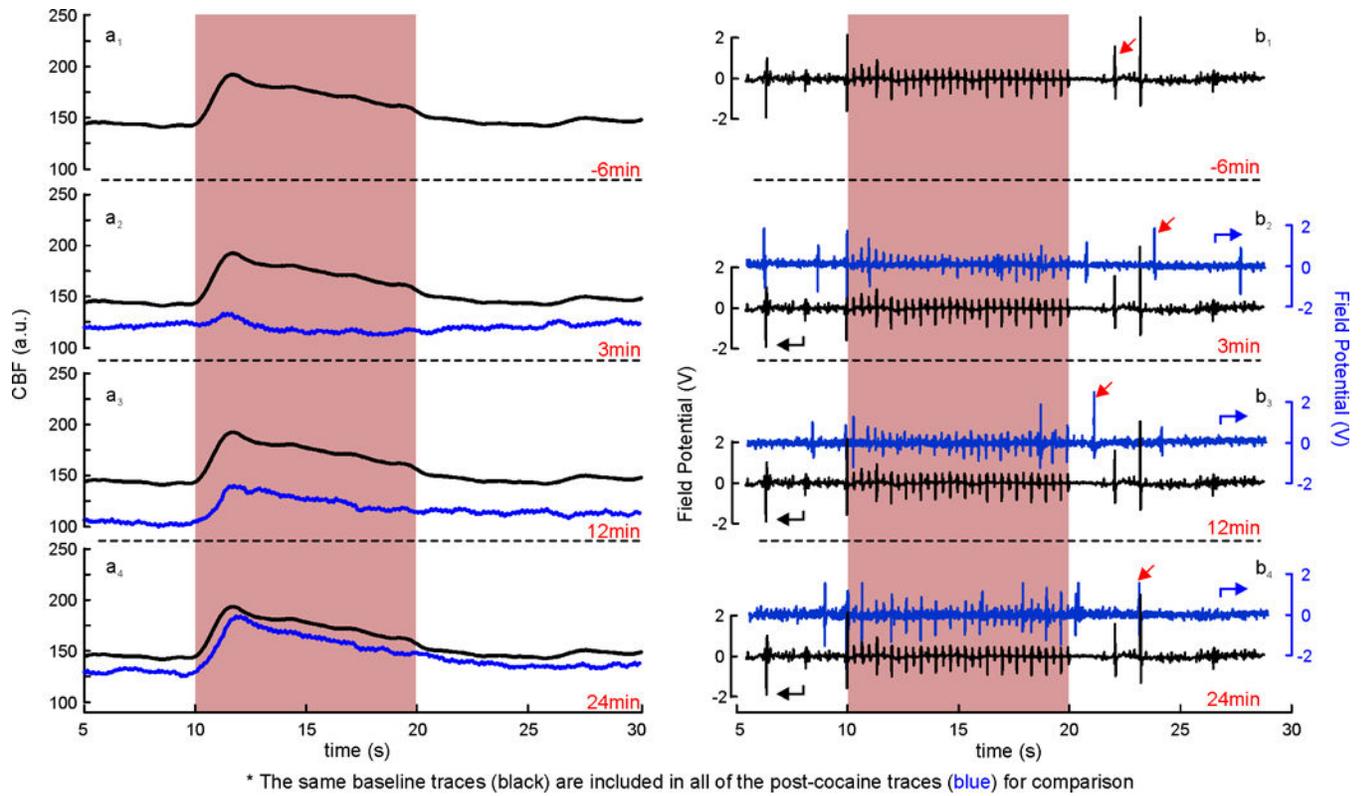
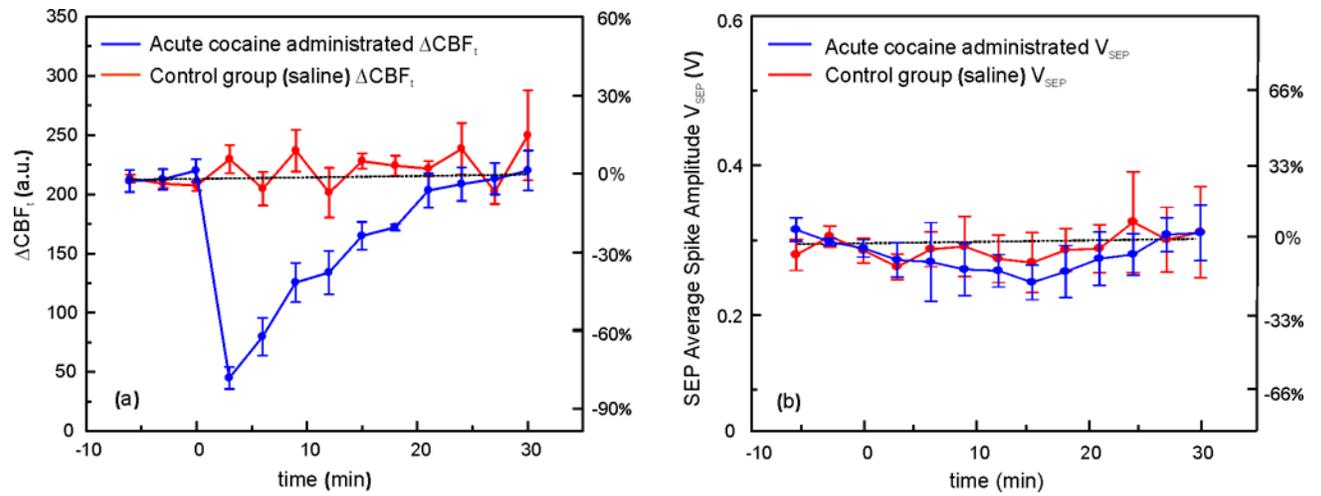


Fig.2.

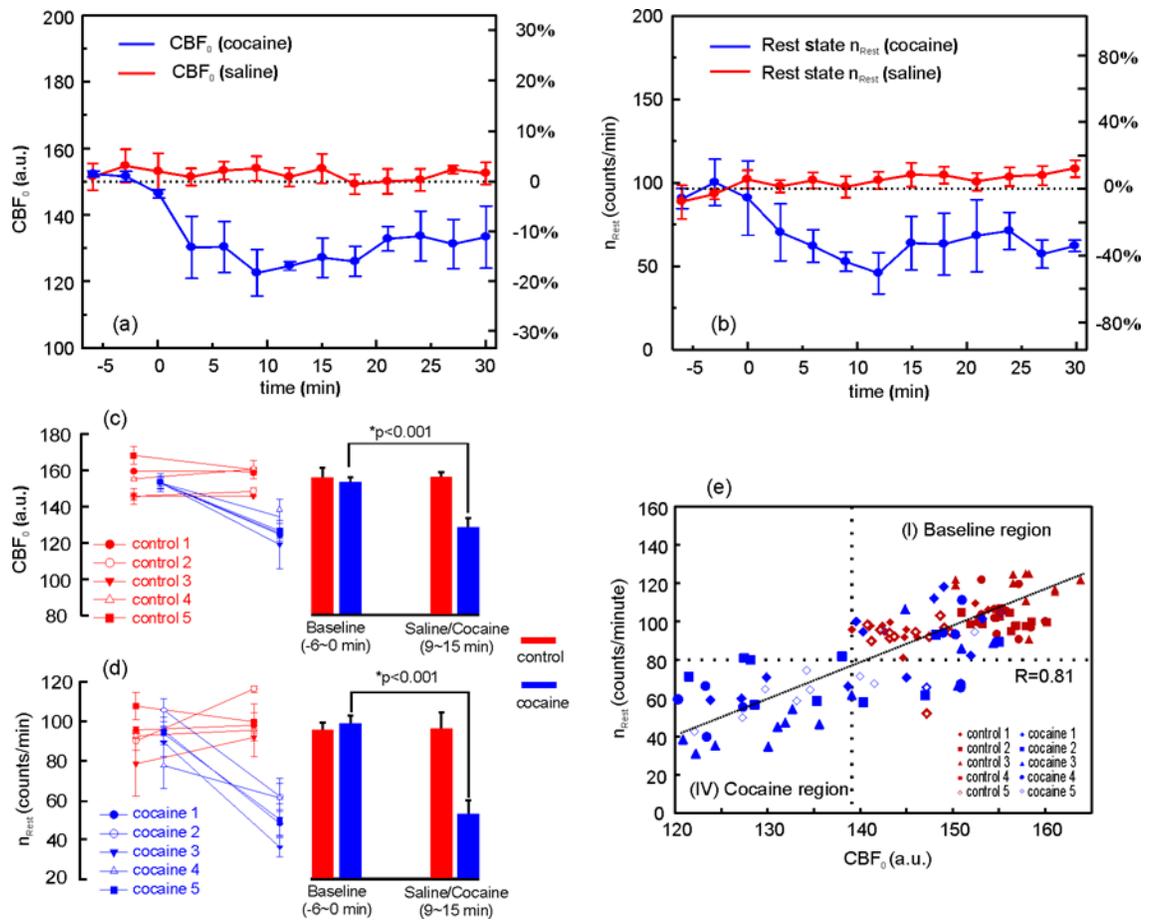
Field potential (a) and CBF (b) traces measured before and after cocaine administration (1mg/kg, *i.v.*, at t=0min) during which forepaw stimulation was performed every 3min from -9min (baseline period) to 30min after cocaine injection. Panels (c, d) show the detailed field potential and CBF signals just before and after cocaine injection (e.g., from -4min to 3min).

**Fig.3.**

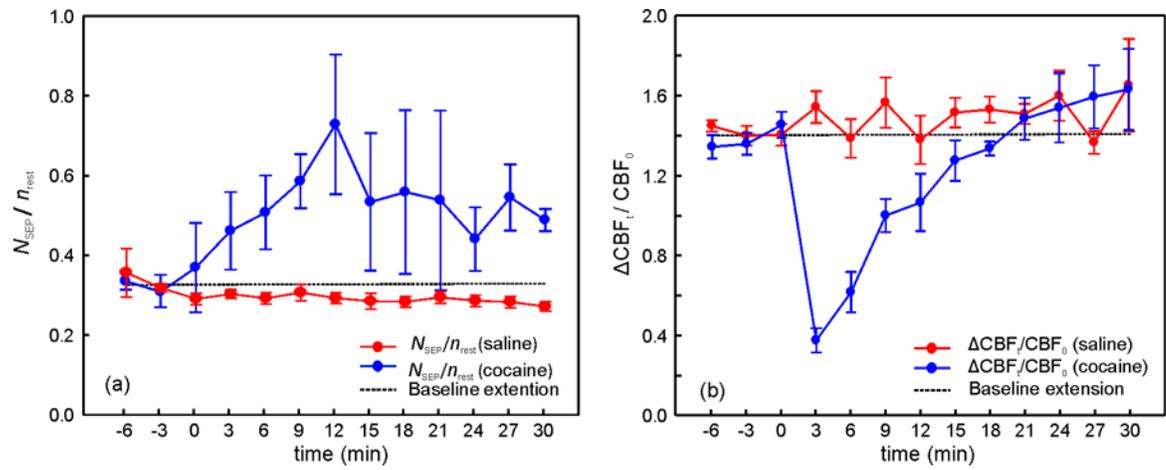
(a₁–a₄) Time traces of CBF₀ ($t < 0$ s) and forepaw stimulation-evoked cerebral blood flow (CBF) and **(b₁–b₄)** SEP potential changes during baseline period (e.g., $t_b = -6$ min shown in panels **a₁–b₁**; black traces) and after acute cocaine administration (e.g., 3-, 12-, and 24-min as shown in **a₂–b₂**, **a₃–b₃**, **a₄–b₄**, respectively; blue traces). Note that the red arrows indicate spontaneous neuronal activity. The baseline traces (black) for the field potential had the same voltage (v) as the post cocaine traces, i.e., no change before and after cocaine.

**Fig.4.**

Forepaw stimulation evoked CBF and field potential changes in somatosensory cortex of rats before and after acute cocaine administration. (a) $CBF_t(t)$ in response to forepaw stimulation for rats injected with acute cocaine and saline groups; (b) Average SEP intensity between cocaine and saline groups. Data represent means \pm standard errors; Blue and orange traces refer to cocaine and saline groups, respectively.

**Fig.5.**

Resting-state CBF and field potential changes in response to cocaine administration (at $t=0$ min). (a) Time dependent CBF₀ after cocaine (blue trace, $n=5$) vs saline (red trace, $n=5$) administrations, (b) Time-dependent resting-state field potential activities (n_{rest}) after cocaine (blue) vs saline (red) administrations, (c) Individual resting CBF₀ ($n=5$) for control/cocaine at baseline (-6-0min) and after saline/cocaine (9-15min), (d) Individual resting neuronal activity ($n=5$) for control/cocaine at baseline (-6-0min) and after saline/cocaine (9-15min), (e) Regression slope between CBF₀ and n_{rest} changes ($n=5$ for both control and cocaine groups).

**Fig.6.**

Time dependent changes in stimulation/background field potential counts and activated/resting-state CBF before and after cocaine administration (at $t=0$ min). (a) forepaw activated SEP counts (N_{SEP}) vs background field potential counts (n_{rest}) showing that the stimulation to background neuronal activity ratio was increased after cocaine; (b) $\Delta CBF_t/CBF_0$ showing that the stimulation to background cerebral blood flow ratio was decreased after cocaine.

Table 1Forepaw stimulation elicited peak change (CBF_p) before and after acute cocaine

| Time point t (min) | Baseline average (t _b ≤0) | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 |
|-------------------------|--------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|--------------------|
| CBF _p (a.u.) | 49.47 ± 1.52 | *24.52 ± 1.32 | *29.06 ± 1.08 | *33.47 ± 2.97 | *34.09 ± 1.06 | *35.58 ± 2.60 | *38.63 ± 2.57 | 43.13 ± 2.38 | 43.16 ± 1.92 | 46.16 ± 3.28 | 50.87 ± 0.97 |

* p < 0.05 with measures at t (>0) versus baseline values at t_b (paired t-test)