Published in final edited form as:

Brain Res. 2020 November 01; 1746: 147008. doi:10.1016/j.brainres.2020.147008.

Cocaine added to heroin fails to affect heroin-induced brain hypoxia

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Abstract

Heroin and cocaine are both highly addictive drugs that cause unique physiological and behavioral effects. These drugs are often co-administered and cocaine has been found in ~20% of cases of opioid overdose death. Respiratory depression followed by brain hypoxia is the most dangerous effect of high-dose opioids that could result in coma and even death. Conversely, cocaine at optimal self-administering doses increases brain oxygen levels. Considering these differences, it is unclear what pattern of oxygen changes will occur when these drugs are co-administered. Here, we used high-speed amperometry with oxygen sensors to examine changes in oxygen concentrations in the nucleus accumbens (NAc) induced by intravenous (iv) cocaine, heroin, and their mixtures in freely-moving rats. Cocaine delivered at a range of doses, both below (0.25 mg/kg) and within the optimal range of self-administration (0.5 and 1.0 mg/kg) modestly increased NAc oxygen levels. In contrast, heroin increased oxygen levels at a low reinforcing dose (0.05 mg/kg), but induced a biphasic down-up change at higher reinforcing doses (0.1 and 0.2 mg/kg), and caused a strong monophasic oxygen decrease during overdose (0.6 mg/kg). When combined at moderate doses, cocaine (0.25, 0.5 mg/kg) slightly increased and prolonged oxygen increases induced by heroin alone (0.5 and 0.1 mg/kg), but oxygen decreases were identical when cocaine (1 mg/kg) was combined with heroin at large doses (0.2 and 0.6 mg/kg). Therefore, health dangers of speedball may result from de-compensation of vital functions due to diminished intra-brain oxygen inflow induced by high-dose heroin coupled with enhanced oxygen use induced by cocaine.

Keywords

speedball; opioids; respiratory depression; neural activation; vasoconstriction/vasodilation; nucleus accumbens; overdose; rats

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Conflict of Interest: The Authors report no conflict of interest

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1. Introduction

Cocaine and heroin are frequently abused in a combination known as speedball. The individuals who use this drug mixture report a more intense, long-lasting high than is experienced from taking either drug alone. While cocaine and heroin are both highly addictive, each drug induces distinct pattern of physiological and behavioral effects. Cocaine, a psychostimulant, induces sympathetic activation and hyperlocomotion, while heroin induces CNS depressive effects, with respiratory depression followed by brain hypoxia as the most dangerous complication of drug overdose. Due to these opposing patterns in effects, it is assumed that the addition of cocaine could balance or cancel out the negative side effects of heroin exposure. However, clinical data suggest that the combination of these substances is more dangerous than each drug alone because of the amplification of negative effects of each drug.

Our previous studies have revealed that intravenous (iv) cocaine and heroin have distinct effects on brain oxygen levels as assessed in freely moving rats using oxygen sensors coupled with high-speed amperometry (Solis et al., 2017; 2018). Cocaine delivered at an optimal self-administering dose modestly increases brain oxygen levels and heroin induces a biphasic oxygen change, with a rapid decrease followed by a rebound-like increase. With subcutaneous oxygen recordings, we confirmed that the initial drop in oxygen induced by heroin results from respiratory depression followed by a decrease in blood oxygen. Considering these between-drug differences in the pattern of oxygen response, it is unclear what pattern of oxygen changes will occur when these drugs are used in combination.

This study was designed to examine how different heroin-cocaine mixtures will affect brain oxygen levels and to compare these changes with those induced by each drug alone. While respiratory depressive effects of opioids are often assessed in rats by plethysmographic monitoring of breathing activity, we directly assessed drug-induced changes in brain oxygen levels—the functional output of breathing and a clinically important parameter for brain metabolic activity. Similar to our previous studies, our experiments were conducted in awake, freely moving rats using nucleus accumbens (NAc) as an oxygen recording location. In this study, cocaine was used at a wide range of doses both below (0.25 mg/kg) and within the optimal range of rat self-administration (0.5 and 1.0 mg/kg) (Pickens and Thompson, 1968; Mandl et al., 2012). The effects of heroin and its combination with cocaine were assessed both at low, self-administering doses (0.05, 0.1 and 0.2 mg/kg) and at a high dose within the range of possible drug overdose (0.6 mg/kg).

2. Results

2.1. NAc oxygen responses induced by iv cocaine and heroin at low, self-administering doses

As shown in Figure 1A–C, iv cocaine increased NAc oxygen levels at each dose tested. In each case the increase was rapid, and its magnitude and duration were dose-dependent. The increase was relatively weak in term of magnitude (5–10% above baseline) and, at each dose, it occurred with short onset latencies (**D**). Rapid time-course analysis also revealed that the NAc oxygen response induced by cocaine at 1 mg/kg is in fact bimodal, showing

rapid but transient rise immediately after the injection followed by a more prolonged increase. In each case, the maximum increase in oxygen occurred either within or immediately after the injection.

Changes in brain oxygen induced by iv heroin (Figure 2) differed from those induced by cocaine. At the lowest reinforcing dose (0.05 mg/kg), NAc oxygen significantly increased (**A**). At higher doses, the effect became biphasic, with an initial slight decrease at a 0.1 mg/kg dose (**B**) and more profound decrease at the largest dose (**C**). When data were analyzed with rapid time-course resolution (**D**), the increase elicited by low-dose heroin developed with 175-s onset latency. In contrast, the drop in oxygen elicited by a large-dose injection was more rapid (~95-s latency), stronger in magnitude (~65% of baseline), and was followed by slowly developed oxygen increase.

2.2. NAc oxygen responses induced by a heroin-cocaine mixture at self-administering doses

Unexpectedly, the addition of cocaine had minimal effects on heroin-induced NAc oxygen levels (Figure 3). In each of three data sets, mean changes in oxygen levels analyzed with 1-min time resolution were virtually identical (A-C) and only minimal between-group differences were found following rapid time-course analysis (D-F). At the lowest drug combination (A), oxygen increase in cocaine-heroin group was larger and more prolonged. At the medium drug combination (B, E), weak post-injection drop in oxygen seen after heroin injection was fully abolished when heroin was mixed with cocaine. No differences in oxygen dynamics were found at the higher (heroin: 0.2 mg/kg + cocaine 1.0 mg/kg) drug combination (C, F).

2.3. Influence of cocaine on NAc oxygen response induced by heroin at a large dose

Heroin at the largest dose (0.6 mg/kg) induced robust and prolonged brain hypoxia, with a rapid, ~50% drop in concentration (Figure 4). The decrease became evident from ~30 s from the injection start, reached nadir at ~180 s, and slowly returned to baseline. When heroin at this dose was mixed with cocaine (1 mg/kg), the response was virtually identical, with no significant differences in both slow and rapid time resolution.

3. Discussion

Heroin is often used simultaneously with cocaine to experience novel, more intense psycho-emotional effects (see testimonials from [crowid.org](https://www.crowid.org)). It has been found that one in five heroin overdose death also involved cocaine (Warner et al., 2016). Since respiratory depression followed by brain hypoxia is the most dangerous effect of opioid drugs (Baud, 2009; Dahan et al., 2005; Jaffe et al., 1997; Pattinson, 2008), this study was designed to examine how brain hypoxic effects of heroin are affected by co-injected cocaine. Similar to our previous studies (Solis et al., 2017a,b; 2018a,b), drug-induced effects on brain oxygen were assessed by directly monitoring oxygen levels in the NAc by using oxygen sensors coupled with high-speed amperometry in freely moving rats. The drugs were used in doses within both self-administration and “overdose” ranges. Contrary to the expectations, our results revealed that addition of cocaine has minimal effects on heroin-induced brain

hypoxia within the wide range of doses. However, our results provided several novel findings regarding the effects of each drug, their underlying mechanisms, and their interactions occurring during drug co-administration.

3.1. Cocaine

As shown here, iv cocaine at a wide range of doses (0.25, 0.5 and 1.0 mg/kg) increased NAc oxygen levels. At each dose, the effect was very rapid (10–20 s onset latencies), correlating with the immediate motor response occurring during the injection. The increase was modest (5–10% above baseline) at each dose but it became more prolonged with dose increases. Rapid time-course analysis revealed that at higher doses (0.5 and 1.0 mg/kg), the increase is bimodal, with a phasic but transient phase immediately after the injection and a more prolonged tonic phase thereafter. This pattern of brain oxygen response mirrors the pattern of EEG activity, suggesting causal relationships cocaine-induced neural activation and increased oxygen entry into brain tissue. Within the same dose range, iv cocaine induced EEG desynchronization manifesting in rapid and strong drop in EEG total power coupled with the rise of high-frequency β (beta)- and γ -(gamma)-frequencies (Kiyatkin and Smirnov, 2010). Similar to oxygen dynamics, the immediate effects of cocaine on EEG activity were similar within a wide-range of doses (0.25–1.0 mg/kg) and, similarly to oxygen, the duration of changes increased with dose increases. Therefore, generalized neural activation appears to be the cause of rapid increase in brain oxygen induced by cocaine. While the rapid changes in oxygen are consistent with the direct neural action on cerebral blood vessels (neuro-vascular coupling), cocaine also induces rapid and strong skin vasoconstriction (Kiyatkin and Bae, 2008; Brown and Kiyatkin, 2005) that results in redistribution of blood flow from the periphery to the brain and its enhanced entry into brain tissue.

3.2. Heroin

Acute brain hypoxia occurring due to respiratory depression is the most dangerous effect of large-dose heroin exposure. This effect was weak and transient after heroin at 0.1 mg/kg, clearly larger and more prolonged at 0.2 mg/kg, and it became robust at the largest dose tested (0.6 mg/kg). However, heroin at our lowest dose (0.05 mg/kg) induced the opposite effect, a modest increase in NAc oxygen. This effect is similar to that induced by cocaine, but heroin-induced oxygen increase had longer latencies and a lesser acceleration. Therefore, the increase in brain oxygen is the initial effect of heroin that appears at low doses. As shown previously with subcutaneous oxygen recordings, this effect results from cerebral vasodilation and increased cerebral blood flow independently of respiratory depression, which occurs at larger doses and results in drop of blood oxygen and its diminished entry into the brain tissue (Solis et al., 2018). Oxygen increases also occurred at higher heroin doses as a second phase of the response, and these increases were larger than those seen with heroin at a low dose. This potentiation of post-hypoxic oxygen increases can result from accumulation of CO₂, a strong dilator of cerebral vessels (Schmidt and Kety, 1947; Battisti-Charboney et al., 2011).

3.3. Cocaine-heroin interactions

Cocaine is often found in samples obtained from patients with opioid overdose (Warner et al., 2016), which may suggest that a mixture of cocaine with heroin is more dangerous,

increasing the likelihood of an overdose death. Since acute hypoxia is the primary effect of heroin responsible for overdose-induced coma and death, our primary goal was to examine whether and how addition of cocaine affects heroin-induced changes in brain oxygen.

Breathing is the primary factor determining fluctuations of brain oxygen levels and respiratory depression induced by opioid drugs is the ultimate cause for brain hypoxia (Pattinson, 2008; Yadon and Kitchen, 1989 Jaffe et al., 1997; Kiyatkin, 2019). However, the brain differs from other peripheral locations due to its ability to induce cerebral vasodilation that enhances oxygen entry from arterial blood independently of brain-blood concentration gradient. Therefore, the actual change in brain oxygen is determined by these two opposing factors. As shown here, brain hypoxia was absent when the rats received heroin at a low, still reinforcing dose. At this dose, the addition of cocaine only slightly increased the duration of this response likely due to summation of cerebral vasodilatory effects typical to each drug (Bola and Kiyatkin, 2017). While no significant differences were revealed between the effects of heroin and heroin-cocaine mixture at a moderate dose, the oxygen increase induced by cocaine seem to influence the small decrease seen with heroin, resulting in a more rapid oxygen increase. Therefore, at this ratio of heroin-cocaine mixture, cocaine's rapid effects overpower mild hypoxia induced by heroin at this dose. At larger self-administering dose (0.2 mg/kg), heroin induced larger hypoxia decreasing NAc oxygen levels to ~70% of baseline. At this dose, heroin and heroin+cocaine curves were virtually identical. Opposite to what was seen with lower doses, heroin now overpowers cocaine and causes hypoxia to the level seen in control heroin-exposed rats. Similarly, no differences were found when heroin was used at a very high dose (0.6 mg/kg) that mimicked drug overdose. In this case, brain hypoxia was profound, resulting in ~50% oxygen drop lasting for ~20 min. The lack of the effect of cocaine on heroin-induced oxygen changes could be explained by the strength and duration of its opposite effects. While cerebral vasodilation appears at low doses when respiratory depression is absent, this effect is mild and has its natural physiological limits. In this case, cocaine, which has similar central vasodilatory effects, is able to only slightly enhance oxygen increases. In contrast to vascular effects, heroin-induced respiratory depression is a stronger effect, appearing at higher doses and is greatly enhancing with dose increases. In this case, weak cocaine stimulatory action combined with a similar action of heroin is unable to affect brain oxygen dynamics.

3.4. Conclusions

Oxygen dynamics following exposure to cocaine-heroin mixture seem to be more complex than initially hypothesized. Contrary to our assumptions, the addition of cocaine has minimal or non-existing effects on heroin-induced brain hypoxia in both the low, reinforcing, doses and during drug overdose. Obviously, potentially harmful effects of each drug differ, but the stimulatory effects of cocaine and its ability to stimulate respiration and slightly increase oxygen levels is unable to affect respiratory depression and brain hypoxia induced by opioid drugs. Contrary to our expectations, cocaine slightly enhanced brain oxygen increases elicited by heroin at low reinforcing doses. This effect could be explained by summation of similar actions of each drug on vascular tone, which could be responsible for the enhanced oxygen inflow from arterial blood independently of concentration gradient. Though our results demonstrate that the addition of cocaine does not increase brain hypoxia

induced by heroin, higher health danger of speedball compared to cocaine or heroin alone may result from de-compensation of vital functions due to diminished intra-brain oxygen inflow induced by high-dose heroin coupled with the enhanced oxygen use and known disturbances of cardiovascular functions (i.e., tachycardia, arrhythmias, hypertension) induced by cocaine. This danger could also result from contamination of cocaine-heroin mixtures by synthetic opioids such as fentanyl or carfentanil that have much higher potency to inhibit breathing than heroin (Solis et al., 2018). Alternatively, the injection of two potentially dangerous drugs can increase the chance of overdosing with either drug. With the subjective effects being opposite, users of speedball at times inject more of either drug to decrease the effect of the opposite drug (erowid.org). This can quickly lead to drug overdose.

3. Experimental Procedures

3.1. Subjects

11 adult male Long-Evans rats (Charles River Laboratories) weighing 440 ± 40 g at the time of surgery were used in this study. Rats were individually housed in a climate-controlled animal colony maintained on a 12–12 light-dark cycle with food and water available ad libitum. All procedures were approved by the NIDA-IRP Animal Care and Use Committee and complied with the Guide for the Care and Use of Laboratory Animals (NIH, Publication 865–23). Maximal care was taken to minimize the number of experimental animals and any possible discomfort or suffering at all stages of the study.

3.2. Overview of the study

In this study, we describe the results of two electrochemical experiments with direct monitoring of drug-induced changes in brain oxygen levels. In the first experiment, we examined how iv cocaine and heroin at three doses within the rat self-administration range affect NAc oxygen levels when delivered alone or in combination. This experiment allowed us to characterize the effects of cocaine, the effects of heroin, and the effects of cocaine-heroin mixture. In the second experiment, we examined how the addition of cocaine to heroin at a high dose, within the range of possible overdose, affects NAc oxygen response. Our recordings were conducted in the NAc, a critical structure involved in natural and drug reinforcement (Di Chiara, 2002; Wise and Bozarth, 1987). This integrative structure has been a focus of our previous electrophysiological and neurochemical studies

3.3. Surgical preparations

Surgical procedures for electrochemical assessment of oxygen have been described in detail elsewhere (Solis et al., 2017, 2018). Briefly put, under general anesthesia (Equithesin, a mixture of sodium pentobarbital and chloral hydrate), rats were chronically implanted with a Pt-Ir oxygen sensor (Model 7002–02; Pinnacle Technology, Inc., Lawrence, KS, USA) into the NAc shell. Target coordinates for the right NAc were: AP +1.2 mm, ML ± 0.8 mm, and DV +7.2–7.6 mm from the skull surface, according to coordinates of the rat brain atlas (Paxinos and Watson, 1998). The sensor was secured with dental acrylic to three stainless steel screws threaded into the skull. During the same surgery, rats were also implanted with a chronic jugular catheter, which ran subcutaneously to the head mount. Rats were allowed a minimum of 5 days of post-operative recovery and at least 3 daily habituation sessions (~6 h

each) to the recording environment. Jugular catheters were flushed daily with 0.2 ml heparinized saline to maintain patency.

3.4. Electrochemical detection of oxygen

For *in vivo* oxygen detection we used Pinnacle oxygen sensors coupled with high-speed amperometry. These sensors were prepared from 180 μm Pt-Ir wire and had a sensing area of 0.025 mm^2 at the tip. The active electrode was incorporated with an integrated Ag/AgCl reference electrode. Dissolved oxygen is reduced on the active surface of these sensors, held at a stable potential of -0.6 V vs. the reference electrode, producing an amperometric current. The current from the sensor is then transmitted via a potentiostat (Model 3104, Pinnacle Technology) to the computer and recorded at 1-s intervals, using PAL software (Pinnacle Technology).

Oxygen sensors were calibrated at 37°C by the manufacturer (Pinnacle Technology) according to a standard protocol described elsewhere (Bolger et al., 2011). The sensors produced linear current changes with increases in oxygen concentrations within a wide range of brain oxygen concentrations (0–40 μM). Substrate sensitivity of oxygen sensors varied from 0.61 to 1.05 $\text{nA}/1\mu\text{M}$ (mean=0.83 $\text{nA}/1\mu\text{M}$). Oxygen sensors were also tested by the manufacturer for their selectivity toward electroactive substances such as dopamine (0.4 μM) and ascorbate (250 μM), none of which had significant effects on current measurements.

3.5. Experimental procedures

At the onset of each experiment, rats were anesthetized ($<2\text{ min}$) with isoflurane and the electrochemical sensor was connected to the recording instrument via an electrically shielded flexible cable and a multi-channel electrical swivel. A catheter extension mounted on the cable was used to allow for stress and cue-free drug delivery from outside the cage. Testing began approximately 120 min after the electrochemical sensors were connected to the recording instruments, allowing for baseline currents to stabilize.

In the first experiment, rats ($n=7$) underwent three treatment sessions (cocaine, heroin, cocaine+heroin), during which they received three drug injections at increasing doses (cocaine: 0.25, 0.5, 1.0 mg/kg ; heroin: 0.05, 0.1, 0.2 mg/kg ; heroin+cocaine: 0.05+0.25 mg/kg ; 0.1+0.50 mg/kg ; 0.2+1.0 mg/kg , respectively). To minimize the possible changes in drug responses during repeated sessions, the order of drug presentation was counter-balanced. Drugs were dissolved in saline and injected at 0.15, 0.3, and 0.6 ml volumes during 10, 20, and 40 s. Two rats were recorded simultaneously, drug injections in each rat were made with 2-h time intervals, and a total duration of recording in each rat was $\sim 8\text{ hrs}$. At the end of a session, rats were injected with Equithesin (0.6–0.7 ml) to safely disconnect the animals from the recording instruments.

In the second experiment, each of the 4 rats underwent four treatment sessions, during which they received a single injection of heroin at 0.6 mg/kg (2 sessions) or a single injection of heroin 0.6 mg/kg + cocaine 1.0 mg/kg (2 sessions). Treatment sessions were counter-balanced.

3.6. Histological verification of electrode placements

When experiments were completed, rats were deeply anesthetized with isoflurane, decapitated, and the brains were extracted, and stored in 10% formalin solution. Later, the brains were sectioned and analyzed for verification of the locations of cerebral implants and possible tissue damage at the area of electrochemical recording.

3.7. Data analysis

Electrochemical data was analyzed with slow (1-min) and rapid (10-s) resolution. Because each individual sensor differed in substrate sensitivity, currents were converted into concentrations according to sensitivity calibrations provided by the manufacturer. Data were then converted into percent (%) changes in concentration of oxygen. Values from one minute prior to injection were averaged and used to set the 100% baseline. One-way repeated measure ANOVAs (followed by Fisher LSD post-hoc tests) were used to evaluate statistical significance of drug-induced current responses and two-way ANOVA was used to assess between-drug differences in oxygen dynamics. For text clarity, quantitative results of statistical evaluations are shown in figure captions.

ACKNOWLEDGEMENTS

The study was supported by the Intramural Research Program of the NIH, NIDA (# 1ZIADA000566-09 for EAK).

FUNDING AND DISCLOSURE

None of the authors have any conflicts of interests to declare.

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Highlights

- Iv cocaine modestly increases brain oxygen levels
- Iv heroin at a low reinforcing dose similarly increases brain oxygen levels
- At higher reinforcing doses, iv heroin induces down-up brain oxygen change
- Powerful and prolonged brain hypoxia occurs during heroin overdose
- Cocaine does not alter brain hypoxia induced by heroin at high doses

Figure 1. Mean ($\pm$ SEM) changes in NAc oxygen levels induced by iv cocaine delivered to freely moving rats at different doses within the self-administration range.

Data are shown with 1-min time resolution with respect to the pre-injection baseline (100%).

Filled symbols show values significantly different from baseline ($p < 0.05$; one-way repeated measure ANOVA followed by post-hoc Fisher test; $F_{5,200} = 1.46, 1.78$ and 1.97 for $0.25, 0.5$, and 1.0 mg/kg, $p < 0.05$ for each case). D shows oxygen changes analyzed with high (10-s bin) time resolution for the first 10 min after the injection. Standard errors are not shown for clarity. In each graph, n represents the number of averaged tests (rats).

Figure 2. Mean ($\pm$ SEM) changes in NAc oxygen levels induced by iv heroin delivered to freely moving rats at different doses within the self-administration range.

Data are shown with 1-min time resolution with respect to the pre-injection baseline (100%).

Filled symbols show values significantly different from baseline ($p < 0.05$; one-way repeated measure ANOVA followed by post-hoc Fisher test; $F_{5,250}=4.35$ and 4.41 for 0.05 and 0.2 mg/kg; $p < 0.001$; $F_{6,300}=1.57$, $p < 0.05$ for 0.1 mg/kg). D shows oxygen changes analyzed with high (10-s bin) time resolution for the first 10 min after the injection. Standard errors are not shown for clarity. In each graph, n represents the number of averaged tests (rats).

Figure 3. Differences in mean ($\pm$ SEM) changes in NAc oxygen levels induced by a mixture of cocaine and heroin delivered to freely moving rats at different doses within the self-administration range.

Control data (heroin at the same doses) are superimposed with grey circles. Data are shown with 1-min (A-C) and 10-s (D-F) time resolutions with respect to the pre-injection baseline (100%). Filled symbols for a drug mixture show values significantly different from baseline ($p < 0.05$; one-way repeated measure ANOVA followed by post-hoc Fisher test). Between-group differences assessed by two-way ANOVA with repeated measures were not significant for each dose combination. For clarity, data from control heroin injections are shown without standard errors. In each graph, n represents the number of averaged tests (rats).

A. Original Records

B. Mean Changes

Figure 4. Mean ($\pm$ SEM) changes in NAc oxygen levels induced by iv heroin delivered to freely moving rats at a high dose (0.6 mg/kg) either alone or with addition of cocaine (1 mg/kg). **Figure A** represents original records of oxygen changes induced by heroin and its mixture with cocaine. **Figure B** represents mean changes in oxygen analyzed with slow (1-min bin) and rapid (10-s bin) time resolution. Filled symbols show values significantly different from baseline ($p < 0.05$; one-way repeated measure ANOVA followed by post-hoc Fisher test). The effects of drug were significant in each case ($F_{6,540} = 12.6$ and $= 13.36$ for heroin and heroin + cocaine, respectively), but difference between drugs was not significant. The graph means represent the average of 7 injections obtained in 4 rats.