HHS Public Access

Author manuscript

Drug Alcohol Depend. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Drug Alcohol Depend. 2018 September 01; 190: 20–28. doi:10.1016/j.drugalcdep.2018.05.021.

Normal glutathione levels in autopsied brain of chronic users of heroin and of cocaine*

Junchao Tong[a,b,c], **Paul S Fitzmaurice**[d], **Anna Moszczynska**[e], **Gausiha Rathitharan**[b,c], **Lee-Cyn Ang**[f], **Jeffrey H Meyer**[g], **Romina Mizrahi**[h], **Isabelle Boileau**[c], **Yoshiaki Furukawa**[i], **Tina McCluskey**[b], **Napapon Sailasuta**[j], and **Stephen J. Kish**[b]

[a]Preclinical Imaging Unit, Research Imaging Centre, Centre for Addiction and Mental Health, Toronto, ON, Canada [b]Human Brain Laboratory, Research Imaging Centre, Centre for Addiction and Mental Health, Toronto, ON, Canada [c]Addiction Imaging Research Group, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada [d]Gribbles Pathology, HealthScope, New Zealand [e]Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, MI, USA [f]Division of Neuropathology, London Health Sciences Centre, University of Western Ontario, London, ON, Canada [g]Research Imaging Centre and Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, ON, Canada [h]Research Imaging Centre, Centre for Addiction and Mental Health; Institute of Medical Science, Department of Psychiatry, University of Toronto, Toronto, ON, Canada [i]Department of Neurology, Juntendo Tokyo Koto Geriatric Medical Center, Faculty of Medicine, University and Post Graduate University of Juntendo, Tokyo, Japan [j]Research Imaging Centre, Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Abstract

Background: Animal studies suggest that exposure to either of the two widely used drugs of abuse, heroin or cocaine, causes depletion of the antioxidant, reduced glutathione, a hallmark of oxidative stress, in the brain. However, the relevance of the animal findings to the human is uncertain and clinical trials with the antioxidant GSH precursor n-acetylcysteine have produced mixed results in cocaine dependence.

Correspondence: Junchao Tong, Preclinical Imaging Unit, Research Imaging Centre, Centre for Addiction and Mental Health, 250 College Street, Toronto, ON, Canada M5T 1R8., Telephone: 416-535-8501 ext. 36422 Junchao.tong@camh.ca.

Author Disclosures

Contributors

SJK, JT and PSF designed the study; JT, PSF, AM and TM conducted experiments, data collection and analysis; LCA performed neuropathology; JT, PSF and SJK wrote initial drafts; AM, GR, JHM, RM, IB, YF and NS contributed to revisions; all authors approved the final manuscript.

Conflict of Interest

No conflict declared.

Methods: Our major objective was to compare glutathione levels, determined by an HPLC-coulometric procedure, in autopsied brain of chronic heroin (n=11) and cocaine users (n=9), who were positive for the drugs in the brain, to those of matched controls (n=16). Six brain regions were examined, including caudate, hippocampus, thalamus and frontal, temporal and insular cortices.

Results: In contrast to experimental animal findings, we found no statistically significant difference between mean levels of reduced or oxidized glutathione in the drug user vs. control groups. Moreover, no correlation was found between levels of drugs in the brain and those of glutathione.

Conclusions: Acknowledging the many generic limitations of an autopsied human brain study and the preliminary nature of the findings, our data nevertheless suggest that any oxidative stress caused by heroin or cocaine in chronic users of the drugs might not be sufficient to cause substantial loss of stores of glutathione in the human brain, at least during early withdrawal. These findings, requiring replication, might also have some relevance to future clinical trials employing glutathione supplement therapy as an anti-oxidative strategy in chronic users of the two abused drugs.

Keywords

Glutathione; Oxidative stress; Heroin; Cocaine; Human brain; Postmortem

1. Introduction

It is generally assumed that chronic exposure of some recreational drugs of abuse (e.g., stimulants, heroin) likely “injuries” the human brain to some extent and that neurotoxic injury might be caused at least in part by oxidative stress (Sharma et al., 2007; Cunha-Oliveira et al., 2008; Yamamoto et al., 2010; Sajja et al., 2016). To date, evidence supporting this possibility is largely driven by results of experimental animal investigations. For example, animal (rodent) studies show that high doses of the dopaminergic stimulant methamphetamine can produce increased brain levels of malondialdehyde or malondialdehyde-like lipoperoxidation substances, cause structural damage to (at least) brain dopamine nerve endings, and with reduction of the dopamine neuronal markers lessened by antioxidant treatment (for review see Kish, 2014).

The relevance of animal model findings to the human condition is always uncertain. Further, a practical consideration is that, at present, few tools can or have been used to assess oxidative stress or damage in the human brain directly. This is particularly relevant because of increasing interests in employing antioxidants such as n-acetylcysteine (Baker et al., 2003a,b; Ng et al., 2008; Zhou and Kalivas, 2008; Moussawi et al., 2009; Berk et al., 2013; McClure et al., 2014; Deepmala et al., 2015; Trivedi and Deth, 2015; Duailibi et al., 2017; Nocito Echevarria et al., 2017; Schulte et al., 2017) as a treatment strategy for drug dependence partly based on the assumption that there exists oxidative stress in brain of drug users. One indirect approach has been a measurement in the brain of the tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine; GSH, the reduced form), a major antioxidant defense. GSH is converted to oxidized glutathione (GSSG) as a consequence of oxidation

catalyzed by glutathione peroxidase and can be recycled from the oxidized form to the reduced form by glutathione reductase (Dringen, 2000). One of the most consistent consequences of severe oxidative stress observed in a variety of experimental conditions affecting different organ systems is depletion of GSH (Sen and Packer, 2000; Gu et al., 2015; Won et al., 2015). In this regard, finding of a below normal concentration of GSH can be suggestive of the presence of oxidative stress (Di Monte et al., 1992; Jenner and Olanow, 1996; Won et al., 2015; Ren et al., 2017). Thus, our previous finding, consistent with animal data (Moszczynska et al., 1998), of a trend for GSH reduction in postmortem brain of a subgroup of human methamphetamine users (Mirecki et al., 2004) who had a marked dopamine loss suggests (but does not prove) that some oxidative stress might have occurred in methamphetamine-exposed human brain.

Emerging data, although not always consistent, shows that GSH concentration can be below normal in brain of experimental animals exposed to two other drugs of abuse, namely the heroin metabolite morphine (Goudas et al., 1997; Qiusheng et al., 2005; Guzman et al., 2006, 2009a,b; Ozmen et al., 2007; Abdel-Zaher et al., 2010, 2013a,b; Sumathi et al., 2011; Deng et al., 2012; Hu et al., 2012; Joshi et al., 2014; Motaghinejad et al., 2015a,b; Singh et al., 2015; Yun et al., 2015; Famitafreshi and Karimian, 2017) and the dopaminergic stimulant cocaine (Muriach et al., 2010; Uys et al., 2011; Lopez-Pedrajas et al., 2015; Vitcheva et al., 2015; Hu et al., 2016; Zhang et al., 2016; but see Wiener and Reith, 1990) (see Table 1 for a review). Overall, the findings show that chronic systemic and acute intra-cerebral-spinal morphine exposure consistently deplete brain GSH, with the exception of one study in rat pups showing increased GSH after repeated morphine injection (Traudt et al., 2012). However, results of acute effects of systemic morphine on brain GSH are mixed (increase, Guzman et al., 2009b; Joshi et al., 2014; decrease, Guzman et al., 2006, 2009a,b; and no change, Bien et al., 1992; Goudas et al., 1997). For cocaine, most studies (Muriach et al., 2010; Uys et al., 2011; Lopez-Pedrajas et al., 2015; Vitcheva et al., 2015; Hu et al., 2016; Zhang et al., 2016) except one (no change, Wiener and Reith, 1990; in mice) of chronic cocaine exposure in adults show decreased levels of brain GSH or ratio of GSH/GSSG although the effects of acute cocaine treatment can also be mixed (Wiener and Reith, 1990; Macedo et al., 2010; Uys et al., 2011). Discrepancies in the literature might be explained by the species employed, age at drug exposure (e.g., adolescent cocaine exposure did not result in GSH abnormality measured in adults; Zhu et al., 2016, 2017), brain regions examined, dose regimen and the GSH assay used (Table 1; see also Discussion).

Investigations of GSH in central nervous system of human users of heroin and cocaine appear to be limited to a postmortem brain study in heroin users reporting markedly below normal GSH throughout the brain (Gutowicz et al., 2011) and a preliminary report of low GSH in cerebrospinal fluid of two (of three examined) patients receiving intracerebroventricular doses of morphine for intractable cancer pain (Goudas et al., 1999). Given the sparse literature on whether the two widely used drugs of abuse might cause oxidative stress in human brain, as suggested by animal data, our objective was to establish whether levels of GSH (primary outcome measure) are lower than normal (and by inference oxidative stress above-normal) in a regionally extensive sampling of well-characterized autopsied human brain of chronic cocaine and chronic heroin users (Wilson et al., 1996; Kish et al., 1999, 2001; Kalasinsky et al., 2000; McLeman et al., 2000; Worsley et al., 2000;

Siegal et al., 2004; Frankel et al., 2008). For comparison, using an HPLC-electrochemical procedure, we measured the following compounds as secondary outcome measures which also appeared on the chromatogram: GSSG (oxidized GSH), GSH-Cysteine (GSH-CYS; the mixed disulfide), uric acid (UA), a xanthine catabolite and potential antioxidant and neuroprotective agent, reported to act via GSH (Mirecki et al., 2004; Bakshi et al., 2015), and methionine, the latter as an index sensitive to postmortem time (Mirecki et al., 2004). Our working hypothesis was that, based on the above-mentioned animal data, GSH levels would be below normal throughout the brain of users of either heroin or cocaine.

2. Subjects and Methods

2.1 Subjects

Postmortem brain from a total of 11 chronic users of heroin (1 female), 9 cocaine (2 females) and 16 controls (2 females) was obtained from medical examiner offices in USA/Canada using a standardized protocol. The study was approved by the Research Ethics Board of the Centre for Addiction and Mental Health at Toronto. Subject information, drug histories, and brain drug and dopamine levels are summarized in Table 2, with the information previously reported (Wilson et al., 1996; Kish et al., 1999, 2001; Kalasinsky et al., 2000; McLeman et al., 2000; Siegal et al., 2004). There were no statistically significant differences in age (control, 37.0±3.1 years; heroin, 36.2±2.5 years; cocaine, 35.4±4.8 years; mean±SEM), postmortem intervals (PMI, interval between death and freezing of the brain; control, 14.6±1.8 hours; heroin, 13.4±2.0 hours; cocaine, 17.0±2.4 hours), or freezer storage time at the time when the biochemical assays were performed in 2001–2003 (control, 5.4±0.5 years; heroin, 6.5±0.4 years; cocaine, 7.0±0.6 years) between the control and drug users. At autopsy, one half-brain was fixed in formalin fixative for neuropathological analysis, whereas the other half was immediately frozen until dissection for neurochemical analysis. Blood samples were obtained from all of the drug users and controls for drug screening. Sequential scalp hair samples for drug analyses could be obtained from 14 of 16 controls, 10 of 11 heroin users, and five of 9 cocaine users. Levels of drugs of abuse in blood and other bodily fluids were measured by the local medical examiner whereas drug analyses in brain and hair samples were conducted at the Armed Forces Institute of Pathology (Washington, DC, USA). Heroin users met the following criteria: 1) presence of heroin metabolites (6-acetylmorphine, morphine, or morphine glucuronide) on toxicology screens in blood and autopsied brain; 2) absence of other drugs of abuse in bodily fluids with the exception of ethanol (see below) or other opioid drugs (two subjects #H5 and #H7 had blood samples positive for the opioid drug propoxyphene and its metabolite norpropoxyphene; see Table 2); 3) evidence from the case records of primary use of heroin for >1 year prior to death; and 4) absence of evidence of neurological illness or, at autopsy, brain pathology unrelated to use of the drug. Five of the heroin users had recently used alcohol as indicated by the presence of ethanol in blood (Table 2). Available hair analysis of the (10 of 11) heroin users revealed presence of only heroin metabolites in eight of the users. The suspected cause of death was heroin intoxication (seven), mixed drug intoxication (two), and cardiovascular disease with heroin as a contributing factors (two). Cocaine users met the following criteria: 1) presence of cocaine or metabolite benzoylecgonine in blood or (one subject) urine; autopsied brain, and, if available, scalp hair by GC-MS; 2) absence of other drugs of abuse

in bodily fluids, with the exception of ethanol (see below), or in brain; 3) evidence from the case records or interview with next of kin of use of cocaine as the primary drug of abuse for >1 year prior to death; and 4) absence of neurological illness or, at autopsy, brain pathology unrelated to use of the drug. Two of the cocaine users had recently used alcohol as indicated by the presence of ethanol in blood or cocaethylene in brain. Available hair analysis of the (five of nine) cocaine users revealed presence of only cocaine and/or metabolites in four of the users. Known or suspected causes of death of the cocaine users were cocaine intoxication (five), cardiovascular disease with cocaine as a contributing factors (two), carotid artery aneurysm with cocaine as a contributing factor (one) and chest trauma (one).

All control subjects (for which brain GSH levels have been previously reported in (Mirecki et al., 2004)) were neurologically normal and had no evidence of brain pathology on neuropathological examination. All had no history of drug use and tested negative for drugs of abuse in blood, autopsied brain, and in sequential scalp hair samples where available. The cause of death for the controls were electrocution (n=1), morbid obesity (n=1), trauma (n=3), pulmonary embolism (n=2), and cardiovascular disease (n=9).

2.2 GSH, GSSG, GSH-Cys, UA and Methionine Analysis

Brain regions were dissected as previously described (Kish et al., 1988), using the Atlas of Riley (Riley, 1943) for the caudate, hippocampal Ammon's horn and medial pulvinar thalamus and Brodmann classification for frontal (BA9), temporal (BA21) and insular cortices. Levels of GSH, GSSG, GSH-Cys, methionine, and UA in tissue homogenates were measured by a coulometric method using HPLC and electrochemical detection with coulometric cells as previously described (Fitzmaurice et al., 2003; Mirecki et al., 2004; Tong et al., 2016) (see Supplementary Methods for more details). Protein concentration was determined using the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, USA) with bovine plasma albumin as the standard.

2.3 Statistical Analyses

Statistical analyses were performed using StatSoft STATISTICA 7.1 (Tulsa, Oklahoma, USA). Differences in levels of GSH, GSSG, GSSG-Cys, UA and methionine among controls and drug groups in brain regions examined were conducted using ANCOVA ($p<0.05$) with age and PMI as the covariates, given influences of age and PMI on some of the outcome measures (Mirecki et al., 2004; Tong et al., 2016), followed by *post-hoc* Bonferroni adjustments ($p<0.05$). Correlations were examined by Pearson product moment correlation or Spearman ranking order correlation as indicated in the text.

3. Results

A one-way ANCOVA with age and PMI as the covariates disclosed that GSH, GSSG, GSH-Cys, UA and methionine levels (Table 3) were normal in all examined brain regions of the two drug user groups versus the controls ($p>0.05$), with the exception of significantly lower levels of methionine in the caudate nucleus of users of heroin (−26%) and cocaine (−33%). Scatter plots of GSH levels (Figure 1) and those of GSSG, GSH-Cys, UA, and methionine

(see Supplementary Figures 1–4)[1] showed overlapped data range between drug users and control subjects.

No significant correlation was observed between levels of GSH, GSSG, GSH-Cys, UA or methionine and available drug use parameters of the heroin and cocaine users including duration of use (Pearson) and composite blood and brain drug levels (Spearman). Three cocaine users (#C1, #C4 and #C6 in Table 2) demonstrated a markedly higher level of cocaine and metabolites in brain than other cocaine users (>150 *versus* <50 nmol/g tissue); however, the three cocaine users did not have abnormally low levels of GSH (Figure 1) or out-of-range values of GSSG, GSH-Cys, UA or methionine (see Supplementary Figures 1–4)[2]. Two heroin users (Table 2) had high blood levels of the opioid drug propoxyphene and its metabolite norpropoxyphene (5.76 μM and 15.21 μM for #H5 and #H7, respectively); however, the two were not particularly affected with respect to brain levels of GSH (see Figure 1), GSSG, GSH-Cys, UA or methionine (see Supplementary Figures 1–4)[3].

Blood tested positive for ethanol in five heroin users (0.05–0.12%; #H2, #H4, #H8, #H9 and #H11 in Table 2) and two cocaine users (0.01% and 0.02% for #C3 and #C4, respectively, in Table 2); however, the presence or absence of ethanol did not differentiate the outcome measures of brain GSH, GSSG, GSH-Cys, UA or methionine. Cocaine users showed a moderate loss of striatal dopamine (Wilson et al., 1996) whereas dopamine levels were normal in heroin users (Kish et al., 2001); however, dopamine levels in the caudate were not significantly correlated (Pearson) with those of GSH, GSSG, GSH-Cys, UA or methionine in the drug users.

In this sample of controls and/or drug users, we found no significant correlation (Pearson) between levels of GSH, GSSG, GSH-Cys, UA or methionine and age or PMI of the subjects (all subjects included or in individual groups).

4. Discussion

The main finding of our study is that levels of GSH are normal in autopsied brain of chronic users of heroin and cocaine. Further, we found no correlation between recent drug exposure (as suggest by brain drug levels) and levels of the tri-peptide antioxidant.

4.1 Limitations

There are many limitations to postmortem human brain studies. Because, as we have shown previously (Mirecki et al., 2004), levels of both reduced and oxidized glutathione are decreased in autopsied vs. biopsied human brain, postmortem time must have influenced to some degree concentration of our major outcome measures. Nevertheless, we feel it reasonable to expect that qualitative differences or lack thereof, found in the autopsied brain would be generally similar to those occurring in living brain. Further, there were no statistically significant differences amongst mean PMI for the drug and control groups (note: PMI was included as a covariate in the statistical analysis), and mean levels of the amino acid methionine, which increase quite markedly after death (Mirecki et al., 2004), were also

[1–3]Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi: …

mostly similar in the three groups. Further, we did not observe any significant negative correlations between levels of GSH and methionine among brain regions and groups; including methionine levels as a covariate did not change any statistical outcomes of the other analytes.

Little information on drug doses, precise duration of chronic use, medication history, or history of alcohol consumption, which is known to be able to deplete brain GSH (Uys et al., 2014), was available for the subjects of our study. In this regard, the possibility cannot be excluded that unknown medications or other drugs used by the subjects of our study might have influenced GSH levels in the brain. However, we can be certain that the users of cocaine and heroin must have used these drugs, at least as recently as 72 hours before death as they all tested positive for the drug in the brain and/or blood.

Arguing against the notion that we might not have been able to detect a small/modest change in postmortem brain GSH concentrations are our previous findings demonstrating a modest GSH reduction (19 to 30%) in autopsied substantia nigra of patients with three different degenerative Parkinsonian conditions (n=10–16 per group; Fitzmaurice et al., 2003) and the observation of slightly decreased (by 17%) GSH levels in striatum of rodents exposed to a binge dose of methamphetamine (Moszczynska et al., 1998). Previously we reported a trend for a modest reduction (by 35%) in autopsied brain of human methamphetamine users, but which was restricted to the subgroup having severe loss of the neurotransmitter dopamine (Mirecki et al., 2004).

4.2 Comparison with Literature (Human)

To our knowledge, there have been no previous studies of glutathione in postmortem or living brain of human cocaine users. However, in autopsied brain of users of heroin, Gutowicz and colleagues (Gutowicz et al., 2011) reported markedly (by about 20–40%) lower GSH in the cerebral cortex, hippocampus, brain stem and white matter. Assuming in their report that brain “heroin level” means total concentration in brain of the major heroin metabolites (6-acetylmorphine, morphine, morphine glucuronide), the brain drug levels in heroin users of the Gutowicz study were generally similar to those in our investigation, suggesting that the extent of recent drug exposure to the heroin users was also similar in both studies. Possibly the discrepancy might be explained by differences in methodologies for glutathione (HPLC with electrochemical detection in our study vs. colorimetric assay; see Tong et al., 2016 for discussions), differences in drug history of the heroin users, or be related to the very long PMI (two-four days) in the Gutowicz study vs. a much shorter mean of 14 hours for both heroin users and controls in our investigation, and uncertainty whether drug and control groups were matched for PMI in the earlier investigation. In this regard, GSH levels reported by Gutowicz study in control brains (2.3–3.4 mM, assuming a protein/tissue ratio of 0.05 (Tong et al., 2016) and a brain unit weight of 1 g/mL) were higher than those reported in the literature for autopsied human brain (generally < 2 mM) (Perry et al., 1982; Slivka et al., 1987; Sofic et al., 1992; Sian et al., 1994) including our studies (Fitzmaurice et al., 2003; Mirecki et al., 2004; Tong et al., 2016) despite much longer PMI (>48 hrs. vs ≤26 hrs.). It is also possible that the autopsied brains in the Gutowicz study had suffered from more severe pathology (not reported), e.g., hypoxic/ischaemic lesions

(Andersen and Skullerud, 1999; Buttner et al., 2000), than those in our study as some animal data suggest that prolonged ischemia might cause glutathione depletion in brain (Rehncrona et al., 1980; Slivka and Cohen, 1993). In this respect, qualitative brain neuropathological examination in our cases did not reveal obvious abnormalities (cell loss or gliosis) in the drug users with the exception of some hypoxic/ischaemic neuronal changes in CA1 of hippocampus (#C3), mild diffuse gliosis in midbrain (#C4) and acute subarachnoid hemorrhage (#C9) in three cocaine users, respectively, and mild ventricular dilatation (#H5) and mild diffuse gliosis in diencephalon and lower brainstem (#H11) in two heroin users, respectively.

4.3 Why Did Animal GSH Findings on Morphine/Cocaine Not Translate into The Human?

Although, as mentioned above, the experimental animal literature is generally consistent (below normal GSH following chronic heroin or cocaine exposure; Table 1), we found no significant change in GSH levels in brain of humans chronically exposed to either of the drugs. Possibly the difference could be explained by different extent of drug exposure in the animal studies vs. that in our human investigation and by different redox response in animals vs humans as exemplified by reported GSH depletion in animal ischemia (Slivka and Cohen, 1993) versus compensatory GSH elevation in human brain stroke (An et al. 2012). The possibility has to be considered that acute/sub-chronic exposure to the drugs, e.g., as suggested by experimental reports of GSH depletion by morphine and cocaine (Table 1) and by a report of depletion of cerebrospinal fluid GSH levels in the human after acute intracerebroventricular morphine for cancer pain (Goudas et al., 1999), might have resulted in excessive GSH utilization and depletion, but that tolerance (compensatory increase in GSH synthesis) occurred in the users of our investigation who likely had been exposed to the drugs for years. Most animal studies employed passive drug administration, and it is conceivable that this might have produced a different profile of brain redox response and disturbance as compared to that of drug self-administration (e.g., see Pomierny-Chamiolo et al., 2013), more relevant to the human condition. The study by Uys et al. (2011) shows that rats repeatedly exposed to cocaine had decreased levels of GSH in brain (nucleus accumbens) at three weeks withdrawal versus saline-treated animals whereas an acute challenging dose of cocaine restored brain GSH levels to that of the controls, suggesting a possible effect of abstinence although some of the studies examined GSH within hours of final drug administration (Abdel-Zaher et al., 2010, 2013a,b). The age of initial drug exposure could be another variable as cocaine exposure during adolescence, which is common in many human users, was not associated with GSH loss in rats later in adults (Zhu et al., 2016, 2017). Interestingly, a recent study (Joshi et al., 2014) showed that morphine could counteract chronic restraint stress-induced GSH depletion in rat brain, suggesting some interactions between drugs of abuse and stress-induced redox disturbance (Madrigal et al., 2001; Ahmad et al., 2010; Kumar et al., 2011; Moretti et al., 2012; Filho et al., 2015; Bouvier et al., 2017; Famitafreshi and Karimian, 2017). Perhaps some of the above factors might help to explain why available preclinical animal data demonstrating a brain GSH reduction following morphine or cocaine exposure do not translate to the human.

4.4 Glutathione and Oxidative Stress in Heroin and Cocaine Users

We emphasize that the “negative” results of our investigation do not imply that chronic use of the abused drugs cocaine and heroin do not cause oxidative stress in human brain, but rather that exposure to the drugs at the doses used by the subjects of our study, which are probably within the dose regimen employed in experimental animal studies (e.g., see Nayak et al., 1976; Djurendic-Brenesel et al., 2010), might not cause oxidative stress of a magnitude that produces some brain depletion of the antioxidant glutathione. Here we caution also that the extent of oxidative stress necessary to cause GSH depletion in human brain is not known and that mild to moderate oxidative stress could induce compensatory increase in GSH synthesis (cf. Tong et al., 2016). Recently, clinical trials of the antioxidant n-acetylcysteine, a prodrug to the rate-limiting GSH precursor cysteine, were performed in a variety of human addiction conditions including cocaine with mixed outcomes (see Berk et al., 2013; McClure et al., 2014; Deepmala et al., 2015; Trivedi and Deth, 2015; Duailibi et al., 2017; Nocito Echevarria et al., 2017; Schulte et al., 2017 for reviews). However, N-acetylcysteine was employed primarily as a modulator of glutamate neurotransmission, with its antioxidant property as a possible secondary mechanism (LaRowe et al., 2013; McClure et al., 2014). In retrospect, our findings of normal brain GSH in heroin and cocaine users provide no support to use of this GSH prodrug to address a GSH deficiency in brain, at least during early withdrawal when the drugs of abuse were tested positive. Future trials of N-acetylcysteine in opiate and cocaine dependence, aiming at redox homeostasis (Trivedi and Deth, 2015), might take our autopsied brain finding of normal brain GSH levels into consideration.

5. Conclusions

The main finding of our study is that, in contrast to results of animal studies, levels of glutathione were found to be normal in autopsied brain of chronic users of heroin and of cocaine, suggesting that any oxidative stress caused by the drugs might not be sufficient to deplete substantially tissue stores of the antioxidant. Our findings, although suggestive, must be considered preliminary especially given the limitations associated with autopsied brain investigations including large variability of GSH levels and a small sample size. Future studies might also consider measurement of GSH in living human brain using a magnetic resonance imaging approach in which the influence of heroin and cocaine (and also opiates for therapeutic purposes) can more easily, e.g., longitudinally, be examined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Role of Funding Source

This study was supported in part by the US NIDA/NIH DA07182 (SK), the New Zealand Institute of Environmental Science and Research, Ltd. (PF, SK), and the Centre for Addiction and Mental Health Foundation.

References

Abdel-Zaher AO , Abdel-Rahman MS , FM EL , 2010 Blockade of nitric oxide overproduction and oxidative stress by Nigella sativa oil attenuates morphine-induced tolerance and dependence in mice. Neurochem. Res 35, 1557–1565.20552271

Abdel-Zaher AO , Mostafa MG , Farghaly HS , Hamdy MM , Abdel-Hady RH , 2013a Role of oxidative stress and inducible nitric oxide synthase in morphine-induced tolerance and dependence in mice. Effect of alpha-lipoic acid. Behav. Brain Res 247, 17–26.23470902

Abdel-Zaher AO , Mostafa MG , Farghly HM , Hamdy MM , Omran GA , Al-Shaibani NK , 2013b Inhibition of brain oxidative stress and inducible nitric oxide synthase expression by thymoquinone attenuates the development of morphine tolerance and dependence in mice. Eur. J. Pharmacol 702, 62–70.23376567

Ahmad A , Rasheed N , Banu N , Palit G , 2010 Alterations in monoamine levels and oxidative systems in the frontal cortex, striatum, and hippocampus of the rat brain during chronic unpredictable stress. Stress 13, 355–364.20536337

An L , Dani KA , Shen J , Warach S ; Natural History of Stroke Investigators, 2012 Pilot results of in vivo brain glutathione measurements in stroke patients. J. Cereb. Blood Flow Metab 32, 2118–2121.23010948

Andersen SN , Skullerud K , 1999 Hypoxic/ischaemic brain damage, especially pallidal lesions, in heroin addicts. Forensic Sci. Int102, 51–59.10423852

Baker DA , McFarland K , Lake RW , Shen H , Tang XC , Toda S , Kalivas PW , 2003a Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. Nat. Neuroscience 6, 743–749.12778052

Baker DA , McFarland K , Lake RW , Shen H , Toda S , Kalivas PW , 2003b N-acetyl cysteine-induced blockade of cocaine-induced reinstatement. Ann. N.Y. Acad. Sci 1003, 349–351.14684458

Bakshi R , Zhang H , Logan R , Joshi I , Xu Y , Chen X , Schwarzschild MA , 2015 Neuroprotective effects of urate are mediated by augmenting astrocytic glutathione synthesis and release. Neurobiol. Dis 82, 574–579.26341543

Berk M , Malhi GS , Gray LJ , Dean OM , 2013 The promise of N-acetylcysteine in neuropsychiatry. Trends Pharmacol. Sci 34, 167–177.23369637

Bien E , Vick K , Skorka G , 1992 Effects of exogenous factors on the cerebral glutathione in rodents. Arch.Toxicol 66, 279–285.1514927

Bouvier E , Brouillard F , Molet J , Claverie D , Cabungcal JH , Cresto N , Doligez N , Rivat C , Do KQ , Bernard C , Benoliel JJ , Becker C , 2017 Nrf2-dependent persistent oxidative stress results in stress-induced vulnerability to depression. Mol. Psychiatry 22, 1795.27801891

Buttner A , Mall G , Penning R , Weis S , 2000 The neuropathology of heroin abuse. Forensic Sci. Int 113, 435–442.10978659

Cunha-Oliveira T , Rego AC , Oliveira CR , 2008 Cellular and molecular mechanisms involved in the neurotoxicity of opioid and psychostimulant drugs. Brain Res. Rev 58, 192–208.18440072

Deepmala , Slattery J , Kumar N , Delhey L , Berk M , Dean O , Spielholz C , Frye R , 2015 Clinical trials of N-acetylcysteine in psychiatry and neurology: A systematic review. Neurosci. Biobehav. Rev 55, 294–321.25957927

Deng Y , Bu Q , Hu Z , Deng P , Yan G , Duan J , Hu C , Zhou J , Shao X , Zhao J , Li Y , Zhu R , Zhao Y , Cen X , 2012 (1) H-nuclear magnetic resonance-based metabonomic analysis of brain in rhesus monkeys with morphine treatment and withdrawal intervention. J. Neurosci. Res 90, 2154–2162.22847893

Di Monte DA , Chan P , Sandy MS , 1992 Glutathione in Parkinson’s disease: A link between oxidative stress and mitochondrial damage? Ann. Neurol 32, S111–115.1510368

Djurendic-Brenesel M , Mimica-Dukic N , Pilija V , Tasic M , 2010 Gender-related differences in the pharmacokinetics of opiates. Forensic Sci. Int 194, 28–33.19913374

Dringen R , 2000 Metabolism and functions of glutathione in brain. Prog. Neurobiol 62, 649–671.10880854

Duailibi MS , Cordeiro Q , Brietzke E , Ribeiro M , LaRowe S , Berk M , Trevizol AP , 2017 N-acetylcysteine in the treatment of craving in substance use disorders: Systematic review and meta-analysis. Am. J. Addict 26, 660–666.28898494

Famitafreshi H , Karimian M , 2017 Socialization alleviates burden of oxidative-stress in hippocampus and prefrontal cortex in morphine addiction period in male rats. Curr. Mol. Pharmacol [Epub ahead of print]

Filho CB , Jesse CR , Donato F , Giacomeli R , Del Fabbro L , da Silva Antunes M , de Gomes MG , Goes AT , Boeira SP , Prigol M , Souza LC , 2015 Chronic unpredictable mild stress decreases BDNF and NGF levels and Na(+), K(+)-ATPase activity in the hippocampus and prefrontal cortex of mice: Antidepressant effect of chrysin. Neuroscience 289, 367–380.25592430

Fitzmaurice PS , Ang L , Guttman M , Rajput AH , Furukawa Y , Kish SJ , 2003 Nigral glutathione deficiency is not specific for idiopathic Parkinson's disease. Mov. Disord 18, 969–976.14502663

Frankel PS , Alburges ME , Bush L , Hanson GR , Kish SJ , 2008 Striatal and ventral pallidum dynorphin concentrations are markedly increased in human chronic cocaine users. Neuropharmacology 55, 41–46.18538358

Goudas LC , Carr DB , Maszczynska I , Marchand JE , Wurm WH , Greenblatt DJ , Kream RM , 1997 Differential effect of central versus parenteral administration of morphine sulfate on regional concentrations of reduced glutathione in rat brain. Pharmacology 54, 92–97.9088042

Goudas LC , Langlade A , Serrie A , Matson W , Milbury P , Thurel C , Sandouk P , Carr DB , 1999 Acute decreases in cerebrospinal fluid glutathione levels after intracerebroventricular morphine for cancer pain. Anesth. Analg 89, 1209–1215.10553836

Gu F , Chauhan V , Chauhan A , 2015 Glutathione redox imbalance in brain disorders. Curr. Opin. Clin. Nutr. Metab. Care 18, 89–95.25405315

Gutowicz M , Kazmierczak B , Baranczyk-Kuzma A , 2011 The influence of heroin abuse on glutathione-dependent enzymes in human brain. Drug Alcohol Depend 113, 8–12.20709470

Guzman DC , Brizuela NO , Alvarez RG , Garcia EH , Mejia GB , Olguin HJ , 2009a Cerebrolysin and morphine decrease glutathione and 5-hydroxyindole acetic acid levels in fasted rat brain. Biomed. Pharmacother 63, 517–521.18993027

Guzman DC , Osnaya-Brizuela N , Garcia-Alvarez R , Hernandez-Garcia E , Juarez-Olguin H , 2009b Oxidative stress induced by morphine in brain of rats fed with a protein deficient diet. Hum. Exp. Toxicol 28, 577–582.19744972

Guzman DC , Vazquez IE , Brizuela NO , Alvarez RG , Mejia GB , Garcia EH , Santamaria D , de Apreza M , Olguin HJ , 2006 Assessment of oxidative damage induced by acute doses of morphine sulfate in postnatal and adult rat brain. Neurochem. Res 31, 549–554.16758364

Haycock JW , Becker L , Ang L , Furukawa Y , Hornykiewicz O , Kish SJ , 2003 Marked disparity between age-related changes in dopamine and other presynaptic dopaminergic markers in human striatum. J. Neurochem 87, 574–585.14535941

Hissin PJ , Hilf R , 1976 A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem 74, 214–226.962076

Hu P , Zhu W , Zhu C , Jin L , Guan Y , Guan X , 2016 Resveratrol fails to affect cocaine conditioned place preference behavior, but alleviates anxiety-like behaviors in cocaine withdrawn rats. Psychopharmacology 233, 1279–1287.26790673

Hu Z , Deng Y , Hu C , Deng P , Bu Q , Yan G , Zhou J , Shao X , Zhao J , Li Y , Zhu R , Xu Y , Zhao Y , Cen X , 2012 (1)H NMR-based metabonomic analysis of brain in rats of morphine dependence and withdrawal intervention. Behav. Brain Res 231, 11–19.22391120

Jenner P , Olanow CW , 1996 Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 47, S161–170.8959985

Joshi JC , Ray A , Gulati K , 2014 Differential modulatory effects of morphine on acute and chronic stress induced neurobehavioral and cellular markers in rats. Eur. J. Pharmacol 729, 17–21.24508522

Kalasinsky KS , Bosy TZ , Schmunk GA , Ang L , Adams V , Gore SB , Smialek J , Furukawa Y , Guttman M , Kish SJ , 2000 Regional distribution of cocaine in postmortem brain of chronic human cocaine users. J. Forensic Sci 45, 1041–1048.11005179

Kish SJ , 2014 Chapter 08: The pathology of methamphetamine use in the human brain, in Madras BK , Kuhar M (Eds.), The effects of drug abuse on the human nervous system Elsevier Inc., Amsterdam, pp. 203–297.

Kish SJ , Kalasinsky KS , Derkach P , Schmunk GA , Guttman M , Ang L , Adams V , Furukawa Y , Haycock JW , 2001 Striatal dopaminergic and serotonergic markers in human heroin users. Neuropsychopharmacology 24, 561–567.11282256

Kish SJ , Kalasinsky KS , Furukawa Y , Guttman M , Ang L , Li L , Adams V , Reiber G , Anthony RA , Anderson W , Smialek J , DiStefano L , 1999 Brain choline acetyltransferase activity in chronic, human users of cocaine, methamphetamine, and heroin. Mol. Psychiatry 4, 26–32.10089005

Kish SJ , Shannak K , Hornykiewicz O , 1988 Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. N. Engl. J. Med 318, 876–880.3352672

Kumar B , Kuhad A , Chopra K , 2011 Neuropsychopharmacological effect of sesamol in unpredictable chronic mild stress model of depression: Behavioral and biochemical evidences. Psychopharmacology 214, 819–828.21103863

LaRowe SD , Kalivas PW , Nicholas JS , Randall PK , Mardikian PN , Malcolm RJ , 2013 A double-blind placebo-controlled trial of N-acetylcysteine in the treatment of cocaine dependence. Am. J. Addict 22, 443–452.23952889

Lopez-Pedrajas R , Ramirez-Lamelas DT , Muriach B , Sanchez-Villarejo MV , Almansa I , Vidal-Gil L , Romero FJ , Barcia JM , Muriach M , 2015 Cocaine promotes oxidative stress and microglial-macrophage activation in rat cerebellum. Front. Cell. Neurosci 9, 279.26283916

Macedo DS , Vasconcelos SM , Andrade-Neto M , Belchior LD , Honorio Junior JE , Goncalves DO , Fonteles MM , Silva MI , Aguiar LM , Viana GS , de Sousa FC , 2010 Cocaine-induced status epilepticus and death generate oxidative stress in prefrontal cortex and striatum of mice. Neurochem. Int 56, 183–187.19822180

Madrigal JL , Olivenza R , Moro MA , Lizasoain I , Lorenzo P , Rodrigo J , Leza JC , 2001 Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. Neuropsychopharmacology 24, 420–429.11182537

McClure EA , Gipson CD , Malcolm RJ , Kalivas PW , Gray KM , 2014 Potential role of N-acetylcysteine in the management of substance use disorders. CNS Drugs 28, 95–106.24442756

McLeman ER , Warsh JJ , Ang L , Li PP , Kalasinsky KS , Ross BM , Tong J , Schmunk G , Adams V , Kish SJ , 2000 The human nucleus accumbens is highly susceptible to G protein down-regulation by methamphetamine and heroin. J. Neurochem 74, 2120–2126.10800957

Mirecki A , Fitzmaurice P , Ang L , Kalasinsky KS , Peretti FJ , Aiken SS , Wickham DJ , Sherwin A , Nobrega JN , Forman HJ , Kish SJ , 2004 Brain antioxidant systems in human methamphetamine users. J. Neurochem 89, 1396–1408.15189342

Moretti M , Colla A , de Oliveira Balen G , dos Santos DB , Budni J , de Freitas AE , Farina M , Severo Rodrigues AL , 2012 Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. J. Psychiatr. Res 46, 331–340.22154133

Moszczynska A , Turenne S , Kish SJ , 1998 Rat striatal levels of the antioxidant glutathione are decreased following binge administration of methamphetamine. Neurosci. Lett 255, 49–52.9839724

Motaghinejad M , Karimian M , Motaghinejad O , Shabab B , Yazdani I , Fatima S , 2015a Protective effects of various dosage of Curcumin against morphine induced apoptosis and oxidative stress in rat isolated hippocampus. Pharmacol. Rep 67, 230–235.25712644

Motaghinejad M , Karimian SM , Motaghinejad O , Shabab B , Asadighaleni M , Fatima S , 2015b The effect of various morphine weaning regimens on the sequelae of opioid tolerance involving physical dependency, anxiety and hippocampus cell neurodegeneration in rats. Fundam. Clin. Pharmacol 29, 299–309.25846801

Moussawi K , Pacchioni A , Moran M , Olive MF , Gass JT , Lavin A , Kalivas PW , 2009 N-Acetylcysteine reverses cocaine-induced metaplasticity. Nat. Neurosci 12, 182–189.19136971

Muriach M , Lopez-Pedrajas R , Barcia JM , Sanchez-Villarejo MV , Almansa I , Romero FJ , 2010 Cocaine causes memory and learning impairments in rats: involvement of nuclear factor kappa B and oxidative stress, and prevention by topiramate. J. Neurochem 114, 675–684.20477932

Nayak PK , Misra AL , Mule SJ , 1976 Physiological disposition and biotransformation of (3H) cocaine in acutely and chronically treated rats. J. Pharmacol. Exp. Ther 196, 556–569.1263111

Ng F , Berk M , Dean O , Bush AI , 2008 Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int. J. Neuropsychopharmacol 11, 851–876.18205981

Nocito Echevarria MA , Andrade Reis T , Ruffo Capatti G , Siciliano Soares V , da Silveira DX , Fidalgo TM , 2017 N-acetylcysteine for treating cocaine addiction -A systematic review. Psychiatry Res 251, 197–203.28213190

Ozmen I , Naziroglu M , Alici HA , Sahin F , Cengiz M , Eren I , 2007 Spinal morphine administration reduces the fatty acid contents in spinal cord and brain by increasing oxidative stress. Neurochem. Res 32, 19–25.17151918

Perry TL , Godin DV , Hansen S , 1982 Parkinson's disease: a disorder due to nigral glutathione deficiency? Neurosci. Lett 33, 305–310.7162692

Pomierny-Chamiolo L , Moniczewski A , Wydra K , Suder A , Filip M , 2013 Oxidative stress biomarkers in some rat brain structures and peripheral organs underwent cocaine. Neurotox. Res 23, 92–102.22791409

Qiusheng Z , Yuntao Z , Rongliang Z , Dean G , Changling L , 2005 Effects of verbascoside and luteolin on oxidative damage in brain of heroin treated mice. Pharmazie 60, 539–543.16076083

Rehncrona S , Folbergrova J , Smith DS , Siesjo BK , 1980 Influence of complete and pronounced incomplete cerebral ischemia and subsequent recirculation on cortical concentrations of oxidized and reduced glutathione in the rat. J. Neurochem 34, 477–486.7354324

Ren X , Zou L , Zhang X , Branco V , Wang J , Carvalho C , Holmgren A , Lu J , 2017 Redox signaling mediated by thioredoxin and glutathione systems in the central nervous system. Antioxid. Redox Signa 27, 989–1010.

Riley RA , 1943 An atlas of the basal ganglia, brain stem and spinal cord The Williams and Wilkins Company, Baltimore, MD.

Sajja RK , Rahman S , Cucullo L , 2016 Drugs of abuse and blood-brain barrier endothelial dysfunction: A focus on the role of oxidative stress. J. Cereb. Blood Flow Metab 36, 539–554.26661236

Schulte MHJ , Wiers RW , Boendermaker WJ , Goudriaan AE , van den Brink W , van Deursen DS , Friese M , Brede E , Waters AJ , 2017 The effect of N-acetylcysteine and working memory training on cocaine use, craving and inhibition in regular cocaine users: Correspondence of lab assessments and Ecological Momentary Assessment. Addict. Behav 79, 24–31.29241082

Sen CK , Packer L , 2000 Thiol homeostasis and supplements in physical exercise. Am. J. Clin. Nutr 72, 653S–669S.10919972

Sharma HS , Sjoquist PO , Ali SF , 2007 Drugs of abuse-induced hyperthermia, blood-brain barrier dysfunction and neurotoxicity: Neuroprotective effects of a new antioxidant compound H-290/51. Curr. Pharm. Des13, 1903–1923.17584116

Sian J , Dexter DT , Lees AJ , Daniel S , Agid Y , Javoy-Agid F , Jenner P , Marsden CD , 1994 Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann. Neurol 36, 348–355.8080242

Siegal D , Erickson J , Varoqui H , Ang L , Kalasinsky KS , Peretti FJ , Aiken SS , Wickham DJ , Kish SJ , 2004 Brain vesicular acetylcholine transporter in human users of drugs of abuse. Synapse 52, 223–232.15103689

Singh P , Sharma B , Gupta S , Sharma BM , 2015 In vivo and in vitro attenuation of naloxone-precipitated experimental opioid withdrawal syndrome by insulin and selective KATP channel modulator. Psychopharmacology 232, 465–475.25059539

Slivka A , Cohen G , 1993 Brain ischemia markedly elevates levels of the neurotoxic amino acid, cysteine. Brain Res 608, 33–37.8495346

Slivka A , Spina MB , Cohen G , 1987 Reduced and oxidized glutathione in human and monkey brain. Neurosci. Lett 74, 112–118.3561870

Sofic E , Lange KW , Jellinger K , Riederer P , 1992 Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci. Lett 142, 128–130.1454205

Sumathi T , Nathiya VC , Sakthikumar M , 2011 Protective Effect of Bacoside-A against Morphine-Induced Oxidative Stress in Rats. Indian J. Pharm. Sci 73, 409–415.22707825

Tong J , Fitzmaurice PS , Moszczynska A , Mattina K , Ang LC , Boileau I , Furukawa Y , Sailasuta N , Kish SJ , 2016 Do glutathione levels decline in aging human brain? Free Rad. Biol. Med 93, 110–117.26845616

Traudt CM , Tkac I , Ennis KM , Sutton LM , Mammel DM , Rao R , 2012 Postnatal morphine administration alters hippocampal development in rats. J. Neurosci. Res 90, 307–314.21971612

Trivedi MS , Deth R , 2015 Redox-based epigenetic status in drug addiction: a potential contributor to gene priming and a mechanistic rationale for metabolic intervention. Front. Neurosci 8, 444.25657617

Uys JD , Knackstedt L , Hurt P , Tew KD , Manevich Y , Hutchens S , Townsend DM , Kalivas PW , 2011 Cocaine-induced adaptations in cellular redox balance contributes to enduring behavioral plasticity. Neuropsychopharmacology 36, 2551–2560.21796101

Uys JD , Mulholland PJ , Townsend DM , 2014 Glutathione and redox signaling in substance abuse. Biomed. Pharm 68, 799–807.

Vitcheva V , Simeonova R , Kondeva-Burdina M , Mitcheva M , 2015 Selective nitric oxide synthase inhibitor 7-nitroindazole protects against cocaine-induced oxidative stress in rat brain. Oxid. Med. Cell. Longev 2015, 157876.26576217

Wiener HL , Reith ME , 1990 Differential effects of daily administration of cocaine on hepatic and cerebral glutathione in mice. Biochem. Pharmacol 40, 1763–1768.2242012

Wilson JM , Levey AI , Bergeron C , Kalasinsky K , Ang L , Peretti F , Adams VI , Smialek J , Anderson WR , Shannak K , Deck J , Niznik HB , Kish SJ , 1996 Striatal dopamine, dopamine transporter, and vesicular monoamine transporter in chronic cocaine users. Ann. Neurol 40, 428–439.8797532

Won SJ , Kim JE , Cittolin-Santos GF , Swanson RA , 2015 Assessment at the single-cell level identifies neuronal glutathione depletion as both a cause and effect of ischemia-reperfusion oxidative stress. J. Neurosci 35, 7143–7152.25948264

Worsley JN , Moszczynska A , Falardeau P , Kalasinsky KS , Schmunk G , Guttman M , Furukawa Y , Ang L , Adams V , Reiber G , Anthony RA , Wickham D , Kish SJ , 2000 Dopamine D1 receptor protein is elevated in nucleus accumbens of human, chronic methamphetamine users. Mol. Psychiatry 5, 664–672.11126397

Yamamoto BK , Moszczynska A , Gudelsky GA , 2010 Amphetamine toxicities: Classical and emerging mechanisms. Ann. N.Y. Acad. Sci 1187, 101–121.20201848

Yun J , Lee Y , Yun K , Oh S , 2015 Bergenin decreases the morphine-induced physical dependence via antioxidative activity in mice. Arch. Pharmacal. Res 38, 1248–1254.

Zhang X , Chiu VM , Todd RP , Sorg BA , Hill HH , 2016 Neuronal metabolomics by ion mobility mass spectrometry in cocaine self-administering rats after early and late withdrawal. Anal. Bioanal. Chem 408, 4233–4245.27108279

Zhou W , Kalivas PW , 2008 N-acetylcysteine reduces extinction responding and induces enduring reductions in cue-and heroin-induced drug-seeking. Biol. Psychiatry 63, 338–340.17719565

Zhu W , Ge X , Gao P , Li M , Guan Y , Guan X , 2017 Adolescent cocaine exposure induces prolonged synaptic modifications in medial prefrontal cortex of adult rats. Brain Struct. Funct 223, 1829–1838.29247260

Zhu W , Mao Z , Zhu C , Li M , Cao C , Guan Y , Yuan J , Xie G , Guan X , 2016 Adolescent exposure to cocaine increases anxiety-like behavior and induces morphologic and neurochemical changes in the hippocampus of adult rats. Neuroscience 313, 174–183.26621120

Highlights

- Effects of morphine and cocaine on brain GSH in animal studies are reviewed.
- Exposure to morphine or cocaine can deplete brain GSH in animals.
- GSH was measured in autopsied brains of chronic heroin and cocaine users.
- Extensive toxicology confirmed chronic use of heroin or cocaine.
- Human chronic heroin and cocaine users have normal levels of brain GSH.

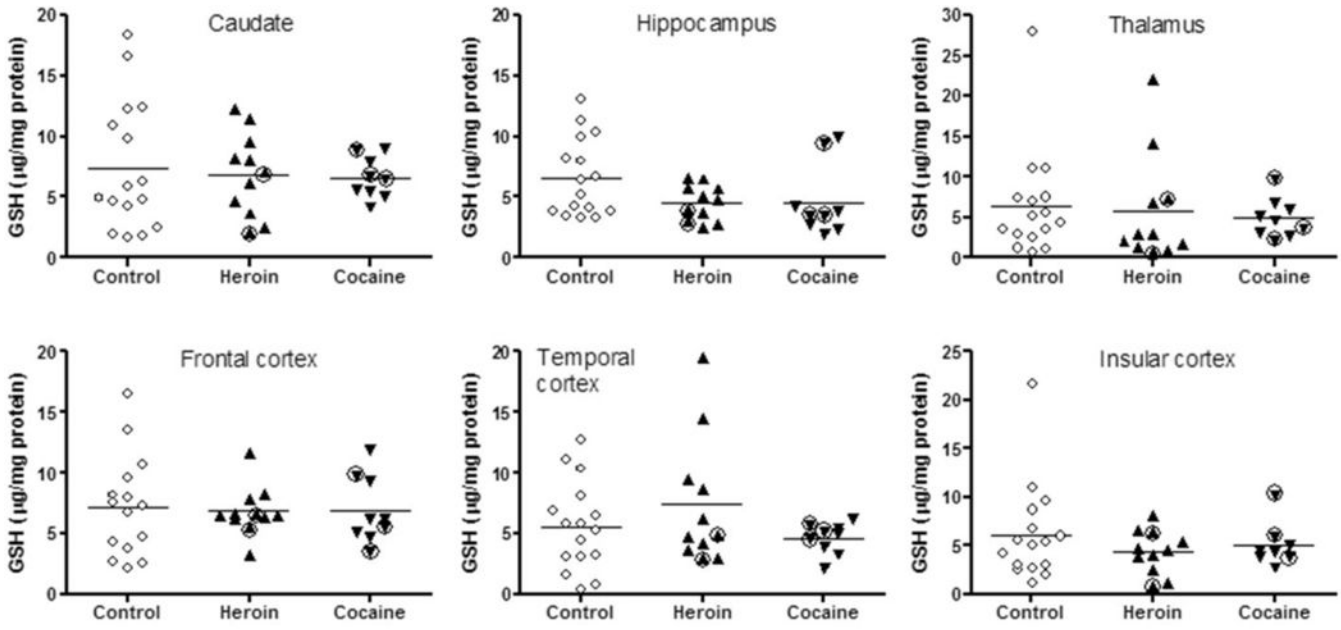

Figure 1.

Scatter plots of levels of reduced glutathione (GSH) in brain of users of heroin and cocaine and control subjects. Circled up-triangles identify two heroin users with high blood levels of propoxyphene and metabolite norpropoxyphene; circled down-triangles identify three cocaine users with high levels of cocaine and metabolites in brain.

Table 1.

Review of animal literature on morphine- or cocaine-induced changes in brain levels of glutathione (GSH)

Reference	Methods[a]	Species[b]	Treatment[c]		Main findings of GSH changes and comments[d]
			Chronicity	Dose regimen	
Morphine:					
Bien et al. 1992	DTNB	Rats ♂, Wistar	Acute	100 mg/kg IP, i3 h	GSH ↔ WB ($[GSH]_C \approx 1.5$ mM)
Goudas et al. 1997	HPLC-ECD	Rats ♂, SD	Acute	80 mg/kg SC i1–4 h 100 μg ICV i1–5 h	GSH ↔ cn/ctx/cereb/pons; GSH ↓30% cn/ctx at 3 h; ↔ cereb/pons ($[GSH]_C$ = 1.1 – 2.7 mM)
Qiusheng et al. 2005	OPT	Mice♀♂, Kunming	Chronic	5–35 mg/kg x 40d IP, escalating dose	GSH/GSSG ↓40% WB (the ratio decreased along with treatment duration; GSH levels not reported; the OPT fluorescence method might over-estimate GSSG levels (Hissin and Hilf, 1976))
Guzman et al. 2006, 2009a,b	HPLC or OPT	Rats ♀♂, SD, Wistar	Acute	3–12 mg/kg, IP i1 h	GSH ↓31–36% WB in adult rats ($[GSH]_C$ = 4.7 mM or 0.27 mM in 48h fasted rats); ↓42–88% WB in weaned Wistar rats ($[GSH]_C$ = 2.0 mM); ↓24% WB at 3 mg/kg but ↑10% at 6-12 mg/kg in young malnourished rats at P60 ($[GSH]_C$ = 0.41 mM)
Ozman et al. 2007	DTNB	Rabbits ♂	Acute	200 μg IT i8 d	GSH ↓48% ctx ($[GSH]_C$ = 1.9 mM)
Abdel-Zaher et al. 2010, 2013a,b	DTNB	Mice ♂, Swiss-Webster	Chronic	2×5 mg/kg x 1–7d SC, i2 h ± NAL 5 mg/kg	GSH ↓20–40% WB with 3–7 d morphine treatment; GSH ↓45% WB in naloxone (NAL) challenged ($[GSH]_C$ = 2.8 mM)
Sumathi et al. 2011	DTNB	Rats♂, Wistar	Chronic	10–160 mg/kg x 21d IP	GSH ↓46% WB ($[GSH]_C$ = 1.0 mM)
Hu et al. 2012	NMR in vitro	Rats ♂, SD	Chronic	5–40 mg/kg x 14d IP, escalating dose, i48 h	GSH ↓prefrontal ctx; ↔ cn/nac/hippocampus (the metabonomic study did not report GSH levels or percentage of changes)
Deng et al. 2012	NMR in vitro	Monkeys ♀♂, rhesus	Chronic	3×3–15 mg/kg x 90d SC, escalating dose, i8d	GSH ↓hippocampus; ↔ prefrontal ctx (the metabonomic study did not report GSH levels or percentage of changes)
Joshi et al. 2014	DTNB	Rats ♂, Wistar	Acute Chronic	1 and 5 mg/kg IP 1 and 5 mg/kg x 15d	GSH ↑57% and 105% WB by acute morphine, respectively; GSH ↔ and ↓51% WB by chronic morphine, respectively (Note: morphine reversed GSH depletion induced by restraint stress).
Singh et al. 2015	DTNB	Mice ♀♂, Swiss-albino	Chronic	2×5 mg/kg x 6d IP, i2h ± NAL 8 mg/kg	GSH ↔ WB by morphine alone (Note: non-significant 41% loss) GSH ↓65% with naloxone (NAL) challenge ($[GSH]_C$ = 3.4 mM)
Yun et al. 2015	DTNB	Mice ♂, C57BL/6	Chronic	10 mg/kg x 7d IP, i6h + NAL 5 mg/kg	GSH ↓36% frontal ctx ($[GSH]_C$ = 8 mM) (non-naloxone-challenged condition was not assessed)

Reference	Methods[a]	Species[b]	Treatment[c]		Main findings of GSH changes and comments[d]
Motaghinejad et al. 2015a,b	OxisResearch kit (?)	Rats ♂, Wistar	Chronic	15–45 mg/kg x 21d, i24h + NAL 3mg/kg [a]; 45 mg/kg x 28d SC [b]	GSH ↓61–65% hippocampal mitochondria preparation (Note: the method of GSH assay employed was not clear; high GSSG levels were also reported).
Famitafreshi et al. 2017	DTNB	Rats ♂, SD	Chronic	5 mg/kg x 14d IP	GSH ↓30% hippocampus (ns) ↓22% prefrontal ctx (ns) (isolation stress exacerbated GSH loss to −54–76%; $[GSH]_C$ = 2.1–3.2 mM)
Traudt et al. 2012	MRS in vivo	Rat pups ♀♂, SD	Chronic	2×2 mg/kg x 5d IP, i24h	GSH ↑43% hippocampus on P8 following morphine on P3-P7 (LCModel for spectra analysis; a volume of interest of 5 μl)
Cocaine:					
Wiener et al. 1990	HPLC	Mice ♂, C57BL/6 ByJ	Acute Chronic	25 mg/kg x 14d IP, i24h ± 50 mg/kg	GSH ↔ cn/ctx by either acute or chronic cocaine, with or without challenge ($[GSH]_C$ = 1.5 mM)
Macedo et al. 2010	DTNB	Mice ♂, Swiss	Acute	90 mg/kg IP, i1 h [SE] or 5–15 min [death]	GSH ↔ after status epilepticus [SE] or ↑28–50% prefrontal ctx/cn after death ($[GSH]_C$ = 0.0025 mM; note possible error in unit)
Muriach et al. 2010	FDNP-HPLC	Rats ♂, Wistar	Chronic	15 mg/kg x 20d IP, i24h	GSH ↓22% hippocampus; ↔ frontal ctx ($[GSH]_C$ = 2–2.2 mM)
Uys et al. 2011	UPLC-MS	Rats ♂	Acute Chronic	15–30 mg/kg x 7d IP, i21d ± 15 mg/kg	GSH ↓23% by 7d cocaine; ↑62% by acute cocaine in nac; but ↔ in cocaine challenged rats ($[GSH]_C$ = 0.55 mM)
López-Pedrajas et al. 2015	FDNP-HPLC	Rats ♂, Wistar	Chronic	15 mg/kg x 18d IP	GSH ↔ cerebellum although GSSG ↑56% so the ratio of GSH vs GSSG decreased.
Vitcheva et al. 2015	DTNB	Rats ♂, Wistar	Chronic	15 mg/kg x 7d IP, i24h	GSH ↓44% WB; also GSH ↓55% in mitochondria preparations ($[GSH]_C$ = 0.0017 mM; note possible error in unit)
Zhang et al. 2016	HPLC-IMMS	Rats ♂, SD	Chronic	SAD FR1 × 3d + FR3 × 7d, i24h and i21d	GSH ↓45% and ↓57% cn at i24h and i21d, respectively; GSH ↔ prefrontal ctx (the metabonomic study did not report GSH levels or the cumulative extent of cocaine exposure)
Hu et al. 2016	DTNB	Rats ♂, SD	Chronic	10 mg/kg x 6d IP, i15d	GSH ↓47% hippocampus; ↔ prefrontal ctx ($[GSH]_C$ unit unclear)
Zhu et al. 2016, 2017	DTNB	Rats ♂, SD P28	Chronic	15 mg/kg x 15d IP, i35–38d	GSH ↔ hippocampus, medial prefrontal ctx (adolescent cocaine exposure; GSH measured in adults; $[GSH]_C$ unit unclear)

[a] DTNB, referring to a variety of GSH assay using Ellman's reagent 5,5′-dithiobis(2-nitrobenzoic acid); OPT = *o*-phthaldehyde; ECD = electrochemical detection; FDNP = Sanger reactant 1-fluoro-2,4-dinitrobencene; IMMS = ion mobility mass spectrometry;

[b] SD = Sprague-Dawley. Adult animals were used unless otherwise indicated;

[c] The dose regimen shows daily single or multiple doses by total days, administration route (IP = intra-peritoneal; SC = subcutaneous; ICV = intracerebroventricle; IT = intrathecal; SAD = intravenous self-administration; FR = fixed ratio), the interval (i) between final drug administration and sacrifice or drug challenge, if reported (see the cited references for more details);

[d] Brain regions are caudate nucleus or striatum (cn), nucleus accumbens (nac), cortex (ctx), cerebellum (cereb), or whole brain (WB); ns = non-significant; $[GSH]_C$ denotes reported concentrations of GSH in the control group, with 1 mM = 1 μmol/g wet tissue or 307 μg/g wet tissue or 20 nmol/mg protein or 6.1 μg/mg protein by assuming a protein/tissue ratio of 0.05 (Tong et al., 2016) and a brain unit weight of 1 g/mL.

Table 2.

Characteristics and drug use histories of the 11 heroin (H1-H11) and 9 cocaine (C1-C9) users[a].

	Age		Duration				Toxicology			
Case	(yrs), Sex	PMI (h)	of use (yrs)	Recent drug use pattern	Route of drug administration	Suspected/known cause of death	Hair	Blood drug level[b]	Brain drug level[c]	Caudate DA level[d]
H1	36,M	8	10	$200 per month	Intravenous	CVD/Narcotic intoxication	—	1.93	1.79	+0.1%
H2*	43,M	13	27	Unknown	Intravenous	Narcotic intoxication	+	0.60	1.79	-11%
H3	34,M	23	>1	Unknown	Intravenous	Narcotic intoxication	+	0.67	1.19	-30%
H4*	34,M	10.5	>1	Unknown	Intravenous	Narcotic intoxication	+	0.42	0.80	-21%
H5	40,M	5	20	Daily	Intravenous	Mixed drug intoxication	+	0.32	0.61	+34%
H6	44,F	18.5	23	Daily, sometimes 1 g per day	Intravenous	CVD/Narcotic intoxication	+	0.11	0.10	-16%
H7	43,M	21	>1	Daily	Intravenous	Mixed drug intoxication	+	1.47	1.79	-5%
H8*	35,M	19.5	>1	Unknown	Unknown	Narcotic intoxication	ND	1.09	1.80	-47%
H9*	28,M	8	4	Daily	Intravenous	Narcotic intoxication	+	0.39	0.46	-0.4%
H10	19,M	9.5	>1	Unknown	Unknown	Narcotic intoxication	ND	0.39	0.37	+69%
H11*	42,M	11	10	Unknown	Intravenous	Narcotic intoxication	+	0.70	0.76	+10%
C1	26,M	18	1–2	Unknown	Oral; smoked	Cocaine intoxication	—	424.0	179.7	-53%
C2	21,M	6	2–3	$150/mo, weekend binges	Nasal	CVD/cocaine intoxication	+	ND	20.7	-38%
C3*	26,F	18	8	Binge/limited only by funds	Oral; smoked	Cocaine intoxication	ND	0.26	12.4	-19%
C4*	36,M	24	3	Binge/limited only by funds	Nasal	Cocaine intoxication	—	27.0	157.6	-79%
C5	39,M	26	8	Considered heavy user	Intravenous; nasal	Cocaine intoxication	+	0.43	11.0	+9%
C6	31,M	22	>2	Limited only by funds	Nasal; smoked	Cocaine intoxication	—	39.2	164.4	-31%
C7	40,M	9	>10	Binge every 2–3 wk	Nasal; smoked	Gunshot wound to chest	+	12.34	40.4	-54%
C8	70,M	10	55	$60/mo, 1st wk of a month	Smoked	CVD	—	19.44	15.7	+50%
C9	30,F	20	>1	Unknown	Smoked	CVD/cocaine intoxication	+	17.16	19.4	+5%

M = male; F = female; PMI = postmortem interval; DA = dopamine; CVD = cardiovascular disorder.

*Cases with ethanol detected in blood. + Drug hair analyses confirmed. ND = not detected. For cases H1, H6, C2 and C9, heroin or cocaine toxicity was considered to be a possible contributing factor to the cause of death; high levels of propoxyphene (0.63 and 1.2 mg/L, respectively) and norpropoxyphene (1.26 and 3.8 mg/L, respectively) were also detected in blood of cases H5 and H7 and could have contributed to the death.

[a]Information on the cases including brain drug levels has been published previously in (Wilson et al., 1996; Kish et al., 1999; Kalasinsky et al., 2000; McLeman et al., 2000; Kish et al., 2001; Siegal et al., 2004);

[b]Measured in μM of levels of the heroin metabolite morphine or cocaine plus metabolite benzoylecgonine;

[c]Measured in nmol/g tissue of total levels of heroin (morphine plus 6-acetylmorphine plus morphine glucuronide) in occipital cortex or cocaine (cocaine plus metabolites benzoylecgonine, ecgonine methyl ester, norcocaine, cocaethylene) in caudate;

[d]Measured as percentage decrease of the control mean (6.62 ng/mg wet tissue; see (Wilson et al., 1996)) with the exception of case#C8, for which a control mean of 4.20 ng/mg wet tissue for aged subjects (mean 69 y; see (Haycock et al., 2003)) was used.

Table 3.

Levels of glutathione (reduced [GSH], oxidized [GSSG], and cysteine-bound [GSH-cysteine]), uric acid, and methionine in brain of users of heroin and cocaine and control subjects.

Region/Group	GSH	GSSG	GSH-cysteine	Uric acid	Methionine
Caudate nucleus					
Controls	7.46 ± 1.32 (16)	0.38 ± 0.04 (16)	0.67 ± 0.08 (16)	0.47 ± 0.06 (16)	1.37 ± 0.08 (16)
Heroin	6.86 ± 1.04 (11)	0.26 ± 0.04 (11)	0.63 ± 0.07 (11)	0.42 ± 0.07 (11)	1.01 ± 0.09 * (11)
Cocaine	6.60 ± 0.57 (9)	0.24 ± 0.06 (9)	0.85 ± 0.06 (9)	0.59 ± 0.11 (9)	0.92 ± 0.06 * (9)
F	*0.17* (2, 31)	*3.06* (2, 31)	*3.01* (2, 31)	*0.76* (2, 31)	***9.13*** (2, 31)
p	*0.85*	*0.06*	*0.06*	*0.48*	***0.001***
Hippocampus, ammon's horn					
Controls	6.60 ± 0.80 (16)	0.06 ± 0.01 (16)	0.30 ± 0.04 (16)	0.44 ± 0.07 (16)	2.03 ± 0.27 (16)
Heroin	4.58 ± 0.44 (11)	0.05 ± 0.02 (11)	0.48 ± 0.13 (11)	0.44 ± 0.07 (11)	1.70 ± 0.21 (11)
Cocaine	4.61 ± 0.99 (9)	0.03 ± 0.01 (9)	0.34 ± 0.12 (9)	0.41 ± 0.13 (8)	1.33 ± 0.24 (9)
F	*2.32* (2, 31)	*0.94* (2, 31)	*1.40* (2, 31)	*0.03* (2, 30)	*1.85* (2, 31)
p	*0.12*	*0.40*	*0.26*	*0.97*	*0.17*
Thalamus, medial pulvinar nucleus					
Controls	6.44 ± 1.64 (16)	0.24 ± 0.08 (16)	1.78 ± 0.28 (16)	0.74 ± 0.11 (16)	3.24 ± 0.40 (16)
Heroin	5.71 ± 2.03 (11)	0.18 ± 0.07 (9)	1.27 ± 0.48 (11)	1.05 ± 0.29 (11)	3.53 ± 0.70 (11)
Cocaine	4.89 ± 0.80 (9)	0.09 ± 0.05 (8)	0.97 ± 0.21 (9)	0.72 ± 0.12 (9)	2.79 ± 0.27 (9)
F	*0.19* (2, 31)	*1.27* (2, 28)	*1.28* (2, 31)	*0.84* (2, 31)	*0.37* (2, 31)
p	*0.83*	*0.30*	*0.29*	*0.44*	*0.69*
Frontal cortex					
Controls	7.24 ± 1.07 (15)	0.25 ± 0.06 (15)	0.59 ± 0.07 (14)	0.61 ± 0.14 (15)	1.56 ± 0.16 (15)
Heroin	6.85 ± 0.62 (11)	0.11 ± 0.05 (9)	0.66 ± 0.12 (11)	0.32 ± 0.05 (11)	1.46 ± 0.23 (11)
Cocaine	6.94 ± 0.93 (9)	0.15 ± 0.04 (9)	0.62 ± 0.11 (9)	0.31 ± 0.06 (9)	1.19 ± 0.15 (9)
F	*0.05* (2, 30)	*1.56* (2, 28)	*0.47* (2, 29)	*2.36* (2, 30)	*0.69* (2, 30)
p	*0.95*	*0.23*	*0.63*	*0.11*	*0.51*
Temporal cortex					
Controls	5.60 ± 0.91 (16)	0.25 ± 0.08 (16)	0.23 ± 0.05 (16)	0.60 ± 0.14 (15)	0.94 ± 0.16 (15)

Region/Group	GSH	GSSG	GSH-cysteine	Uric acid	Methionine
Heroin	7.45 ± 1.60 (11)	0.42 ± 0.15 (11)	0.21 ± 0.05 (11)	1.00 ± 0.29 (11)	1.65 ± 0.42 (11)
Cocaine	4.62 ± 0.43 (9)	0.17 ± 0.03 (9)	0.15 ± 0.02 (9)	0.43 ± 0.05 (9)	0.77 ± 0.10 (9)
F	*1.04* $_{(2, 31)}$	*1.54* $_{(2, 31)}$	*0.52* $_{(2, 31)}$	*1.98* $_{(2, 30)}$	*3.02* $_{(2, 30)}$
p	*0.36*	*0.23*	*0.60*	*0.16*	*0.06*
Insular cortex					
Controls	6.14 ± 1.25 (16)	0.36 ± 0.09 (15)	0.37 ± 0.06 (16)	0.78 ± 0.13 (16)	1.76 ± 0.29 (16)
Heroin	4.38 ± 0.68 (11)	0.40 ± 0.04 (11)	0.31 ± 0.03 (11)	0.80 ± 0.09 (11)	1.87 ± 0.10 (11)
Cocaine	5.08 ± 0.81 (8)	0.31 ± 0.05 (8)	0.30 ± 0.05 (8)	0.68 ± 0.10 (8)	1.48 ± 0.15 (8)
F	*0.69* $_{(2, 30)}$	*0.48* $_{(2, 29)}$	*0.59* $_{(2, 30)}$	*0.17* $_{(2, 30)}$	*0.46* $_{(2, 30)}$
p	*0.51*	*0.62*	*0.56*	*0.84*	*0.63*

Data (mean ± SEM) are in μg/mg protein with the number of cases in parentheses. One-way analysis of co-variance [ANCOVA, *F (df)*] with age and postmortem interval as the covariates did not reveal any significant difference ($p > 0.05$) among heroin, cocaine users and control subjects except that of methionine in the caudate (*$p < 0.05$, heroin or cocaine users vs. controls, following *post hoc* Bonferroni adjustment).