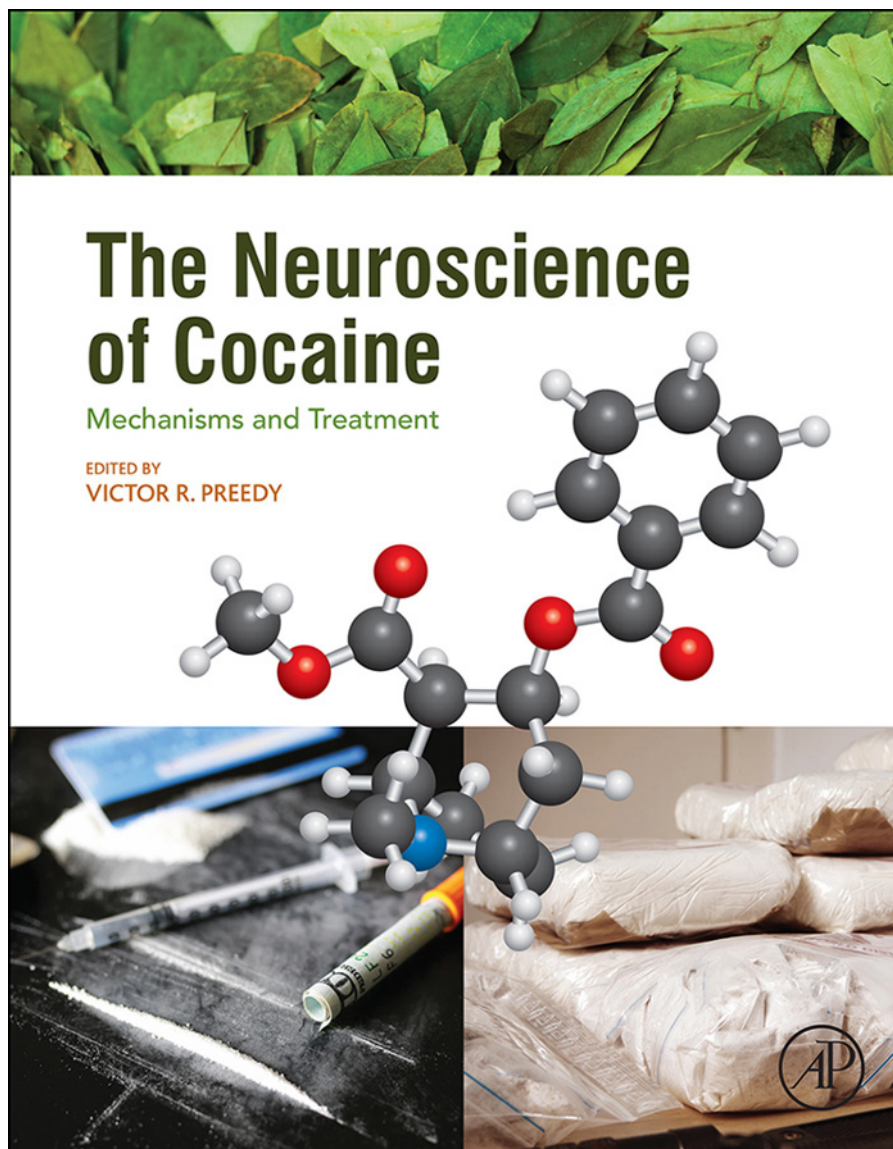


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From S.P. Pereira and T. Cunha-Oliveira, Role of Mitochondria on the Neurological Effects of Cocaine.
In: Victor R. Preedy, editors, *The Neuroscience of Cocaine*. : Academic Press, 2017, pp. 205-218.

ISBN: 978-0-12-803750-8

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Chapter 21

Role of Mitochondria on the Neurological Effects of Cocaine

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SUMMARY POINTS

- Cocaine is a lipophilic weak base with a positive charge at physiological pH.
- Cocaine dissipates mitochondrial membrane potential and impairs mitochondrial respiration.
- Acute cocaine exposure activates mitochondrial apoptotic signaling.
- Cocaine exposure induces oxidative stress in the brain, which may be a cause or a consequence of mitochondrial dysfunction.
- Long-term cocaine exposure induces adaptation to oxidative stress.
- Cocaine affects epigenetic mechanisms that may account for some of its long-term effects on the users or in their progeny.

KEY FACTS

- Mitochondria are the cellular powerhouses, providing energy for cellular activities.
- Mitochondria have their own genome, encoding core subunits of the oxidative phosphorylation system.
- Mitochondria regulate intracellular calcium homeostasis and participate in apoptotic signaling.
- Mitochondria are both a source and a target of oxidative stress.
- Mitochondrial metabolism modulates the epigenetic landscape.
- Mitochondrial dysfunction is involved in many neurologic diseases.

LIST OF ABBREVIATIONS

$\Delta\Psi_m$	mitochondrial transmembrane electric potential
AcH3	acetylated histone H3
AIF	apoptosis-inducing factor

BDNF	brain-derived neurotrophic factor
CART	cocaine and amphetamine regulated transcript
DA	dopamine
DAQ	dopamine quinones
DNMT	DNA methyltransferase
FCCP	carbonylcyanide p-trifluoromethoxyphenylhydrazone
GPx	glutathione peroxidase
GRed	glutathione reductase
GSH	reduced glutathione
H3K4me3	H3 lysine 4 trimethylation
HDAC	histone deacetylase
MDA	malondialdehyde
MSK1	mitogen and stress-activated protein kinase 1
NAc	nucleus accumbens
NAD⁺	nicotinamide adenine nucleotide
NADH	nicotinamide adenine nucleotide reduced form
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
NMDA	<i>N</i> -methyl-D-aspartate
nNOS	nitric oxide synthase
NO	nitric oxide
O₂⁻	superoxide
OH	hydroxyl radical
ONOO⁻	peroxynitrite
OXPHOS	oxidative phosphorylation system
PARP1	poly (ADP-Ribose) polymerase 1
PFC	prefrontal cortex
ROS	reactive oxygen species
SAM	<i>S</i> -adenosyl methionine
SOD	superoxide dismutase

21.1 INTRODUCTION

Cocaine (or benzoylmethylecgonine) is a lipophilic weak base, positively charged at physiological pH, rapidly absorbed, except when ingested or applied topically, being quickly transported across the blood–brain barrier (Dinis-Oliveira, 2015). After administration, cocaine

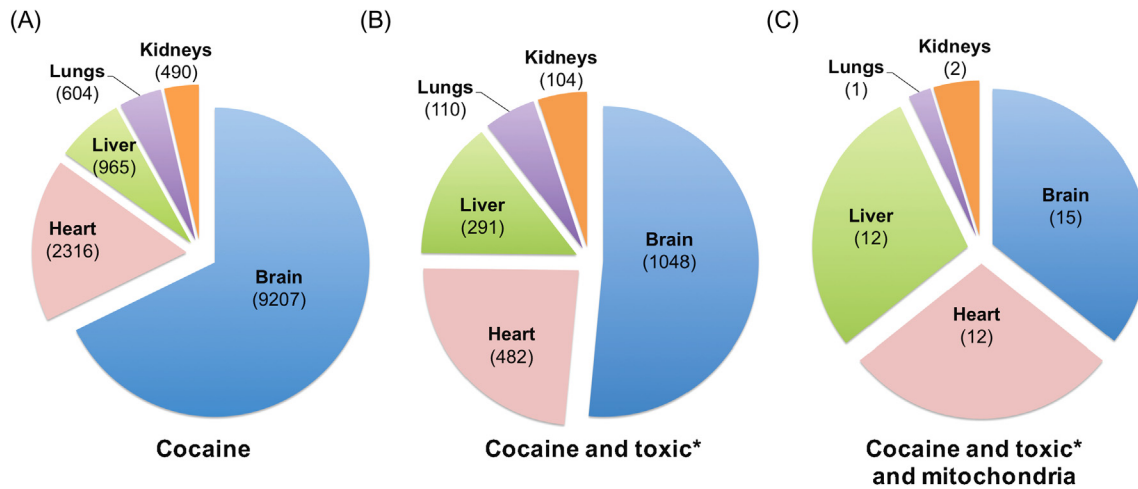


FIGURE 21.1 Distribution of PubMed results describing cocaine effects in different organs. (A) Number of papers describing cocaine effects on the brain, heart, liver, lungs, and kidneys. (B) Number of papers describing toxic cocaine effects on the same organs. (C) Number of papers describing toxic mitochondrial effects of cocaine in different organs. Search was performed using the keywords ((brain) OR neuro*) for brain; ((heart) OR cardi*) for heart; ((liver) OR hepat*) for liver; ((lung) OR pulmon*) for lungs; ((kidney) OR (renal)) for kidneys; cocaine; toxic* (in B,C); and mitochondria (in C), excluding reviews. Search was performed on December 31, 2015.

concentration becomes several times higher in the brain than in peripheral tissues (Heard, Palmer, & Zahniser, 2008) and it may be even more concentrated inside cells upon specific uptake, or by binding to intracellular components (Sulzer & Rayport, 1990).

Cocaine is typically used intravenously, intranasally, or smoked in the form of “crack.” Cocaine abuse is particularly worrisome among pregnant women, being associated with increased rate of premature births and decreased birth weight (Gouin et al., 2011).

Cocaine has a plasma half-life of about 1 hour, being metabolized in the liver within 2 hours of administration, mainly into ecgonine methyl ester, benzoylecgonine, and to a lesser extent to norcocaine, which is bioactive (Cunha-Oliveira, Rego, Carvalho, & Oliveira, 2013a).

Cocaine directly binds to monoamine transporters, and thus its main targets are the brain and the cardiovascular system. When used topically, cocaine also acts as a local anesthetic, inhibiting nerve impulse transmission by binding to sodium and potassium channels and inhibiting the inflow of sodium and the outward flow of potassium ions (Heard et al., 2008). Cocaine effects on the brain are responsible for its addictive effects and behavioral consequences, but toxicological evidences are also documented for other tissues (Fig. 21.1).

In addition to its direct effects, cocaine also has some important indirect effects that may contribute to its neurotoxicity, such as vasoconstriction, which leads to tissue hypoxia and deficient nutrient supply; and stimulation of epinephrine release from adrenal medulla, with consequent hyperglycemia (Repetto et al., 1997).

21.2 COCAINE AND BIOENERGETICS

Since cocaine can accumulate inside cellular compartments due to the weak base effect (Sulzer & Rayport, 1990), it can reach high concentrations inside mitochondria and produce direct effects on mitochondrial function, such as the disruption of mitochondrial transmembrane electric potential ($\Delta\Psi_m$) (Cunha-Oliveira et al., 2006a, 2013d).

Cocaine abuse results in a long-term reduction in glucose metabolism in the human frontal brain (Volkow et al., 1992), also affecting the transcription of genes associated with mitochondrial function and energy metabolism in the hippocampus (Zhou, Yuan, Mash, & Goldman, 2011) and prefrontal cortex (PFC) (Lehrmann et al., 2003). Studies in humans are very relevant, but they present many challenges and limitations. Cocaine abusers are a very heterogeneous population, encompassing many variables, such as the amount and frequency of drug intake, poly drug abuse, life style, or the presence of comorbidities. In addition, since for research purposes human brain tissue may only be collected after death, even more variables are introduced due to different causes of death, and variable quality of the samples, which is highly dependent on the amount of time between death and tissue collection (Lull, Freeman, Vrana, & Mash, 2008). Thus, animal and in vitro models are very helpful to uncover the mechanisms elicited by cocaine exposure under controlled conditions.

In vitro studies have shown that acute cocaine cytotoxicity partially requires the presence of a functional respiratory chain (Fig. 21.2), as suggested by the decreased metabolic activity observed in cocaine-exposed NT-2

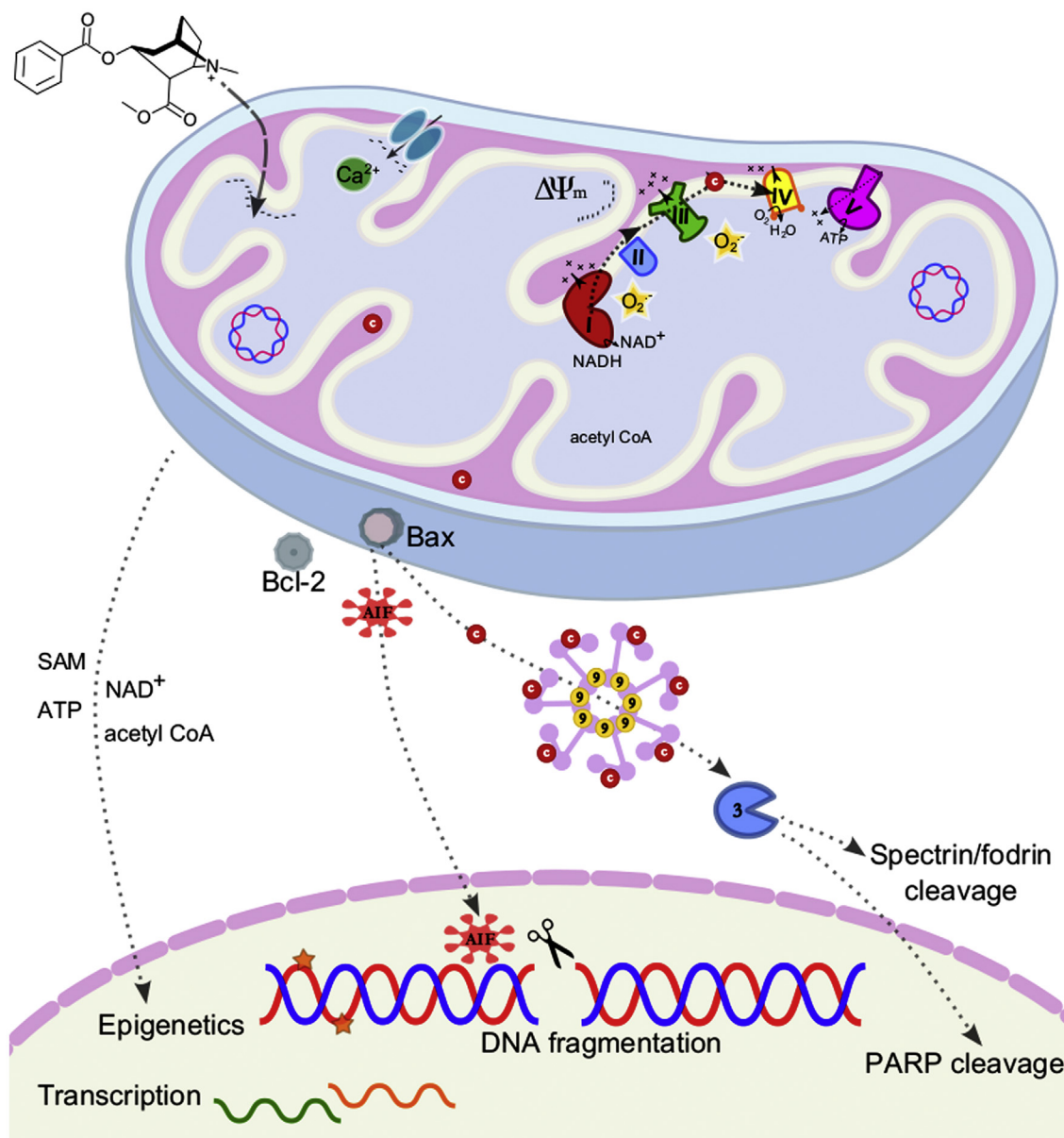


FIGURE 21.2 Interactions of cocaine with mitochondrial functions. Mitochondrial respiratory chain pumps protons to the intermembrane space, creating a pH and charge gradient. Cocaine has a positive charge at physiological pH, which facilitates its accumulation in negatively charged organelles. Cocaine impairs complex I activity, which may result in increased production of O_2^- and in compromised bioenergetics, including changes in ATP, acetyl CoA, NAD^+ , and SAM levels, impairing epigenetic regulation and changing transcriptional patterns. Mitochondrial dysfunction may also affect mitochondrial calcium buffering capacity, and lead to the activation of apoptotic signaling cascades. Increased Bax/Bcl-2 levels result in the formation of pores that mediate the release of proapoptotic factors, such as AIF that is translocated to the nucleus inducing DNA fragmentation, or cytochrome c that binds to APAF-1 and procaspase-9 in the presence of ATP, leading to the activation of the initiator caspase-9, which in turn activates the effector caspase-3, leading to the cleavage of important cellular proteins, including spectrin/fodrin and PARP1.

cells, compared to the same cell line when devoid of functional mitochondria (rho zero) (Cunha-Oliveira et al., 2006a). In addition, acute *in vivo* exposure to cocaine modified the expression of several genes encoded by the mitochondrial genome in the rat cingulate cortex, including several complex I and complex IV subunits (Dietrich, Poirier, Aunis, & Zwiller, 2004).

Complex I seems to be a direct target of cocaine in rat brain mitochondria (Cunha-Oliveira et al., 2013d; Dietrich et al., 2004), and also in heart (Vergeade et al., 2010; Yuan & Acosta, 1996, 2000) and liver mitochondria (Cunha-Oliveira et al., 2013c; Devi & Chan, 1997). *In vitro* exposure to cocaine was shown to affect the respiratory chain in different manners in liver and brain

mitochondria, but both tissues shared an effect on complex I-mediated respiration (Cunha-Oliveira et al., 2013d). Cocaine decreased state-3 respiration in brain, but not liver, mitochondria energized with complex I substrates, indicating a tissue-specific interaction with the oxidative phosphorylation system (OXPHOS) under complex I energization. Cocaine also decreased FCCP-stimulated respiration and $\Delta\Psi_m$ in complex I-energized brain and liver mitochondria, but not in mitochondria energized with complex II substrates, suggesting an important role of complex I in mediating cocaine effects in both tissues. A decrease in $\Delta\Psi_m$ also occurred in cocaine-exposed liver mitochondria energized through complex II, suggesting that, at least in the liver, cocaine effect on $\Delta\Psi_m$ is independent of mitochondrial respiration impairment (Cunha-Oliveira et al., 2013d), probably due to a dissipation of charge associated with the weak base effect, as described for other intracellular compartments (Sulzer & Rayport, 1990). Cocaine effect on complex I was shown to be independent of disrupted nicotinamide adenine nucleotide reduced form (NADH) supply, because the drug also decreased oxygen consumption in mitochondrial fractions energized with NADH. The possibility of a dysfunction on other components of the respiratory chain was discarded since cocaine did not affect succinate-mediated oxygen consumption in the same mitochondrial fractions (Cunha-Oliveira et al., 2013d). These data suggest a direct effect of cocaine on complex I activity, although the complete mechanism still needs to be elucidated.

Cocaine impact on mitochondrial function has been associated with the activation of apoptotic signaling pathways (Cunha-Oliveira, Rego, & Oliveira, 2014) (Table 21.1). Although the evidence suggests the occurrence of adaptive mechanisms related with the mitochondrial apoptotic signaling in postmortem brains of cocaine abusers, abnormal cell death seems to occur, as indicated by the appearance of cleaved PARP1, possibly due to oxidative stress and nuclear translocation of apoptosis-inducing factor (AIF) (Alvaro-Bartolome, La Harpe, Callado, Meana, & Garcia-Sevilla, 2011).

In vitro studies showed that cocaine activates apoptotic signaling pathways. Cocaine exposure was shown to dissipate $\Delta\Psi_m$ in rat cortical neurons (Cunha-Oliveira et al., 2006a, 2010), in isolated brain mitochondria (Cunha-Oliveira et al., 2013d), and in C6 astroglia cells (Badisa, Darling-Reed, & Goodman, 2010). Further evidence for the activation of the intrinsic apoptotic signaling by cocaine includes alterations in Bcl-2 and Bax protein levels (Dey, Mactutus, Booze, & Snow, 2007; Lepsch et al., 2009; Xiao & Zhang, 2008), cytochrome c (Cunha-Oliveira et al., 2006a, 2010), and AIF (Alvaro-Bartolome et al., 2011) release from mitochondria, and caspase-9 activation (Cunha-Oliveira et al., 2006a). This

apoptotic signaling is associated with mitochondrial dysfunction in rat cortical neurons, occurring simultaneously with decreased ATP levels and increased intracellular calcium concentration (Cunha-Oliveira et al., 2010), which may be partially explained by the loss of $\Delta\Psi_m$ that drives mitochondrial calcium uptake. However, the induction of structural apoptotic hallmarks by cocaine is not consensual in the literature. Many studies failed to find structural features of neuronal apoptosis upon in vivo (Dietrich et al., 2005) or in vitro (Cunha-Oliveira et al., 2006a, 2010; Oliveira, Rego, Morgadinho, Macedo, & Oliveira, 2002) cocaine exposure, while others have observed apoptotic morphology in cultured fetal mouse cortical neurons exposed to cocaine (Nassogne, Louahed, Evrard, & Courtoy, 1997). Apoptotic neurons were also observed in the brain of rat fetuses exposed to cocaine in utero, whereas the maternal brain seemed to be spared (Xiao & Zhang, 2008).

In human neuronal progenitor cells, cocaine-induced cell death seemed to be preceded by oxidative stress (Poon, Abdullah, Mullan, Mullan, & Crawford, 2007). Oxidative stress may be a consequence of cocaine-induced mitochondrial dysfunction, but it may also be a factor involved in cocaine's mitochondrial effects (Vergeade et al., 2010).

21.3 COCAINE AND OXIDATIVE STRESS

Several transcripts associated with apoptosis and oxidative stress regulation were shown to be altered in human cocaine abusers (Mash et al., 2007). In addition, apoptotic hallmarks detected in the brains of human cocaine abusers seem to be a consequence of oxidative stress and nuclear AIF translocation (Alvaro-Bartolome et al., 2011).

Cocaine may induce an imbalance between oxidants and antioxidants due to an increase in reactive species associated with oxidative drug metabolism, monoamine oxidation, mitochondrial dysfunction, or excitotoxicity, or by impairment of cellular antioxidant systems (Cunha-Oliveira, Rego, & Oliveira, 2013b) (Table 21.2).

Cocaine binds to monoamine transporters and inhibits the reuptake of monoamines, particularly dopamine (DA), inducing an increase in their extracellular concentrations, which may induce oxidative stress in dopaminergic and neighboring cells (Fig. 21.3).

Evidence for oxidative stress in the brain after cocaine exposure includes increased hydrogen peroxide (H_2O_2) in the PFC and in the striatum of rats (Dietrich et al., 2005), and high levels of lipid peroxidation in the hippocampus of rats exposed to cocaine in utero (Bashkatova, Meunier, Vanin, & Maurice, 2006). Moreover, oxidized proteins were found in cocaine-exposed human neuronal progenitor cells (Poon et al., 2007) and elevated malondialdehyde (MDA) and nitrite levels were observed in the PFC and

TABLE 21.1 Summary of Studies Presenting Evidence of Mitochondrial Dysfunction and Neuronal Apoptosis Induced by Cocaine

References	Dose	Biological Model	Treatment Duration	Type of Exposure		Mechanisms
Nassogne et al. (1997)	0.5 mM	Mouse cortical cocultures	48–108 h	Acute, prolonged	In vitro	DNA fragmentation, chromatin condensation
Repetto et al. (1997)	0.1–10 mM	Mouse neuroblastoma cells Neuro-2a	24–72 h	Acute	In vitro	Increased intracellular LDH activity, decreased SDH activity
Oliveira et al. (2002)	3 mM	PC12 cells	48 h	Acute	In vitro	Decreased metabolic activity without apoptotic morphology
Oliveira et al. (2003)	0.3 mM	PC12 cells	5 h	Acute, low serum	In vitro	Caspase-3 activation, loss of mitochondrial cytochrome c
Mitchell and Syder-Keller (2003)	3 × 15 mg/kg, s.c.	Newborn Sprague-Dawley rats at 0, 3, and 24 h after C-section	1 h between injections, E22	Binge	In vivo, in utero	Increased caspase-3 immunoreactivity in several brain areas at 24 h
Su et al. (2003)	0.001–1 mM	Cerebral vascular muscle cells	12–48 h	Acute, low serum	In vitro	Nuclear apoptotic morphology, increased terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells
Dietrich et al. (2005)	20 mg/kg, i.p.	Male Wistar rats	3–15 h post injection	Acute (single injection); chronic (1 injection/day × 10 days)	In vivo	Downregulation of mitochondrial genome transcripts
Imam et al. (2005)	0.5 mM	Differentiated PC12 cells	24 h	Acute	In vitro	Cleaved caspases -9 and -3
Novikova et al. (2005)	20 mg/kg, s.c.	Fetal mouse cerebral wall	Twice a day, E8–E18	Repeated	In vivo, in utero	Transcriptional changes in several apoptotic genes, including cytochrome c, AIF, and caspases -9 and -3
Cunha-Oliveira et al. (2006a)	1 mM	Rat cortical neurons (Wistar, E16), rho zero and parental NT-2 cells	24 h	Acute	In vitro	Loss of $\Delta\Psi_m$ loss of mitochondrial cytochrome c, activation of caspases-9, -2, and -3, no significant increase in apoptotic morphology, maintenance of AIF levels and distribution, decreased cytotoxicity in rho-zero cells
Dey et al. (2007)	1.5–3 μ M	Fetal LC and SN neurons (Long Evans rats E14)	0.5–48 h	Acute	In vitro	In LC neurons: decreased metabolic activity, upregulation of Bax, caspases -9 and -3 activation, spectrin/fodrin cleavage, PARP cleavage, TUNEL-positive cells, chromatin condensation and DNA fragmentation; in SN neurons: increased Bcl-2/Bax
Poon et al. (2007)	1 μ M	Human neuronal progenitor cells	30 min + 48–72 h recovery	Acute	In vitro	Loss of membrane integrity occurs after oxidative stress peak

(Continued)

TABLE 21.1 (Continued)

References	Dose	Biological Model	Treatment Duration	Type of Exposure		Mechanisms
Xiao and Zhang (2008)	30–60 mg/kg/day	Fetal and maternal brains of Sprague-Dawley rats	E15–E21	Repeated	In vivo, in utero	In fetal brain: apoptotic nuclei, DNA fragmentation, upregulation of Bcl-2 and Bax with increased Bax/Bcl-2, caspases -9 and -3 activation; in maternal brain: no alteration in caspase activity
Lepsch et al. (2009)	1 mM	PC12 cells	24 h	Acute, no serum	In vitro	Decreased metabolic activity, downregulation of Bcl-2, caspase-3 activation
Lee et al. (2009)	0.1 mM	Primary human fetal CNS cell lines (~20 w gestation)	24 h	Acute	In vitro	Upregulation of Bax in microglial cultures
Yao et al. (2009)	1 μ M	Rat primary striatal and cortical neurons, Sprague-Dawley E18-E19	24 h	Acute	In vitro	Condensation and fragmentation of nuclei, caspase-3 activation, increase in Bax/Bcl-xL, loss of $\Delta\Psi_m$
Badisa et al. (2010)	3–6 mM	Rat C6 astrogloma cells	24–48 h	Acute	In vitro	Loss of $\Delta\Psi_m$
Cunha-Oliveira et al. (2010)	1 mM	Rat cortical neurons (Wistar, E16)	24 h	Acute	In vitro	Increased intracellular calcium levels, decreased ATP levels, loss of mitochondrial membrane potential, caspase-3 activation
Alvaro-Bartolome et al. (2011)	Variable	Human PFC	Variable	Chronic	Human	Decreased cytochrome c content, increase in cytosolic, membrane, and nuclear AIF levels
Badisa and Goddman (2012)	2–7 mM	Rat C6 astrogloma cells	24 h	Chronic	In vitro	Decreased metabolic activity
Numa et al. (2011)	1.5 mM	PC12 cells	48 h	Acute	In vitro	Decreased metabolic activity, increased mitochondrial superoxide levels
Costa et al. (2013)	1–100 μ M	BV2 human microglial cell line	48–72 h	Acute	In vitro	Decreased metabolic activity, increased Bax/Bcl-xL, caspase-3 cleavage
Cunha-Oliveira et al. (2013d)	1 mM	Isolated rat brain mitochondria	5 min	Acute	In vitro	Decreased complex I-mediated state-3 respiration, respiratory control ratio, FCCP-stimulated respiration, $\Delta\Psi_m$ and NADH induced oxygen consumption
Lepsch et al. (2015)	1 mM	Mesencephalon and striatum primary cultures (Sprague-Dawley rats E17)	24 h	Acute	In vitro	Increased nuclear apoptotic morphology and TUNEL labeling
Zhou et al. (2011)	variable	Human postmortem hippocampal tissue	Variable	Chronic	Human	Decreased transcript levels for genes associated with mitochondrial oxidative phosphorylation, maintenance of $\Delta\Psi_m$ and antioxidant defenses

TABLE 21.2 Summary of Studies Showing Evidence of Oxidative Stress Induced by Cocaine

References	Dose	Biological Model	Treatment Duration	Type of Exposure		Evidence
Das and Ratty (1987)	5–2500 ug/mL	Isolated brain mitochondria (male Wistar rats)	1 h	Acute	In vitro	Increased MDA levels
Lipton et al. (2003)	30 mg/kg	Fetal rat brain (Sprague-Dawley rats E20)	1 h after injection E20	Acute: 1 injection E20 chronic/ repeated 2 injections/day E7–E19 + 1 injection E20	In vivo, in utero	Acute: decreased GSH levels; GSSG levels dependent on fetal uterine position (possibly due to vasoconstriction); chronic: decreased alphatocopherol and increase in alphatocopherol quinone
Dietrich et al. (2005)	20 mg/kg, i.p.	Male Wistar rats	3–15 h after last injection	Acute (single injection); chronic (1 injection/day × 10 days)	In vivo	Acute: increased H ₂ O ₂ levels in mitochondrial fractions from frontal cortex; acute and chronic: increased H ₂ O ₂ , increased lipid peroxides, and increased GPx and SOD activities in frontal cortex and striatum
Macedo et al. (2005)	10–90 mg/kg, i.p.	Male Swiss mice	1 h	Acute	In vivo	Decreased catalase activity PFC (only higher dose) and striatum
Novikova et al. (2005)	20 mg/kg, s.c.	Frontal and occipital cerebral wall; Swiss Webster mice	2 injections/day E8–E18, 1 h after the last injection	Repeated	In vivo, in utero	Transcriptional changes in several oxidative stress-related factors
Bashkatova et al. (2006)	20 mg/kg, i.p.	Sprague-Dawley	1 injection/day E17–E20	Repeated	In vivo, in utero	Increased hippocampal thiobarbituric acid reacting substances (TBARS) and NO
Cunha-Oliveira et al. (2006b)	Chronic: 30 μM; acute: 3 mM	PC12 cells	Chronic: 7–12 months; acute: 24–96 h	Chronic; acute	In vitro	Chronic cocaine-induced partial resistance to H ₂ O ₂ acute toxicity
Poon et al. (2007)	1 μM	Human neuronal progenitor cells	30 min + 48–72 h recovery	Acute	In vitro	Increased protein carbonyls and hydroxynonenal (HNE) levels, and decreased GSH levels
Numa et al. (2008)	In vivo: 30 mg/kg; in vitro: 10 μM	PFC, NAc homogenates or slices	In vivo: 30 min after injection; in vitro: 15 min	Acute	In vivo; in vitro	Increased MDA and nitrite levels in PFC and NAc, in vitro: decreased total antioxidant content in PFC and NAc; in vivo: increased total antioxidant content in NAc
Yao et al. (2009)	1 μM	Rat primary striatal and cortical neurons, Sprague-Dawley E18–E19	3 h	Acute	In vitro	Increased ROS production

(Continued)

TABLE 21.2 (Continued)

References	Dose	Biological Model	Treatment Duration	Type of Exposure		Evidence
Muriach et al. (2010)	15 mg/kg, i.p.	Male Wistar rats	20 days	Repeated	In vivo	Decreased GSH, GPx, and NFκB in the hippocampus
Numa et al. (2011)	1.5 mM	PC12 cells	24 h	Acute	In vitro	Increased mitochondrial superoxide and intracellular peroxide levels, decreased total scavenging capacity, protection of cell death by the antioxidant tempol
Uys et al. (2011)	15–30 mg/kg, i.p.	Male rats	7 days: days 1, 7 (15 mg/kg); days 2–6 (30 mg/kg) + withdrawal or cocaine challenge (15 mg/kg) at day 28	Repeated	In vivo	Increased redox potential, increase in protein S-glutathionylation, increased glutaredoxin, decreased expression of glutathione S-transferase (GST)pi
Pomierny-Chamiolo et al. (2013)	Self-administration: 0.5 mg/kg/infusion, i.v.; passive administration: paired with self-administration, between 15 and 18 mg/kg/day, 14 days	Male Wistar rats	Acquisition: minimum 10 days; maintenance: until response stabilization; extinction: until extinction was achieved	Repeated, self-administration	In vivo	SA (without extinction): increased SOD activity in hippocampus, frontal cortex and dorsal striatum; decreased MDA in hippocampus, frontal cortex and dorsal striatum; passive cocaine: decreased MDA in dorsal striatum; SA + extinction: increased SOD activity in hippocampus, frontal cortex and dorsal striatum; increased MDA in hippocampus and frontal cortex and decreased MDA in dorsal striatum
Costa et al. (2013)	10 μM	BV2 human microglial cell line	24 h	Acute	In vitro	Increased ROS production
Peraile et al. (2013)	30 mg/kg, i.p.	Adult male NIH/Swiss mice	1–3 h	Acute	In vivo	Decreased striatal SOD activity after 1 h and increased GPx activity after 3 h
Walker et al. (2014)	Variable	Human plasma and erythrocytes	Variable	Chronic	Human	Decreased antioxidant capacity and SOD activity
Sordi et al. (2014)	Variable	Male human blood	Variable	Chronic	Human	Positive correlation between TBARS levels and severity of drug use
Badisa et al. (2015)	2–4 mM	Rat c6 astroglia-like cell line	1 h	Acute	In vitro	Increased ROS, decreased GSH levels. Attenuation of cocaine toxicity by N-acetyl cysteine pretreatment
Jang et al. (2015)	0.25 mg/kg/injection	Male Sprague-Dawley rats	FR1 reinforcement schedule	Repeated	In vivo, self-administration	Increased 8-OHG in NAc neurons

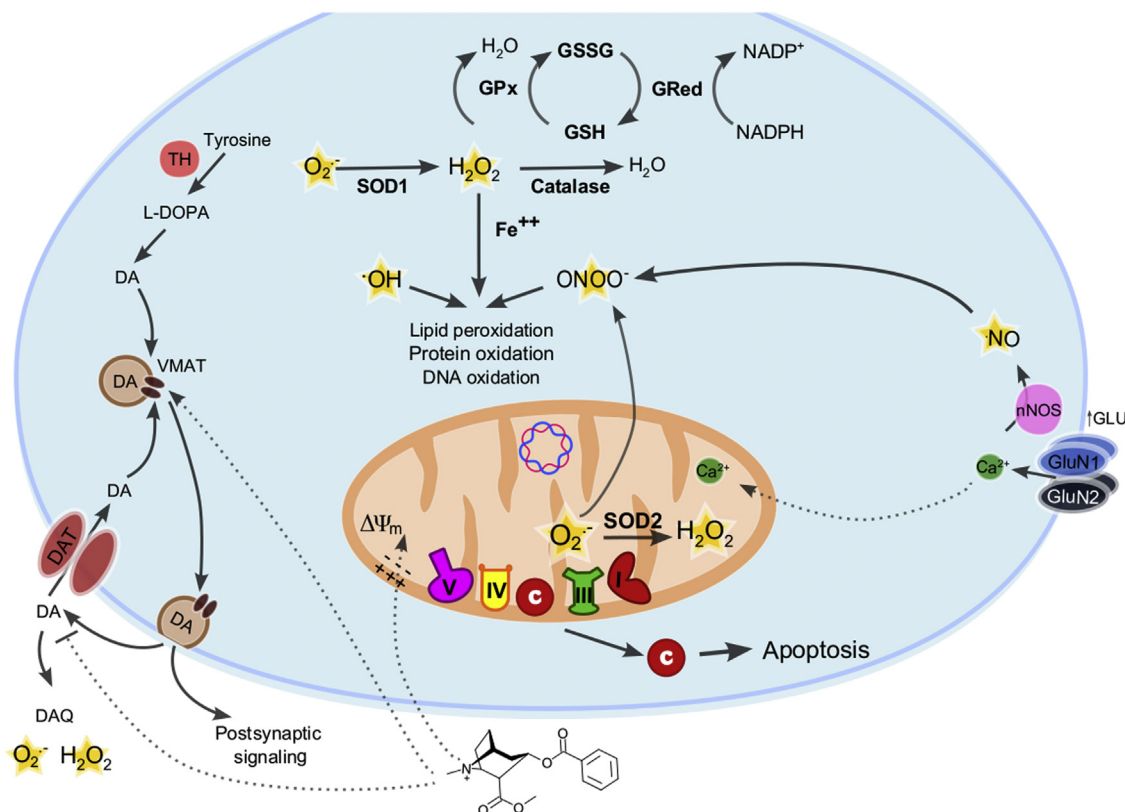


FIGURE 21.3 Cocaine and oxidative stress. Cocaine exposure induces an increase in oxidative species and an impairment of cellular antioxidant systems. Cocaine inhibits DA uptake, increasing extracellular DA, which may suffer auto-oxidation. Cocaine also induces mitochondrial dysfunction, leading to an increase in O_2^- formation by the mitochondrial respiratory chain complexes I and III. Overstimulation of NMDA glutamate receptors after drug exposure may also induce oxidative stress. Reactive species are detoxified by cellular antioxidant systems. Imbalance between oxidant production and antioxidant activity may culminate in cell dysfunction and death. *L-DOPA*, L-3,4-dihydroxyphenylalanine; *VMAT*, vesicular monoamine transporter; *DAT*, dopamine transporter; *TH*, tyrosine hydroxylase; *GSSG*, oxidized glutathione. For other abbreviations see text.

nucleus accumbens (NAc) in rat brain slices acutely exposed to cocaine (Numa, Kohen, Poltyrev, & Yaka, 2008). Curiously, rats exposed to cocaine self-administration exhibited decreased MDA brain levels, but withdrawal from the drug increased MDA in the hippocampus (Pomierny-Chamiolo, Moniczewski, Wydra, Suder, & Filip, 2013).

Cocaine exposure also induced a lower catalase activity in the PFC and striatum in mice (Macedo et al., 2005), but higher superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in the same brain areas in rats (Dietrich et al., 2005). Nonenzymatic antioxidant levels such as reduced glutathione (GSH) or vitamin (vit) E, were also decreased upon cocaine exposure (Lipton et al., 2003; Poon et al., 2007). GSH concentration and GPx activity were decreased in the hippocampus of cocaine-treated animals (Muriach et al., 2010). A decrease in total antioxidant content occurred together with increased markers of oxidative damage in the PFC and NAc of rats exposed to cocaine, and both were prevented by the antioxidant tempol (Numa et al., 2008), which was also able

to attenuate cocaine-induced cell death in PC12 cells (Numa, Baron, Kohen, & Yaka, 2011).

Repeated cocaine exposure decreased GSH concentration and GPx activity in the hippocampus of rats, which also presented learning and memory impairments, associated with lower nuclear factor kappa B (NF κ B) activity in the frontal cortex (Muriach et al., 2010). NF κ B is a sensor of oxidative stress that also participates in memory formation, and may be involved in drug toxicity and addiction mechanisms. The same study found a high neuronal nitric oxide synthase (nNOS) activity in the hippocampus, which was associated with impaired memory retrieval of experiences acquired prior to cocaine administration. In contrast, learning of new tasks was enhanced and correlated with the increase of nNOS activity and the decrease of GPx (Muriach et al., 2010). nNOS activity is stimulated upon activation of *N*-methyl-D-aspartate (NMDA) glutamate receptors (Fig. 21.3), which may result from mitochondrial dysfunction, because energy metabolism impairment affects the maintenance of the resting potential, increasing glutamate release.

Adaptation to oxidative stress was found after chronic exposure to a nontoxic concentration of cocaine induced in PC12 cells, which became resistant to H₂O₂ acute toxicity, but were more susceptible to acute cocaine toxicity (Cunha-Oliveira, Rego, Morgadinho, Macedo, & Oliveira, 2006b). In accordance, repeated cocaine administration in rats induced an increase in antioxidant enzyme activities in the frontal cortex and striatum (Dietrich et al., 2005). Self-administration of cocaine also increased SOD activity in the hippocampus, frontal cortex, and dorsal striatum, associated with decreased MDA levels (Pomierny-Chamiolo et al., 2013). Adaptation to oxidative stress may represent a compensatory mechanism against the increase in ROS induced by cocaine. Cocaine-induced adaptation to cellular redox seems to contribute to its enduring behavioral plasticity (Uys et al., 2011).

The peptide encoded by the cocaine and amphetamine regulated transcript (CART) is upregulated by psychostimulants, and was shown to localize in mitochondria and to have antioxidant effects (Mao, Meshul, Thuillier, Goldberg, & Reddy, 2012). Thus, cocaine may also have some indirect effects on mitochondria through upregulation of CART, which was shown to interact with succinate dehydrogenase subunit B, stimulating its activity and increasing ATP production (Mao et al., 2007). Upregulation of CART could be a neuroprotective mechanism contributing to some adaptive changes observed after repeated cocaine exposure.

21.4 COCAINE, MITOCHONDRIA, AND EPIGENETICS

Cocaine addiction involves persistent changes in gene expression that can be explained by interference with epigenetic mechanisms. Mitochondrial DNA is subjected to epigenetic regulation (Shock, Thakkar, Peterson, Moran, & Taylor, 2011), and nuclear histone epigenetic modifications, chromatin remodeling, and nucleosome positioning depend on key substrates provided by mitochondria (Cyr & Domann, 2011). Mitochondrial energetics links the nuclear epigenome to calorie availability through intermediate metabolites, such as ATP, that can regulate the phosphorylation of cytosolic and nuclear signal transduction proteins; acetyl CoA, which regulates the acetylation of chromatin and signal transduction proteins; NAD⁺, that can modulate sirtuins to deacetylate proteins; and SAM, involved in histone and DNA methylation reactions (Wallace & Fan, 2010) (Fig. 21.4). Since cocaine interferes with mitochondrial function it is reasonable to postulate that cocaine consumption can trigger nuclear epigenetic changes mediated, at least, by altered production of mitochondrial energy intermediates.

Cocaine exposure can cause alterations in DNA methylation (Anier, Malinovskaja, Aonurm-Helm, Zharkovsky,

& Kalda, 2010) and in chromatin enzymatic machinery, leading to changes in histone methylation, phosphorylation, and acetylation and changes the protein levels of histone deacetylases (HDACs) and the histone lysine methyltransferase G9a (Brami-Cherrier, Roze, Girault, Betuing, & Caboche, 2009; Feng et al., 2014; Maze et al., 2010).

Long-term cocaine users presented changes in hippocampal expression of DNA methyltransferase (DNMT)3a and HDAC2, and alterations on histone H3 lysine 4 trimethylation (H3K4me3) (Zhou et al., 2011). In addition, an early-phase response to cocaine in mice striatal neurons involved histone H3 phosphorylation, mediated by mitogen and stress-activated protein kinase 1 (MSK1). H3 phosphorylation by MSK1 is critically involved in c-fos transcription, and cocaine-induced locomotor sensitization, showing that cocaine alters gene regulation by activation of transcription factors and by chromatin remodeling (Brami-Cherrier et al., 2009). Studies in humans and nonhuman primates have also shown altered expression of genes directly related to MAPK signaling (Freeman et al., 2001; Lehrmann et al., 2003). On the other hand, recurrent cocaine administration reduced global levels of H3K9 dimethylation in mice NAc, mediated through the repression of G9a, which was regulated by the transcription factor deltaFosB (Δ FosB) (Covington et al., 2011; Maze et al., 2010).

Maternal cocaine abuse may affect epigenetic patterns in the offspring. In mice, maternal cocaine administration resulted in altered DNA methylation and gene expression in male offspring hippocampal neurons, producing potentially profound structural and functional modifications in the epigenomic programs of neonatal and prepubertal mice (Novikova et al., 2008). In particular at postnatal day 3, hypomethylation was observed in the promoter region of the gene encoding for coenzyme Q7, a cofactor in mitochondrial respiration essential for mitochondrial integrity and normal neurogenesis (Nakai et al., 2001), although no alteration was detected in its mRNA expression (Novikova et al., 2008).

Paternal chronic cocaine exposure may also affect the progeny, causing impaired working memory in the female offspring (He, Lidow, & Lidow, 2006). Although no significant damage in the DNA of the mature male reproductive cells was observed, the expression of DNMT1 and DNMT3a was modified in the germ cell-rich seminiferous tubules of the testis. Since DNMTs create and preserve a parental gene imprinting in germ cells through DNA methylation, paternal-derived neuronal birth defects can be transmitted by male spermatozoon (He et al., 2006). More recently, Vassoler et al. showed that male rats that self-administered cocaine had hyperacetylated brain-derived neurotrophic factor (BDNF) promoters in their

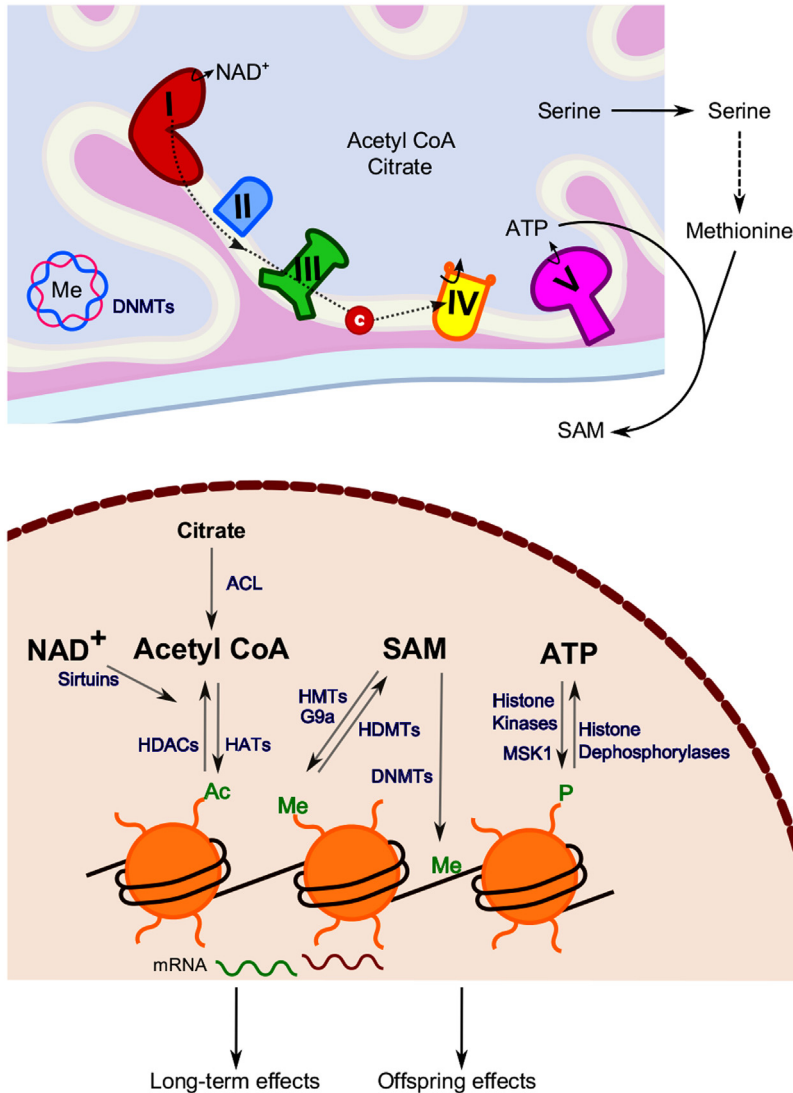


FIGURE 21.4 Mitochondria and epigenetics. Mitochondrial metabolites such as NAD^+ , acetyl CoA, ATP, and SAM regulate histone posttranslational modifications and DNA methylation. These epigenetic modifications regulate long-term changes in gene transcription and may be transmitted to the offspring. *ACL*, ATP citrate lyase; *HMTs*, histone methyl transferases; *HDMTs*, histone demethylases. For other abbreviations see text.

sperm, and this epigenetic mark was also observed in the medial PFC of their male (but not female) progeny. This was associated with increased BDNF protein levels and with decreased response to cocaine, measured as a delay in the acquisition of drug-seeking behavior, in the sons (Vassoler, White, Schmidt, Sadri-Vakili, & Pierce, 2013).

Thus, epigenetic changes induced by cocaine may be important in the context of the direct users, explaining the persistent nature of drug addiction, but may also be important for their progeny, when the drug is taken by male progenitors before fertilization or by female progenitors before or during gestation and breastfeeding, impacting fetal development and future life. Mitochondrial dysfunction may be a cause of these epigenetic changes, but it may also be a consequence of altered gene expression patterns caused by epigenetic regulation.

MINI-DICTIONARY OF TERMS

- *Apoptosis*: A highly regulated programmed cell death process characterized by the elimination of cells without releasing proinflammatory substances.
- *Bioenergetics*: Energy transformations within living systems, including cellular respiration and other metabolic processes.
- *Epigenetics*: Alterations caused by external or environmental factors that affect gene expression patterns without alterations in the DNA sequence.
- *FCCP-stimulated respiration*: Mitochondrial respiration in the presence of substrate and the uncoupler FCCP. In an attempt to reestablish the $\Delta\Psi_m$, mitochondria increase the respiratory chain activity and oxygen consumption, reaching the maximal respiratory capacity.
- *Mitochondrial DNA*: Genetic material located inside mitochondria with a double-stranded circular

organization that encodes key polypeptides of the OXPHOS complexes, mitochondrial rRNAs, and tRNAs. Recently it was suggested that mtDNA may also codify thousands of small ncRNAs.

- **Mitochondrial respiration:** Transformation of macronutrient energy into ATP using oxygen. Electrons from energy donors (reducing equivalents) are transported by the OXPHOS complexes and transferred to water, generating a proton gradient that is used by F1Fo-ATP synthase to drive the phosphorylation of ADP into ATP.
- **Mitochondrial uncoupling:** Electron transport without generation of the electrochemical gradient that fuels ATP synthesis, eliminating the coupling between the respiratory chain and ATP synthase.
- **Oxidative stress:** Imbalance between the generation of reactive oxygen species and the ability of antioxidant defenses to neutralize them.
- **State-3 respiration:** Mitochondrial respiration in the presence of an excess of substrate, saturating oxygen levels, and high concentration of ADP to stimulate ATP synthesis.
- **Weak base effect:** Weak bases are protonated at physiological pH and may accumulate in negatively charged intracellular environments, including the mitochondrial matrix, disrupting intracellular pH gradients and transmembrane potentials.

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