Chapter V

Neurotoxicity of Cocaine

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Abstract

Cocaine is a psychostimulant drug with sympathomimetic properties that is widely abused. In the central nervous system, cocaine interacts with monoaminergic systems, which mediate many of the drug's effects. However, the interaction with the dopaminergic system is the main cause of cocaine's addictive effects. Cocaine shares chemical similarities with dopamine and binds to the dopamine transporter at the plasma membrane of dopaminergic terminals, blocking dopamine re-uptake, resulting in increased synaptic dopamine. Excess dopamine levels may induce oxidative stress through dopamine auto-oxidation, generating reactive oxygen species. Cocaine also affects glutamate levels and the expression of glutamate receptors, which may mediate excitotoxic cell damage. Interactions with these neurotransmission systems may underlie cocaine neurotoxicity.

Cocaine has also been shown to impair mitochondrial function in several models, namely through the inhibition of mitochondrial respiratory chain complex I and may induce the activation of mitochondrial apoptotic pathways. Cocaine is frequently co-abused with heroin in a combination known as speedball. Indeed, heroin may modulate or modify cocaine's effects.

A chemical interaction between cocaine and morphine was found in drug mixtures similar to those used by speedball abusers, which may have a specific neurotoxic profile. In addition, cocaine abuse shares some common aspects with neurodegenerative disorders such as Parkinson's disease (PD), which also involves a dysfunction in the dopamine system. Thus, although neurotoxicity of cocaine may not be very pronounced, it may underlie brain dysfunction in cocaine and poly-drug abusers, which may predispose the brain to the development of neurodegenerative diseases.

Introduction

Cocaine is a widely abused psychostimulant drug with sympathomimetic properties that was first isolated in 1855. This alkaloid is extracted from the plant *Erythroxylum coca,* which is cultivated in the South American countries of Bolivia, Colombia, and Peru.

Worldwide, cocaine is one of the most abused illicit drugs, being used annually by 0.3-0.4% of the world population aged 15-64 (United Nations Office on Drugs and Crime 2010). Cocaine abuse mainly affects the brain and the cardiovascular system (Heard *et al.* 2008). Cocaine is highly lipophilic and rapidly crosses the blood-brain barrier, as opposed to its main metabolites ecgonine methyl ester and benzoylecgonine (Buttner *et al.* 2003). In this chapter, we discuss the literature describing the neurotoxic effects of cocaine, and the similarity between cocaine-induced neuronal dysfunction and hallmarks of Parkinson's disease (PD).

Neurological Impairments

Cocaine addiction may be described as a neurological disorder (Majewska 1996) because cocaine abusers present several neurological impairments, such as seizures (Koppel *et al.* 1996), cerebral ischemia, cerebral hemorrhages, infarction, optic neuropathy, cerebral atrophy, cognitive impairment, and mood and movement disorders (Majewska 1996). Brain lesions and cerebral atrophy are mainly observed in the prefrontal cortex and basal ganglia of cocaine abusers (Bartzokis *et al.* 1996;Langendorf *et al.* 1996). Moreover, cocaine abusers present specific dysfunction of executive functions associated with prefrontal brain regions (Bolla *et al.* 1998). Some authors defend that the primary cause of brain dysfunction induced by this drug abuse is related with cocaine-induced vasoconstriction and consequent hypoxia (Olsen 1995). Importantly, prenatal brain toxicity constitutes a serious negative effect of cocaine (Nassogne *et al.* 1998), leading to structural, metabolic, and functional brain abnormalities (Roussotte *et al.* 2010).

Cocaine and Neurotransmission

Cocaine and other drugs of abuse affect brain areas that include the ventral pallidum (involved in reward), orbitofrontal cortex and subcallosal cortex (involved in motivation/drive), amygdala and hippocampus (involved in memory and learning), and prefrontal cortex and anterior cyngulate gyrus (involved in impulse control)(Volkow *et al.* 2003). The addictive effects of drug abuse may be explained by the activation of the brain reward pathway by virtually all of these substances. This pathway is composed of dopaminergic neurons projecting from the ventral tegmental area to the nucleus accumbens (NAc) in the ventral striatum, and to the prefrontal cortex (Hyman *et al.* 2006). In the reward pathway, cocaine interacts directly with the dopamine transporters (DAT) in dopaminergic neurons (and in astrocytes), inhibiting the reuptake of dopamine and increasing its concentration in the synaptic cleft (Cunha-Oliveira *et al.* 2008). Moreover, glutamatergic neurotransmission has also been implicated in addiction, in the processes of reinforcement,

sensitization, habit learning, context conditioning, craving, and relapse (Tzschentke and Schmidt 2003).

Cocaine and Dopamine/Glutamate

Cocaine and other psychostimulant drugs share structural similarities with monoamines, and thereby interfere with the activity of these neurotransmitters.

Monoamine neurotransmitters include the catecholamines dopamine and noradrenaline and also serotonin. Dopamine and noradrenaline are synthesized from tyrosine by the highly regulated enzyme tyrosine hydroxylase (TH) and serotonin is synthesized from tryptophan by the enzyme tryptophan hydroxylase. The monoamines are actively transported by vesicular monoamine transporters (VMATs) into synaptic vesicles where they are stored in high millimolar concentrations (Staal et al. 2004). When the neurons depolarize, the synaptic vesicles fuse with the plasma membrane releasing the neurotransmitters into the synaptic cleft, where they interact with pre- and post-synaptic receptors. The neurotransmitters are then reuptaken by the pre-synaptic neuron for further release, or metabolized, in order to terminate their synaptic activity. Reuptake is performed by specific transporters in the plasma membrane of the synaptic terminal. DAT, noradrenaline transporter (NET), and serotonin transporters (SERT) are the specific proteins involved in the reuptake of monoamines. The effects of cocaine on the central nervous system are mainly due to the blockade of monoamine reuptake (Ki:02-0.7 µM)(Han and Gu 2006). However, at higher concentrations (>5 µM) cocaine may also act as a local anesthetic by binding to voltage-gated ion channels and inhibiting inward sodium and outward potassium currents (Heard *et al.* 2008). These concentrations are in the range of those found in cocaine addicts, who usually present cocaine plasma concentrations between 0.3-1 mM, and have brain concentrations that may exceed the plasma concentration by several times (Heard *et al.* 2008). Besides inhibiting the DAT, cocaine also interacts with VMAT-2, favoring the storage of catecholamines inside synaptic vesicles (Brown *et al.* 2001). It was suggested that cocaine-induced inhibition of the DAT and increased vesicular sequestration of dopamine causes a shift in the ratio of cytosolic to vesicular dopamine, increasing the amount of neurotransmitter packaged in each vesicle before its release. This effect on the VMAT may contribute to a further increase in synaptic dopamine, upon a depolarizing stimulus (Brown et al. 2001). Importantly, the reinforcing effects of cocaine were shown to be largely dependent on its effect on dopaminergic neurotransmission (Ritz *et al.* 1987).

Evidence suggests the involvement of glutamatergic neurotransmission in the mechanisms of drug dependence mediated by the dopaminergic reward circuit in the brain (Tzschentke and Schmidt 2003). Cocaine was shown to increase extracellular glutamate concentrations in brain areas such as the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC), or striatum (You *et al.* 2007;Williams and Steketee 2004;Kalivas and Duffy 1998;Reid *et al.* 1997). Plasticity of glutamatergic synaptic transmission in the VTA is one of the brain adaptations induced by cocaine, and contributes to the development of addictive behaviors (Schilstrom *et al.* 2006;Wolf 2010). The striatum plays an important role in action selection, execution, in reward-dependent learning, and

dopamine in the NAc controls the efficacy of glutamatergic corticostriatal synapses (Wickens *et al.* 2007).

However, an excessive increase in extracellular glutamate has been associated with excitotoxic processes due to increased activation of NMDA receptors and a subsequent increase in intracellular Ca^{2+} concentration (Rego and Oliveira 2003).

Changes in the expression of N-methyl-D-aspartate (NMDA) receptor subunits were observed in the brains of cocaine-exposed rats (Huber *et al.* 2001;Schilstrom *et al.* 2006;Yamaguchi *et al.* 2002;Scheggi *et al.* 2002;Hemby *et al.* 2005). In VTA neurons, cocaine induced an increase in NR1 and NR2B (which is mainly extrasynaptic) subunit expression and their redistribution to synaptic membranes (Schilstrom *et al.* 2006). NR2B expression was also shown to be increased in the NAc and hippocampus of cocaine-exposed rats. This effect was prevented in rats exposed to cocaine and MK-801, a NMDA receptor antagonist (Scheggi *et al.* 2002). Cocaine-induced changes in the expression of NMDA receptor subunits may be mediated by extracellular dopamine, via stimulation of dopamine receptors (Schilstrom *et al.* 2006). Interactions between NR2B subunits of the NMDA receptor and the D2 dopamine receptor were observed in the neostriatum of cocaine exposed rats (Liu *et al.* 2006) and may contribute to the stimulant effect of cocaine. Indeed, it seems that D1 dopamine receptors, NMDA receptors, and extracellular-signal-regulated kinase (ERK) contribute significantly to neuronal morphological changes induced by repeated exposure to cocaine (Ren *et al.* 2010). NMDA receptor activation in the striatum was shown to recruit D1R from the cytoplasm to the plasma membrane and dendritic spines (Missale *et al.* 2006;Sun *et al.* 2008). Both D1 and NMDA receptors regulate the extent of dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP32) phosphorylation, which forms a potent inhibitor of protein phosphatase 1, but in opposite directions. D1 receptor activation induces cAMP formation, activation of protein kinase A (PKA), and phosphorylation of DARPP32, whereas NMDA receptor stimulation induces an increase in intracellular calcium, leading to the activation of calcineurin and dephosphorylation of DARPP32 (Nakano *et al.* 2010). Activation of the ERK pathway in the striatum has been pointed out as an essential event for chromatin remodeling and gene expression associated with long-term behavioral adaptations induced by drug abuse. Interestingly, cocaine-induced ERK activation occurs selectively in dopamine D1 receptor-expressing medium spiny neurons (MSNs), and requires the coincident stimulation of D1 and NMDA receptors (Pascoli *et al.* 2011). Interplay between D1 and NMDAR resulting in ERK activation in MSNs upon exposure to cocaine seems to be mediated by two pathways. The first is D1-mediated PKA activation, which leads to phosphorylation of DARPP-32, inhibiting PP1, and preventing ERK dephosphorylation. The second is D1R mediated phosphorylation (activation) of SFK (Fyn), which leads to the phosphorylation of NR2B subunits, increasing Ca^{2+} entry through these receptors, and leading to the activation of Ras protein-specific guanine nucleotide-releasing factor 1 (Ras-GRF1), and mitogen-activated protein kinase kinase(MAPKK)/MEK and ERK. This represents a powerful mechanism of NMDA receptor potentiation (Pascoli *et al.* 2011).

This data suggests that the interaction between glutamate and dopamine signaling in striatal MSNs is critical for long-term plasticity in the striatum and behavioral alterations induced by the drug abuse, particularly cocaine.

Cocaine Neurotoxicity

Cocaine and Oxidative Stress

As described previously, cocaine directly induces an increase in extracellular dopamine in specific regions of the brain.

Dopamine is easily oxidized and may induce oxidative stress in dopaminergic and neighboring cells, which may contribute to the neurotoxicity of the drugs of abuse, particularly cocaine due to its direct effect in increasing synaptic dopamine concentration. Dopamine has been shown to be neurotoxic *in vitro* (Graham *et al.* 1978;McLaughlin *et al.* 1998) and *in vivo* (Hastings *et al.* 1996). Dopamine has the ability to form reactive metabolites by enzymatic and non-enzymatic mechanisms. It may be metabolized intracellularly by monoamine oxidase A (MAO_A) (Fornai *et al.* 2000) (and in a lower extent by MAO_B (Youdim *et al.* 2006)), a mitochondrial enzyme that is present in the cytoplasmic side of the outer mitochondrial membrane in neurons and astrocytes. This enzyme generates 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is highly toxic and rapidly metabolized by aldehyde dehydrogenase, producing 3,4-dihydroxyphenylacetic acid (DOPAC) and hydrogen peroxide (H_2O_2) (Marchitti *et al.* 2007).

In addition, auto-oxidation of the catechol ring of dopamine produces the superoxide anion ($O_2^{\cdot-}$) and H_2O_2, which may react with transition metal ions, such as iron, via the Haber-Weiss/Fenton reactions, creating the highly toxic hydroxyl radical ($^{\cdot}OH$). $O_2^{\cdot-}$ may also react with nitric oxide, forming the highly toxic peroxynitrite. The autoxidation of dopamine also originates the electron-deficient dopamine quinone and may be facilitated by the presence of transition metal ions (Hastings 2009). Electron-deficient dopamine quinones readily react with cellular nucleophiles, such as the reduced sulfhydryl group on protein cysteinyl residues, and covalently modify protein structure. These cysteinyl residues are often localized at the active site of proteins and, thus, covalent modification by DA quinones often leads to inactivation of protein function, which may result in compromised cell survival (Hastings 2009).

Antioxidants are an important line of defense against free radicals and other reactive species. The main antioxidant enzymes involved in H_2O_2 inactivation are glutathione peroxidase (GPx) and catalase (present in the peroxisomes). Superoxide dismutase (SOD), another antioxidant enzyme, may contribute to increased H_2O_2 levels by a detoxification of $O_2^{\cdot-}$ (Cunha-Oliveira *et al.* 2008). The levels and the activity of these enzymes are regulated by the cells and are important in maintaining cellular homeostasis, explaining why the effects of chronic exposure to oxidants normally contrasts with the effects of acute exposure. For example, acute exposure to H_2O_2 induces apoptotic cell death (Benedi *et al.* 2004;Jang and Surh 2004), whereas chronic exposure to low concentrations of H_2O_2 induces cellular resistance to the acute toxicity of this compound (Cunha-Oliveira *et al.* 2006c).

The occurrence of oxidative stress in neurons upon exposure to cocaine has been suggested to occur due to an accumulation of dopamine and its metabolites in the brain following cocaine exposure (Cunha-Oliveira *et al.* 2008), or due to the presence of oxidized metabolites of cocaine (Kovacic 2005), which may result in increased production of reactive oxygen species (ROS). Cocaine exposure has been reported to increase H_2O_2 in the prefrontal cortex and in the striatum of rats (Dietrich *et al.* 2005), and to decrease catalase activity in the

prefrontal cortex and striatum of mice (Macedo *et al.* 2005), but it was shown to increase SOD and GPx activities (Dietrich *et al.* 2005) in the same brain structures in rats. The levels of antioxidants such as reduced glutathione (GSH) or vitamin E were also shown to be decreased upon cocaine exposure (Poon *et al.* 2007;Lipton *et al.* 2003). Furthermore, cocaine exposure also results in oxidative injury in the brain, as indicated by an increase in lipid peroxidation in the hippocampus of rats exposed *in utero* to cocaine (Bashkatova *et al.* 2006) and by the oxidation of proteins in human neuronal progenitor cells exposed to cocaine (Poon *et al.* 2007). Acute cocaine exposure was shown to elevate malondialdehyde (MDA) and nitrite levels in the PFC and NAc in rat brain slices. The increase in oxidative damage markers was accompanied by a decrease in total antioxidant content in these regions, and both were prevented by the antioxidant tempol (Numa *et al.* 2008). GSH concentration and GPx activity were also found to be decreased in the hippocampus of cocaine treated animals (Muriach *et al.* 2010). Interestingly, adaptation to oxidative stress induced by cocaine may explain the partial resistance against H_2O_2 toxicity observed in PC12 cells chronically exposed to cocaine (Cunha-Oliveira *et al.* 2006c), suggesting the involvement of oxidative stress in the chronic effects of cocaine.

Similarities between Cocaine and Neurodegenerative Disorders – The Case of PD

Cocaine and PD

Cocaine abuse shares some common aspects with neurodegenerative disorders such as PD, which also involves a dysfunction in the dopamine system. Chronic cocaine use leads to increased motor-stimulant response upon repeated, intermittent exposure, designated by locomotor sensitization (Liu and Steketee 2011). This motor phenomenon is thought to underlie drug-craving and relapses to drug use. Chronic use may also lead to subtle parkinsonian features, such as a resting tremor that persists when the drug is withdrawn. However, cocaine use has not been reported to be a risk factor for the development of idiopathic PD (San Luciano and Saunders-Pullman 2009).

Alpha-synuclein was the first protein found to be mutated in genetic cases of PD, and its accumulation in intracellular filamentous aggregates is a pathological feature of PD and other neurodegenerative disorders (Perfeito and Rego 2008). Interestingly, cocaine abusers present an overexpression of alpha-synuclein in dopamine neurons (Mash *et al.* 2003), similar to those that occur with amphetamine (Fornai *et al.* 2005) or MPTP treatment (Vila *et al.* 2000) in mice. Overexpression of alpha-synuclein in the NAc was also observed in rats for high-dose cocaine exposure (Brenz Verca *et al.* 2003). Increased alpha synuclein protein levels were also found in serum from recently abstinent cocaine abusers (Mash *et al.* 2008). Cocaine abuse was suggested to concomitantly regulate alpha-synuclein and dopamine transporter binding and function, due to the presence of elevated levels of the two proteins in the striatum of human cocaine abusers (Qin *et al.* 2005). Thus, overexpression of alpha-synuclein may play a role in cocaine-induced plasticity and regulation of dopamine synaptic tone (Qin *et al.* 2005). Accordingly, alpha-synuclein overexpression in rat NAc was suggested to modulate cocaine-induced locomotion and self-administration (Boyer and Dreyer 2007).

Another common characteristic observed in cocaine abuse and in different models of PD is mitochondrial dysfunction and apoptosis, as described in the next section.

Cocaine and Mitochondrial Dysfunction/Apoptosis

Cocaine neurotoxicity has been associated with the induction of biochemical features of apoptosis, such as activation of caspases (Dey *et al.* 2007;Cunha-Oliveira *et al.* 2006a;Imam *et al.* 2005;Oliveira *et al.* 2003;Mitchell and Snyder-Keller 2003), loss of mitochondrial potential, and cytochrome c release (Oliveira *et al.* 2003;Cunha-Oliveira *et al.* 2006a). Interestingly, morphological features of neuronal apoptosis were not always evident upon *in vivo* (Dietrich *et al.* 2005) or *in vitro* (Oliveira *et al.* 2002;Cunha-Oliveira *et al.* 2006a) exposure to cocaine. However cocaine-induced apoptotic morphology was found in cultured fetal mouse cortical neurons (Nassogne *et al.* 1997), and maternal cocaine administration lead to the appearance of apoptotic neurons in the fetal brain, whereas the maternal brain was not affected (Xiao and Zhang 2008). Interestingly, cell death involving cytochrome c release induced by cocaine in human neuronal progenitor cells seems to be preceded by oxidative stress (Poon *et al.* 2007).

Cocaine was previously suggested to influence mitochondrial function in multiple cell types. Studies from our laboratory have shown that a functional respiratory chain seems to be required for cocaine's toxicity (Cunha-Oliveira *et al.* 2006a). Similar to what occurs in PD, complex I activity and subunit expression were reduced after cocaine exposure (Yuan and Acosta, Jr. 2000;Devi and Chan 1997;Dietrich *et al.* 2004). Moreover, mitochondrial gene expression was found to be down-regulated in rat cingulate cortex upon exposure to cocaine (Dietrich et al. 2004). In vivo cocaine administration decreased state 3 respiration and the respiratory control ratio (RCR) of hepatic mitochondria and decreased activity of complexes I, II/III, and IV (Devi and Chan 1997). Unpublished results from our lab also show that cocaine inhibits complex I driven-respiration in isolated liver and brain mitochondria (Silva et al., unpublished results). Furthermore, microarray data suggested that mitochondrial functions and the energy metabolism are affected in brains of human cocaine abusers (Lehrmann et al. 2003).

Oxidative stress seems to be involved in cocaine's mitochondrial effects, since cocaine-induced cardiac mitochondrial dysfunction was reported to be prevented by the mitochondrial-targeted antioxidant MitoQ (Vergeade *et al.* 2010).

Combinations of Cocaine and Other Substances

Effects of Speedball (Cocaine + Heroin)

Cocaine may be abused together with other drugs that may modify its effects. A relatively common combination of drugs is the *speedball* (European Monitoring Center for Drugs and Drug Addiction 2009), which consists in concurrent administration (by injection) of cocaine and heroin. *Speedball* has been reported to cause more rewarding effects in rats than cocaine or heroin alone (Ranaldi and Munn 1998). This may be explained by the reduction of the unwanted side-effects of one drug by the other, which has different mechanisms of action, or by the fact that one drug enhances the effect of the other (Leri *et al.* 2003).

This drug combination has serious consequences. Cocaine use during methadone maintenance treatment may contribute to re-initiation of heroin use, leading to serious medical, social, and criminal problems (European Monitoring Center for Drugs and Drug Addiction 2008;Leri and Rizos 2005). Moreover, the use of cocaine in combination with heroin is associated with the presence of a mental illness and may aggravate underlying psychological problems such as bipolar disorder (European Monitoring Center for Drugs and Drug Addiction 2008). Thus, speedball abusers seem to exhibit a more severe psychopathology as compared to other cocaine addicts, and are more likely to fail in drug abuse treatment (Bandettini Di *et al.* 2006).

The pharmacological mechanisms underlying speedball abuse are not fully understood, but they seem to involve an increase in the reward obtained from the drug combination (Rowlett and Woolverton 1997). Interestingly, cocaine self-administration was found to be enhanced by (otherwise) inactive doses of heroin in rhesus monkeys (Rowlett and Woolverton 1997). Self-administration of cocaine and heroin produces synergistic elevations in extracellular dopamine concentration in the reward pathways (Hemby *et al.* 1999;Smith *et al.* 2006) that are thought to be mediated by dopamine and μ-opioid receptors (Cornish *et al.* 2005). This suggests that the dynamics of dopamine and other neurotransmitters affected in drug addiction are altered upon exposure to the drug combinations.

We have previously demonstrated that both cocaine (Cunha-Oliveira et al. 2006a) and heroin (Cunha-Oliveira et al. 2007) induce neurotoxicity in rat cortical neurons, upon an acute exposure, in a process involving mitochondrial dysfunction and cell death by apoptosis. We also showed that chronic exposure of dopaminergic PC12 cells to cocaine (Cunha-Oliveira *et al.* 2006c) or to street heroin (Cunha-Oliveira *et al.* 2006b) leads to a potentiation of extracellular dopamine accumulation in response to an acute exposure to the same drug, in comparison with PC12 cells not previously exposed to any of the drugs. As previously discussed, dopamine oxidation may be the primary mechanism of psychostimulant toxicity in dopaminergic cells (Cunha-Oliveira *et al.* 2008). However, despite the high prevalence and severe effects of speedball, little is known about the toxicity of this drug combination.

Heroin is an indirect agonist of the μ-opioid receptor, acting at these receptors through its metabolites 6-acetylmorphine and morphine (Selley *et al.* 2001). Acute activation of opioid receptors leads to inhibition of neurotransmitter release via activation of Gi/o proteins, resulting in inhibition of adenylyl cyclase, and an activation of K^+ outward and inhibition of Ca^{2+} inward conductances (Williams *et al.* 2001). However, after prolonged exposure to opioids, compensatory changes in these systems occur, leading to tolerance. Besides the effects mediated by opioid receptors, it is also possible that heroin, due to its lipophilicity, enters into the cells to interact directly with intracellular targets, such as DNA (Li and Dong 2009). This mechanism could explain the opioid receptor-independent apoptosis observed upon street heroin exposure in rat cortical neurons (Cunha-Oliveira *et al.* 2007). Cocaine may also interact directly with intracellular targets, such as the sigma receptor (Heard *et al.* 2008), probably entering the cell due to the weak base effect, since at physiological pH, cocaine is positively charged.

Cocaine and morphine were previously shown to interact at the molecular level, giving rise to a new chemical entity, a cocaine-morphine adduct (Garrido *et al.* 2007). Therefore, chemical interactions between cocaine, heroin, or their metabolites, such as cocaine-morphine adducts, may play a role in the effects of cocaine-heroin combinations, especially when the co-abuse occurs simultaneously and chemical interactions may occur. A recent study from

our lab was designed to understand if, besides pharmacodynamic interactions, chemical interactions between drugs could also play a role in speedball neurotoxicity. For this purpose, we used primary cultures of rat cortical neurons subjected to heroin and/or cocaine, administered sequentially or simultaneously (Cunha-Oliveira *et al.* 2010). In the presence of a mixture of the two drugs, but not upon sequential exposure, the effects of cocaine seemed to prevail over heroin effects (Cunha-Oliveira *et al.* 2010). Thus, the interaction of the mixed drugs with opioid receptors may be lower, compared to a sequential administration of the two drugs, probably due to the presence of cocaine:morphine adducts. Unpublished results from our lab also show that a cocaine-morphine combination affects mitochondrial functions in isolated mitochondria (Silva *et al.*, unpublished data)**.** Our data suggests that the pathways of neurotoxicity caused by co-administration of cocaine and heroin (1:1) are distinct from those evoked by a sequential administration of these drugs, possibly related with chemical interactions between the two drugs (or their metabolites), which may interfere with cell death mechanisms. Thus, it is possible that the formation of cocaine:morphine adducts, or other chemical interactions occurring between cocaine and heroin, will affect the capacity of cocaine to interact with its cellular targets, such as the monoamine transporters or mitochondria.

Our results show that co-use of cocaine and heroin may lead to different effects depending on the mode of co-exposure, and suggest that poly-drug abusers are more prone to neurotoxic damage than single drug abusers.

Cocaine and Ethanol

Another toxic drug combination involves cocaine and ethanol. When these two substances are co-consumed, the euphoric effects of cocaine are enhanced. However, this combination also increases the toxic effects of both drugs because the drugs are combined in the liver to form a very toxic metabolite – cocaethylene. Cocaethylene is a very lipophilic compound and is able to cross the blood-brain barrier. The effects of cocaethylene are similar to those of cocaine but the metabolite has a longer half-life, prolonging the acute effects of cocaine (Henry 2007). The concomitant use of cocaine and alcohol may have additive negative effects on the brain as compared to the use of only one of these two substances (Bolla *et al.* 2000).

Conclusions

In summary, cocaine addiction may be described as a neurological disorder (Majewska 1996) because cocaine abusers present several neurological impairments. The interaction with the dopaminergic system is the main cause of cocaine's addictive effects, leading to excess synaptic dopamine levels that may induce oxidative stress through dopamine auto-oxidation, generating reactive oxygen species. Cocaine also affects glutamate levels and the expression of glutamate receptors, which may mediate excitotoxic cell damage and long-term neuroadaptive effects. Interaction with these neurotransmission systems may underlie cocaine neurotoxicity.

Cocaine abuse shares some common aspects with neurodegenerative disorders such as PD, which also involves a dysfunction in the dopamine system. Cocaine exposure has been

suggested to concomitantly regulate alpha-synuclein and dopamine transporter binding and function. Moreover, cocaine has been shown to impair mitochondrial function in several models, namely through the inhibition of mitochondrial respiratory chain complex I, as occurs in PD, and cocaine toxicity has been shown to require a functional respiratory chain. Cocaine may also induce the activation of mitochondrial apoptotic pathways in several models.

Cocaine is frequently co-abused with heroin, in a combination known as speedball, and heroin may modulate or modify cocaine's effects. A chemical interaction between cocaine and morphine was found in drug mixtures similar to those used by speedball abusers, which may have a specific neurotoxic profile.

Cocaine induces neurotoxicity *in vivo* and *in vitro*, though sometimes not very pronounced, which may underlie brain dysfunction in cocaine and poly-drug abusers and may predispose the brain to the development of neurodegenerative diseases.

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