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Drug-Induced Neurotoxicity Mechanisms Underlie Addiction Symptoms

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Abstract. The molecular mechanisms contributing to substance use disorder (SUD) have gained significant attention in recent years. In the last decade, people have found that addiction is hardly a decision of willpower but can be attributed to multiple molecular changes occurring in the brain after repeated drugs administration. The current research is focusing on understanding the mechanisms through which drugs administrations modify the brain and cause addicted individuals to become more susceptible to relapse. Notably, neurological impairments were found in drug addicts, suggesting potential drug-induced neurotoxicity. From studies conducted between 1992 to 2022, this paper analyzes the neurotoxicity of three commonly abused substances: methamphetamine (MA or METH), opiate, and cocaine at a cellular and molecular level, and the mechanisms through which they trigger neurotoxic effects. One shared neurotoxicity mechanism among the three substances is oxidative stress, as all drugs lead to an elevated DA level that renders oxidation feasible. Nonetheless, each substance exhibits unique mechanisms in addition to oxidative stress. For MA, additional neurotoxicity mechanisms include alteration in glutamate (Glu) and glial cells. Opiates could induce programmed cell death (apoptosis). Last but not the least, cocaine administration leads to decreased brain-derived neurotrophic factor (BDNF) and norepinephrine (NE) facilitation. By contributing to allostasis and withdrawal symptoms, these various neurotoxic effects play a crucial role in the addiction process, making addicts vulnerable to relapse.

Keywords: Substance use disorder; Neurotoxicity; Methamphetamine; Opiate; Cocaine.

1. Introduction

Addiction is a chronic relapsing disease accompanied by impaired cognitive controls [1]. Previously stigmatized as a moral failure, latest research has shed light on the underlying mechanisms of the SUD, resolving to establish better diagnostic criteria and therapeutic approaches [2]. In recent studies, it was found that despite the initial voluntary behavior of drug abuse, the later administration may have been influenced and reinforced by the neurotoxicity effect brought out by repeated drugs administration. To illustrate, common neurotoxic effects of substance administration include decreased white matter and DA terminal degeneration.

Though sharing certain similarities, the specific mechanisms leading to neurotoxicity vary by substances. This research aims to find out the specific neurotoxic effects induced by repeated MA, cocaine, and opiate administration and their relationship with addiction symptoms.

1.1 Substance Related Addiction

The blood brain barrier (BBB) is a protective barrier of the brain that allows certain substances to pass through. Addictive substances invade the BBB to induce neurotoxicity effect that ultimately leads to the SUD [3]. There are multiple factors putting individuals at greater risk, including but not limited to environmental influences, early drugs exposure, and stress [2].

The mechanism considered most relevant in addiction is the reward pathway, also known as the mesolimbic pathway, which connects the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and is mostly driven by DA. DA plays a critical role in multiple brain functions, such as reward, addiction, and even voluntary movements.

The addictive substances passed through the BBB would target at the reward pathway and induce a phasic DA firing with the activation of a DA receptor with low affinity, known as D1R, in the NAc. This phasic firing would then reinforce behaviors associated with the rewards [4].

In addition to the positive reinforcement, addiction is reinforced by the "dark side of addiction". Repeated D1R phasic firing leads to a low DA baseline and form an allostasis state where individuals experience dysphoria during drug withdrawal. Further, during drug administration, the stress response associated with NE is activated simultaneously with the reward pathway. Both the continuous low level of DA inside the body and the upregulation of kappa opioid and NE are implicated in the "dark side of addiction" where addicted individuals would experience extreme stress without drug administration [2].

For the diagnosis criteria, addiction is diagnosed when compulsive use is continued in the presence of adverse consequences including severe social impacts on one's life [5].

1.2 Oxidative Stress

Oxidative stress is one of the common causes of neurotoxicity. It generates free radicals including reactive nitrogen species (RNS) and reactive oxygen species (ROS), which, in turn, triggers apoptotic proteins that are associated with the self-destruction sequence in the DNA [6]. When the equilibrium between the ROS and antioxidants is broken, the oxidative stress impairs the integrity of receptors. Studies have demonstrated that the pathway thereby oxidative stress triggers apoptosis mediates by mitochondria [7]. Mitochondria plays an essential role in oxidative phosphorylation maintenance, and adenosine triphosphate (ATP) production and consumption. The dysfunctional mitochondria release pro-caspases, cytochrome c (cyt c), apoptosis-induing factor (AIF), and apoptotic-activating factor-1 into the cytosol [8].

1.3 Apoptosis

Apoptosis is an active physiological process of programmed cell death that can be triggered by oxidative stress, serving as a means to maintain homeostasis with the genetic elimination of cell populations in tissues, and a defense mechanism in immune reactions [9, 10]. Apoptosis responds to various stimuli and conditions, and is characterized by morphological changes including cell shrinkage, fragmentation into membrane-bound apoptotic bodies, and DNA condensation and fragmentation [3].

2. Methodology

The review provides an analysis of neurotoxicity mechanisms and their contributions to specific addiction symptoms. The research method of this study is literature reading and analysis. The references are primarily selected from PubMed. The keywords that are used in the advanced search include neurotoxicity, addiction, methamphetamine, opiate, cocaine, oxidative stress, locus coeruleus (LC) neuron, BNDF, and mechanisms.

3. Analysis of neurotoxicity mechanisms

3.1 Methamphetamine (METH)-induced Neurotoxicity

3.1.1 Glutamate release and METH neurotoxicity

Multiple studies have provided empirical implications of the role of glutamate (Glu) release in the neurotoxic effect of MA. Given that Glu is widely distributed in the central nervous system (CNS), cell death could be caused by the overactivity of glutamatergic neurotransmission [11]. Furthermore, enhanced Glu concentration is implicated in dopaminergic neurotoxicity. In a study comparing the effects of repeated administration of MA on the striatum (ST) and nucleus accumbens, repeated administration of MA has been shown to decreases the tissue concentration of DA, but dopaminergic

damage was observed only in the ST. The ST was where delayed Glu release occurred, whereas in NAc, there was no additional Glu release [12]. Another study observing the different DA levels, Glu levels, and DA content after repeated MDMA and MA administration by J. Frank Nash and Bryan K. Yamamoto showed congruent results. Repeated MDMA administration in the ST where there was no Glu efflux showed an intact DA content, whereas repeated MA administration with an increased Glu level showed a decrease in the striatal DA content, supporting that an elevated level of Glu could be the cause of the impaired DA axon terminals [11].

In spite of its dopaminergic damage, Glu release is also responsible for other neuronal damage through binding to glutamate receptors (GluR), inducing calcium influx and producing RNS [5]. Multiple studies have demonstrated that Ca^{2+} influx is primarily responsible for excitotoxic damage, and the activation of Glu by MA, accompanied by multiple intracellular signaling cascades, is associated with an increase in Ca^{2+} influx.

3.1.2 Meth-induced oxidative stress

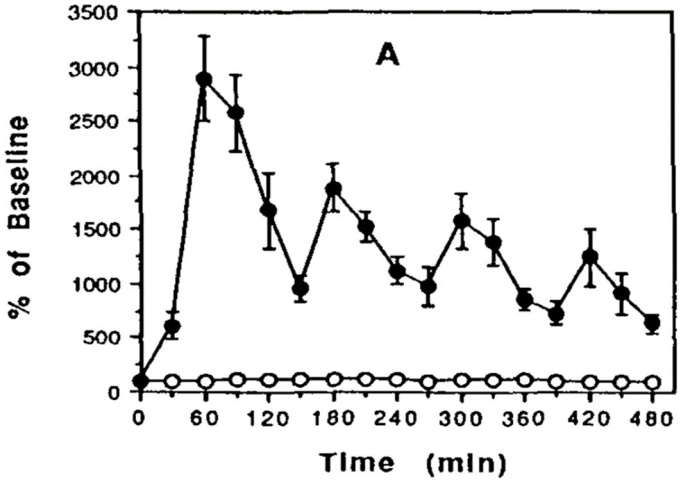

Figure 1. Effect of MA on extracellular concentration of DA(A) in the striatum [12].

The administration of MA can lead to an accumulation of DA. Figure 1 presents the effect of repeated METH administration on DA concentration. With each administration, the percent baseline rises, and the effect of the drug diminishes following each administration [12]. Excessive DA can induce auto-oxidation in interneural and extracellular spaces [11]. Additionally, METH can also redistribute DA and increase DA auto-oxidation and DA terminal injury [13]. In turn, with increased production of ROS and RNS, when DA decreases anti-oxidant systems and increases levels of oxidative stress markers, cellular toxicity can be triggered [14]. The macromolecules that can be damaged by excessive ROS include DNA, lipid membranes, and proteins. Damage to DNA can then lead to loss of genetic information, triggering inhibition of the complex II electron transport chain and then accelerating mitochondrial dysfunction, which is associated with striatal dopaminergic terminal damage [15]. Furthermore, the activation of NADPH oxidase triggered by METH could induce a pro-oxidant effect, damaging the BBB permeability [5].

DA oxidation can also produce hydroxyl radicals, one of the key components leading to the METH-induced cell death cascade. Hydroxyl radicals in METH addiction can be generated through either the Fenton reaction ($O_2^- + H_2O_2 \rightarrow HO + O_2 + HO^-$) or the superoxide anion produced by the increased cytosolic DA oxidation. The hydroxyl radical generated can induce lipid peroxidation and activation of proteases [15].

Oxidative stress is implicated in the degeneration of DA terminals, which leads to an inhibition of DA synthesis. Another cause of oxidative stress by METH is the over-expression of α-synuclein oligomers. α-synuclein is a protein responsible for regulating DA release and maintaining the supply

of synaptic vesicles in presynaptic terminals. In addition, within the inner membrane of neuronal mitochondria, the protein can predispose some neurons to degeneration [15].

3.1.3. METH-induced changes in glial cells

Glial cells, primarily microglial, are the main modulators of inflammation in the CNS. DA has a bidirectional relationship with microglial cells, so the elevated DA level during MA administration would potentially alter the biology of microglial cells [16]. Large doses of METH have been shown to cause microglial and astrocyte activation in the brain regions including ST, hippocampus, and substantial nigra (SN), exclusively in the DA-innervated areas. These activations are associated with the secretion of pro-inflammatory cytokines, triggering neurodegeneration, and activating apoptotic signaling [15]. Microglial activation is observed in the regions where DA terminal degeneration occurs, showing that the activation leads to minimal DA damage [16].

3.2 Cocaine-induced Neurotoxicity

3.2.1 Cocaine-induced oxidative stress

Cocaine exposure leads to an accumulation of DA in the synaptic cleft. Metabolism, which could remove the DA in the extra neuronal space, has been shown to produce ROS, H_2O_2, inducing cell death [17]. Moreover, the metabolism mechanisms, auto-oxidation and oxidation by monoamine oxidase, decrease catalase activity, the main antioxidant enzymes implicated in H_2O_2 inactivation, increase superoxide dismutase (SOD) activities, an antioxidant enzyme that could detoxify O_2^-, and thus lead to H_2O_2 increase [3, 18]. Furthermore, the metabolism can also generate hydroxyl radicals through the Fenton-Haber Weiss reaction ($O_2^- + H_2O_2 \rightarrow HO + O_2 + HO^-$). In conclusion, cocaine exposure could lead to oxidative stress by having ROS on the loose without proper inhibitions [19].

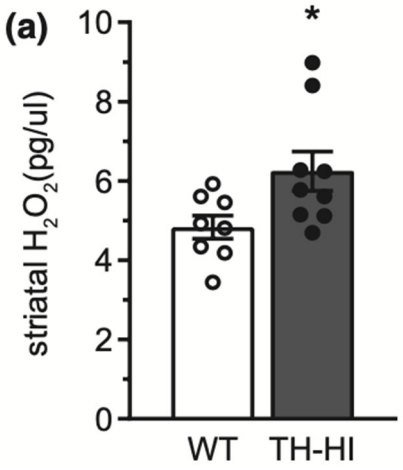

Figure 2. Genomic copy number of tyrosine hydroxylase (TH) in TH-HI mice relative to wild-type (WT) littermates [13].

Increasing tyrosine hydroxylase (TH) activity is another mechanism by which repeated cocaine administration triggers oxidative stress [20]. TH is the rate-limiting enzyme in DA biosynthesis and is able to trigger oxidative stress unique to catecholamine neurons. In Figure 2, the concentration of H_2O_2 in ST was found elevated in TH-over-expressing mice (TH-HI) [13].

3.2.2 Cocaine-induced apoptosis

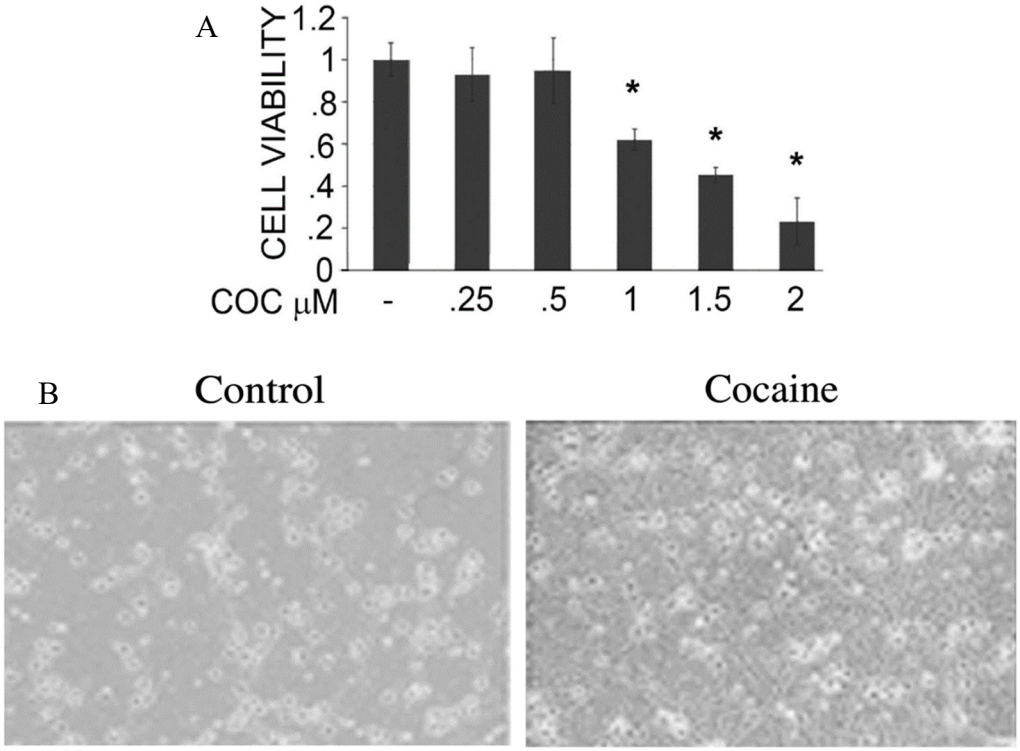

Figure 3. A. MTT Viability assay of dose-dependent effect of cocaine on rat primary hippocampal neurons. B. Contrast photomicrographs of the treated rat primary hippocampal neurons showing the cytopathic effects of cocaine [21].

MTT reduction has shown that cocaine exposure induces a dose-dependent decrease in cell viability with a significant decrease in cell reduction capacity (See Figure 3 A). Upon further studies, cocaine toxicity effects can lead to mitochondrial dysfunction and activate mitochondria-initiated programmed cell death, which is shown to be mediated through the electron transport chain [19]. To illustrate the neurotoxic effects of cocaine, Figure 3 B compares primary hippocampal neurons of untreated (control) rat with rats treated with cocaine and demonstrates the ability of cocaine to produce pathological changes in cells. Additionally, Cytochrome c, a hemeprotein that plays an important role in apoptosis, is found to decrease after cocaine exposure, indicating the neurotoxic effect of cocaine. It is an important electron carrier between mitochondrial complex III and complex IV that could indicate mitochondrial integrity [22].

Studies have demonstrated that cocaine exposure decreases the survival of embryonic LC neurons. The cocaine-induced apoptosis of the LC neurons is characterized by pro-apoptotic Bax levels, decreased anti-apoptotic Bcl-2 levels, activation of initiator caspase-9, and activation of effector caspase-3 [23]. Cocaine exposure to the LC neurons also triggers tumor necrosis factor alpha expression, which is an important modulator of apoptotic cell signaling. It is reported that tumor necrosis factor alpha triggers cell death of nerve growth factor-deprived sympathetic and sensory neurons [23].

3.2.3 Cocaine-induced elevated norepinephrine

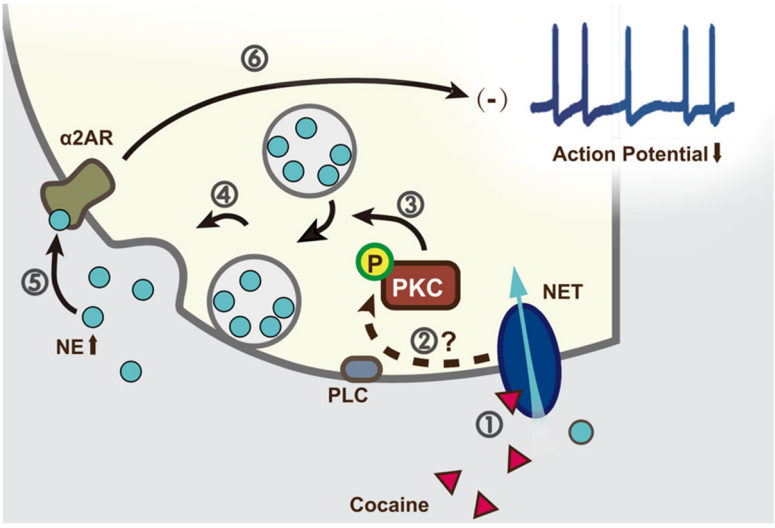

Figure 4. Model of cocaine on quantal NE release [24].

Cocaine modulates the extracellular level of NE in the LC, the sole source of NE in the cortex. The facilitation of NE can be caused by multiple pathways. First, the cocaine-NET-protein kinase c (PKC) pathway prompts NE release (see Figure 4). Following the Ca^{2+} influx triggered by cocaine, LC-NE neurons substantially increase the release of their transmitters due to increased releasable vesicles, enhanced phosphorylation level of PKC, and increased quantal NE release frequency [24]. Additionally, cocaine can block the reuptake into NEnergic neurons via the NE transporter (NET) to increase NE overflow. In a study of the effects of pharmacologically relevant doses, cocaine causes inhibition of NEnergic neuronal firing, which also contributes to NE accumulation [25].

3.3 Opiate-induced Neurotoxicity

3.3.1 Opiate-induced oxidative stress

Similar to a variety of drugs, opiates induce an elevation of DA concentration that can lead to oxidative stress [3]. Synaptic DA can be oxidized by monoamine-oxidase-B (MAO-B), then increase 3,4 dihydroxyphenylacetic acid (DOPAC) level [26]. Heroine exposure was shown to decrease the activity of glutathione peroxidase (GPx), a main antioxidant enzyme involved in H_2O_2 inactivation [27]. H_2O_2 is also increased with the elevated synaptic DA and can give rise to highly toxic hydroxyl radicals.

Opiates can induce ROS formation and decrease antioxidant capacity and antioxidant enzymes, including SOD, CAT, and GSH peroxidase. Opiate drug administration demonstrated a high level of unsaturated FA content in particular brain areas sensitive to neurodegeneration mediated by the uneven distribution of ROS [28].

3.3.2 Opiate-induced modification in brain derived neurotrophic factor (BDNF) expression

Studies comparing opiate use disorder (OUD) with a control group have shown a decreased BDNF expression in VTA for the OUD group [29]. BDNF plays an important role in maintaining midbrain dopaminergic neurons, so decreased BDNF can have a deleterious effect on CNS neurons. In opiate addiction, BDNF reverses GABA current in VTA from inhibitory to excitatory, associates opiate rewards with DA, and leads to downregulation of TrkB in D1-neurons [30].

3.3.3 Opiate-induced apoptosis

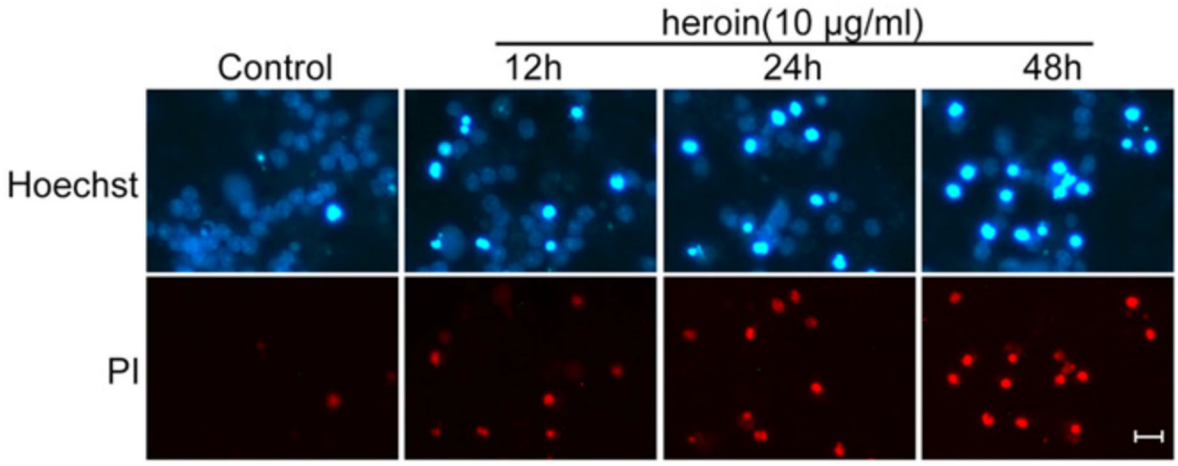

Figure 5. Heroin induces apoptosis of CGCs. Neurons were stained with Hoechst or propidium iodide (PI) to visualize condensed nuclei after heroin treatments, comparing to a control group [31].

In human addicts, opiates have been shown to cause neuronal apoptosis (See Figure 5) and it is indicated to be mediated by the c-Jun N-terminal Kinase (JNK) pathway. The activation of the JNK pathway is triggered through affecting gene expression and mitochondrial mediated apoptosis [32]. The activation would increase the activity of the apoptotic protein Bax, and release cyt c into the cytoplasm. This activity disrupts the Bax/Bcl-2 ratio in cortical neurons and cerebellar granule cells (CGCs). Further, a study on heroin-induced apotosis indicated that Bim, which can directly activate Bax, was upregulated as one of the targets of the JNK pathway. Then, upregulated Bim is reported to translocate to mitochondria with Bax activation leading to the decrease of mitochondrial membrane potential [31]. Moreover, the activation opens the mitochondrial permeability transition pore. Then, the cyt c would initiate a protease cascade reaction [32].

4. Analysis of neurotoxicity mechanisms contributing to addiction

4.1 Excessive dopamine

Glutaminergic neurotoxicity is suggested to be strongly associated with the addictive effects of the drug, memory impairment and psychotic effects [15]. Glu is associated with a decreased DA level in substance-related addiction. DA is a crucial neurotransmitter for assessing the value of a given situation, updating the value, and influencing the willingness to exert effort [33]. With the decreased DA terminals in the brain, the old established habitual rewards by the previous phasic DA firing dimmish in their effect, leaving addicted individuals less sensitive to non-habitual rewards. With substance-related addictions, the decreased DA receptors greatly contribute to withdrawal symptoms by depriving addicted individuals of their motivation. Decreased motivational arousal has been shown in animals with DA system lesions, meaning a decrease in the amount of effort the animals are likely to exert for a reward [34].

4.2 Oxidative stress

Oxidative stress is implicated in the degeneration of DA terminals, leading to an inhibition of DA synthesis. Based on studies concerning the effects of oxidative stress, the mice observed with oxidative damage showed a decline in brain functions including cognitive control, motor capacity, and response to stress stimuli [35]. In another rodent study, oxidative metabolism is shown to be associated with anxiety regulation. Consequently, oxidative stress can lead to irrational and excessive anxiety [36]. In conclusion, the altered DA baseline, heightened suspensibility to stress, increased anxiety, and impaired cognitive control collectively contribute to the dysphoric experience that

addicts endure during drug withdrawal. Moreover, these factors serve as key indicators of the cause of relapse.

4.3 Locus coeruleus

Repeated administration of cocaine has been shown to reduce survival rate of LC neurons which regulates arousal, attentional functions, and stress response [23]. Additionally, the LC contains NE-synthesizing neurons which can contribute to the "dark side of addiction" [37]. NE is associated with pain modulation, motor control, and energy homeostasis. The phasic firing of the LC, in particular, is essential for stimulus-induced attentional shifts and cognitive flexibility, the ability to generate appropriate behavioral responses [37]. Studies have shown that chronic cocaine sensitizes PC12 cells to acute cocaine exposure, so chronic exposure to cocaine can contribute to the behavioral sensitization of drug addicts after acute exposure [19]. The attentional deficit is also characterized by altered LC-NE activity. According to Yerkes-Dodson theory, the level of arousal regulated by LC has to be kept at a narrow 'range' for optimal performance on cognitive and behavioral tasks. Therefore, anxiety during withdrawal featured with excessive avoidance behavior and deficits attention can be attributed to the cocaine-induced alteration in the LC [38].

4.4 Modification in brain-derived neurotrophic factor (BDNF) expression

Tracking the BDNF expression, several studies have shown that elevated BDNF expression efficiently prompts abstinence [29]. BDNF is also responsible for memory consolidation and hippocampal functions. Hence, the decreased BDNF expression after repeated opiate exposure results in a decrease cognitive flexibility, primarily due to an incapability of effective hippocampal functions [39]. Further, with reduced GABA currents, opiate-induced modification in BDNF expression possibly contributes to the aversive effects during withdrawal [30].

5. Discussion

Substance abuse has been shown to have vital neurotoxic consequences. The increasing prevalence of addiction lays importance on understanding the mechanisms that underlie the symptoms. In the review, the major mechanisms associated with drug-induced neurotoxicity include oxidative stress, apoptosis, and modification at different molecular concentrations. Each mechanism contributes to the behavioral abnormalities during addiction. Excessive DA and toxic hydrogen radicals are shared neurotoxic effects among the three substances discussed, interrupting the DA baseline, and contributing to allostasis. Similarly, oxidative stress is another important contributor to the neurotoxicity of drugs that alter homeostasis by causing dopaminergic damage. The DA lesions would, in turn, contribute to the decreased motivation commonly seen in withdrawal. With deeper understanding of the mechanism of neurotoxicity induced by different drugs, a better therapeutic approach could be developed for substance use addiction [5]. Concerning the importance of oxidative stress in addiction, blocking oxidative stress could become an effective method in new therapies. To illustrate, studies have focused on the role of different antioxidant enzyme expression and treatments with antioxidants have been shown to prevent drug-induced anxiety in rodents [26]. However, more studies, like rodents' models, are required to see the effect of treatments targeting at different signals and pathways before adopting the treatments on to human subjects.

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