Opioid-Induced Neuroplasticity: Insights from Animal Models

Zhaolin Yang[1,a,*]

[1]*Department of Psychology, McGill University, Montreal, Canada*
a. Zhaolin.yang@mail.mcgill.ca
**corresponding author*

Abstract: Synaptic plasticity is defined as the modification of the transmission of synapses. It has been proven to be strongly associated with learning. Thus, drug-evoked synaptic plasticity in brain reward circuits can establish persistent learning of addictive drugs, reflecting the neural basis underlying addiction. The mesolimbic dopamine pathway has been widely indicated to be strongly associated with opioid use disorder (OUD) and other drug addictions. The paper focuses on discussing the drug-evoked neural plasticity underlying two important stages called intoxication and withdrawal which are critical for addiction and reinstatement of drug use respectively. The paper first explores the neural basis of OUD, emphasizing drug-induced plasticity at glutamate and Gamma-aminobutyric acid (GABA) synapses on neurons of key substrates in the pathway and how they influence mesolimbic dopamine (DA) neuron transmission. Then, the review discussed withdrawal-induced neuroplasticity and reorganization of associated neuron circuits, which explain deficits led by withdrawal from opioid administration. An overall understanding of drug-evoked synaptic plasticity in key brain circuits in the development of addiction helps find possible therapeutic methods to prevent the initiation of OUD and reinstatement.

Keywords: opioid use disorder, mesolimbic pathway, dopamine, synaptic plasticity.

1. Introduction

The hallmark of opioid use disorder (OUD) is the persistent use of opioids notwithstanding the negative consequences. Specifically, OUD is defined as the result of cumulative exposure to opioid drugs, characterized by impulsive preparatory and consummatory motivations for drugs that reflect role dysfunction. OUD is a well-recognized severe problem since it severely affects public and individual health. It is estimated that around 26.8 million people globally were affected by Opioid Use Disorder (OUD) in 2016, and more than 100,000 individuals perish each year from opioid overdoses [1]. In the early stage of addiction, addicts consume drugs to obtain positive effects. Abrupt drug discontinuation or gradual tarping tends to produce withdrawal syndrome, resulting in physical and psychological deficits and increasing the risk of relapse [2].

Positive and negative reinforcement, as two key contributors to addiction, all reflect presynaptic and postsynaptic plasticity, at least in part [3]. The former is defined as increased drug-seeking triggered by drug-induced subject euphoria, while the latter is defined as the alleviation of adverse effects caused by withdrawal. Both types of reinforcement contribute to the development of addiction. Positive reinforcement is essential in the association with recreational drug use, whereas negative

reinforcement is increasingly essential in the presence of addiction to drive compulsive drug-seeking [4]. because both types of reinforcement are strongly associated with reward, it is unequivocal that mesolimbic dopamine (DA) pathways from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) are crucial for developing addiction since they are essential for incentive salience. They are pivotal for the reinforcing and rewarding properties of opiates and other drugs [4]. Meanwhile, this system is also pivotal for regulating motivational aspects of cessation of drug exposure, contributing to the reinstatement of drug use. A plausible explanation is that DA is the candidate for increasing the brain stimulation reward threshold. Withdrawal from opioid exposure, resulting in a state of anhedonia contributing to relapse although the specific neurochemical basis remains undefined. Together, these findings imply that the disturbance of the mesolimbic DA neuron activity level may lead to maladaptive motivation and encourage reinforcement [5].

Although reinforcement undoubtedly depends on mesolimbic DA neurons, the capacity of glutamate to impact the activity of DA neurons has been increasingly recognized [3]. The development of addiction requires the participation of glutamate to alter the synaptic strengthening of DA neurons to impact the activity of DA neurons. Or glutamate alters the synaptic excitability of gamma-aminobutyric acid (GABA) neurons, reducing DA neurons' activity, and enabling an indirect regulation of the activity of DA neurons.

The paper presumes that opioid exposure induces synaptic plasticity with reflected behavioral alternations. This paper attempts to review previous studies on animal models and combine them to understand how modifications in neuron activities contribute to maladaptive motivation. Investigation into the animal models (mice) provides an opportunity to study it, since the activity of neurons in people with addiction cannot be tested directly because of limitations of technology and ethical issues. In short, this review summarizes associated studies on opioid-evoked synaptic plasticity to establish a coherent understanding of the biological basis underlying OUD, which is beneficial to neurobiological and pharmacological fields.

2. Learning Mechanism

Many studies have demonstrated the important role the mesolimbic DA pathway from the VTA to the NAc and other associated limbic brain regions play in the development of addiction [6]. Mesolimbic DA neurons have been recognized to play a critical role in regulating the rewarding and incentive value of opioids [4, 6]. It has been recognized that regardless of their chemical makeup or molecular targets, all drugs initially enhance DA concentrations in the VTA itself and VTA projection regions in the mesolimbic pathway.

Glutamate, as the primary modulator of the synaptic plasticity underlying learning phenomena, has its role in mediating the activity of DA neurons by altering the synapse excitability [7]. Transportation of glutamate plays a fundamental role in inducing and maintaining LTP and LTD at synapses, which are two important learning and memory mechanisms that can impact how DA neurons respond. It indicates that glutamatergic neurotransmission is a necessary part of developing and preserving opioid-associated memories [8]. It has been well-recognized that opioid-evoked LTP and LTD are two learning processes mainly reflecting synaptic plasticity. Although LTP and LTD were discovered initially in the hippocampus and are thought particularly essential for the formation of memories with the capacity to store a huge amount of information [9]. Their participation in other brain circuits, including the mesolimbic DA pathway, has been increasingly recognized. The former refers to the persistent strengthening of synaptic excitability, while the latter refers to the selective weakening of synapses [10].

Ionotropic glutamate receptor-dependent LTP and LTD can be found in the synapses receiving glutamate inputs on VTA and NAc neurons. Indeed, LTP and LTD can be exhibited at inhibitory and excitatory synapses to alter the synaptic strength to facilitate or suppress synaptic transmission by

strengthening or weakening synapses respectively. In the mesolimbic pathway, opioids evoke synaptic plasticity at inhibitory and excitatory synapses of key brain substrates including the VTA and the NAc. The latter is recognized to be innervated by glutamatergic inputs mainly from the prefrontal cortex, lateral hypothalamus, and laterodorsal tegmental nucleus [6]. Now, how opioid exposure changes the synapses of VTA and NAc neurons respectively, and induces long-lasting alterations in synaptic transmission will be discussed specifically.

2.1. Plasticity at Excitatory Synapse in VTA

Previous studies have well recognized that to affect VTA DA neurons' activity in a direct approach, opioid exposure is sufficient to induce LTP and LTD at the excitatory synapses [6]. Administration of N-methyl-D-aspartate (NMDA) receptor antagonists prevented the excitatory synapses' potentiation of the VTA DA neurons [11]. It implied that opioid exposure potentially induces an NMDAR-dependent LTP at the excitatory synapse of VTA DA neurons [6]. The NMDA receptor-dependent LTP requires the activation of NMDA receptors to insert α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors through activating intracellular signaling cascades as the postsynaptic membrane is dramatically depolarized to remove magnesium block of NMDA receptors. The LTP-evoked synaptic strengthening is reflected by increased AMPA/NMDA receptors excitatory postsynaptic currents (EPSCs) ratio [6].

A significant increase in this ratio was shown 24 hours after only a single opioid administration [12]. It was potentially because of an upregulated conductance of AMPA receptors. Opioid-evoked enhanced levels of GluR1-AMPARs (GluR2-lacking) incorporated at glutamate synapses on VTA DA neurons [10]. AMPA receptors lacking GluR1 were observed to replace a portion of synaptic receptors constitutively [13]. Finally, they show a great capacity to change synaptic plasticity since GluR2-lacking AMPA receptors have a greater Ca+ permeability. Additionally, the opioid-evoked decrease in NMDA receptor-regulated EPSCs might also provide a plausible explanation of why this ratio was increased [10]. Or both the former and the latter result in the increase of this ratio.

Postsynaptic plasticity is also reflected by the alteration in metabotropic glutamate receptors. The mGluR-LTD was presumed to be exhibited at excitatory synapses of VTA DA neurons. Activation of mGluR1 receptors in the VTA was recognized to sufficiently reverse the replacement of the GluR2-containing AMPA receptor with the GluR2-containing AMPA receptor. Hence, persistent synaptic strengthening possibly implies that the signaling of mGluR2 is depressed by opioid exposure, facilitating the induction of NMDA receptor-dependent LTP at glutamate synapses on VTA DA neurons [10].

2.2. Plasticity at Inhibitory Synapse in VTA

Around 30%-35% of the cells in the VTA are GABAergic neurons inhibiting the DA neurons locally [14]. Hence, controlling GABA activities can indirectly impact the activity of VTA DA neurons. Drug-evoked alterations in GABAA receptor synapse on VTA DA neurons are thought to regulate DA neurons' activity indirectly. Repeated cocaine exposures over five to seven days daily significantly reduced GABAA receptor-regulated inhibitory postsynaptic currents (IPSCs) on VTA DA neurons. This enhanced discharge of DA neurons in response to a fixed stimulus is facilitated by the reduction in inhibition [15].

Furthermore, chronic opioid exposure promotes endocannabinoid-dependent LTD at GABAA receptor synapses, disinhibiting the activity of DA neurons [16]. Animals previously exposed to morphine exhibited an absence of robust NMDAR-dependent GABAA LTP which was originally exhibited in slices from naïve animals [6, 10]. This LTP requires the postsynaptic excitatory terminal participants to produce nitric oxide synthase (NOS), which can further produce nitric oxide (NO).

NO then moves to nearby presynaptic inhibitory terminals and turns on guanylate cyclase (GC). This raises cyclic GMP (cGMP), which in turn greatly increases the release of GABA at the presynaptic site. Therefore, the decreased presynaptic transmission of GABA caused by disruption of the interaction between postsynaptic NOS and presynaptic GC potentially explains why this LTP was removed with exposure to opioids. This was thought to happen because either GC was no longer able to sense diffusible NO. Or it has been lost from presynaptic GABA-releasing terminals [6]. Intriguingly, this LTP was also observed to be absent in VTA 24 hours after in vivo administration of other drugs, such as ethanol and nicotine [17]. Together, NMDA receptor-dependent LTP at excitatory synapses on VTA DA neurons, and the depression at GABA synapses all contribute to the enhancement of activity of VTA DA neurons [6].

2.3. Synaptic Plasticity in the NAc

Synaptic plasticity which contributes to maladaptive reparatory and consummatory responses to drugs can also be found in the NAc, a substrate appearing essentially for mediating the expression of drug-seeking behavior. Current studies have suggested that the NAc integrates the glutamatergic signals from the cortex and the dopaminergic signals from the VTA. The synaptic plasticity capacity is progressively altered to a persistent functional form of rigidity ('crystallization') of drug-associated memory because of the progressive modification of drug-associated information processing that is induced by chronic exposure to drug abuse [8].

Unlike the measured upregulation at glutamate synapses on VTA DA neurons, a significantly decreased AMPA/NMDA ratio was observed in the NAc 24 hours after the last administration of opioids, whereas this ratio exhibited a significant increase conversely in protracted withdrawal (10-14 days) [10]. The decreased ratio is thought to be a result of the persistent increase of this ratio in the VTA since enhanced excitation of VTA projections is widely postulated to potentially modify the threshold for the initiation of NAc plasticity by influencing the excitability of the neural circuit [10, 18]. However, this reduction is also assumed to be the outcome of the exposure of NR2B-containing NMDA receptors on the corresponding postsynaptic membrane. This subunit is known to have higher single-channel conductance with a longer opening time. therefore, the insertion of NR2B-containing NMDA receptors decreases this ratio by increasing the denominator of this ratio [10].

Due to the insertion of NR2B-containing NMDA receptors, silent synapses were observed to dramatically express [10]. Silent synapses are characterized by the capacity to express currents that were mediated by NMDA receptors, despite the lack of involvement of AMPA receptors. This feature offers enhanced synaptic transmission, contributing to LTP [10]. This is possibly one explanation for the upregulated proportion of AMPA/NMDA in protracted withdrawal. It was found that after 24-48 hours of withdrawal from repeated cocaine injections that were noncontingent, high amounts of synapses with remarkable activity were preferentially produced in the NAc shell with GABAergic neurons mediating drug-seeking [10]. Moreover, drug-evoked altered astrocytic-mediated synaptogenic signaling pathway, including the drug-evoked release of thrombospondin-2 activating its corresponding receptor alpha2delta-1, which is also necessary for generating these new silent synapses [19]. Opioid-evoked plasticity in neurocircuits during withdrawal will be further discussed below.

3. Neural plasticity Underlying Withdrawal

The NAc shows distinct structural properties from the VTA [20]. The Nucleus NAc contains approximately 95% of GABAergic medium spiny neurons (MSNs). Certain limbic areas including the prefrontal cortex, the ventral subiculum, the hippocampus, and the basolateral amygdala provide glutamatergic inputs to it [10]. Furthermore, the location of MSNs and what they contain can be

further divided into the direct pathway (dMSNs) or the indirect pathway (iMSNs). Direct pathway mainly expresses MSNs containing D1 receptors (D1-MSNs). Habenula-projecting globus pallidus (GPh) is a region that is important for evaluating action outcomes contributing to reward learning is indicated to be essential for drug-seeking, and it receives inputs from striosomal dMSNs [21-22].

One to three days after withdrawal from morphine administration in a repetitive manner, optogenetics observed a substantial increase in the amplitude of this pathway's inhibitory postsynaptic currents, implying a withdrawal-induced enhancement of striatopallidal GABAergic transmission [23]. The same result was also observed in withdrawal from chronic fentanyl self-administration. However, withdrawal from chronic voluntary fentanyl administration recruited the entire striatal dMSNs, evidenced by increased tagged dMSNs and their ratio to all dMSNs. Fentayl-induced potentiation of inhibitory inputs to midbrain DA neurons was also found, evidenced by a significant decrease in the firing of substantia nigra (SNc) as D1-MSNs received optogenetic stimulation. Therefore, this withdrawal-induced striatonigral plasticity tends to suppress DA transmission and eventually induces negative psychological effects, and partly contributes to negative reinforcement. Besides, it was found that the fentanyl's recruitment of striatal dMSN tended to evoke long-lasting opioid-related conditional memories because all mice receiving fentanyl administration showed significantly higher conditional place preference (CPP) scores, compared to mice receiving saline administration after 10 and 31 days of withdrawal. This may explain why OUD is prone to be triggered when opioid-associated cues exist [23].

Cell-type-specific plasticity can be induced by opioid exposure and withdrawal. Electrophysiological data revealed that 24 hours after five-day morphine exposure, the frequency of miniature excitatory postsynaptic currents (mEPSCs) on D2-MSNs but not D1-MSNs, was significantly reduced. In contrast, the intrinsic membrane excitability of D2-MSN was significantly increased during abstinence. The final functional output of D2-MSNs in response to synaptic inputs, which encodes aversion to natural stimuli in withdrawal, remained unchanged since it was observed that D2-MSNs of mice receiving saline and morphine showed no significant difference in the number of action potential (AP), as well as the rheobase (minimal current to elicit AP) and chronaxie. (time eliciting AP by using a current double the rheobase) [24]. These findings together, on the one hand, reveal cell-type-specific intrinsic and extrinsic changes in circuits mediating reward. On the other hand, they demonstrate the existence of a possible neural homeostasis that maintains basal levels of functional output in NAc D2-MSNs. However, whether this balance results in deficits when abstinence duration increases remains unclear [24].

It is recognized that projection from the paraventricular thalamus (PVT) to the NAc is essential for driving aversion and opioid-related contextual memories [25]. Mice that received heroin self-administration and then received abstinence and extinction training respectively. The latter is defined as a condition with broken contingency between preparatory responses to drugs and the practical delivery of the drug reward [25]. D1-MSN activity is in general associated with drug-seeking behavior. Related synaptic plasticity was found in another withdrawal model called extinction. It is recognized that projection from the paraventricular thalamus (PVT) to the NAc is essential for driving aversion and opioid-related contextual memories [25]. Activating this pathway optogenetically still drove aversion, contributing to drug seeking. This was evidenced by the induction of real-time conditional place aversion (rtCPA) and significantly increased seeking for heroin in mice that received abstinence, but not extinction. This was because the extinction itself had already depressed D1-MSNs, evidenced by significantly decreased EPSCs of D1-MSNs, but not D2-MSNs after the LTD protocol in the condition of abstinence, but not in the condition of extinction. Together, these findings suggests that extinction training potentially induced neural plasticity within the PVT-NAc pathway, which is originally an important contributor to the reinstatement of heroin, to alter the function of this pathway by depressing D1-MSN transmission [25].

4. Conclusion

This paper aims to provide an overall understanding of opioid-evoked synaptic plasticity by reviewing associated studies in this field. Studies have demonstrated that opioid-evoked synaptic plasticity on neurons of key substrates, mainly the VTA and NAc, is causally associated with drug abuse since the alterations converge to mediate the activity of mesolimbic DA neurons directly by upregulating excitatory synapses or indirectly by downregulating inhibitory synapses in the development of addiction. This is especially true for the VTA because the VTA contains both DA neurons and GABA neurons. Opioid exposure is adequate to induce LTP at the excitatory synapses of VTA DA neurons and LTD at the synapses of VTA GABA neurons. Together, the upregulation of VTA excitatory synapses and the downregulation of inhibitory synapses contribute to facilitating mesolimbic DA transmission. Opioid-induced plasticity in the NAc is characterized by alterations in the ratios of AMPA/NMDA receptors as well as silent synapses' formation, which facilitate the formation of LTP in protracted withdrawal, and partly contributing to the negative reinforcement and facilitating the reinstatement of opioids. Withdrawal-induced neural plasticity is also reflected in the alterations in neurocircuits important for reinforcement and reward learning. For instance, withdrawal from chronic opioid administration potentiates the transmission of GABAergic neurons in the NAc-GPh pathway. In addition, withdrawal can also induce plasticity in the subtypes of NAc MSNs, suggesting the capacity of withdrawal to induce cell-type specific neural plasticity.

Other factors that potentially affect the expression of synaptic plasticity are required in the future. The likelihood that the withdrawal-induced neuroplasticity persists with the increase in withdrawal durations and the alternate effect of synaptic plasticity in the protracted withdrawal should be further studied. Moreover, the role of other factors, such as gender and opioid exposure history in impacting withdrawal symptoms and neuroplasticity, possibly contribute to reinstatement of drug use.

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