CHAPTER II

REVIEWS OF RELATED LITERATURE AND RESEARCH

Reward system

The reward system is located in ventral tegmental area (VTA) and in the shell of the nucleus accumbens (NAcc). The reward system in the brain is activated when a person performs an action necessary to survival such as eating, water, and sex. Rewards are positive stimuli with primary motivational value as they predict events useful for survival without requiring an associative learning process. Activation of the reward system provides pleasurable feelings, giving positive feedback for necessary actions. Most drugs of abuse stimulate the reward system and often provide greater pleasure than natural stimulation. The reward system underlies addiction to cocaine, speed, angel dust, morphine, alcohol and tobacco [19, 20].

The neuronal substrates of brain reward involve neural condition and mechanisms associated with the medial forebrain bundle (MFB) which located primarily in the ventral limbic midbrain-forebrain. Reward substrates consist of first-stage, second-stage, and third-stage reward-related neurons cooperated with one another. First-stage neurons are activated directly by rewarding electrical brain stimulation. Second-stage dopaminergic (DAergic) neurons project through the MFB to synapse in the NAcc then third-stage neurons carry the reward signal to ventral pallidum (VP). Some of third-stage neurons use the neurotransmitter γ-aminobutyric acid (GABA), and GABAergic medium spiny NAcc output neurons are implicated in brain reward functions [21]. A simple diagram of rodent brain reward circuit is shown in Figure 1.

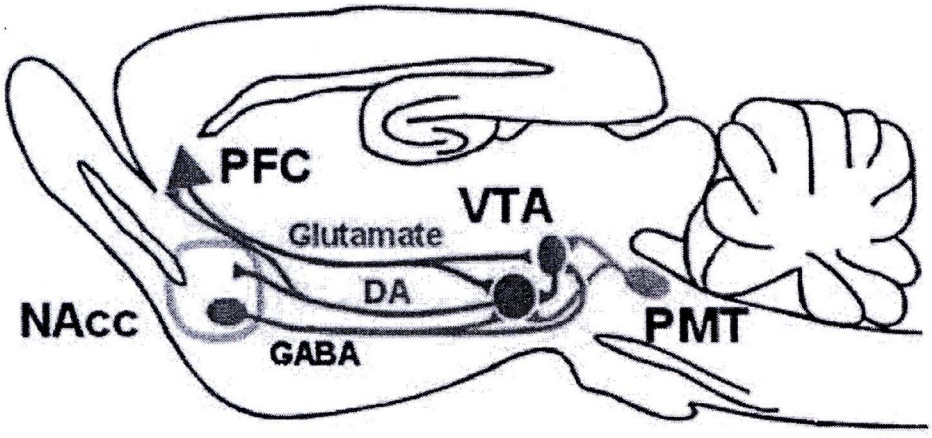

Figure 1 A simple diagram of rodent brain reward circuit [22]. Abbreviations: PMT, pontomesencephalic tegmental; VTA, ventral tegmental area; NAcc, nucleus accumbens; PFC, prefrontal cortex; DA, dopamine; GABA, gamma aminobutyric acid.

Drug addiction

Drug addiction is a complex chronic relapsing disorder. The major feature of drug addiction is the compulsive use of drugs with aspects of impulse control and compulsive disorders, which may lead to relapse of drug-seeking behaviors even after long periods of abstinence [23].

In a normal natural stimulation, neurotransmitter releases are stimulated in reward system and a negative feedback loop exists in the synapses of the reward system to maintain the neurotransmitter concentration at a set value. When take a drug of abuse, the neurotransmitter concentration is shifted over a set value. Nevertheless, long-term of strong use of the same drug can change the set point on a semi-permanent basis. Hence, it takes a larger dose to acquire the same effect leading to tolerance. Repeated drugs use affecting the reward system cause a neuroadaptation and tolerance.

Many drugs cause physical addiction by affecting the levels of neurotransmitters, especially dopamine (DA) and glutamate, in the reward system of the VTA. Addictive drugs (such as opioids, alcohol, cocaine and amphetamines) act by stimulating this system, which is more usually activated by natural rewards.

Cocaine increases the amount of DA by inhibiting the DA transporter thus preventing re-uptake of DA from the synaptic cleft, whereas opioids act by inhibiting inhibitory GABA interneurons in the VTA to increase DA release [24]. These mechanisms increase the DA concentration of the synapse causing pleasurable feelings greater than normal. Therefore, DA was the key neurotransmitter involved in addiction. However, Kalivas et al. [25] have shown that glutamate play a larger role than DA does and the brain structures rich in glutamate are involved in learning processes which play a part in developing drug cravings, one of the central aspects of compulsive drug seeking and use.

Nicotine and addiction

Nicotine plays a key role in maintaining smoking of tobacco. Nicotine has been found in a wide variety of plants. However, the principal source of nicotine exposure is through the use of tobacco (*Nicotiana tabaccum*). Nicotine is an amine composed of pyridine and pyrrolidine rings (Figure 2). It can cross the biological membranes including the blood brain barrier [26].

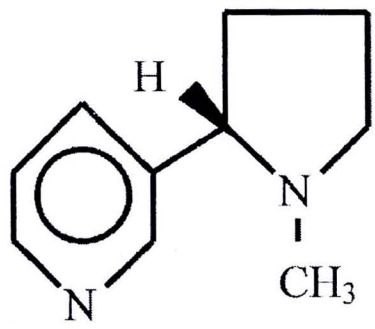

Figure 2 A chemical structure of nicotine

The predominant effects of nicotine in animal or human consist of an increase in pulse rate, blood pressure, plasma free fatty acids, blood catecholamine level, mobilization of blood sugar, and anorexic effect [27]. Moreover, nicotine interacts with nicotinic acetylcholine receptors (nAChRs) causing activation of the reward system in the central nervous system (CNS). Absorption of tobacco smoke at

the lung produces arterial bolus reaches the brain within a few seconds. Nicotine binds to nAChRs located on the dopaminergic cell bodies in the VTA [28]. Stimulation of these nAChRs by nicotine produces increases in DA levels in the NAcc [29]. Afterward, reinforcing effect and enhanced mood are produced. Smokers maintain level of nicotine throughout the day and for long-term use of tobacco smoking, smoker could become addict to nicotine [26].

Nicotinic and muscarinic receptors in drug addiction

Nicotinic receptor is a main target of nicotine exposure. Currently, scientists many nicotinic receptor subtypes are involved with nicotine addiction including alpha7 containing nicotinic acetylcholine receptors (α7 nAChRs) and this subtype contribute to withdrawal symptoms of nicotine [30]. In addition, M5 muscarinic receptors (M5 mAChRs) may also play an important role in nicotine addiction [31].

1. Role of α7 nAChR in drug addiction

Hippocampus and pontomedullary are the brain regions that play important role in memory [32] and behavior regulation [33], respectively. Hippocampus contain numerous of GABAergic [34, 35] and glutamatergic neurons [36, 37] involved in nicotine addiction. Activation of α7 nAChRs presenting on these neurons by nicotine can promote release of the neurotransmitters and enhance hippocampal activities such as memory. Expression of α7 nAChRs on both GABAergic and glutamatergic neurons either pontomedullary region or hippocampus modulate neurotransmitters signaling in these area [38-40]. Chronic nicotine exposure can induce α7 upregulation and contribute to withdrawal effects of nicotine [39]. Moreover, serotonergic neurons also express α7 nAChRs [41] and serotonin system in the brain are importance in drug addiction-related behaviors [42, 43]. Thus, α7 nAChRs could play a role in nicotine addiction and nicotine withdrawal-related behavior.

2. Role of M5 mAChR in nicotine addiction

M5 mAChRs play roles in a variety of neuronal functions including regulation of neurotransmission, reward response, and behavior [44]. Variation in muscarinic 5 (CHRM5) locus, the gene that codes for the M5 mAChRs, is involved with tobacco addiction in human [31]. The laterodorsal tegmentum (LDT) cholinergic inputs to dopaminergic neurons in the VTA, via activation of mAChRs (probably of the M5 subtype), are involved in modulating the facilitatory effects of cocaine on NAcc-DA neurotransmission [45]. Modulation effects of addictive drugs by M5 receptor were also confirmed by amphetamine-induced hyperactivity in mice and DA release [46] as well as inhibition of morphine-induced locomotion [47], reduced reward and withdrawal responses following morphine administration in mice lacking of M5 receptor [48].

Recent studies indicate that M5 mAChRs play a role in modulating the behavioral of both morphine reward and withdrawal, probably by stimulating DA release in the NAcc via activation of mesolimbic dopaminergic neurons [45, 46]. Some of reinforcing properties of other drugs of abuse including cocaine and nicotine occur via mesocorticolimbic dopaminergic pathways. Thus, it is likely that M5 mAChRs have a role in nicotine addiction [49].

The effect of nicotine on animal behaviors

1. Anxiolytic effects of nicotine

Anxiety is psychological and physiological states determined by emotional, somatic, and behavioral signs. Anxious patient feel fear, worry, and nervous. Anxiety in animal model can occur after withdrawal of drug of abuse such as nicotine, which is an anxiolytic or antianxiety agent. Nicotine reduced approach–avoidance conflict and increased willingness to enter the center of an open field. The substance can produce anxiolytic properties and that such effects may serve as an important factor in the persistence of smoking behavior [50]. Levin et al. [51] have shown that nicotine induced anxiolytic effect in zebrafish by significant decrease in diving session and also improved learning and memory. On the other hand, nicotine withdrawal had a significant anxiogenic effect in rat, shown by specific decreases in the percentage of time spent on the open arms and in the percentage of open-arm

entries in elevated plus maze test [52]. Indeed, nicotine exerts some effects in the brain that seen in animals exposed to a stressful stimulus. It is effects on noradrenaline (NA) secretion from NA-secreting neurons which innervate the hippocampus from the locus coeruleus, and this mediates the calming effects. It seems to be reasonable to suggest that nicotine exerts anxiolytic effect [27].

2. Nicotine withdrawal (abstinence of nicotine)

Chronic nicotine exposure and nicotine withdrawal can induce profound alterations in the mesolimbic DA system that supports a variety of functions including emotion and behavior. It is possible that chronic nicotine exposure causes a long-term reduction of the baseline level of DA-mediated signaling as a compensatory response after heightened DA-mediated transmission during continued nicotine exposure. Thus, medications that increase DA concentrations have proven efficacious in preventing nicotine relapse and craving in smokers [53]. Cessation of chronic nicotine exposure in human resulting in abstinence syndrome characterized by irritability, anxiety, difficulty concentrating, restlessness, craving and weight gain [54]. However, withdrawal from chronic continuous nicotine administration in rodents, especially the mouse, has been reported to produce a mild somatic syndrome, opiate abstinence-like syndrome, and to decrease brain reward function. Grunberg [55] suggested that there was a strong correlation between stress and cigarette smoking as well as an anxiolytic effect of nicotine [50].

Smoking cessation

Nowadays, several effective pharmacotherapies for the treatment of nicotine addiction are available. The first line therapies approved by the Food and Drug Administration (FDA) are nicotine replacement therapy (NRT), sustained-release bupropion and varenicline and the second line therapies is clonidine.

NRT maintains plasma nicotine level that is sufficient to decrease the desire to smoke. NRT also provides a background level of nicotine that reduces craving and withdrawal symptoms. NRT products have several dosage forms that available on the market, for example; patches, gum, and nasal spray. NRT has many side effects such as local irritation at the site of administration, throat irritation and coughing but has no serious adverse effect [56].

Bupropion is an antidepressant that inhibits re-uptake of both NA and DA. Bupropion works as for aid for smoking cessation by maintaining reinforcement of DA in the mesolimbic system. Bupropion has associated with more frequent side effects than NRT such as insomnia, dry mouth, and nausea [57].

Varenicline, a newly approved agent for smoking cessation, is an $\alpha 4\beta 2$ nAChR partial agonist [58]. It mimics the effect of nicotine and hence reduces craving when smokers stop. Clinical trials have demonstrated that varenicline appears to be safe and well tolerated in healthy smokers and produces mild to moderate nausea [59, 60].

However, many researchers try to find pharmacotherapeutic properties of some medicinal plants in order to decrease smoking simultaneously have low adverse effects and also find alternative treatment with cost savings for smoking cessation. For example, *Hypericum perforatum*, a natural antidepressant effective for treating mild to moderate depression, attenuates nicotine withdrawal signs in mice [61] while ginseng total saponin (GTS) presents attenuating effect on nicotine-induced enhancement of DAergic transmission [62]. The essential oils from *Angelica gigas* NAKAI inhibit nicotine-induced behavioral and neurochemical sensitization [63] and a Thai traditional medicinal plant, *Vernonia cinerea* Less, has also been documented for smoking cessation. Now, *V. cinerea* becomes products for smoking cessation in several marketing forms including tea and mouth wash [17, 18].

***Vernonia cinerea* Less.**

Classification

Class:	Magnoliopsida
Order:	Asterales
Family:	Asteraceae
Genus:	*Vernonia*
Species:	*Vernonia cinerea* Less.

Figure 3 *Vernonia cinerea* Less.

Source: http://en.wikipedia.org/wiki/File:Vernonia_cinerea_(Ash_Fleabane)_in_Talakona_forest,_AP_W_IMG_8553.jpg

Vernonia cinerea Less. is a small annual herb classified in the Asteraceae family. It has a slender stem and variable in leaf shape with small purple flowers [18] (Figure 3). *V. cinerea* is a medicinal plant which has been reported to have many medicinal properties. Chemicals constituents of *V. cinerea* were identified to be (+)-lirioresinol B, stigmasterol, stigmasterol-3-O-beta-D-glucoside, 4-sulfo-benzocyclo butene, (-)-clovane-2, 9-diol, caryolane-1,9beta-diol, apigenin, chrysoeriol, luteolin, thermopsoside, luteolin-7-O-beta-D-glucoside, quercetin, apigenin-4'-O-beta-D-glucoside, hyperin, beta-amyrin acetate, and lupeol acetate [64, 65]. Different parts of the plant have different therapeutic values (Table 1). For examples, *V. cinerea* has anti-inflammatory properties [8, 66] that might be resulted from effect of *V. cinerea* on down regulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) level and gene expression in macrophages and reduced the lipopolysaccharide (LPS) induced elevated levels of nitric oxide (NO) and proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 β (IL-1β), and interleukin 6 (IL-6) in mice [67]. *V. cinerea* also exhibited a broad spectrum of antibacterial activities against *Bacillus subtilis* and *Psudomonas aeruginosa* [15]. The chloroform extract of *V. cinerea* induced significant diuresis, while the methanol and aqueous extracts induced significant antidiuresis in rats [68]. However, the methanol extract of *V. cinerea* did not produce any toxic effects in mice and brine shrimp [69].

Currently, Thai researchers establish *V. cinerea* properties in smoking cessation that reduced smoking rate may be related to oxidative stress and beta-endorphine levels [18]. Wongwiwatthananukit et al. [17] were given a 4-gram tea bag which contained the whole dried crushed *V. cinerea* to smoker for 15 days. Continuous abstinence rate (CAR) and prevalence abstinence rate (PAR) tended to be greater in *V. cinerea* than placebo over 24-week follow-up period without any statistical significance. *V. cinerea* has been used for smoking cessation in several forms in Thailand such as coffee, tea, and sprays.

Table 1 Application of *Vernonia cinerea* Less.

Pharmacological properties	***Models***	***References***
1. Anti-inflammation	- Rat	[8, 10, 66]
	- Activated polymorphonuclear leucocytes	[11]
	-Mouse/ Macrophages	[12, 67, 70]
2. Antibacterial activity	- *Pseudomonas aeruginosa, Bacillus subtilis*	[14, 15]
3. Antipyretic	- Rat	[8, 9]
4. Analgesic activity	- Mouse	[8]
5. Antimalarial Activity	- *Plasmodium falciparum*	[13]
6. Diuretic and antidiuretic activity	- Rat	[68]
7. Toxicity of VE	- Mice and brine shrimp	[69]
8. Anticancer Cytotoxicity of Sesquiterpene Lactones	- Human KB, DLD-1, NCI-661, and Hela tumor cell lines	[16]
9. Smoking cessation	- Human	[17, 18]