CHAPTER II

Review of Related Literature and Research

Methamphetamine

Methamphetamine, an amphetamine derivative, is an abused psychostimulant that produces potent effects on both central (CNS) and peripheral nervous systems (PNS) (Nestler et al., 2001; Nordahl et al., 2003; Pagliaro and Marie-Pagliaro, 2004). Other names of methamphetamine also known on the street are METH, speed, crank, zip, go, chalk, ya-khayan and yaba (Fields, 2001; Meyer and Quenzer, 2005; Sherman et al., 2007). Methamphetamine is typically ingested, smoked, snorted, or injected intraperitoneally, intravenously or subcutaneously (Nordahl et al., 2003; Pagliaro and Marie-Pagliaro, 2004). Amphetamine and methamphetamine are metabolized by the liver. Metabolites, and some unmetabolized drug molecules, are mainly excreted in the urine (Meyer and Guenzer, 2005).

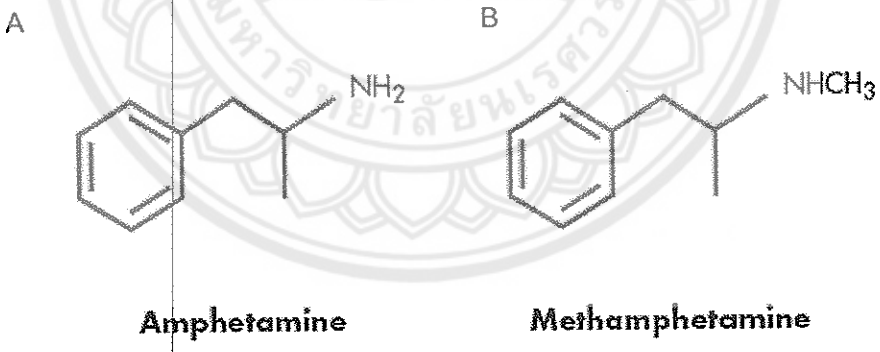

Figure 1 Schematic representation chemical structures of amphetamine and amphetamine derivative. Amphetamine (A) Methamphetamine (B) (Nordahl et al., 2003)

Methamphetamine is chemically similar to amphetamine, as shown in Figure 1. In contrast to the amphetamine, methamphetamine composes of the methyl group that is responsible for its potent effects when compared to the related compound amphetamine (Nordahl et al., 2003; Nestler et al., 2001). Methamphetamine has a

longer half life than amphetamine (13.3-15.0 hours versus 10.2-10.7 hours (Mendelson et al., 2006)) and may be more rapidly addicting. Additionally, methamphetamine has higher lipid solubility, thus, larger amounts of the drugs rapidly and efficiently cross the blood-brain barrier than the amphetamine and other CNS stimulants (Cho and Kumagai, 1994; Fields, 2001). Therefore, methamphetamine has higher potent effects than amphetamine on the central nervous system and is favored by stimulant drug abuser.

Effects of methamphetamine

Effects of methamphetamine can range from mild to severe toxicity or overdosage. Low dose administration of methamphetamine produced a sense of heightened alertness, attentiveness, and energy. Higher dose intoxication produced self-esteem that can approach hypomania and grandiosity (Nordahl et al., 2003; Niesink, 1999). Repeated and intermittent low dose administration of amphetamine (Fiorino and Phillips, 1999a; 1999b) and methamphetamine may increase sexual activity and pleasure (Nordahl et al., 2003), although longer use is associated with impaired sexual functioning (Nordahl et al., 2003). Repeated use of methamphetamine can produce tolerance and dependence (Segal and Kuczenski 1997b; Fields, 2001; Nordahl, 2003). Abrupt cessation of long term use produces withdrawalsyndrome which induces symptoms of fatigue, dysphoria, irritability, agitation, abnormalities in brain waves, prolonged sleep, a voracious appetite, stomach cramps, and depression (Niesink, 1999; Olson, 2004).

Acute toxicity effects, usually begin within 20 to 60 minutes or as long as 24 hours after administration. These may be characterized by signs and symptoms such as aggressiveness, euphoric grandinosity, hostility, hyperactivity, hyperalertness, hypervigilance, impaired judgment, irritability, loquacity extending to pressured speech, mental distraction, cardiac dysrhythmias, chest pain, hypertension, hyperpyrexia confusion, diaphoresis, dizziness, delirium, hallucinations, irritability, palpitations and tremor. The regular long term and short term use of large-dose methamphetamine may produce severe toxicity which is characterized by abdominal pain, anorexia, blurred vision, chest pain, diarrhea, dizziness, headache, urinary retention, emotional lability, tremor, automatic jerking movement, stereotype,

repetitive behavior that may lead to toxic psychosis (Grilly, 2002; Olson, 2004; Pagliaro and Marie-Pagliaro, 2004).

Several studies reported that behavioral sensitization appear in parallel the development of methamphetamine-induced psychosis including hallucination and delusions (Chantarasak, 2000; Yui et al., 2002; Srisurapanont et al., 2003). Clinical studies using perfusion magnetic resonance (pMRI) and functional magnetic resonance imaging (fMRI) showed that dysfunction in the brain of methamphetamine is related to cognitive impairment (Chang et al., 2002; Paulus et al., 2002). Furthermore, behavioral studies demonstrated deficits of verbal memory and manipulation of information in methamphetamine users (Volkow et al., 2001a; Simon et al., 2002). However, there is little documentation of methamphetamine-induced cognitive impairment in experimental animals. Animals treated with methamphetamine have been observed impairments of spatial and non-spatial memories (Friedman et al., 1998; Bisagno et al., 2002; Schroder et al., 2003), recognition memory (Bisano et al., 2002; Kamei et al., 2006), and object recognition (Bisagno, 2003; Belcher et al., 2006) accompanied by neurotoxicity to neurotransmitter system. Yet it is not clear whether neurotoxicity found following methamphetamine administration is associated with learning and memory processes.

Drug dependence

Drug dependence (sometimes also called addiction) is a complex neuro-behavioral disorder (Tzschentke and Schmidt, 2003). It is characterized by behavioral and other responses that always include a compulsion to regularly take the drug in order to maintain psychic effects, and sometimes to avoid the discomfort of its absence. Determinants of addictive behavior comprise of factors including unconditioned reward, conditioned reward, sensitization process, reinforcement learning, withdrawal and relapse after periods of abstinence (Miller and Gold, 1995; Fields, 2001; Ghodse, 2002; Meyer and Quenzer, 2005). Drug dependence can be divided in two important components compose of physical dependence and psychological dependence. The psychological dependence is a subjective state that drug abusers attempt to continue taking drug for maintaining a sense of well-being and avoidance a discomfort that it becomes extremely difficult to abstain. The physical

dependence is an altered state that develops when a drug abuser cannot stop taking a certain abused drug without suffering from withdrawal. The sudden drug withdrawal is followed by specific effects known as withdrawal or abstinence syndrome which represent symptoms and sign tend to be opposite of own drug effects when it is acutely administered (Fields, 2001; Ghodse, 2002; Grilly, 2002; Meyer and Quenzer, 2005). Furthermore, repeated administration causes a reduction in sensitivity to a drug and leads to increase doses for producing the same effect as previously produced by smaller doses, this phenomenon is also called tolerance (Miller and Gold, 1995; Fields, 2001; Ghodse, 2002; Grilly, 2002; Pagliaro and Marie-Pagliaro, 2004). In term of mechanisms underlying drug dependence, it is clear that neurotransmitter especially dopamine is involved in this mechanism (Miller and Gold, 1995; Nestler et al., 2001; Sidiropoulou et al., 2001; Meyer and Quenzer, 2005). However, there is increasingly evidence that not only dopamine that is related to drug dependence, glutamate is also involved in drug addiction which will be discussed below (Abekawa et al., 1994; Tzschentke and Schmidt, 2003; Kelley, 2004; Reid and Lingford-Hughes, 2006). However, the mechanism of glutamate transmission in drug dependence remains unknown.

Mechanism in drug dependence

The mechanism in addiction has been believed to be involved in the interaction between dopamine and glutamate transmissions, as demonstrated in Figure 2. When drug taking is begun, glutamatergic projections from corticolimbic regions such area as prefrontal cortex, amygdala, and hippocampus facilitate dopamine transmission into the nucleus accumbens (NAS) directly or via the ventral tegmental area (VTA). The abnormal high level of dopamine in the nucleus accumbens is critical in mediating rewarding effects or reinforcement behaviors which can be developed into drug dependence. Repeated drug taking may lead to an alteration in dopamine-glutamate interaction that finally leads to compulsive drug-taking behavior. During withdrawal and drug-free period, dopaminergic and glutamatergic activities within the mesocorticolimbic system return to normal levels but these remain in a hypersensitive state. Exposure to drug or stress may induce overactivation of glutamatergic system, thereby producing a relapse (Tzschentke and Schmidt, 2003; Reid and Lingford-

Hughes, 2006). Although, these studies have also provided evidence that the abnormalities of dopaminergic transmission are implicated in the drug dependence, while, the mechanisms of other neurotransmitters as well as glutamatergic transmission in drug dependence have also been pointed out and determined.

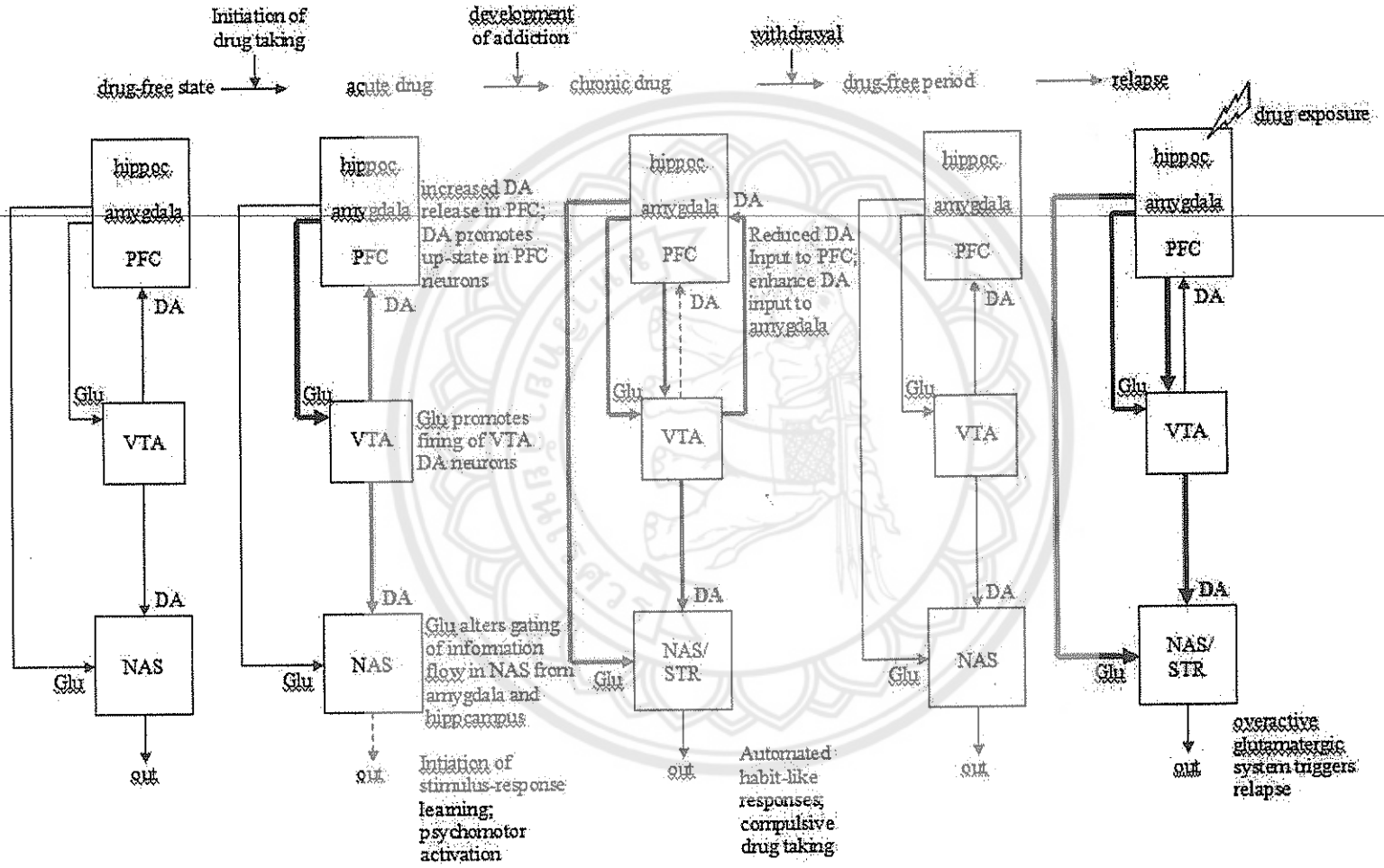

Figure 2 Diagrammatic representation of mechanism in drug dependence (Adapted from Tzschentke and Schmidt, 2003)

The effects of methamphetamine on neurotransmitter system

Recently, there is a growing body of evidence which was pointed to a role of glutamate and interaction between dopamine and glutamate in neuropharmacology of psychostimulants. The neurotransmission abnormalities related to the methamphetamine administration are discussed below.

The effects of methamphetamine on dopaminergic transmission

The dopamainergic system has been indicated to play an important role in the process of addiction (Reid and Lingford-Hughes, 2006 Moore, 1997; Butcher et al., 1988; Leyton, 2007). It has been suggested that amphetamine is a dopamine agonist which directly affects on dopaminergic system (Moore, 1997; Butcher et al., 1988). The dopamine transporter, which re-uptakes extracellular dopamine into presynaptic neurons, plays a primary role in the behavioral pharmacology of psychostimulants. Amphetamine and methamphetamine can elevate dopamine signaling by interfering with dopamine transporter function (Figure 3). Firstly, these substrates block the dopamine transporter, inhibiting re-uptake of dopamine into presynaptic nerve terminal, thereby increase dopamine levels in the extracellular space (Fleckenstein et al., 2000; Rothman and Baumann, 2003; Reid and Lingford-Hughes, 2006). Secondly, they also bind to transporter proteins and subsequently transport into the cytoplasm of the presynaptic nerve terminal which then lead to an increase of extracellular dopamine levels through reversing the process of the transport. Finally, they also enhance cytoplasmic concentrations of dopamine by disrupting with vesicular storage and consequently depletion of dopamine from vesicles to cytosolic and extracellular spaces (Rudnick and Clark, 1993; Nestler, 2001). However, the mechanism of amphetamine and methamphetamine in enhancing extracellular dopamine by reversing of the transporter process is stronger than the blockage of dopamine reuptake (Howell and Kimmel, 2008).

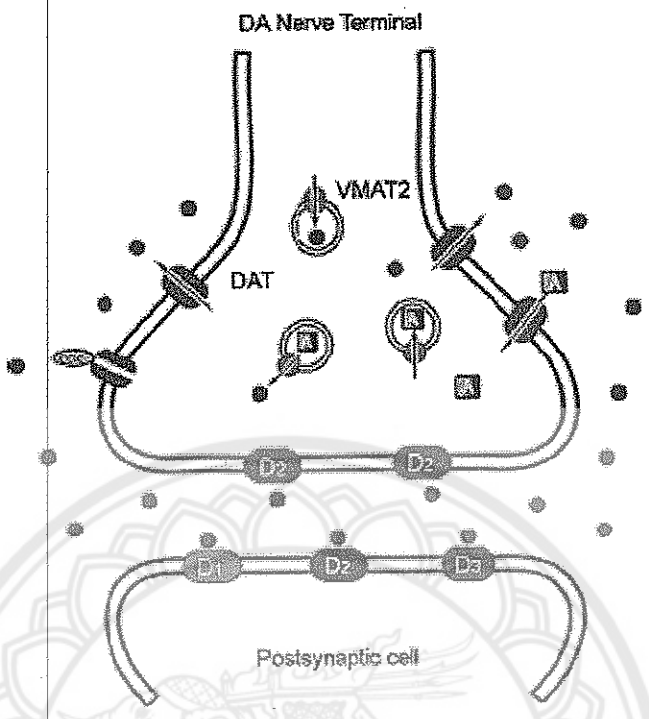

Figure 3 Schematic representation of the influence of amphetamine on dopaminergic transmission abnormalities by disrupting with transporter function. (Adapted from Howell and Kimmel, 2008). A, amphetamine; DAT, dopamine transporter; VMAT2, vesicular monoamine transporter 2; D1, D2, D3, dopamine receptor type 1, 2, 3

Recent study showed that increased levels of dopamine in nucleus accumbens (NAS) are critical in mediating rewarding effects or positive reinforcement for all drug abuse (Koob and Le Moal, 2001). Previous studies reported that methamphetamine administration leads to reductions in a number of dopaminergic axonal marker including dopamine content (O'Dell et al., 1991; Fukumura et al., 1998), the dopamine synthesizing enzymes (tyrosine hydroxylase (TH) (Fukumura et al., 1998; Cappon et al., 2000), the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Robinson, et al., 1990a; Robinson, et al., 1990b) dopamine transporter (DAT) (Eisch et al., 1992; McCann et al., 1998; Volkow et al., 2001b) and vesicular monoamine transporters type-2 (VMAT-2) (Guilarte 2003). Morphological studies demonstrated that a loss of dopaminergic axonal

markers is related to degeneration of dopaminergic nerve terminals and axons in brain regions such as striatum (Fukumura et al., 1998; Bowyer and Schmued, 2006) and nucleus accumbens (Broening et al., 1997). These studies provided strong evidence that neurochemical abnormalities in the dopaminergic system are implicated in methamphetamine dependence.

The effects of methamphetamine on serotonergic transmission

In addition to dopaminergic system, serotonergic system may be affected by methamphetamine administration. Methamphetamine administration in rats has been shown to produce long-term reduction in serotonin content (Friedman et al., 1998), serotonin synthesizing enzymes such as tryptophan hydroxylase (Gibb and Hotchkiss, 1980), serotonin metabolites (5-hydroxyindoleacetic acid (5-HIAA) (Robinson et al., 1990a) and serotonin transporter (Eisch et al., 1992). Moreover, methamphetamine has been reported to cause serotonergic nerve terminals degeneration (Axt and Molliver, 1991). The mechanism of methamphetamine damage to serotonergic nerve terminals is still unknown, while the release of dopamine is believed to be an intermediate step in the cause of serotonergic degeneration (Sonsalla et al., 1986; Johnson et al., 1987). The relationship between dopamine release and serotonergic nerve terminals degeneration is complex and may involve other neurotransmitter systems (Callahan et al., 2001). There is a study that found the degeneration of serotonergic nerve terminals in several brain regions. This study demonstrated that the frontal cortex and hippocampus were affected at lower dose of methamphetamine in comparison to the striatum and parietal cortex (Zhou et al. 1996).

The effects of methamphetamine on glutamatergic transmission

Methamphetamine is a dopamine agonist that also directly impact on dopaminergic system (Moore, 1997; Butcher et al., 1988). In drug addiction, the interaction between dopaminergic and glutamatergic systems is associated with rewarding, reinforcing or pleasurable experiences from abused drugs as well as stimulant drugs (Sidiropoulou et al., 2001; Reid and Hughes, 2006). The role of glutamate in addiction has been suggested to be involved in the activity of the

dopaminergic system (Gorelova and Yang, 1997). However, the mechanism of methamphetamine on glutamatergic system is unknown.

The effects of methamphetamine lead to alterations in levels of glutamate in several brain regions such as striatum (Nash and Yamamoto, 1992; Abekawa et al., 1994; Stephan and Yamamoto, 1994; Yamamoto et al, 1999; Mark, 2004), nucleus accumbens (Abekawa et al., 1994), prefrontal cortex (Staphan and Yamamoto, 1995; Shoblock, 2003) and hippocampus (Rocher and Gardier, 2001). Recent studies demonstrated that alterations in mRNA and protein levels for AMPA, NMDA and metabotropic glutamate receptor after withdrawal from chronic amphetamine exposure (Lu et al., 1997; Lu et al., 1999; Lu and Wolf, 1999). On the other hand, the effects of amphetamine on the glutamate transporter have been reported that both glial (GLT-1) and neuronal glutamate (EAAT3/EAAC1) transporter levels do not alter in rat midbrain, nucleus accumbens, striatum and prefrontal cortex (Sidiropoulou et al., 2001).

Glutamatergic system

Glutamate is an excitatory amino acid that acts as a major neurotransmitter in the central nervous system (Nestler, 2001). It has a role in normal synaptic transmission, synaptic plasticity and brain development, including learning and memory (Bliss and Collingridge, 1993; McEntee and Crook, 1993). A typical glutamatergic transmission represented as Figure 4.

A typical glutamatergic transmission is represented in Figure 4. Glutamate is synthesized in the presynaptic neuron, stored in synaptic vesicle, and released by exocytosis. After release, glutamate then acts at metabotropic or ionotropic receptors, and is removed from the synaptic cleft by diffusing away from the cleft or uptake in neurons and glial cells. This uptake process is carried out by plasma membrane glutamate transporter (Nestler, 2001). Glutamate which is taken up by cells may be used for metabolic processes (protein synthesis, energy metabolism, ammonia fixation) or be reused as transmitters that have many metabolic pathways. These pathways occur in both nerve terminals and astrocytes.

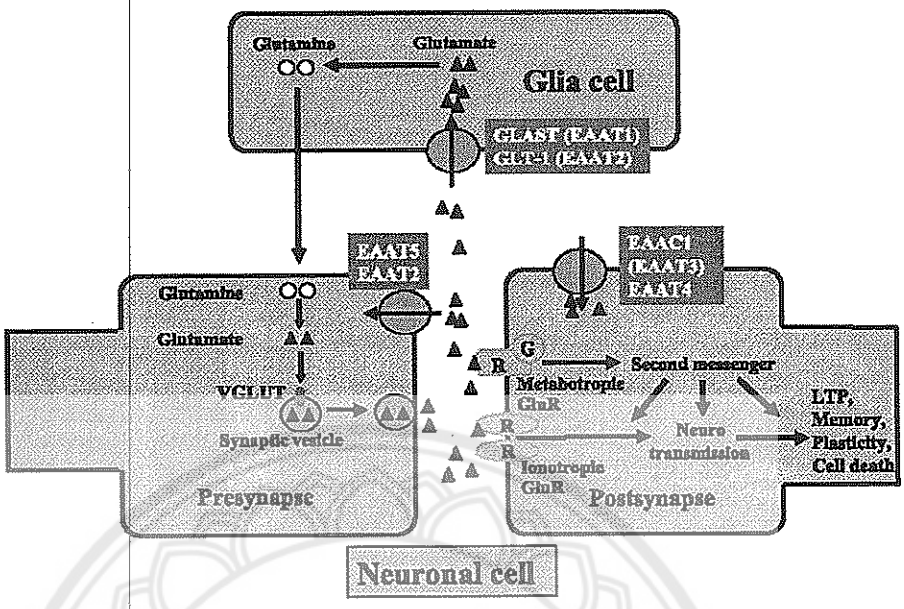

Figure 4 Schematic representation of the glutamate system (Adapted from Shigeri, 2004)

Glutamine (gln) or α-ketogutarate is used as a major precursor of glutamate in nerve terminals. In glutamatergic nerve terminals, glutamine is converted to glutamate (glut) using glutaminase enzyme, while α-ketogutarate is transaminated into glutamate by aminotransferase enzyme (Daikhin and Yadkoff, 2000). Additionally, aspartate (asp) is also converted to glutamate by transaminase (Nestler, 2001). Newly synthesized glutamate is packaged into synaptic vesicles for further release from nerve terminals in response to nerve impulses. In astrocytes, glutamate is taken up from the extracellular and it is then metabolized by glutamine synthase enzyme into glutamine or metabolized into α-ketogutarate by either glutamate oxaloacetate transaminase or glutamate dehydrogenase (Anderson and Swanson, 2000; Meldrum et al., 2000). Both glutamine and α-ketogutarate are then actively transported out of astrocytes and back into the pre-synaptic nerve terminals for subsequent re-synthesis of glutamate (Meldrum et al., 2000; Nestler, 2001). The brain contains large amounts of free glutamate (~10 mM), which is almost contained intracellularly and the highest concentration is found at nerve terminals (Shupliakov et al., 1992; Mathisen et al., 1992). In extracellular space, the concentration of glutamate is generally low (1-3 mM) except during impulse transmission (Clements et al., 1992; Nicholls, 1993).

Long-lasting high levels of glutamate at nerve terminals may produce overactivation of glutamate receptor, resulting neuronal excitotoxicity (Choi, 1988; Regan and Choi, 1991).

Glutamate receptor

Glutamate act on glutamate receptors that are concentrated in the postsynaptic or presynaptic complex of central synapses (Tovar and Westbrook, 2002). These receptors have been divided into two classes including the ionotropic (iGluRs), and the metabotropic glutamate receptors (mGluRs). The metabotropic glutamate receptors are coupled via G-proteins to second messenger systems. They are divided into three groups: group I (mGluR1, mGluR5), group II (mGluR2, mGluR3), and group III (mGluR4, mGluR6, mGluR7 and mGluR8). In contrast, the ionotropic glutamate receptors are ligand-gated ion channels that act as postsynaptic receptors to mediate the vast majority of excitatory neurotransmission in the brain. They are subdivided into three pharmarcologically distinct classes: the N-methyl-D-aspartate (NMDA) receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors and kainate receptors (Asztely and Gustafasson, 1996; Maren and Baudry, 1995). The ionotropic glutamate receptors are tetra-or pentameric ion channels formed by class-specific subunits. Each subunit has an extracellular N-terminal and intracellular carboxy terminus. Four hydrophobic domains (M1-M4) have been characterized, three of them (M1-M3) form transmembrane segments while M2 forms a loop within the membrane (Hollmann et al., 1994).

The N-methyl-D-aspartate receptors (NMDARs) play a central role at excitatory synapses where it has been implicated in multiple functions associated with synaptic plasticity (Wenthold et al., 2003) especially learning and memory (Harris et al., 1984; Morris et al., 1986). NMDA receptors are heteromeric protein complexes that form ligand-gated ion channels. They are tetra- or pentamers composed of different subunits including NMDAR1, NMDAR2 (A-D) or NMDAR3 (A-B) subunits which presented the specific binding site (Dingledine et al., 1999; Nishi et al., 2001). The glycine, co-agonist, binding-site locates on NMDAR1, while NMDAR2 and NMDAR3 subunits contain glutamate-binding site (Riedel et al., 2003). Functional

NMDA receptors are only formed by combination of at least one NMDAR1 subunit and various amounts of NMDAR2 subunits (Danysz and Parsons, 1995; Dingledine et al., 1999; Nishi et al., 2001).

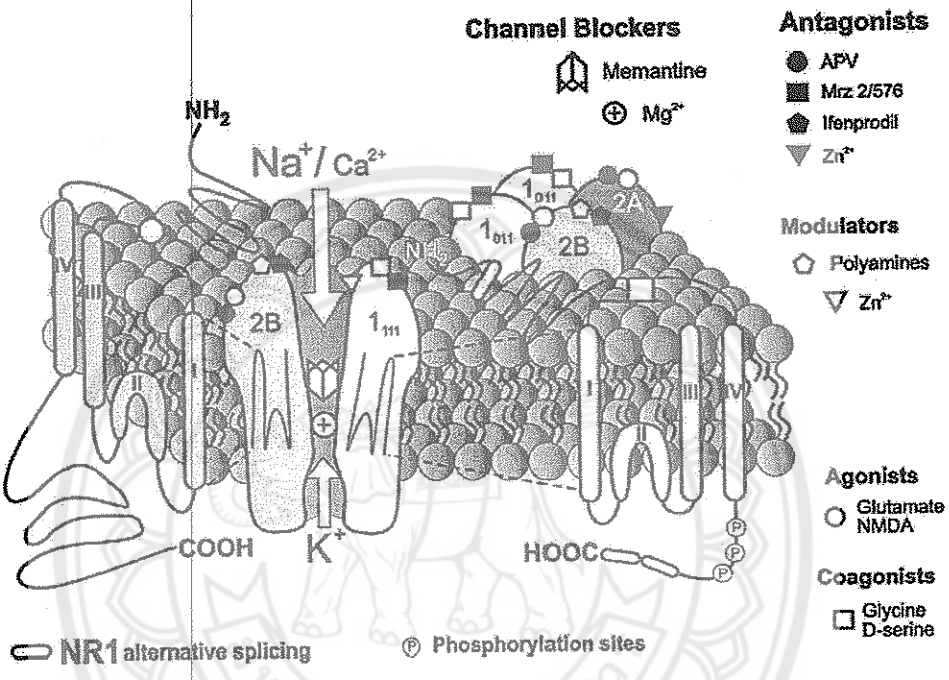

Figure 5 Schematic representation of NMDA receptors organization (Adapted from Danysz and Parsons, 1998)

The NMDA channel is permeable to cations including K^+, Na^+, and Ca^{2+}. At resting membrane potential, the NMDA receptors are inactivated by blocking of Mg^{2+} ion at channel pore. Activation of the NMDA receptor requires glutamate released from presynaptic neuron and depolarization of the postsynaptic membrane to relieve Mg^{2+} ion from the ion pore of the channel. This activation results in opening of the channel, leading to the flow of Na^+ and Ca^{2+} into the neuron and K^+ out of the neuron. Calcium influx through NMDA receptor is a critical role in synaptic plasticity and cellular mechanism for learning and memory (Danysz and Parsons, 1998; Riedel et al., 2003; Saal and Malenka, 2005).

The subunits of NMDA receptor have different physiological and pharmacological properties, and different distribution throughout the central nervous system. The highest levels of NMDAR1 mRNA in the adult rat and mouse CNS are found in the olfactory bulb, and the lowest levels are expressed in the spinal cord. Intermediate levels are discovered in frontal cortex, hippocampus, cerebellum, and whole brain (Benke et al., 1995). The expression of NMDAR2A, NMDAR2B, NMDAR2C, and NMDAR2D mRNA is found predominantly in hippocampus, forebrain, cerebellum, and brainstem, respectively (Monyer et al., 1992; McBain and Mayer, 1994). The distribution of NMDAR3A subunit is predominant in spinal cord, brainstem, hypothalamus, thalamus, hippocampus, and amygdala (Ciabarra and Stevarino, 1997).

Excessive stimulation of NMDA receptors, also known as glutamate excitotoxicity, can lead to neuronal cell death and may be a common final pathway in several pathological conditions including stroke, head injury, epilepsy (Rothman and Olney, 1987; Choi, 1988; Choi and Rothman, 1990) and in neurodegenerative diseases such as Huntington's disease (Young et al., 1988) and Alzheimer's disease (Ulas and Cotman, 1997). Recent studies also indicated a role for NMDA receptors in drug addiction. Accumulating data from experimental models and human brain studies suggest that NMDA receptor function upregulates after chronic ethanol administration (Hu and Ticku, 1995; Krystal et al., 2003a; Reid and Lingford-Hughes, 2006). Previous study also demonstrated that the activity of the glutamate system is functionally decreased in the hippocampus of the methamphetamine sensitization rats, whereas the glutamate system in the striatum of methamphetamine sensitization rats shows adaptive and functional changes in the NMDA receptors (NMDAR1, NMDAR2A and NMDAR2B) in response to an increase of the glutamate release (Yamamoto et al., 1999). NMDAR2A mutant mice has exhibited a malfunction of NMDA receptors, as evidenced by a reduction of [3H]MK-801 binding in an autoradiographic receptor binding assay. NMDAR2A receptor mutant mice showed an attenuation of sensitization by repeated treatment with phencyclidine (PCP) and methamphetamine-induced hyperlocomotion (Reynolds et al., 1998). Moreover, the development of morphine induced analgesic tolerance and naloxone precipitated morphine withdrawal symptoms are also attenuated (Miyamoto et al., 2004).

Furthermore, withdrawal from repeated amphetamine administration reduces NMDAR1 expression in the rat substantia nigra, nucleus accumbens and medial prefrontal cortex (Lu et al., 1999). However, the cellular and molecular mechanisms underlying methamphetamine dependence are not fully understood.

Glutamate transporter

Large amounts of glutamate are presented in the brain tissue where this neurotransmitter acts as the principle excitatory neurotransmitter. Excessive amounts of extracellular glutamate can induce overactivation of glutamate/NMDA receptor, leading to neuronal damage or neuronal death (Grant et al., 1997; Grimwood et al., 1996; Hartley et al., 1993; Hyrc et al., 1997). Although there are no enzymes to metabolize the extracellular glutamate, while the simple diffusion of glutamate only occurs at synapse with small diameters. Thus, the clearance of glutamate from the synaptic cleft majorly depends on glutamate transporters (Danbolt, 2001). Glutamate transporters are believed to prevent excitotoxicity of glutamate by maintenance of low and non-toxic concentrations of glutamate (Rothstein et al., 1996., Tanaka et al., 1997), and help to terminate the excitatory signal (Mennerick and Zorumski, 1994., Otis et al., 1997). Additionally, glutamate transporters can also supply the precursors for glutamate in central nervous system (Palacin et al., 1998; Danbolt, 2001). Glutamate transporter can be classified into two types as the vesicular membrane glutamate transporters and the plasma membrane glutamate transporters according to their electrochemical gradient and site of action (Figure 6). In glutamatergic nerve terminals, vesicular membrane glutamate transporters: VGLUT1, VGLUT2, and VGLUT3 collect glutamate into synaptic vesicles for further release into synaptic cleft. The vesicular uptake is dependent of a proton gradient which is created by hydrolysing adenosine triphosphate (ATP), and/or the membrane potential. The plasma membrane glutamate transporters present in the plasma membrane of both glial cells and neurons. In contrast to vesicular membrane glutamate transporters, the plasma membrane glutamate transporters are dependent on electrochemical gradient of sodium ion (Masson et al., 1999; Danbolt, 2001).

Plasma membrane glutamate transporters (also known as EAATs; excitatory amino acid transporters) are responsible for the high-affinity uptake of glutamate by

neurons and glial cells at the level of their plasma membrane. These membrane-bound proteins are all dependent on the Na+ intracellular/extracellular gradient for their activity. Five mammalian EAATs isoforms have been cloned and characterized electrophysiologically and pharmacologically: EAAT1 (GLAST) (Arriza et al., 1994; Kawakami et al., 1994), EAAT2 (GLT-1) (Arriza et al., 1994), EAAT3 (EAAC1) (Kanai and Hediger, 1992; Arriza et al., 1994), EAAT4 (Fairman et al., 1995) and EAAT5 (Arriza et al., 1997). EAAT family members display ~50-55% amino acid sequence identity and almost identical hydrophobicity pattern, suggesting that each transporter exhibits its functional properties on the basis of similar characteristics. They each contain 10 hydrophobic domains, with both the N-and C-termini in the cytoplasmic side (Wahle and Stoffel, 1996).

Glutamate transporter EAAT1 (glutamate and aspartate transporter, GLAST, in rodents) is expressed in astrocytes and found mainly in the molecular layer of the cerebellar cortex (Storck et al., 1992; Chaudhry et al., 1995; Schmitt et al., 1997) as well as in the heart, skeletal muscle, and placenta (Schmitt et al., 1997; Storck et al., 1992). EAAT2 (glutamate transporter 1, GLT-1, in rodents) is expressed in astrocytes and highly expressed in the forebrain (Pines et al., 1992). EAAT3 (excitatory amino acid carrier 1, EAAC1, in rodents) is the neuronal glutamate transporter that is the most widely distributed in brain including the hippocampus, cerebral cortex, red nucleus, substantia nigra, and striatum, and is also presented in the peripheral tissue (intestine, kidney, liver, and heart) (Kanai and Hediger, 1992; Rothstein et al., 1994). EAAT3 is found in both glutamatergic (such as granule cells in the dentate gyrus and pyramidal cells in the hippocampus and cerebral cortex) and GABAergic (such as Purkinje cells in cerebellum and medium spiny neurons in striatum) system. EAAT4 is neuronal glutamate transporter that is heavily enriched in cerebellar Purkinje cells (Fairman et al., 1995; Gegelashvili and Schousboe, 1997; Tanaka, 2000), and EAAT5 is expressed primarily in the retina (Gegelashvili and Schousboe, 1997; Arriza et al.,1994, 1997), however, very low level of EAAT5 expression has been observed in the brain, heart, and muscle (Arriza et al., 1997).

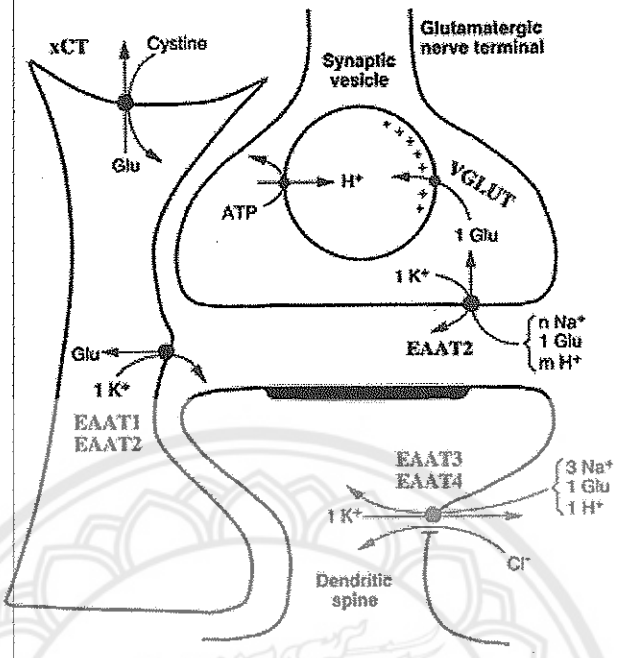

Figure 6 Schematic representation of the glutamate transporters at glutamatergic synapses. (Adapted from Danbolt, 2001)

Glutamate transporters, EAAT2 (GLT-1) EAAT1 (GLAST) and EAAT3 (EAAC1) and EAAT4, are located on the plasma membranes of neurons and/ or glial cells throughout the central nervous system. These transporters play functions as both a glutamate transports and a glutamate-gated chloride channels (Eskandari et al., 2000; Danbolt, 2001). EAATs transport glutamate across the membrane using translocation of three Na^+, one proton (H^+) and one negative charge of glutamate ion into the cells together with exchange of one K^+ to the exterior (Gegelashvili and Schousboe, 1997; Dalbolt, 2001). Additionally, some of glutamate transporters, EAAT3 (EAAC1) and EAAT4, are associated with Cl^- channel activity. Activation of these transporters triggers the Cl^- conductance which is occurred independently with the transport process of glutamate (Danbolt, 2001). The glutamate transporter in glutamatergic nerve terminals has still not been molecularly identified and the exact stoichiometry of the transporter cycle is not known.

Glutamate transporters are regulated in various pathological states, many of which are thought to involve excitotoxicity and oxidative stress. For example, studies of postmortem human tissue showed that mRNA levels of EAAT3 are reduced in the several areas including the dentate gyrus, subiculum and CA1 subfield of the hippocampus (Bachus et al., 1996) and striatum (McCullumsmith and Meador-Woodruff, 2002). These results suggest that changes in striatal glutamate transporter mRNA expression are restricted to neuronal EAATs and extend the body of evidence implicating abnormal glutamate neurotransmission in schizophrenia. On the other hand, expression of EAAT3 is increased in the hippocampus of epileptic patients (Mathern et al, 1999) and rat with kindling-induced epileptogenesis (Ghijsen et al, 1999; Miller et al., 1997). However, no evidence has been demonstrated whether methamphetamine administration affect the expression of neuronal glutamate transporter (EAAT3/EAAC1).

Learning and memory

Experimental data have provided evidences that learning and memory are based on changes in synaptic plasticity. Synaptic plasticity involves long-lasting changes in the efficacy or strength of synaptic transmission at excitatory synapses. Long-term potentiation (LTP) and long-term depression (LTD), forms of synaptic plasticity, have been used as models of synaptic plasticity, and have been implicated in critical role in several forms of learning and memory. The mechanisms of LTP and LTD related with activation of glutamate receptor that flux ions into the cells, especially AMPA and NMDA receptors (Riedel et al., 2003; Wolf et al., 2004; Saal and Malenka, 2005), as depicted in figure 6. Calcium entering the postsynaptic cell through NMDA receptors can induce LTP and LTD, and the different amount of calcium can trigger different intracellular second messenger systems which in turn increase LTP or LTD synaptic strength (Figure 7). A large amount of calcium ions flow into postsynaptic neuron via NMDA receptors leading to activation of protein kinases which adds phosphate groups to AMPA receptor and consequently insertion of additional AMPA receptors at the synaptic plasma membrane. This process leads to increase synaptic strength also known as LTP. In contrast to LTP, LTD requires a smaller number of calcium ions than those of LTP for removal of AMPA receptors

from synapse. The results of decreased calcium levels activate protein phosphatase which removes phosphate groups from AMPA receptor leading to decrease synaptic strength (Malenka and Nicoll, 1999; Yang Lu et al., 2001; Malinow and Malenka, 2002; Malenka and Bear, 2004).

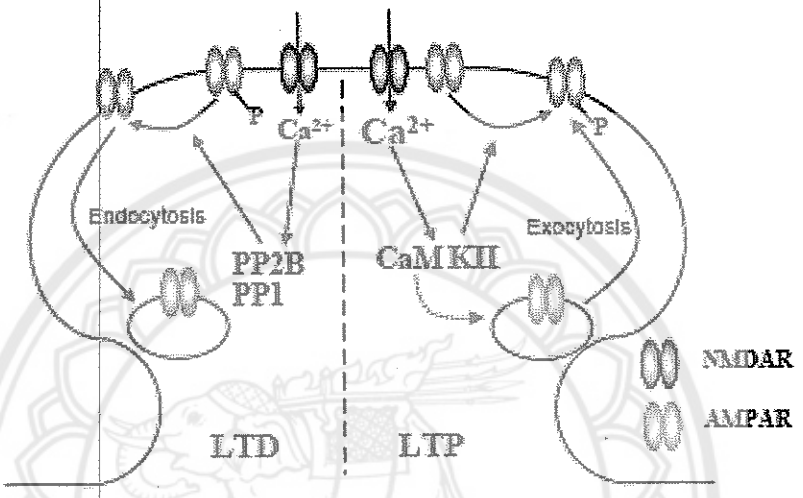

Figure 7 Schematic representation of mechanisms of LTP and LTD (Adapted from Saal and Malenka, 2005). LTP, long-term potentiation; LTD, long-term depression; AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; NMDAR, N-methyl-D-aspartate receptor; Ca^{2+}; calcium ion; CaMKII, calcium/calmodulin-dependent protein kinase type II; PP1, protein phosphatase 1; PP2B, protein phosphatase 2B; P, phosphate groups

Excitotoxicity

Excitotoxicity refers to the toxic action of glutamate on neurons. Under certain condition such as duration time of trauma, ischemia, hypoglycemia and status epilepticus, excessive amounts of glutamate are released and this can trigger neuronal damage and death (Snider and Choi, 2002). NMDA-R activation results in Ca^{++}-influx in postsynaptic regions at times of glutamate release. The neurotoxic effects of glutamate may derive from an increased formation of hydroxyl radicals from excessive activation of the NMDA receptors and downstream enzymes such as nitric

oxide synthase (NOS) and phospholipase A_2 (PLA_2) (Lancelot et al., 1998) (Figure 8). Ca^{++}-influx through the NMDA-R is related to activation of NOS (Schulz et al., 1995; Ueda et al., 2007) and PLA_2 (Ueda et al, 2007) followed by generation of nitric oxide and superoxide anion, respectively. Reactions between nitric oxide and superoxide anions resulted in the formation of peroxynitrite anion, which produces hydroxyl radicals (Lafon Cazal et al., 1993). Hydroxyl radicals abstract hydrogen from neural membrane polyunsaturated lipid acid and initiates lipid peroxidation leading to necrosis.

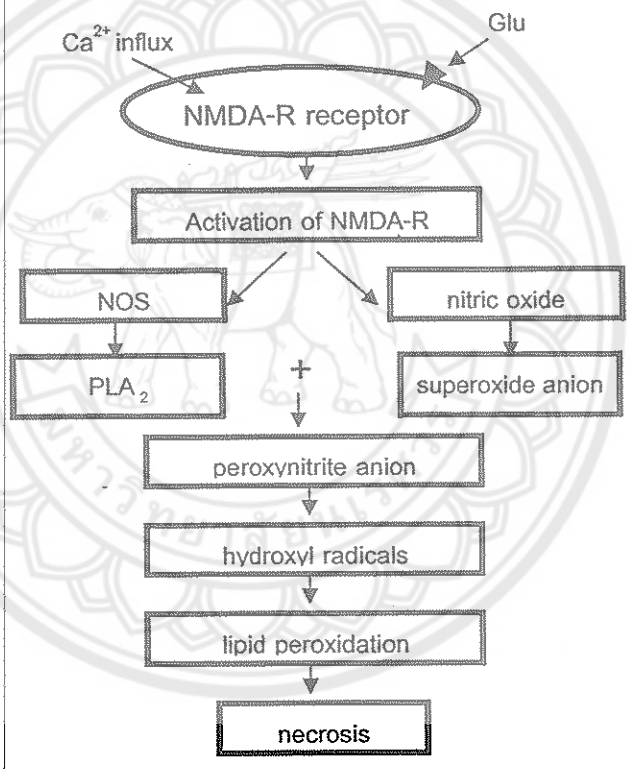

Figure 8 Diagrammatic representation for possibility of the regulation of glutamate is associated with the excitatory neurotransmission in the brain through NMDA-R activation (Adapted from Lancelot et al., 1998)

Degenerative processes in the central nervous system occur through necrosis or apoptosis (McConkey et al., 1996; Vermes and Haanen, 1994). Necrosis often follows severe insults and in early membrane damage, cellular swelling, spilling of intracellular contents and inflammatory responses to damage. In contrast to necrosis, apoptosis is usually caused by mild to moderate toxic injuries, participates in the normal process of tissue regulation and results in DNA fragmentation, clumping of chromatin, cell shrinkage and vacuolar formation, but no inflammatory changes in surrounding cells. Both apoptosis and necrosis can be caused by the same agents depending on the doses used (Bonfoco et al., 1995).

Apoptosis is generally a feature of programmed cell death. Using terminal deoxynucleotidyl transferase (TdT)-mediated dNTP nick end labeling (TUNEL) histochemistry, it was found that methamphetamine exposure can cause DNA strand breaks, chromatin condensation and nuclear fragmentation in several brain regions (Deng et al., 2001). This finding implicates apoptosis as one of molecular mechanisms involved in the influence of methamphetamine. Cell death has been indicated to mediate by the activation of a number of genes. It has been reported that methamphetamine induces an increase of p53 mRNA (Tsao et al., 1998) and p53 protein (Hirata and cadet, 1997). In p53 knockout mice the methamphetamine-induced neurotoxicity in striatal dopaminergic terminals was reduced (Hirata and cadet, 1997). In addiontion to p53 gene, reactive species (O_2^-, H_2O_2, OH and NO) have been proposed in the neurotoxicity induced by methamphetamine (Figure 9) (Tsao et al., 1998; Frost and Cadet, 2000).

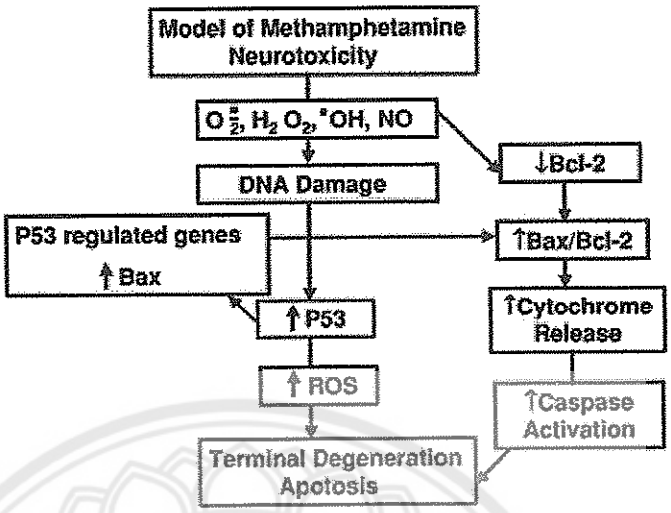

Figure 9 Diagrammatic representation of molecular pathways of methamphetamine neurotoxicity. The model takes into consideration in recent data that have implicated reactive oxygen species, p53 and the bcl2 family of cell death genes in the mechanism of methamphetamine-induced neurodegeneration (Adapted from Frost and Cadet, 2000)

Abnormalities in brain regions related with methamphetamine dependence

There have been many studies indicating the abnormalities of several brain regions in methamphetamine dependence. Although mechanisms of drug dependence have been believed to involve many regions such as frontal cortex, limbic system, and basal ganglia. Some of these brain regions such as frontal cortex, hippocampal formation, and striatum have also been reported to play an important role in mechanisms of drug dependence, as depicted in Figure 2 (Tzschentke and Schmidt, 2003; Kelley, 2004; Saal and Malenka, 2005; Reid, 2006), and in circuits involved in memory and drug dependence (Kelley, 2004). Moreover, several studies showed that neurotoxicity of methamphetamine induced nerve terminal damage or apoptosis in frontal cortex (Ryan et al., 1990; Deng et al., 2001), hippocampal formation (Schmued and Bowyer, 1997; Eisch et al., 1998; Deng, 2001), and striatum (Broening et al., 1997; Fukumura et al., 1998; Deng et al., 2001; Xu et al., 2005; Bowyer and Schmued, 2006). These studies have led to the hypothesis that neurotoxic methamphetamine may

cause learning and memory impairments. Thus, this section points out to the specific brain regions particularly hippocampal formation, frontal cortex, and striatum in methamphetamine dependence.

The hippocampal formation

The hippocampal formation has long been recognized to play a critical role in learning and memory which is associated with synaptic plasticity. Many studies pointed out to a role of hippocampal formation in drug addiction (Tzschentke and Schmidt, 2003; Kelley, 2004; Saal and Malenka, 2005; Reid, 2006). Mechanisms of addiction and natural learning and memory processes are common in that glutamatergic transmission and neuronal plasticity are involved (Wolf, 2002; Kelley, 2004; Robinson and Kolb, 2004; Wolf et al., 2004; Saal and Malenka, 2005). However, the detailed mechanisms of those processes remain to be elucidated.

The hippocampal formation, a part of the brain located under the temporal lobe, consists of three components including dentate gyrus, hippocampus (ammon's horn: field CA1-3) and subiculum (Figure 10) (Nester, 2001; Witter, 2003). The hippocampal formation connects to different bran regions such as unimodal and polymodal association area (frontal, temporal and parietal lobes), parahippocampal, perirhinal, and entorrhinal cortices (Witter, 2003). The major cortical input to subfields of the hippocampal formation arises from the entorhinal cortex, whereas the major output structures, subiculum and CA1 subfield of hippocampus, send a dense direct projection to prefrontal cortex and/or send reciprocal projection to entorhinal cortex which then send information back to hippocampal formation. Within hippocampal formation, there are intrahippocampal connections form a trisynaptic loop, which is involved in long-term potentiation induced memory. First, the perforant pathway, which project from the entorhinal cortex to granaule cells of the dentate gyrus. Second, the mossy fiber pathway, which consists of the axons of the granule cells of the dentate gyrus and projects to the pyramidal cells in CA3 subfield of the hippocampus. This terminal fibers release glutamate, which binds to both NMDA and non-NMDA receptors on the target pyramidal cells. Third, the Schaffer collateral pathway which contains the axonal pyramidal cells in the CA3 and terminates on the pyramidal cells in the CA1 region. These fiber terminals also use glutamate as

transmitter alike the mossy fiber, but LTP produced by Schaffer collateral pathway requires only activation of the NMDA receptor, as represented in Figure 11 (Nester, 2001; Witter, 2003).

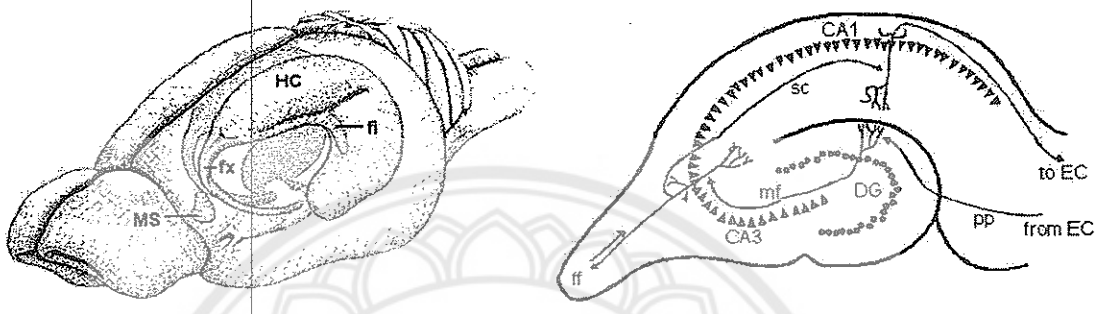

Figure 10 Schematic representation of hippocamal formation. A three-dimensional organisation of the septo-hippocampal system in the rat brain (A) and the trisynaptic loop of the hippocampus (B), the filled triangles represent the pyramidal cell layer (CA1 and CA3) and the filled circles represent the granular cell layer of the dentate gyrus (Adapted from Amaral and Witter 1995). Fx, fornix; fi, fimbria; HC, hippocampus; MS, medial septum; EC, entorhinal cortex; DG, dentate gyrus; pp, perforant pathway; mf, mossy fibers; sc, Schaffer collaterals; ff, fimbria fornix

Since, it is well known that hippocampal formation plays an important role in learning and memory functions. Abnormalities in this region may lead to impairments of learning and memory functions. Behavioral studies have been demonstrated that hippocampal excitotoxic lesion in the rat causes learning and memory impairments (Moser et al., 1995; Ferbinteanu et al., 2003; Zhang, 2004). Recently, clinical studies suggested that chronic use of methamphetamine induces long-term learning and memory impairments (Simon et al., 2002; Kamei et al., 2005). Similarly, recent reports demonstrated that animal exposed to methamphetamine show impaired behavioral task which is involved in learning and memory (Bisagno et al., 2002;

Belcher et al., 2006). However, less is known about methamphetamine used on learning and memory functions, eventhough few studies have found that methamphetamine-induced neuronal cell death widely throughout the brain including hippocampus (Eisch et al., 1998; Deng, 2001) and hippocampal remnants (Schmued and Bowyer, 1997).

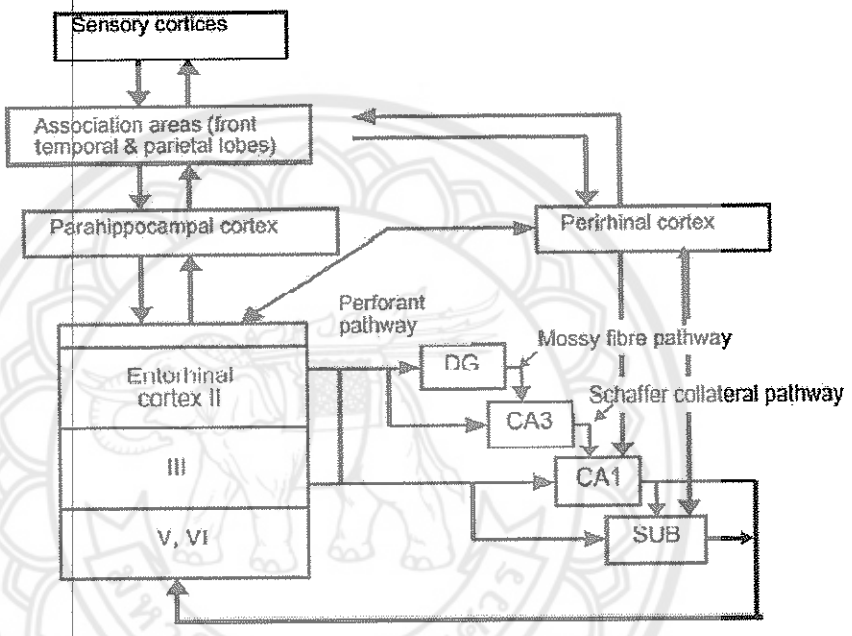

Figure 11 Diagramatic representation of the connections of hippocampal formation (Adapted from O'Mara, 2006)

The frontal cortex

The frontal lobes represent a large area of the brain. The function of the frontal areas is to modulate and control motor function, emotion, attention and cognitive activity, including learning and memory (Witter, 2003; Poldrack and Rodriguez, 2004). The frontal cortex has numerous interconnections with other parts of the brain. The rat prefrontal cortex (PFC) receives a direct projection from hippocampus (Jay et al., 1989). The CA1, which exhibits a long-lasting NMDA receptor-dependent form of LTP, and the subiculum have been reported to give a massive projections to PFC neurons (Figure 12) (Jay and Witter, 1991; Jay et al., 1996; Witter, 2003). These lead to a suggestion that the connection between the

hippocampus and prefrontal cortex play a crucial role in learning and memory (Witter, 2003).

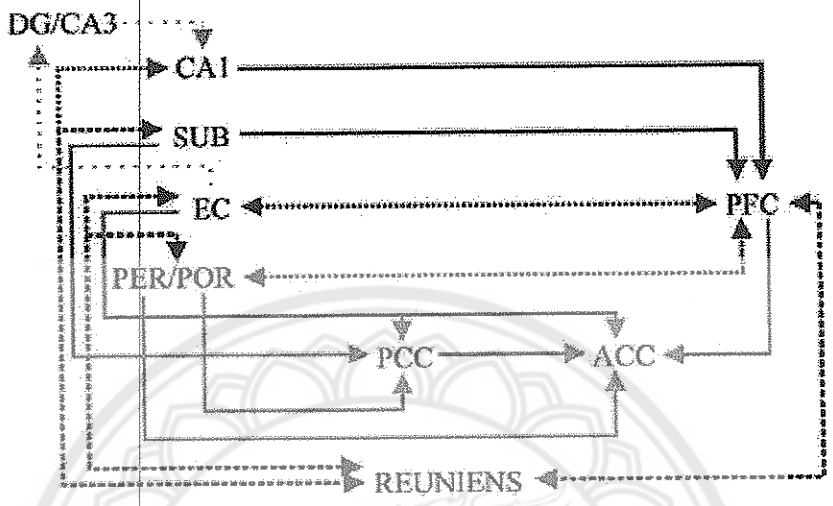

Figure 12 Schematic representation of connection between hippocampus and prefrontal cortex (Adapted from Witter, 2003)

The alterations in the structure and function in the frontal cortex were known to underlie abnormal behaviors. The brain-imaging studies have been indicated that frontal cortex may also be involved in human memory formation (Buckner, 1999). Moreover, a recent study with functional magnetic resonance imaging showed that dysfunction in the prefrontal cortex of methamphetamine abusers is related to cognitive deficits (Paulus et al., 2002). However, the mechanism underlying of methamphetamine exposure in frontal cortex remains unclear but there is a suggestion that abnormalities in frontal cortex could be related to psychostimulant-induced cognitive impairments (Crombag et al., 2005).

Severals studies established a key role for the PFC in neuronal circuits that is involved in dependent behaviors (Jantseh and Taylor, 1999) and reward-related learning (Cardinal et al., 2002). Furthermore, more recent studies pointed out to a role of glutamate in a drug dependence pathway (Wolf, 2002; Tzschentke and Schmidt, 2003; Kelley, 2004; Wolf et al., 2004; Saal and Malenka, 2005). In vivo microdialysis study on rats, methamphetamine has been reported to produce an elevation of extracellular glutamate concentrations in prefrontal cortex (Shoblock et al., 2003).

Histological and ultrastructural studies demonstrated that amphetamine causes neuronal degeneration in rat frontal cortex (Ryan et al., 1990). Moreover, terminal deoxynucleotidyl transferase (TdT)-mediated dNTP nick end labeling (TUNEL) study in methamphetamine treated-rat has found neuronal death in frontal cortex (Deng et al., 2001).

The striatum

The striatum, a major part of the basal ganglia system (Figure 13), is a subcortical region of the brain including the ventral (nucleus accumbens) and dorsal (caudate and putamen) striatum. The striatum is important for initiation, production and sequencing behavior. The major neuronal type is the GABAergic medium spiny neuron which received the major synaptic input both glutamatergic and dopaminergic pathways from several parts of brain, as depicted in Figure 13. Firstly, glutamatergic fibers arise from limbic and cortical regions, such as prefrontal cortex, hippocampus, and amygdala to ventral striatum, while, dopaminergic input to these regions arise primarily from ventral tegmental area. Secondly, medium spiny neurons in dorsal striatum are innervated by glutmatergic projections which are originated in cortex and thalamus, and dopaminergic projections which are derived from substantia nigra pars compacta. The output of striatum are inhibitory signals which project to pallidum and substantia nigra pars reticulata and in turn to be the major output from the basal ganglia and end to cerebral cortex (Alexander et al., 1990; Smith et al., 1998; Swanson, 2000).

The striatum has been studied extensively in animal models of drug dependence. Many studies have reported that striatum is an important regions that is involved in the neuronal circuitry of drug dependence (Wolf, 2002; Tzschentke and Schmidt, 2003; Kelley, 2004; Wolf et al., 2004; Saal and Malenka, 2005). Moreover, striatum seem to be indicated as a target area of drug of abuse responsible for the expression of behavioral sensitization (Tzschentke and Schmidt, 2003).

Adaptations in glutamate system after methamphetamine exposure have also been studied in striatum. More recent studies demonstrated that methamphetamine administration can induce an elevation of the extracellular glutamate concentration in striatum (Deng and Cadet, 2000; Deng et al., 2001; Xu et al., 2005; Zhu, 2006). A

reduction in presynaptic glutamate immunoreactivity has been observed within motor cortex and striatum following methamphetamine treatment (Bowyer et al., 1993; Burrows et al., 1997). In addition, a loss of NMDA receptors has been found after one week of discontinuous administration of methamphetamine (Eisch et al., 1996). Yamamoto and colleagues (1999) demonstrated a reduction in NMDAR1 2A and 2B protein levels in the striatum. Chronic amphetamine treatment has been shown to increase in the expression mGluR1 in striatum, but decrease in expression mGluR1 (Mao and Wang, 2001). Moreover, an elevation of vesicular glutamate transporter 1 (VGluT1) levels (Mark et al., 2007) and the plasma membrane glutamate transporter 2 (EAAT2) (Shiri et al., 1996) have been reported in the striatum after repeated methamphetamine administration. These observations suggeste that methamphetamine may disturb glutamatergic transmission in the striatum. However, mechanism of methamphetamine on glutamatergic transmission is still unknown.

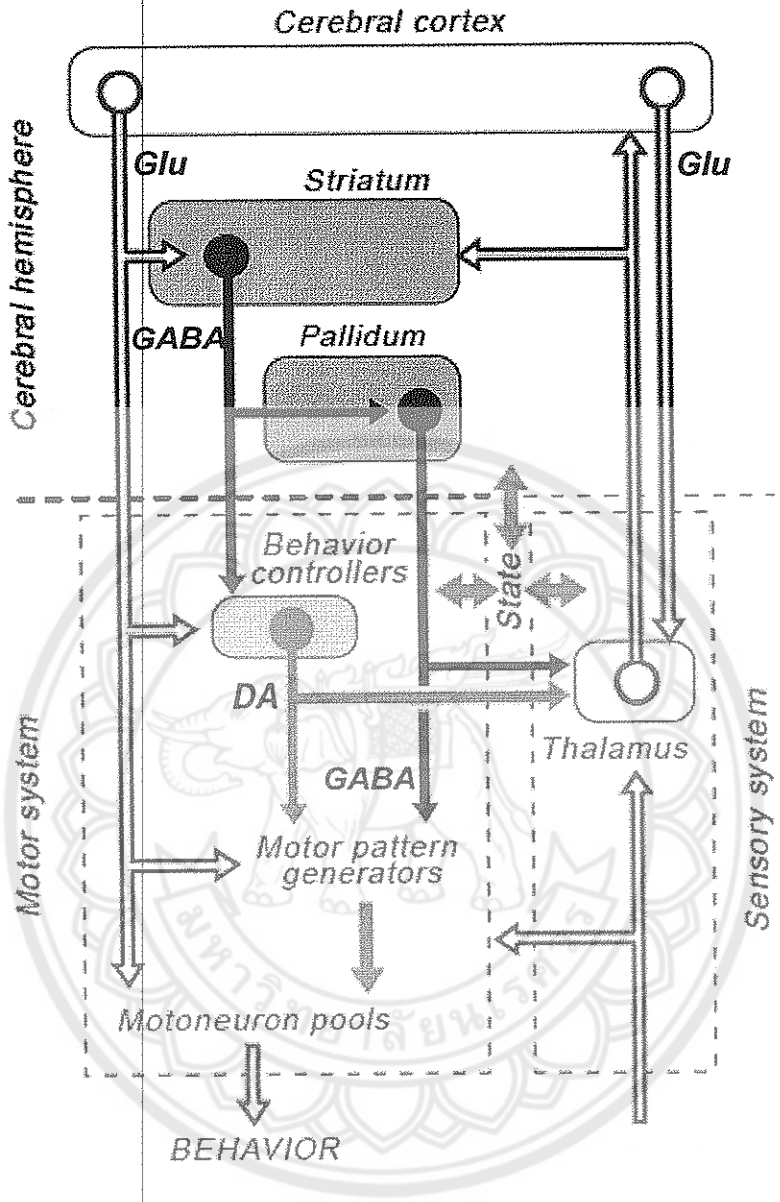

Figure 13 **Diagramatic representation of the complex circuitry between the cortex, striatum, pallidum and thalamus (Adapted from Swanson, 2000). The different color line is indicated in specific neurotransmitter involved in their pathway. The white, gray and black lines represent glutamate (Glu), dopamine (DA) and GABA, respectively**

Burrows and Yamamoto (2000) presented the idea of increase striatal glutamate release indirectly via activation of the basal ganglia output pathways. They found a correlation between an in crease of extracellular glutamate levels in cerebral cortex and an elevation of glutamate levels in striatum. These alterations can be speculated that activation of the corticostriatal pathway may result from a disinhibition of thalamocortical pathway. This is consistent with the observation that methamphetamine increases dopamine receptor (D1)-mediated striatonigral GABAergic transmission which in turn activates GABA-A receptor in substantia nigra, leading to a decrease in GABAergic nigrothalamic activity and subsequent increase in corticostriatal glutamate release and thereby long-term depleting of striatal dopamine (Mark et al., 2004) (Figure 14).

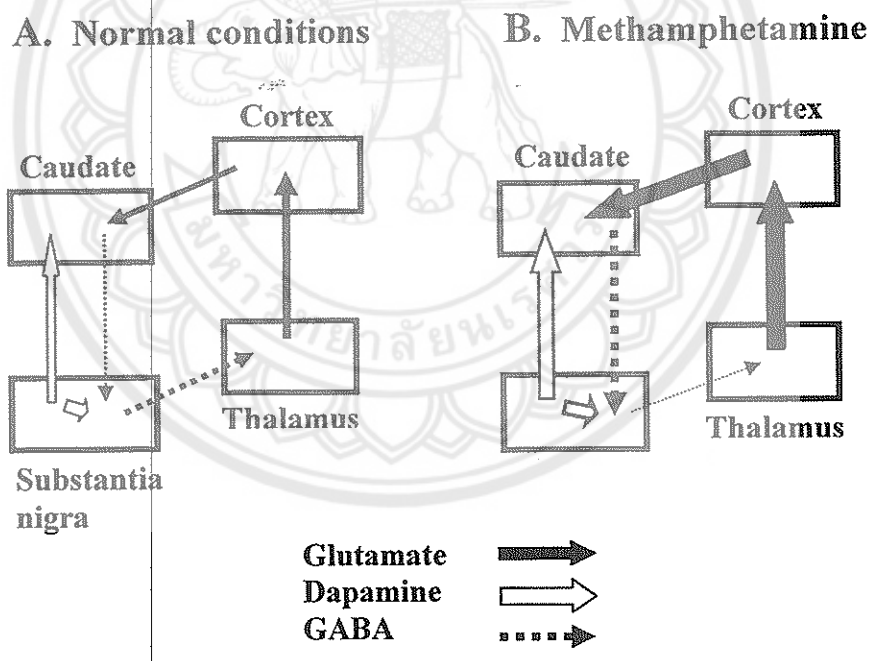

Figure 14 Diagrammatic representation of hypothetical model of METH-induced striatal glutamate release (Adapted from Mark et al., 2004)

Increased extracellular glutamate levels following methamphetamine exposure have been found to mediate excitotoxicity, as discussed above, in several brain regions as well as the striatum. Recent studies showed that methamphetamine

administration leads to long-lasting reductions in nerve terminal markers in striatum (Robinson, et al., 1990a; Robinson, et al., 1990b; Eisch et al., 1992; Cappon et al., 2000; Guilarte 2003). Furthermore, morphological studies have found a loss of nerve terminal markers associated which are ith destruction of axons and axon terminals in striatum (Broening et al., 1997; Fukumura et al., 1998; Bowyer and Schmued, 2006). Using the terminal deoxynucleotidyl transferase (TdT)-mediated dNTP nick end labeling (TUNEL), Deng et al. (2001) found neuronal cell death in the striatum after single high dose of methamphetamine treatment. Moreover, it has also been reported that methamphetamine induces both striatal apoptosis and DA-terminal damage related to a depletion of DA-terminal markers (Xu et al., 2005).